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About TERI

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

The Bioresources and Biotechnology Division

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

The Mycorrhiza Network and the Centre for Mycorrhizal Culture Collection

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

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

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

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Effect of edaphic and climatic factors on development of mycorrhiza in tree nurseries (part III): effect of soil toxicity

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Many unfavourable edaphic factors may influence development of mycorrhiza in tree nurseries. These include toxicity due to metals, release of allelopathic compounds in soil, high salt concentrations in soil, etc.

Toxicity due to metals

Metal toxicity in soil may be induced by discharge of sewage or industrial pollutants into the soil or due to the presence of excessive quantities of metals in certain categories of soils. Information on the effect of heavy metal pollution on mycorrhiza was given in an earlier issue of Mycorrhiza News (Sujan Singh 1996). This issue gives the work done on experimental evidence of the effect of metals on mycorrhiza.

Various physico-chemical reactions in the soil determine the availability of metals. Of specific importance are the adsorption of metals onto soil particles, competition of the metals present in the soil, and interaction of biological factors in the rhizosphere. Increased tolerance of mycorrhizal plants to toxic metal concentrations in the soil makes mycorrhiza significant (Schepp, Dehn, and Sticher 1987).

Aluminium toxicity

Effect of mycorrhiza on aluminium toxicity in plants

Studies were conducted at the Departmento de Genetica Escola, Superior Agricultura Luiz de Queiroz University, Sao Paulo, Brazil, on aluminium-tolerant and -intolerant cultivars of *Leucaena leucocephala*. Height, shoot dry weight, and nutrient accumulation increased in both cultivars, 65 days after the seedlings were transplanted in the pots with washed, sterilized sand, irrigated with nutrient solution containing 0 and 9 ppm Al (aluminium) and inoculated with *Glomus leptotichum*; but the increases were greater in Al-tolerant cultivars. There were no significant differences between seedlings given different Al treatments (Melo, Silveira, and Maluf 1987).

In studies conducted at the Department of Natural Resources and the Boyce–Thompson Institute for Plant Research, Cornell University, USA, pitch pine (*Pinus rigida*) seedlings were grown with or without *Pisolithus tinctorius* and exposed to low levels of Al on sand culture. At 50 μM, Al reduced non-mycorrhizal seedling growth and increased foliar concentrations but did not alter photosynthetic gas exchange or other aspects of seedling nutrition. Non-mycorrhizal seedlings exposed to 200 μM Al exhibited decreased growth, increased transpiration rates, decreased water use efficiency, increased foliar aluminium and sodium, and reduced foliar phosphorus. Mycorrhizal seedlings when exposed to Al exhibited unaltered growth, physiological functions, and ionic relations. The studies suggested that the fungal symbiont modulated ionic relations in the rhizosphere which reduced Al–phosphorus precipitation reactions, Al uptake, and subsequent Al exposure of root and shoot tissue (Cumming and Weinstein 1990a).

Studies were conducted with the seedlings of birch, pine, and spruce at the School of Biological Sciences, University of Birmingham, Birmingham, Great Britain. Mycorrhizal associations with species of *Amanita*, *Paxillus*, *Pisolithus*, *Rhizopogon*, *Scleroderma*, and *Suillus* decreased the toxicity of zinc, copper, nickel, and aluminium. Except with low levels of treatments, the presence of mycorrhiza decreases metal accumulation in

* Compiled from TERI database – Riza

shoots. Concentration in roots is little affected but large amount of metal may be found in extramatrical hyphae (Wilkins 1991).

In studies conducted at the Department of Microbial Ecology, University of Lund, Helgonavagen, Sweden, intact ectomycorrhizal systems containing *Fagus sylvatica*, *Betula pendula*, or *Pinus contorta* plants infected with either *Paxillus involutus* or *Laccaria bicolor* and grown in either peat microcosms or semihydroponic culture showed that elevated concentrations of Al decreased the yield of all plant species and the potassium concentration in roots at high levels. The interactions involving mycorrhizal infection were strongest in *P. contorta*. Yield of mycorrhizal pine plants was largely unaffected by raised Al although mycorrhizal root morphology was altered in peat microcosm and phosphorus and magnesium concentrations significantly increased in the mycorrhizal roots (Finlay, Kottke, and Soderstrom 1993).

Studies were conducted on *Pinus sylvestris* seedlings at the Polish Academy Science Institute, Dendrol, Parkowa, Kornik, Poland. Seedlings were grown on semisterilized peat parlite medium with aluminium ion (Al^{3+}) concentration of 4.0 mM and inoculated with Al-tolerant strain of *Suillus luteus* (No. 14). Mycorrhizal abundance was similar in Al-treated and untreated plants. Of the three mycorrhizal morphotypes (single, dichotomous branched, and coralloid), the coralloid mycorrhizae were rare in Al-treated plants. Also, mycorrhizae in Al-treated plants had thinner mantle and lacked Harting net as compared to untreated controls. The Al^{+3} concentration did not reduce growth. Also, mycorrhizal formation with the selected *S. luteus* strain caused lower Al^{3+} translocation to the upper part of the tested seedlings compared with non-mycorrhizal controls (Kieliszewska-Rokicka et al. 1998).

The fixation and removal of phosphorus in the laterite soil due to iron and Al induced phosphorus deficiency in sweet potato which could be rectified to a certain extent by inoculation with VAM (vesicular–arbuscular mycorrhizal) fungus *Glomus microcarpum* in studies conducted at the Department of Post Graduate Studies and Research in Botany, Sanatana Dharma College, Alappuzha, India (Haricumar and Potty 1999).

Effect of mycorrhiza on aluminium toxicity under different nitrogen sources

Pitch pine (*Pinus rigida*) seedlings were grown in sand culture with NO_3^-, NH_4NO_3, or NH_4^+ and exposed to 0 or 200 µM Al for six weeks in studies conducted at the Department of Botany, University of Vermont, Burlington, USA. Foliar nitrogen concentrations, root nitrate reductase activity, and Al inhibition of nitrate reductase activity were dependent on the proportion of nitrate in the nutrient solution. The association of *Pisolithus tinctorius* with roots of seedlings reduced both root and foliar nitrogen reductase activity compared with uninoculated controls suggesting that symbionts reduced nitrate uptake and translocation to foliage. This was confirmed by using 36 ClO_3^- to measure undirectional plasma membrane nitrate fluxes. Mycorrhizal root tips absorbed 50% less nitrate than non-mycorrhizal root tips. Preferential use of NH_4^+ by ectomycorrhizal roots may thus result in reduced movement of Al into root tissues and amelioration of Al toxicity (Cumming 1990).

In another study conducted at the same university, non-mycorrhizal and mycorrhizal (with *Pisolithus tinctorius*) seedlings of *Pinus rigida* were grown in a-quarter strength Johnson's solution modified to contain NO_3^-, NH_4NO_3, or NH_4^+ and exposed to 0 or 200 µM Al for six weeks. Non-mycorrhizal seedlings grown with NO_3^- showed reductions in height, growth, and final shoot weight in response to Al. Aluminium, for all sources of nitrogen, consistently reduced the root weights. Increasing the proportion of NO_3^- in the nutrient solution raised cation accumulation in roots and shoots and depressed tissue anion concentrations. The coprecipitation of Al and phosphate in roots of Al-treated seedlings further limited phosphorus availability in this treatment. Mycorrhizal infection maintained growth and foliar phosphorus levels under Al exposure suggesting that Al-induced phosphorus limitation was a critical factor in non-mycorrhizal seedlings grown on primarily NO_3^--based nutrient solutions (Cumming and Weinstein 1990b).

In studies conducted at the US Forest Service, United States Department of Agriculture, North East Research Station, Delaware, USA, *Pinus rigida* seedlings, with or without *Pisolithus tinctorius* inoculation, were grown in sand irrigated with nutrient solution (pH 3.8) containing 0, 1, or 20 mg Al per litre (0, 370, or 740 µM Al) to see the effect of elevated nitrate nitrogen or ammonium nitrogen where nitrogen was increased three times above ambient levels. Elevated nitrate nitrogen had no significant effect on Al toxicity in non-mycorrhizal seedlings but Al toxicity at ambient ammonium nitrogen was ameliorated by elevated ammonium nitrogen. Symptoms of Al toxicity in roots of mycorrhizal seedlings at ambient nitrogen level were reduced by elevated ammonium nitrogen and absent in elevated nitrate nitrogen (Schier and McQuattie 1999).

Effect of mycorrhiza collected from different site conditions on aluminium toxicity

In studies conducted at the School of Environmental Biology, Curtin University of Technology, Perth, Western Australia, Al tolerance of *Pisolithus tinctorius* isolates collected from three different localities – a 30-year-old abandoned coal mining site (pH 4.3; Al 164 ppm), a 10-year-old rehabilitated site (pH 5.1; Al 6 ppm), and forest site (pH 5.3; Al 2 ppm), was determined both in

Al-impregnated MMN (Modified Melin-Norkran's) medium and in host tissues. Isolates from abandoned coal mining site demonstrated mycelial growth up to 2000 ppm Al with a threshold of 90 ppm before significant Al accumulation occurred. In contrast, growth of isolates from rehabilitated site and forest site was limited by 22 ppm and 12 ppm Al, respectively; with significant metal accumulation at 1–4 ppm substrate Al. Electron microprobe analysis of eucalyptus seedlings inoculated with Al-tolerant (mine site) or Al-sensitive (forest site) isolates and control plants (without mycorrhizal inoculation) grown in acidic, Al-contaminated soil, demonstrated that Al accumulation within the hyphal wall and presence of metal within the cytoplasm affected the degree of Al tolerance in the host plant. The presence of Al in the cytoplasm coincided with significant accumulations of sulphur, indicating a role of phytochelatins as biochemical (and hence genetic) basis for tolerance to Al in mine site isolates. Retention of Al within the hyphal mantle led to decreased Al levels across the root proper. In contrast, non-mycorrhizal roots demonstrated consistently high Al levels in all root tissues with negligible quantities of calcium and magnesium indicating a degree of cellular necrosis attributable to Al toxicity. Dry-weight analysis confirmed Al accumulation within roots of seedlings inoculated with Al-tolerant mycorrhizae and limited metal translocation to the shoot. In contrast, forest site and control plants contained as much Al in shoot as in root tissues. Also, plants inoculated with Al-tolerant isolates were taller with greater root and shoot dry mass and increased mycorrhizal infection and possessed superior nutrient status with total nitrogen, phosphorus, and potassium double that of seedlings inoculated with isolates from forest site. Control seedlings possessed low nutritional status (Louise, Brendon, and John 1992).

Effect of mycorrhiza on aluminium toxicity under elevated CO_2

In studies conducted at the US Forest Service, North East Forest Research Station, Delaware, USA, *Pinus rigida* seedlings inoculated with *Pisolithus tinctorius* were grown for 13 weeks on sand irrigated with two different levels of nutrients (0.1 or 0.2 strength Clark solution) containing 0, 6.25, 12.5, or 25 mg/litre Al (0, 232, 463, or 927 µM Al) in growth chambers fumigated with 350 (ambient) or 700 (elevated) µlitre/litre CO_2. At ambient CO_2 in the absence of Al, mean total dry weight of seedlings at high nutrient level was 164% higher than that at the low level. Total dry weight at the elevated CO_2, in the absence of Al, was significantly greater than that in the ambient CO_2 at low (+34%) and high (+16%) nutrient levels. Root and shoot dry weight at both nutrient levels decreased with increasing Al concentrations with Al reducing root growth more than shoot growth. Although visible symptoms of Al toxicity in roots and needles were reduced by CO_2 enrichment, there was no significant CO_2–Al interaction for shoot and root dry weight. The percentage of seedlings' roots that became mycorrhizal were negatively related to nutrient level and was greater at elevated than at ambient CO_2 levels (Schier and McQuattie 1998).

Effect of aluminium and calcium on ectomycorrhizal fungi

In studies conducted at the Department of Botany, University of Toronto, Ontario, Canada, isolates of *Hebeloma crustuliniforme, Rhizopogon rubescens*, and *Suillus tomentosus* obtained from basidiospores of different-aged jack pine (*Pinus banksiana*) stands were grown for four weeks in nutrient solutions containing 37, 185, 370, or 740 µM Al combined in a factorial design with 25, 125, 250, or 500 µM calcium and maintained at pH 3.8. Growth of all fungi was reduced at 370 µM Al. In *H. crustuliniforme,* stepwise increments in external Al concentrations resulted in reduced mycelial concentrations of calcium, magnesium, and potassium, and increased mycelial concentrations of Al, phosphorus, and iron. However, stepwise increments in external calcium concentrations from 25 to 500 µM stimulated the growth of *H. crustuliniforme*. In *R. rubescens,* high external Al concentration resulted in reduced mycelial concentration of all elements, except Al and phosphorus, while increments of calcium had no effect on the growth of this fungus. In *S. tomentosus*, high external Al concentration led to increased mycelial concentration of all elements except calcium and sulphur while high external level of calcium acted synergistically with high external Al concentration to reduce the growth of this fungus. The response of *S. tomentosus* to Al and calcium could not be predicted from the soil and basidiocarp analysis. Alterations in calcium–Al ratios of soils may influence the succession of ectomycorrhizal fungi (Browning and Hutchinson 1991).

Effect of aluminium and pH on ectomycorrhiza

Soil pH affects Al toxicity to mycorrhizal fungi and plants. In acidic forest soils, Al and other heavy metals become water soluble, reducing the absorbing capacity of the tree root system, primarily through destruction of mycorrhizal fungi and resulting in death of stress trees (oaks) (Jakucs et al. 1986).

In studies conducted at the Institute für Allgemeine Botanie, Johannes Gutenberg – Universitat, Mainz, Germany, three-year-old seedlings of Norway spruce (*Picea abies*) were held for two years in spray hydroculture and then treated for six months with nutrient solution similar in composition to lysimeter solution obtained from below spruce forests. The pH values were adjusted to 5.0, 3.5, and 2.5, and some of the nutrient solutions with pH 3.5 or 2.5 were

enriched with 1 or 2 mM Al per litre. The acid stress caused damage to fine roots, namely lysis of the peripheral root cell walls (acid holes), destruction of the calyptra, lysis of the root meristem, and, in some cases, collapse of the phloem in Norway spruce (*Picea abies*) seedlings. Aluminium intensified the damage and also led to swelling of the root tips and made the root cell walls brittle (Vogelei and Rothe 1988).

In studies conducted at the Department of Forest Science, College of Forestry, Oregon State University, Corvallis, USA, balsam fir (*Abies balsamea*) seedlings inoculated with *Cenococcum geophilum* (isolate 145A), *Hebeloma crustuliniforme* (isolate S166), and *Laccaria laccata* (isolate 238B), were grown in potting mixture which was supplied with 0, 50, or 100 mg Al per g of substrate and adjusted to pH 3, 4, or 5. Seedlings were watered with one-fifth strength Arnon's solution having a pH of 3, 4, or 5 and containing 0, 50, or 100 mg Al per litre. With increase in solution acidity and Al concentration, ectomycorrhizal formation, root weight, shoot weight, total weight, root–shoot ratio, and boron concentration decreased; iron, manganese, phosphorus, and zinc concentrations increased; and nitrogen, potassium, calcium, and magnesium concentrations did not vary significantly (Entry et al. 1987).

Studies were conducted at the School of Biological Sciences, University of Birmingham, Birmingham, Great Britain, on Norway spruce seedlings obtained from seedlots collected from populations growing in acidic and calcareous soils and then grown on sterile perlite culture containing 0–6 mM Al. Aluminium inhibited hypocotyl extension in seedlings in calcareous soils but not in seedlings in acidic soils. Both types of seedlings were inoculated or not with *Paxillus involutus* and grown for further 10 weeks in perlite. Aluminium sulphate solutions were added to raise Al concentrations to 0–6 mM. The seedlings were harvested after further 10 weeks. The fungus was associated in roots of inoculated plants but did not form mycorrhiza. The growth of uninoculated acidic plants was somewhat stimulated by Al treatment but that of uninoculated calcareous plants was reduced and the plants were chlorotic. The presence of mycorrhizal fungus in rhizosphere enhanced growth of calcareous plants but had little effect on acidic plants. Shoot analysis suggested that greater Al sensitivity of calcareous plants involves an inability to exclude Al or to maintain normal calcium and magnesium uptake in its presence. The presence of rhizospheric fungi reduced the effect of Al (Wilkins and Hodson 1989).

Nickel toxicity

Studies were conducted on birch (*Betula papyrifera*) seedlings at the Department of Botany, University of Toronto, Toronto, Ontario, Canada. The seedlings were either uninoculated or inoculated with *Scleroderma flavidum* (known to increase nickel tolerance) or *Lactarius rufus* (does not increase nickel tolerance), and were exposed to 85 µM Ni (nickel). Taking decreased water uptake by seedlings as an indicator of the progression of Ni toxicity for over 16 weeks, *L. rufus* was found to provide some initial protection against Ni toxicity. However, water uptake in *S. flavidum* infected seedlings was least affected by Ni and was significantly higher than *L. rufus* infected or uninoculated seedlings after week 10. Total dry weight of the *S. flavidum* infected seedlings was higher than that of other seedlings at weeks 12 and 21. The weight of *S. flavidum* tissue per root, as determined by chitin analysis, was significantly greater than that of *L. rufus* in Ni-treated and control seedlings, it increased with time. *L. rufus* did not grow once the roots were exposed to Ni but *S. flavidum* continued to grow following Ni exposure and this may be an important characteristic in distinguishing fungi for enhancing metal tolerance. Nickel reduced photosynthetic rates in seedlings infected with *S. flavidum* relative to *S. flavidum* inoculated seedlings not exposed to Ni but this effect was lacking in uninoculated seedlings. Respiration rates were not affected by metal treatment or mycorrhizal formation (Jones and Hutchinson 1988).

Betula papyrifera was grown with or without Ni for 22 weeks and then both were exposed to ^{63}Ni for two minutes to 48 hours in further studies conducted at the same university. After Ni uptake, the roots were desorbed with 50 mM calcium chloride at 5 °C for 30 minutes to determine exchangeable Ni. Total exchangeable ^{63}Ni uptake in roots was unaffected by inoculation treatments with *Lactarius rufus* or *Scleroderma flavidum* either in the presence or absence of DNP (dinitrophenol), a general metabolic inhibitor. The amount of exchangeable Ni was significantly affected by infection and uptake was least in seedlings inoculated with *S. flavidum*. Application of DNP increased Ni uptake in shoots of uninfected seedlings and those inoculated with *L. rufus* but not in seedlings inoculated with *S. flavidum* or those in the absence of DNP (Jones, Dainty, and Hutchinson 1988).

Cadmium toxicity

Mycorrhizal associations reduced the uptake of heavy metals in shoots of plants growing in soils with high concentrations of readily available metals in experiments conducted at the Eidg. Forschungsanstalt fhr Obst-Wein-und-Gartenhau, Wadensinl, Germany. In soils with low metal content, VAM increased the uptake of zinc but decreased the uptake of highly toxic Cd (cadmium) in shoots (Schepp, Dehn, and Sticher 1987).

Studies were conducted at the Laboratory of Plant Ecology, Katholiecke Universiteit, Lenven, Belgium, on *Pinus sylvestris* plants cultivated in a semihydroponic system that allowed the visual observation of the root system for comparison of the ability of nine ectomycorrhizal fungi (collected from either unpolluted soils or soils polluted with

zinc and Cd) to increase Cd tolerance in plants. Although Cd addition resulted in decreases in transpiration in the non-mycorrhizal plants, disturbances in water relations could be more or less pronounced in the mycorrhizal pines. Several mycorrhizal fungi hardly survived in the polluted substrate. Fungi producing a large mycelial biomass showed the greatest effect in overcoming toxicity. Some strains were able to increase their growth when treated with Cd. In a dense extramatrical mycelium, the potential for Cd retention increases where the individual hyphae are exposed to a lower Cd concentration than in a sparse mycelium. A protective effect against Cd toxicity in the host was observed with all mycobionts since Cd uptake was highest in non-mycorrhizal seedlings. The treatment had no effect on the growth of the seedlings or on the concentrations of other cations probably due to the relatively low toxicity and short observation period (Colpaert and Van Assche1993).

In studies conducted at the University of Wales, School of Agriculture and Forest Science, Bangor, Wales, UK, both mycorrhizal (with *Laccaria bicolor* or *Paxillus involutus*) and non-mycorrhizal seedlings of Norway spruce (*Picea abies*) were exposed to 0 (control), 0.5, or 5 µM $CdSO_4$ for nine weeks in a sand culture system with frequent addition of nutrient solutions. In pure culture, *P. involutus* and *L. bicolor* showed similar Cd tolerance. However, in symbiosis, Cd treatments decreased colonization by *L. bicolor* but not by *P. involutus* which ameliorated the negative effect of 0.5 µM Cd on shoot and root growth and chlorophyll content of old needles while *L. bicolor* did not. Mycorrhizal colonization did not affect Cd concentration of old needles and roots of seedlings. Both mycorrhizal species reduced Cd concentrations of young needles to a similar degree compared with non-mycorrhizal seedlings. However, in the 0.5 µM Cd treatment, Cd content of needles of *P. involutus* colonized seedlings was increased whereas that of *L. bicolor* colonized and non-mycorrhizal seedlings were similar. Also, the amount of Cd translocated to the needles expressed on the root length basis was similar in all treatments. All mycorrhizal seedlings were similarly affected by 0.5 µM Cd and concentrations of phosphorus, potassium, calcium, magnesium, and manganese were decreased to a similar extent in both mycorrhizal and non-mycorrhizal seedlings. In contrast to *L. bicolor*, *P. involutus* increased phosphorus uptake and altered patterns of root branching (Jentschke, Winter, and Godbold 1999).

Copper toxicity

In studies conducted at the Citrus Research and Education Centre, University of Florida, Lake Alfred, USA, carrizo citrange (*Ponicirus trifoliata* × *Citrus sinensis*) seedlings were planted in a sandy soil (pH 6.8) which was amended with eight Cu (copper) concentrations (0–300 µg per g of soil) as basic copper sulphate ($CuSO_4.3Ca(OH)_2.H_2O$) adjusting to double acid extractable Cu concentrations in soil from 3 to 248 µg per g of soil. Seedling growth and colonization by the mycorrhizal fungus, *Glomus intraradices,* reduced logarithmically with Cu concentration. Minimum toxic amount of Cu ranged from 19 to 34 µg per g of soil. Leaf phosphorus content decreased linearly with Cu for mycorrhizal seedlings without added phosphorus but not for non-mycorrhizal seedlings with supplemental phosphorus. The Cu-induced reduction in phosphorus uptake of mycorrhizal plants was more closely related to the inhibition of hyphal development outside the root than to the development of vesicles and arbuscules inside the root. Thus Cu-induced phosphorus deficiency was attributed to inhibition of phosphorus uptake by the mycorrhizal hyphae in the soil. In citrus orchard soil with a double acid extractable Cu 80 µg per g of soil and pH 5, replanted trees were stunted and had less mycorrhizal colonization than unaffected trees (Graham, Timmen, and Fardelmann 1986).

Zinc toxicity

In studies conducted at the Physiologische Pflanzen Anatomie, Universitat Bremen, Germany, *Pinus sylvestris* seedlings were either uninfected or infected with *Suillus bovinus* strain cultured on zinc-enriched medium, *S. bovinus* strain cultured on normal medium, and an unidentified isolate (BP), and exposed to various external Zn (zinc) concentrations. Non-mycorrhizal seedlings had the capability to control uptake and threshold level, where no limitation of uptake is possible. Excess Zn accumulated in the root system to protect the shoot against toxic concentrations. Effect of an ectomycorrhizal fungus on Zn uptake and distribution depended on the fungal species, external Zn concentrations, and Zn content of fungal culture medium. Under conditions of low external Zn supply, mycorrhizal infection with *S. bovinus* led to increased Zn uptake in roots and needles of *P. sylvestris*. In case of high external Zn concentrations, mycobionts varied considerably in their capability to reduce Zn transport to the shoot. The effect could be enhanced only by an infection with *S. bovinus*, pretreated with high Zn concentrations (Bucking and Heyser 1994).

Scandium toxicity

Studies were conducted at the Faculty of Environmental and Forest Biology, College of Environmental Sciences and Forest Biology, Syracuse, USA, on loblolly pine (*Pinus taeda*) and honey locust (*Gleditsia triacanthos*) grown in solution culture at pH 3.95 with 10–100 µM and 10–50 µM Sc (scandium), respectively. After 35 days, root and shoot elongation in both species were significantly reduced at 50 µM Sc or higher. With honey locust, reductions in dry weights of root, leaf, and stem were measurable at 10 µM Sc. In both species, Sc induced chlorosis and

substantially reduced chlorophyll contents of young leaves at all concentrations. At 100 µM Sc, short roots of loblolly pine developed a distinctly mycorrhizal appearance and Sc significantly reduced potassium concentration in tissues but had little effect on the concentrations of calcium and magnesium. Scandium is a transition element known to accumulate in combustion products at concentrations of up to 1000 ppm or in treated sewage sludge. Its average concentrations in soil are 0.5–45 ppm (Yang, Schaedle, and Tepper 1989).

Lead toxicity

In studies conducted at the University of Gottingen, Institute of Forest Botany, Forest Ecosystem Research Centre, Gottingen, Germany, Norway spruce seedlings, non-mycorrhizal or mycorrhizal with *Laccaria bicolor* or *Paxillus involutus*, were grown in root boxes in a quartz sand / nutrient solution culture system supplied with NH_4NO_3 nitrogen or NO_3^- nitrogen for 6 weeks and with 5µM Pb (lead) during the last three weeks. pH was higher with NO_3^- nutrition than with NH_4NO_3 nutrition. No Pb was detected in shoots. Total Pb in roots was reduced by mycorrhizal infection but was not affected by the source of nitrogen. About 47% and 10% Pb could be desorbed from roots by washing in 5 mM $CaCl_2$ or 0.4 mM HCl, respectively. This mainly comprised apoplastic Pb and was neither affected by the source of nitrogen nor by the presence of mycorrhiza. Mycorrhizal infection had no effect on the pH at the rhizoplane. X-ray micro-analysis of semi-thin sections of mycorrhizal and non-mycorrhizal short roots showed that despite the higher binding capacity of cortex cell walls, Pb content was lower with NO_3^- nutrition than with NH_4NO_3 nutrition. Lead content in the stele was also reduced with NO_3^- nutrition. Mycorrhizal infection had no effect on the Pb content of cell walls in the cortex and stele. In an accompanying solution culture experiment conducted under similar conditions, increased solution pH (5 vs 3.2) had a similar effect on Pb concentration in root cortex cell walls as had nitrate nutrition in the sand culture experiment (Jentschke et al. 1998).

Toxicity due to allelopathic compounds

Phenolic compounds identified previously as potentially responsible for allelopathic interferences in spruce forest at high altitude were analysed in canopy leachates and snow and soil solution collected from the three layers of the podsolic soil (OA, E, and B) at the Department of Life Sciences, Laboratory Altitude Ecosystem Dynam, Le Bourget, France. Leachates were characterized by high tanning capacity and by p-hydroxyacetophenone which was also detected as the major monomeric compound in snow. At least 10 phenolic monomers including vanillic, p-hydroxy benzoic, and protocatechuic acids were identified in capillary waters extracted from the OA layer. In soil solutions, significant decreases in phenolic concentrations with depth were observed between the E and B layers; with qualitative modifications of the phenolic pattern. Spruce leachates and soil solutions exhibited high temporal variability resulting in transitory allelopathic potential towards both aerial and subterranean parts of spruce seedlings (Gallet and Pellissier 1997).

Allelopathic effect of associated flora

In studies conducted at the INRA (Institut National de la Recherche Agronomique), Bordeaux Laboratoire d' Ecophysiologie et Nutrition, Cestas, France, the hypothesis that grass, *Molinia caerulea*, has an allelopathic effect on the growth of young red oak seedlings, was tested. Oak seedlings were grown for two years in a greenhouse, with or without fertilizer application and alone or with *M. caerulea*. Stem and root biomass of unfertilized oak seedlings grown with *M. caerulea* were significantly lower (10.3 and 60.0 g dry weights, respectively) compared with those of the seedlings grown alone (53.4 and 294.9 g, respectively). Roots of seedlings grown with *M. caerulea* had less *Laccaria* species (an efficient ectomycorrhizal fungus) and greater proportion of *Cenococcum geophilum* (which is less efficient) as well as more non-mycorrhizal roots and fewer fine roots (Timbal, Gelpe, and Garbeye 1990).

Studies were conducted on Douglas fir (*Pseudotsuga menziesii*) and pine (*Pinus ponderosa*) seedlings at the Department of Forest Science, Oregon State University, Corvallis, USA. The seedlings were grown in replacement series in pasteurized soil with no ectomycorrhizal fungi; two ectomycorrhizal fungi added (Douglas fir specific *Rhizopogon vinicolor* and pine specific *R. ochraceorubens*); and four ectomycorrhizal fungi added (two *Rhizopogon* species and two generalists *Laccaria laccata* and *Hebeloma crustuliniforme*). A replacement series in unpasteurized forest soil was also included. Tree species mutually inhibited each other in treatments without ectomycorrhizal fungi but infected with *Thelephora terrestris*, a greenhouse contaminant which usually colonized control plants. The mutual inhibition disappeared when four ectomycorrhizal fungi species were added. Douglas fir seedlings were significantly larger in mixtures than in monoculture. However, there was no corresponding decrease in the size of pine seedlings. This was solely due to seedlings with *L. laccata* which apparently enhanced nitrogen and phosphorus uptake by Douglas fir at the expense of luxury consumption by pine. Results indicate that ectomycorrhizal fungi can reduce competition between plant species and perhaps increase overall community phosphorus uptake. However, patterns were specific to both ectomycorrhizal fungi and tree species and were different in unpasteurized soils (Perry et al. 1989).

Below-ground competition and phytotoxicity between *Vaccinium myrtillus* (which reduces

survival of tree seedling and growth in boreal forests) and transplanted *Picea abies* was reduced by exclusion tubes in studies conducted at the Swedish University of Agricultural Sciences, Faculty of Forestry, Umea, Sweden. Reduced below-ground competition by *V. myrtillus* increased current-year shoot length, shoot and root biomass, and nutrient concentration and mycorrhizal colonization in seedlings of *P. abies* (Jaderlund et al. 1997).

In studies conducted at the McGill University, Department of Natural Resources Science, Canada, black spruce (*Picea mariana*) seedlings close to (less than one metre) and far from (greater than one metre) *Kalmia angustifolia* were sampled from a clear cut to assess mycorrhizal infection and various growth parameters. *K. angustifolia*, an ericaceous shrub, frequently invades black spruce clear cuts and on many sites where *K. angustifolia* grows, black spruce seedlings become chlorotic and stunted due to allelopathic chemicals of *K. angustifolia*. Seedlings close to *K. angustifolia*, as compared to those growing far from *K. angustifolia*, had significantly lower foliage concentrations of nitrogen and phosphorus, lower rate of mycorrhizal infection, and were more frequently associated with *Phialocephala dimorphospora*, a potential root pathogen of black spruce (Yamasaki et al. 1998).

Allelopathic effect of mulch or extracts of organic matter

Studies were conducted in the Laboratory et Pedologie Biology, University of Savoie, Chambery, Cedex, France, to verify that the initial growth of spruce seedlings is controlled by allelopathic compounds from vegetation and soil organic horizon and enhanced by mycorrhizal development. The action of different aqueous extracts under laboratory conditions was studied at different stages of spruce development. Germination was completely inhibited by bilberry leaves and partly by fern foliage and moss. The first stages of seedling growth were limited by bilberry leaves and rhizosphere bilberry roots. A positive effect was noticed for rotten wood. Mycorrhiza of young seedlings was inhibited by bilberry leaves (Andre et al. 1987).

Studies conducted at the Agricultural University, Biological Station, Kampsweg, Wijster, The Netherlands, showed that aqueous extracts of Scots pine needles negatively affected growth rates of *Laccaria proxima* and *Rhizopogon luteolus* whereas only high concentrations of needle extracts sufficiently inhibited the growth rates of *Paxillus involutus* and *Xerocomus badius*. The needle extracts significantly enhanced the growth rate of *L. bicolor*. Shoot extracts of the grass, *Deschampsia flexuosa,* inhibited the growth rates of *L. proxima, P. involutus*, and *R. luteolus*, and significantly enhanced the growth rate of *L. bicolor*. The shoot extracts contained about 3–5 times more high molecular weight components, aliphatic acids, and phenolics, than the root extracts (Baar et al. 1994).

Potential allelopathic (phytotoxic or beneficial) effect of barley, oat, and wheat straw mulches on growth, mineral nutrition, and mycorrhizal status of black spruce seedlings was evaluated under greenhouse conditions in studies conducted at the Ministere de l' Energie et des Resources, Servie de l' Amelioration des Arbes Sainte Foy, Québec, Canada. The various straws did not affect the height of spruce seedlings over a two-month growth period. The newly formed fine roots of treated and control seedlings were mycorrhizal. Oat and wheat straw significantly enhanced the phosphorus content of foliage as compared to that of controls. All treatments significantly depressed foliar manganese content indicating that the straw could exert a detrimental effect on manganese uptake. Monitoring the status of manganese in planted spruce seedlings when using allelopathic cover straw mulches is suggested as a method for preventing the establishment of weed species (Jobidon 1989).

Allelopathic effect of other fungi on mycorrhiza

Studies were conducted at the University of Wisconsin, Madison, Wisconsin, USA, to observe the possible suppression of mycorrhiza forming fungi by antibiotics excreted by *Coprinus ephemerus* commonly used in the production of composted fertilizers from saw dust and bark and paper mill sludge. Additional inoculation with *C. ephemerus* did not reduce the growth or enzymatic activity of feeder roots of one-year-old *Pinus resinosa* and *P. ponderosa* grown in a greenhouse in sandy soil inoculated with *Cenococcum graniforme* or *Thelephora terrestris*. Several seedlings showed sinuous morphology or serpentinosis of tap root which probably resulted from excretion of antibiotics by *T. terrestris* (Iyer 1981).

Toxicity due to soil salinity

Studies conducted at the Laboratoire de Phytonique, FRD Biotechnologies, Angers, Cedex, France, on 60-day-old culture of two birch (*Betula pendula*) clones, 4 and 5 (isolated in vitro), inoculated with *Paxillus involutus* indicated that the Clone 4 showed the most growth stimulation (120%) as compared to Clone 5 (55%). Clone 4 was also more tolerant to NaCl (sodium chloride) than Clone 5, their respective decreases in biomass being 10% and 40% in a medium containing 50 mM NaCl. Responses to salinity and mycorrhiza were linked to the osmotic and nutritional status of in vitro plants. Translocation of ions towards the shoots thus appeared to be key factor in the observed response (Catinus, Guerrier, and Strullu 1990).

Studies were conducted at the University of Western Australia, Nedlands, Australia, on the effect of NaCl on infection of *Trifolium resupinatum* by hyphae of *Gigaspora decipiens*. Roots, 1 cm from the spore, were infected after 15 days in soils moistened with 0 or 75 mM NaCl indicating that the initial hyphal growth was not inhibited in soil moistened with 75 mM NaCl. With time, NaCl increasingly inhibited hyphal growth as roots, 2.5 cm from the spores, were not infected after 26 days in the soil moistened with 75 mM NaCl but were infected in the soil without added NaCl. In soil moistened with 150 mM NaCl, roots, 1 cm from the spores, were infected after 26 days but roots, 2 cm from the spores, were not infected after 36 days. Increasing concentrations of NaCl inhibits either the hyphal growth or the infectivity of *G. decipiens* hyphae after they have grown through soil. The latter may be unlikely because NaCl did not affect the infectivity of *G. decipiens* hyphae placed directly on to the roots. Increasing concentrations of NaCl also inhibited the spread of infection after the occurrence of initial infection. This is probably due to NaCl in the soil inhibiting hyphal growth as well as possibly affecting supply of carbohydrates from the plant to the fungus (McMillen, Juniper, and Abbott 1998).

In studies conducted at the Department of Ornamental Horticulture, University of Florida, Gainsville, Florida, USA, NaCl tolerance and phosphoorus content in split-root carrizo citrange seedlings (*Ponicirus trifoliata* × *Citrus sinensis*) colonized with *Glomus intraradices* on zero, one, and two root halves and treated with NaCl at 0, 25, 50, and 100 mM doses were examined. The degree of stress was measured as reduction of dry matter accumulation and rise in the level of proline-betaine (stachydrine). Shoot and root dry weight production during this period decreased with increasing levels of salt. Absolute reductions were similar for plants inoculated on one vs two half root systems but percentage decreases were less in the latter due to greater overall growth in all treatments. Betaine levels in leaf tissues were positively related to salt level of the soil for each mycorrhizal treatment. Significant differences in betaine levels were also detected in plants with or without mycorrhizal fungi and mean levels tended to be higher for those colonized on one vs two halves of their root system. In contrast, a half root system and its fungal symbiont supplied enough phosphorus to allow concentration of leaf phosphorus to equal that of fully infected root systems, yet the two groups did not show equal growth under control conditions or percentage reductions with NaCl (Duke, Johnson, and Koch 1986).

The responses of *Pistacia vera* (a non-halophytic salt-tolerant deciduous nut tree) inoculated with *Glomus mosseae* and treated with deionized water and water with electrical conductivities of 3.8, 6.5, and 9.7 ds/m EC at age one week was determined in studies conducted at the Department of Padologie Vegetal and Department de Technologia Agraria, Generalitat de Catalunga, Cabrils, Barcelona, Spain. After 12-week growth, mycorrhizal *P. vera* maintained the leaf turgor and shoot dry weight while uninoculated plants did not maintain leaf turgor when irrigated with water of 9.7 ds/m EC. A significant check in growth with respect to controls was observed in all the non-VAM treatments. The results thus show the improved salt tolerance of VAM plants (Estaun and Save 1990).

Studies were conducted at the Instituto Sperimentale per l' Olive Culture, Casenza, Italy, on 14-month-old olive trees of cultivars Carolea and Nacellaria del Belice in pots irrigated with water with NaCl content of 0%, 0.3%, 0.6%, and 0.9%. After one year or 35 irrigations, there was reduction in root content of phosphorus, potassium, and calcium and rise in sodium and no change in magnesium as the salinity increased in both the cultivars. Difference in mycorrhizal responses were observed between the cultivars regardless of the irrigation water salinity. However, mycorrhizal parameters were affected by increasing salinity more in Carolea (with a reduction in both frequency and function) than in Nacellaria del Belice which showed significant increase in frequency but no significant change in function (Briccoli–Bati et al. 1994).

The response of young mycorrhizal (inoculated with *Glomus mosseae*) and uninoculated olive (*Olea europaea*) plants under saline conditions (25, 50, 75, and 100 mM NaCl) with or without supplemental calcium was studied at the Departmento di Ortoflorofrutticoltura, Universita de Firenze, Firenze, Italy. Depolarization caused by NaCl at the cellular level (cell transmembrane electropotential) was less in mycorrhizal roots than in non-mycorrhizal ones. The opposite was found at the level of the whole root system (transroot electropotential). Supplemental calcium in the saline treatments had protective effect on membrane integrity cancelling or reducing the differences in depolarization (cell transmembrane electropotential and transroot electropotential, respectively) between mycorrhizal and non-mycorrhizal plants. After three months, both mycorrhizal and non-mycorrhizal plants subjected to saline conditions grew less than control plants not exposed to saline conditions. However, mycorrhizal plants when compared with non-mycorrhizal ones demonstrated greater growth of shoots and leaves. Infected roots accumulated greater quantities of sodium, phosphorus, and calcium, and exhibited a lower potassium–sodium ratio; but in leaves, mycorrhizal plants had greater potassium–sodium ratio than non-mycorrhizal plants (Rinaldelli and Mancuso 1996).

Further studies were conducted at the same university to measure the electrical impedance parameters in shoots and leaves of olive plants to

follow variations in extracellular resistance, intracellular resistance, and the state of membrane in mycorrhizal (inoculated with *Glomus mosseae*) and non-mycorrhizal plants subjected to saline stress. A double DCE (double-distributed circuit element) (ZARC) and a single DCE (ZARC) model were used as equivalent circuit for shoots and leaves, respectively. There was a reduction in extra- and intracellular resistance values for non-mycorrhizal plants with increased NaCl concentration. Mycorrhizal infection brought about a noticeable improvement in salt tolerance in olive plants which was clearly demonstrated by trends in impedance parameters (Mancuso and Rinaldelli 1996).

Studies conducted at the Pomology Department, Faculty of Agriculture, Alexandria University, Alexandria, Egypt, on one-year-old sour orange seedlings in black polyethylene bags with clay loam soil showed that irrigation with saline water (up to 4500 ppm salt; NaCl + potassium chloride) reduced total leaf chlorophyll and chlorophyll-a concentrations, peroxidase activity in leaves, and starch and total carbohydrate concentrations in leaves and roots; did not affect chlorophyll-b concentration and polyphenol oxidase (catechol oxidase); and increased sugar concentrations in some cases when compared with controls (irrigated with tap water). Inoculation with *Glomus intraradices* increased total chlorophyll and chlorophyll-b content in the first season, chlorophyll-a in the second season, increased polyphenol oxidase activity, leaf and root sugars, and carbohydrate concentrations but did not affect peroxidase activity (Ezz and Nawar 1994).

Toxicity due to calcareous soil

The term symbiocalcicole is used to define dwarf shrubs or tree species that can only tolerate calcareous soils in association with mycorrhizal fungi (Lapeyrie 1990). Studies conducted at the Department of Agricultural Botany, the Habrew University, Rohovot, Israel, on *Citrus incanus* showed that the plants transplanted in the laboratory degenerated in native dark basaltic soils and in sterilized, terrarosa calcareous soils where infection by mycorrhizal fungi did not occur. They grew in calcareous terrarosa when mycorrhiza developed. Successful development of *C. incanus* in unfertilized soils depended on ectomycorrhizal infection. Plants developed without mycorrhiza in both the soils when irrigated with a complete nutrient solution. It thus suggests that when mycorrhizae are absent, *C. incanus* plants degenerate in unfertilized soils due to mineral nutrient deficiency (Berliner, Jacoby, and Zamski 1986).

In studies conducted at the Zagazig University, Faculty of Agricultural Science, Moshtohor-Tukh, Egypt, sour orange (*Citrus aurantium*) seedlings were transplanted into pots (containing 18 kg calcareous soil), fertilized with three different levels of rock phosphate, and foliar sprayed with micronutrient solution (iron, manganese, molybdenum, zinc, and copper). The VAM inoculation and or phosphorus fertilization generally stimulated plant growth characters, shoot and root length, leaf numbers, and dry matter accumulation. Nitrogen and phosphorus contents of mycorrhizal plants were greater than non-mycorrhizal plants at all soil phosphorus levels. Micronutrient uptake was considerably improved in VAM plants over either phosphorus fertilized, uninoculated, or control plants (El-Maksoud, Boutros, and Lofty 1988b).

Studies conducted at the National Research Centre, Dokki, Egypt, on sour orange (*Citrus aurantium*) grown in sandy and calcareous soils under greenhouse conditions and inoculated with VAM spores showed that plant height, root length, leaf number, leaf area, and dry mass of inoculated plants were always significantly greater than uninoculated controls in both soils. Percentage of mycorrhizal infection significantly increased and significant differences were observed in nitrogen, phosphorus, iron, manganese, and zinc contents of roots and aerial parts as a result of inoculation. Combined treatment with VAM and phosphorus gave more growth responses than phosphorus alone or control although phosphorus fertilized plants were always better than unfertilized plants. The growth characters were significantly larger in calcareous than in sandy soil (El-Maksoud, Boutros, and Lofty 1988a).

Toxicity by overheated soil

Studies conducted at the Instituto de Investigaciones Agrobiologicas de Galicia, Apdo, Santiago de Composieta, Spain, showed that germination rates, root colonization, and intensity of arbuscule formation of *Glomus macrocarpum* were reduced following treatment with aqueous extracts of burnt soil or overheated unburnt soil and enhanced by extracts from unburnt soils compared with the control (distilled water). It is thus concluded that overheated soils contain water soluble substances which affect VAM colonization and growth (Vilarino and Arines 1992).

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Research findings

Effect of VAM on root induction in in vitro sugarcane (*Saccharum officinarum* L.) seedlings – a new technique

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Introduction

Tissue culture technique has been a novel approach for large-scale production of quality plants, establishment of hybrids, and development of varieties resistant to various stresses and diseases. However, one of the constraints still is good rooting in the differentiated shoots of in vitro sugarcane (Islam, Begum, and Haque 1982). The modified MS (Murashige and Skoog) liquid medium containing 5 mg/litre NAA (naphthalene acetic acid) is widely used for root induction in sugarcane (Chen et al. 1988). The plants thus formed have feeble root formation and do not quickly acclimatize to field conditions.

In many instances, VAM (vesicular–arbuscular mycorrhizal) inoculations have been successfully used to increase the survival capacity of micropropagated seedlings (Adholeya and Cheema 1990; Jamaluddin and Chaturvedi 1996). The VAM fungal association has been reported to stimulate rooting (Barrows and Roncadori 1977) and enhance root growth by hormone secretion (Allen, Moore, and Christensen 1980; Powell and Bagyaraj 1986; Whipps and Lumsden 1994). Hence, in this investigation, VAM fungal spore extract and VAM root extract were tested as substitutes to the rooting hormone NAA in MS liquid medium to enhance root induction.

Materials and methods

Source of explant

A high-yielding locally grown sugarcane variety Co. 419 was selected and 3–4 month old sugarcane seedlings were used as the source of explant.

Callus induction

The leaf sheaths of the sugarcane seedlings were carefully peeled up to the first node and the few innermost leaf whorls used. The leaf sheath was excised up to 4–6 cm from the shoot tip and the surface sterilized with a liquid soap solution followed by rinsing thrice in sterile distilled water. Further inoculation procedures were carried out under aseptic conditions (inside laminar airflow). Leaf explants were treated with aqueous mercuric chloride (0.1%) followed by rinsing thrice in sterile distilled water. The innermost whorl was carefully peeled and chopped into small pieces (approximately 0.5 mm) and immediately inoculated in MS medium supplemented with 3mg/litre 2–4 D (2–4 dichlorophenoxyacetic acid) and 10% coconut

milk (Heinz and Mee 1969; Liu, Huang, and Shib 1972) for callus induction. Further subculturing of the callus was done on the same medium to obtain luxuriant growth of friable callus. All the culture tubes were incubated in the dark at 25 ± 2 °C.

Morphogenesis

The friable sugarcane callus was subcultured onto the MS medium supplemented with 2 mg/litre kinetin and 10% coconut milk for shoot induction. The flasks were incubated at 25 ± 2 °C under fluorescent light maintaining a 12-hour photoperiod. The shoots were subcultured on the same medium for profuse tillering (Figure 1). Then regenerator shoots were transferred to MS liquid medium using the following alternatives for root induction.

1 MS medium supplemented with NAA (5 mg/litre) along with 10% coconut milk (Chen et al. 1988)
2 MS medium supplemented with VAM fungal spore (*Glomus aggregatum*) extract (2500 spores/litre) and 10% coconut milk
3 MS medium supplemented with VAM root extract (10 g/litre) and 10% coconut milk
4 MS medium supplemented with non-mycorrhizal root extract (10 g/litre) and 10% coconut milk

Fifteen tubes were used for each treatment and the experiment was done twice.

Preparation of spore and root extracts

The root extract was prepared by homogenizing 10 g of fresh, surface sterilized mycorrhizal or non-mycorrhizal roots of sugarcane in 25 ml distilled water. The homogenate was filtered through Whatman No. 2 filter paper and the filtrate was added to the medium before sterilization.

Spore extract was prepared by harvesting spores of *Glomus aggregatum* from pure soil inoculum using the wet sieving and decanting method (Gerdemann and Nicolson 1963); after surface sterilization it was treated with 10% Chloramine-T for 10 minutes followed by washing in sterile distilled water; and later treated with 200 ppm streptomycin sulphate and again washed in sterile distilled water and crushed in a cavity glass with the help of a glass rod in distilled water. The homogenized mixture was then added to the medium. The pH of the medium was maintained at 5.6–5.8.

Results

Callus induction was observed on explants after three weeks of inoculation and a well-developed friable callus was obtained within two weeks after subculturing. The callus when subcultured on shooting medium produced multiple shoots in 3–4 weeks after incubation. Further subculturing resulted in proliferation of transferable shoots (Figure 1).

Figure 1 Transferable shoots of sugarcane variety Co. 419 for rooting response

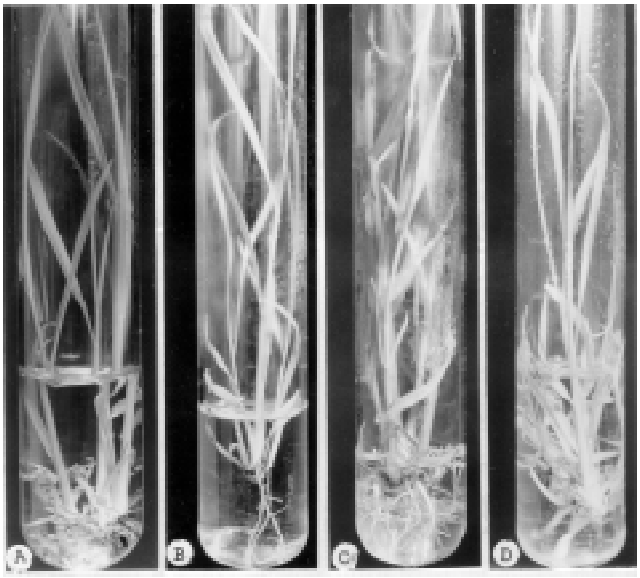
Well-developed, healthy, transferable shoots were excised and transferred into modified MS liquid medium supplemented with four alternatives of rooting stimuli (vide materials and methods) and incubated under controlled conditions of light and temperature. The response of shoots for rooting varied from one treatment to another and more clear measurable variation was obtained after 40 days of transfer (Table 1, Figure 2).

In the shoots transferred in MS liquid medium supplemented with NAA (control), root initiation was observed within 8–12 days of incubation. However, clear root formation and growth

Table 1 Effect of NAA[a], VAM[b] fungal spore extract, VAM root extracts, and non-mycorrhizal root extract as supplements in MS[c] liquid media on rooting response of sugarcane tissue culture shoots

Treatment	*Days taken for root initiation*	*Number of roots per shoot*	*Length of roots* (cm)*	
			after 20 days	*after 40 days*
NAA[a]	8–12	10–15	0.4–0.6	1–2
VAM[b] fungal spore extract	3–6	3–12	1.5–3	3–4
VAM[b] root extract	2–6	30–50	0.5–1	1.5–3
Non-mycorrhizal root extract	No rooting	Nil	–	–

[a]NAA – naphthalene acetic acid; [b]VAM – vesicular–arbuscular mycorrhiza; [c]MS – Murashige and Skoog
*Average length of 10 roots selected randomly



(a) (b) (c) (d)

Figure 2 Rooting response and root proliferation in **(a)** MS + 5 mg/litre NAA; **(b)** MS + VAM fungal spore extract; **(c)** MS + VAM root extract; and **(d)** MS + non-mycorrhizal root extract in liquid medium

(0.4–0.6 cm) could be observed only after 20 days of continuous incubation and 1–2 cm long roots were observed after 40 days (Table 1, Figure 2a). The total number of roots formed were in the range 10–15 per shoot.

In the shoots transferred to MS liquid medium supplemented with VAM spore extract, root initiation was observed within 3–6 days of incubation and the growth of the roots was faster as compared to that in the NAA supplement. The roots were 1.5–3 cm and 3–4 cm long after 20 and 40 days of incubation, respectively (Table 1, Figure 2b). However, fewer roots were produced, 3–12 per shoot.

Similarly, in the shoots transferred to VAM root extract supplemented medium, the rooting response was observed within 2–6 days of incubation. The growth and proliferation of roots were also high, 0.5–1 cm and 1.5–3 cm long roots after 20 and 40 days of incubation, respectively (Table 1, Figure 2c). Also, 30–50 roots per shoot were produced.

The shoots transferred to the medium supplemented with non-mycorrhizal root extract did not show rooting response even after 40 days of continuous incubation (Table 1, Figure 2d).

Discussion

In this study, the meristem leaf explants of sugarcane variety Co. 419 when cultured on modified MS medium produced profuse callus and shoot formations. This is in accordance with the earlier reports by Heinz and Mee (1969), Liu, Huang, and Shib (1972), Hendre et al. (1983), Pant and Hapase (1987), and Burner and Grisham (1995), in which the same combination of media for callus induction and shoot formations in sugarcane were used. Islam, Begum, and Haque (1982) have observed that despite rooting in differentiated sugarcane seedlings with NAA, subsequent adaptability upon transfer to pots seems to be difficult. As an alternative to NAA, VAM fungal spore extract or VAM root extract showed better rooting response. These substitutes not only hastened the rooting initiation but also appreciably enhanced root growth and proliferation. This increased response is attributed to VAM fungal influence since non-mycorrhizal root extract failed to induce rooting. Barrows and Roncadori (1977) have observed the stimulation of rooting due to the association and synthesis of a hormone by VAM fungus *Gigaspora margarita* in *Poinsettia*.

The enhanced rooting response in sugarcane with either VAM fungal spore extract or VAM root extract substitutes in MS liquid medium could make them a better solution than the NAA supplement. These seedlings on transfer to pots have also shown increased vigour as compared to control plants.

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Distribution of an ectomycorrhizal fungus (*Pisolithus tinctorius*) in association with different forest tree species in Andhra Pradesh, southern India

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Introduction

Mycorrhizal association is essential to forest trees. Among the different mycorrhizae, ectomycorrhizal fungi occur in 10% of the world's flora, which include most of the gymnosperms (especially conifers) and few angiosperms. Many species of acacia viz. *Acacia auriculiformis*, *A. holosericea*, and *A. mangium*, and eucalypti viz. *Eucalyptus camaldulensis* and *E. tereticornis*, native to Australia, have been successfully introduced in different parts of India by the state forest departments. These tree species grow on a variety of soils such as red soils, laterite soils, gravely brown soils, sand dunes, shallow to deep, infertile to fertile soils, and from acid to highly alkaline soils and can tolerate the periodic waterlogging in Andhra Pradesh (India). These trees are useful for pulpwood, poles, cheap timber, etc.

Reports on the association of ectomycorrhizal fungi in tropical angiosperm tree species is scanty; hence, the present survey for collection, isolation, and identification of different ectomycorrhizal fungi in association with *Acacia* spp. and *Eucalyptus* spp. The distribution of the ectomycorrhizal fungus Pt (*Pisolithus tinctorius*) in association with different tree species in 10 different plantations of the state forest department in three districts of Andhra Pradesh during the period 1994–96 is presented. This ectomycorrhizal fungus is being reported for the first time in association with different tree species of acacia and eucalyptus plantations in low fertile soils of Andhra Pradesh.

Materials and methods

Periodical surveys were undertaken to study the natural occurrence of the ectomycorrhizal fungus *Pisolithus tinctorius* in association with different economically important forest tree species such as *Acacia auriculiformis, A. holosericea, A. mangium, Eucalyptus camaldulensis,* and *E. tereticornis* in 10 different plantations of three districts of Andhra Pradesh. Basidiomata of Pt were recorded from the tree base in all the plantations (3–11 years old).

Root and rhizosphere soil samples were collected from underneath the fungal basidiomata. Soil samples were analysed for pH, carbon, and available phosphorus and potassium. Root samples were processed for identifying ectomycorrhizal association with the tree species. Pure culture of the ectomycorrhizal fungus was attempted from young, fresh, unopened basidiomata.

Results and discussion

Basidiomata of Pt emerged within a fortnight of the first showers during June and July and continued appearing until October/November. Basidiomata were distributed in irregular patterns at varying distances from the tree base in plantations with or without leaf litter. The frequency distribution of the ectomycorrhizal fungus (Pt) collected and identified from various tree plantations is presented in Table 1. The soil nutrient status of each plantation site is given in Table 2.

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Table 1 Frequency distribution of *Pisolithus tinctorius* in different plantations at various sites in Andhra Pradesh

Name of tree species	Chittoor district						Cuddapah district	Nellore district			Site frequency (%)
	Tirupathi	Tirumala	Kalyani Dam	Punganur	Sathyavedu	Settigunta	Gadela	Dakkili	Dagadarthi	Kavali	
Acacia auriculiformis	–	+	–	+	–	–	–	+	–	–	30
Acacia holosericea	+	+	–	–	–	+	+	+	–	–	50
Acacia mangium	–	–	–	–	–	–	–	+	–	–	10
Eucalyptus camaldulensis	+	+	+	+	+	+	+	+	+	+	100
Eucalyptus tereticornis	+	+	+	+	+	+	+	+	+	+	100

Table 2 Soil nutrient status of different plantation sites in Andhra Pradesh

Sites	Nutrient status			
	pH	Organic carbon (%)	Phosphorus (ppm)	Potassium (ppm)
Tirupathi	6.5–9.0	17	22	150
Tirumala	6.0–8.2	17	7	550
Kalyani Dam	6.7–8.6	Low	Trace	396
Punganur	6.5–9.5	8	9	170
Sathyavedu	6.5–9.0	12	14	360
Settigunta	6.9–9.0	17	4	550
Gadela	7.0–9.0	12	4	330
Dakkili	7.0–10.0	12	22	97
Dagadarthi	6.5–9.0	18	14	400
Kavali	7.0–9.0	13	16	370

The ectomycorrhizal fungus Pt was well distributed in all the plantations (3–11 years old) (Table 1; Figure 1). Maximum distribution was recorded in *E. camaldulensis* (100%), *E. tereticornis* (100%), and *A. holosericea* (50%), and minimum in *A. auriculiformis* (30%) and *A. mangium* (10%) (while *A. mangium* plantation was grown in one site only).

The fungus has been earlier reported in association with *Pinus* spp. (Marx 1977) in the United States; *Hopea odorata* and *Shorea* spp. (Suhardi and Tasimin 1988) in Indonesia; *Pinus kesiya*, *P. merkusii*, and *P. caribaeae* (Nopamornbodi 1988) in Thailand; *Eucalyptus tereticornis* (Natarajan, Mohan, and Kaviyarasan 1988) in India; and *Acacia auriculiformis* (Sampangiramaiah and Bhatta 1996) in India. This fungus is reported from India for the first time in association with *Acacia mangium*, *A. holosericea*, and *Eucalyptus camaldulensis* tree species in low fertile soils of Andhra Pradesh.

Root samples collected from underneath the basidiomata of Pt in association with different tree species were processed for identification of fungal association. All the samples had ectomycorrhizal association in the roots showing fungal mantle and Hartig net structures.

Figure 1 Basidiomata of *Pisolithus tinctorius* near the tree base of *Acacia holosericea* (close-up of the basidiomata)

Conclusion

The acreage under *Acacia* spp. and *Eucalyptus* spp. is increasing both in forest plantations and agricultural lands. Mycorrhization of forest crops has attracted considerable attention over the last few years because of their role as biofertilizer, improving host growth as well as contributing to disease suppression (Marx 1969 and 1973; Sampangiramaiah and Bagyaraj 1989). Hence, the natural association of Pt with acacia and eucalypti is significant and needs further study and exploitation in the nursery and the field.

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Long-term preservation and delivery of ectomycorrhizal fungal inoculum

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Introduction

Research in the field of mycorrhiza requires pure and viable inoculum for investigations at the laboratory level or field level. Reliable storage and delivery materials are required to promote the use of mycorrhiza as a biofertilizer. Germplasm needs to be conserved for a longer duration not only to preserve the culture but also because its delivery into the field along with base or carrier material requires a suitable medium. Ectomycorrhizal fungi which grow as mycelial filaments in vitro are commonly maintained for long durations by conventional storage methods like parafilm oil layering; storage in cold water, silicon gel, hydrogel, and sterile water; freeze-drying or lyophilization. Conventional methods have certain disadvantages like loss of infectivity, contamination, structural damage, variation, and high cost (Anonymous 1997).

Various carrier materials have been tried and tested for delivery of the mycelium into the fields. These include charcoal or lignite-starch, cereal grains, vermiculite, and mycobeads or alginate beads. Cereal grains and vermiculite often contain undesirable levels of residual carbohydrates that can adversely affect the success of the inoculum and also invite contamination (Anonymous 1992).

In this study, base material was screened and used for the ectomycorrhizal inoculum to overcome these problems. Softone, $2MgSi_2O_5.Mg(OH)^{2-}$, was tried as storage as well as carrier material. Also known as talc, it consists of $Si_2O_5^{2-}$ layers with Mg^{2+} and OH^{1-} ions sandwiched in between; the

whole sandwich being electrically neutral. Therefore, talc is very soft; in fact, one of the softest minerals known (Sienko and Plane 1979).

Materials and methods

Softone in fine powder form is used as storage and carrier material. Ectomycorrhizal biomass of papaya (250 g) was homogenized in a blender and mixed vigorously with 750 g of softone under aseptic conditions. This was water saturated using sterile distilled water and stored at 20 °C in a glass beaker. The mixture was tested for viability of mycelium in both liquid as well as solid agar medium at monthly intervals for three months. Inoculated base material was streaked on a PDA (potato dextrose agar) plate as well as an MMN's (Modified Melin Norkran's) liquid medium and kept for further growth at room temperature.

Results and discussion

Ectomycorrhizal association in papaya was confirmed by observing Hartig net and mantle in the short roots. Mycelial growth could be seen in about 4 cm diameter of colony in solid medium and about 0.3 g biomass in 250 ml of MMN liquid medium was generated in 35 days. The mycelium showed viability even after three months; though softone does not contain easily available nutrients, it can hold enough water to sustain mycelial life for a long time without any deleterious effects. Inoculum mixture can turn into brittle cake after some time, which can be broken down into granular form to serve as an easy delivery system. The data favourably suggest the use of softone as storage and carrier material for mycorrhizal biofertilizer.

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New approaches

Molecular tools for generic differentiation in Glomales

A technique involving PCR (polymerase chain reaction) and restriction fragment length polymorphism analysis was used by Redecker D, Thierfelder H, Walker C, and Werner D (1997) to generate specific DNA fragment patterns from spore extracts of AM (arbuscular mycorrhizal) fungi (*Applied and Environmental Microbiology* **63**(5): 1756–1761). DNA fragments that were approximately 500 bp long were amplified from species of *Scutellospora* and *Gigaspora* with the universal primers ITS 1 and ITS 4.

The apparent lengths of the corresponding fragments from *Glomus* spp. varied between 580 and 600 bp. In the genus *Glomus*, Mbo I, Hinf I, and Taq I restriction enzymes were useful for distinguishing species. Depending on the restriction enzyme used, groups of species with common fragment patterns could be found. Five tropical and subtropical isolates identified as *Glomus manihotis* and *G. clarum* could not be distinguished by their restriction patterns, corresponding to morphological similarity of the spores. The variation of internal transcribed spacer sequences among the *Gigaspora* spp. under study was low. Fragment patterns of *Scutellospora* spp. indicated a phylogenetic relationship with *Gigaspora* and revealed only a slightly higher degree of variation.

An improved procedure for mycorrhizal root surface disinfection

A disinfection procedure for mycorrhizal root segment surface was improved by Gryndler M, Hrselova H, and Chvatalova I (1997) by using neomycin and polymyxin B besides sodium hypochlorite, streptomycin, and penicillin G to obtain clean material for observation of proliferation of hyphae of AM (arbuscular mycorrhizal) fungi (*Folia Microbiologica* **42**(5): 489–494). This procedure is more efficient in terms of incidence of contamination compared to the original procedure involving only sodium hypochlorite, streptomycin, and penicillin G. Visually detectable contamination can be further decreased using bacteriostatic concentrations of rolitetracycline which reduces hyphal growth of the AM fungus, *Glomus fistulosum*. This technique was used for studying the uptake of U-C-14 glucose by proliferating hyphae. An active uptake of glucose occurred even in the presence of all the four antibiotics at concentrations of 500 mg per litre.

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Forthcoming events

Conferences, congresses, seminars, symposiums, and workshops

Gent, **Belgium**
9 May 2000

52nd International Symposium on Crop Protection
Prof. Dr P De Clercq

Fax +32 0 9 264 62 39 • *Tel.* +32 0 9 264 61 58
E-mail Patrick.DeClercq@rug.ac.be
Web site http://allserv.rug.ac.be/~hvanbost/symposium or
http://allserv.rug.ac.be/~hvanbost/symposium/index.html

Thunder Bay, Ontario, **Canada**
14–18 May 2000

Forest Sustainability – Beyond 2000
Frances Bennett-Sutton, Conference Coordinator

Fax +1 807 622 4353 • *Tel.* +1 807 622 8228
E-mail fsb2000@flash.lakeheadu.ca

Ed Iwachewski, Program Chair

Fax +1 807 343 4001 • *Tel.* +1 807 343 4106
E-mail iwachewski@mnr.gov.on.ca

Ma'ale Ha'chamisha Kibbutz, **Israel**
14–18 May 2000

World Congress for Soilless Culture on 'Agriculture in the Coming Millennium'
Congress Secretariat, ORTRA Ltd, 1 Nirim Street, PO Box 9352,
Tel-Aviv 61092, Israel

Fax +972 3 6384455 • *Tel.* + 972 3 6384444
E-mail soil@ortra.co.il • *Web site* www.agnic.org/mtg/2000/wcsc.html

Toronto, Ontario, **Canada**
5–8 June 2000

3rd Agricultural Biotechnology International Conference (ABIC 2000)
Sharon Murray, ABIC Conference Coordinator, C/o The Signature Group Inc.,
489 Second Avenue, North Saskatoon, SK, Canada S7K 2C1

Fax +1 877 333 2242 (North America) or 306 664 6615
Tel. +1 877 925 2242 (North America) or 306 934 1772
E-mail siggroup@sk.sympatico.ca • *Web site* www.abic.net/

Bellingham, **Washington**
17–22 June 2000

8th International Symposium on Society and Resource Management
Western Washington University

John C Miles *E-mail* jcmiles@nessie.cc.wwu.edu
Rabel J Burdge *E-mail* burdge@cc.wwu.edu

Québec, **Canada**
18–24 June 2000

6th Congress of International Society for Plant Molecular Biology
International Society for Plant Molecular Biology Quebec 2000, C/o Agora
Communication Inc. 2600, Boulevard Laurier (suite 2680), Sainte-Foy, QC GIV
4M6, Canada

Tel. +1 418 658 6755 • *Fax* +1 418 658 8850
E-mail ispmb@agoracom.qc.ca • *Web site* www.ISPMB-2000.org

Kuala Lumpur, **Malaysia**
7–12 August 2000

21st IUFRO World Congress
Congress Scientific Committee, IUFRO Secretariat, C/o Federal Forest Research
Centre, Seckendorff-Gudent-Weg 8, A-1131 Vienna, Austria

Fax +43 1 8779355 • *Tel.* +43 1 8770151
E-mail iufroxxi.csc@forvie.ac.at

Kuhmo, **Finland**
21–25 August 2000

3rd Workshop on Disturbance Dynamics in Boreal Forests
Workshop on Disturbance Dynamics, Department of Forest Ecology,
PO Box 24, FIN-00014, University of Helsinki, Finland

Fax +358 9 191 7605 • *E-mail* DIST2000@Helsinki.fi
Web site http://honeybee.helsinki.fi/dist2000/

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List of cultures available with the Centre for Mycorrhizal Culture Collection as on 23 March 2000

S No.	Bank code	Name of the fungus	Host
1	EM-1243	*Suillus luteus*	–
2	EM-1245	*Suillus variegatus*	*Sous resineux*
3	EM-1239	*Suillus bovinus*	*Sous resineux*
4	EM-1246	*Suillus variegatus*	*Sous resineux*
5	EM-1136	*Suillus luteus*	*Pinus*
6	EM-1137	*Suillus luteus*	*Picea*
7	EM-1087	*Suillus subluteous*	*Pinus patula*
8	EM-1237	*Suillus bellini*	–
9	EM-1238	*Suillus bovinoides*	*Pinus sylvestris*
10	EM-1236	*Suillus bellini*	–

FORM IV
(as per Rule 8)

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I, R K Pachauri, hereby declare that the particulars given above are true to the best of my knowledge and belief.

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