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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 enhancement and reclamation of barren sites overburdened with fly ash.

About Mycorrhiza Network and CMCC

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

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

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Interaction of mycorrhizae with soil microflora and microfauna—Part II. Interaction with free-living nitrogen fixers and soil microfauna[1]

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Interaction of mycorrhizae with free-living nitrogen fixers

Soil serves as a favourable habitat for a large number of free-living, nitrogen-fixing micro-organisms which do not enter into any organic union with plant roots, and thus, do not form any root nodules. These micro-organisms are known to enhance vesicular–arbuscular mycorrhizal efficiency, resulting in enhanced plant growth. Interaction of these free-living nitrogen fixing micro-organisms with mycorrhizal fungi is discussed in the following account.

Association of free-living nitrogen fixers with mycorrhizal roots

Most of the free-living, non-nodule forming, nitrogen fixing soil micro-organisms are known to be intimately associated with mycorrhizal roots in plants. In studies conducted at the US Department of Agriculture (USDA), Pacific Northwest Research Station, Forestry Science Laboratory, Corvallis, Oregon, USA, nitrogenase activity, measured by acetylene reduction, was detected on nursery grown, surface sterilized, ectomycorrhizae of Douglas fir, formed with *Laccaria laccata, Hebeloma crustuliniforme, Rhizopogon vinicolor*, and *Thelephora* sp.

Detached mycorrhizae were incubated in nitrogen-free liquid medium under microaerophilic conditions. Nitrogenase activity was attributed to *Clostridium* sp. and *Aspergillus* sp. isolated from the mycorrhizae (30). Studies conducted at the Faculty of Soil Science, MV Lomonosov Moscow State University, Russia, on dynamics of populations of *Pseudomonas fluorescence* and *Azospirillum brasilense* in the rhizosphere of potato plants of the cultivar Domodedovskii, showed that during the formation of vesicular–arbusculare mycorrhizae (VAM) by *Glomus mosseae*, the most numerous and active populations of *P. fluorescence* were found in the exorhizosphere while the greatest development of *A. brasilense* occurred in the rhizosphere and endorhizosphere. VAM rapidly stimulated the development of the *P. fluorescence* populations on the surface of the extraradicular mycelium and had the most favourable effect on *A. brasilense* in the endorhizosphere (26).

In studies conducted at the Department of Microbiology, Indian Agricultural Research Institute, New Delhi, India, *Azospirillum* was isolated from the roots of onion (heavily infected with VAM fungi) after surface sterilization of roots with chloramine-T for 30 minutes and incubation at 30 °C on nitrogen-free semisolid medium containing malic acid. Characteristic formation of white pellicles 2–4 mm below the surface of the medium appeared two days after incubation and exhibited acetylene reduction activity (55). Studies conducted at the Instituto Agronomico de Parana, Iapar, Caixa Londrina, Brazil on cassava showed that three diazotrophic bacteria namely

[1] Compiled from TERI database—Riza

Klebsiella sp., *Azospirillum lipoferum*, and Bacterium E were always present in large numbers in stem, tuber, root, and rhizosphere soil (2).

Non-nodule forming, nitrogen-fixers inhabiting sporocarps of ectomycorrhizal fungi

In studies conducted at the USDA, Pacific Northwest Research Station, Forestry Sciences Laboratory, Corvallis, Oregon, USA, nitrogenase activity was detected when tissue fragments from within sporocarps of *Laccaria laccata* and *Rhizopogon vinicolor* were inoculated into nitrogen-free liquid medium and incubated under microaerophilic conditions (99% N_2 plus 1% O_2) at 30 °C. Nitrogenase activity was also detected when tissue homogenates from within the sporocarps of *Hebeloma crustuliniforme Rhizopogon parksii, Barssia oregonensis, Laccinium* sp., and *Hysterangium separabile* were incubated in nitrogen-free, semisolid agar medium. *Azospirillum* spp. were isolated from the nitrogenase-active cultures. *Azospirillum* strains isolated from *R. vinicolor* had higher nitrogenase activity than those isolated from either *H. crustuliniforme* or *L. laccata*. Another study at the above place showed that *Azotobacter chroococcum* grew on spores of *Glomus mosseae* in pots of the tall fescue (*Festuca arundinacea*). *Glomus* spores sieved from the soil were coated with Azotobacter (19, 28, 29).

Studies conducted at the Department of Botany and Plant Pathology, Oregon State University, Corvallis, USA, showed that two different diazotrophic bacterial isolates from within sporocarps of *Rhizopogon vinicolor*, a common ectomycorrhizal associate of Douglas fir (*Pseudotsuga menziesii*), did not differ significantly in their response to extracts from sporocarps of *R. vinicolor, R. subcaerulescens, Leucogaster rubescens, Hymenogaster sublilacinus*, and *Gautieria monticola* under anaerobic or microaerophilic conditions for their effect on nitrogenase activity. Extracts of *L. rubescens, R. vinicolor* and *R. subcaerulescens* enhanced nitrogenase activity under anaerobic conditions but ineffectively or negatively affected nitrogenase activity under microaerophilic conditions. Thus, in a fungal sporocarp, near anaerobic growth conditions plus growth factors provided by the fungus can enhance active nitrogen-fixation by associative diazotrophic bacteria (11).

In the studies conducted at the Department of Microbiology, Indian Agricultural Research Institute, New Delhi, India, Azospirillum was isolated from spores of *Glomus fasciculatum, G. intraradices, G. scientillans, G. mosseae, Gigaspora gilmori*, and *Endogone dusii* after surface sterilization with chloramine-T and incubating at 30 °C on nitrogen-free, semisolid medium containing malic acid. Characteristic formation of white pellicles, 2–3 mm below the medium level appeared after incubation of 60 hours and showed acetylene reduction activity (56).

In studies conducted at the Agricultural University, Uppsala, Sweden, and University of Lund, Sweden, the number of bacteria isolated from the fruit body of *Cantharellus cibarius* was 2.4×10^6/g gross weight, the dominant bacterium being *Pseudomonas fluorescence*. The population of *P. fluorescence* changed from 12% of the total bacterial population in soil to 78% in fruit bodies of *C. cibarius*. No growth stimulating strains were found. Transmission electron microscope studies on ectomycorrhizal system of *C. cibarius* with *Picea abies* showed that the bacteria were embedded in mucus and were proliferating in interhyphal spaces. In mycorrhizae, bacteria were only found at the surface of the mantle (10).

Effect of Diazotrophs on germination of VAM spores

Studies conducted at the Moscow University, Russia, on the effects of inoculation of sterile spores of the endomycorrhiza-forming fungus, *Glomus mosseae*, with bacterial cultures, *Azotobacter chroococcum* and *Pseudomonas fluorescens* and associated cultures of these bacteria with the amoeba, *Hermannella rhysodes*, on the germination of the spores on water agar showed that associated cultures caused greater spore germination, more active growth, and resulted in the higher viability of the saprotrophic mycelium compared with pure bacterial cultures (25). Studies conducted at the University of Florida, Gainesville, Florida, USA, showed that *Klebsiella pneumoniae* increased spore germination and hyphal growth of *Glomus deserticola* compared with control (59). Studies conducted at the Department of Microbiology, Indian Agricultural Research Institute, New Delhi, India, showed that spore germination of *Glomus fasciculatum* was stimulated by cell-free extracts of *Azotobacter chroococcum Azospirillum brasilense, A. lipoforum, Pseudomonas putida*, and *P. fluorescens* (54).

Interaction of VA-mycorrhizal fungi with Azospirillum

Azospirillum spp. are known to fix atmospheric nitrogen. Studies conducted at the USDA, Agricultural Research Service, Albany, USA, showed that in sorghum plants, inoculation with Azospirillum increased plant dry weight and nitrogen assimilation by 25%. Most plant growth responses to Azospirillum were comparable to application of 2.0 mM nitrogen. Increased scavenging of nutrients, altered root permeability or nitrogen-fixation are the possible explanations for these effects (39).

Azospirillum exerts considerable influence on the efficiency of VA-mycorrhizal fungi and plant growth and various aspects of this subject are discussed below.

Effect of dual inoculation with VAM and Azospirillum on plant growth

Studies conducted at the Departamento de Microbiologia, Estacion Experimental del Zaidin, Consejo Superior de Investigaceones Cientificas (CSIC), Granada, Spain, showed that roots from maize (*Zea mays*) and ryegrass (*Lolium perenne*) plants inoculated with *Azospirillum* reduced C_2H_2 but there was no significant effect of inoculation on nitrogen concentration in the roots. In non-mycorrhizal plants, inoculation with *Azospirillum* resulted in greater dry matter production than was achieved by supplying nitrogen as a fertilizer but this trend was reversed at the last harvest in maize. With mycorrhizal plants, *Azospirillum* stimulated the development of VAM and was effective in improving the plant growth at the last harvest of rye grass. In mycorrhizal plants of maize, *Azospirillum* produced similar plant growth and nutrient uptake as compared to that achieved with nitrogen fertilizer. The dual inoculation of maize by *Azospirillum* and *Glomus* produced plants of similar size and nitrogen content and a higher phosphorus content at the last harvest in comparison with those supplied with nitrogen and phosphorus. Shoot production by rye grass was greater at the last two harvests with nitrogen plus phosphorus fertilizers than with dual inoculation (5).

In studies conducted at the USDA, Western Regional Research Centre, Albany, USA, sorghum plants were inoculated with a pigmented strain of *Azospirillum brasilense* on either a high peat or on low organic matter substrates. Half of these plants were inoculated with *Glomus etunicatum* and the other half were fertilized with levels of phosphorus that have been found to be comparable to phosphorus input resulting from mycorrhizal colonization. Total plant dry weights were statistically similar between both the above treatments for each substrate, at all harvests, after the plants were harvested one, three, five, and seven weeks after emergence. The ratio of the total number of inoculated *A. brasilense* cells in the *Glomus* colonized roots as compared to the phosphorus-fertilized roots was positively and significantly correlated with VAM colonization. Colonization of *Sorghum* roots by *G. etunicatum* thus enhanced the establishment and persistence of *A. brasilense* in the endorhizosphere of *Sorghum* (36).

Studies conducted at the Mahatma Phule Agricultural University, Department of Plant Pathology and Agricultural Microbiology, Rahuri, India, showed that dual inoculation of brinjal with *Azospirillum brasilense* and *Glomus fasciculatum* combined with the application of 75% of the recommended phosphorus fertilizer dosage gave the highest yield in field trial on a phosphorus-deficient soil (18). In another study at the above University, results of pot culture experiments on onion (*Allium cepa*) in phosphorus-deficient soil revealed that simultaneous inoculation with *Azospirillum brasilense* and VAM (*Glomus* sp. or *Gigaspora* sp.) significantly enhanced the fresh and dry weights, and nitrogen and phosphorus uptake by shoots and bulbs over their corresponding inoculation with single cultures and controls. Dual inoculation with *Glomus* plus *Azospirillum* was at par with that of *Gigaspora* plus *Azospirillum. Azospirillum* alone in sterile soil enhanced nitrogen and phosphorus uptake in the plants (27).

Studies conducted at the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, India, on screening of 60 sweet potato cultivars showed that dual inoculation of sweet potato in pots with *Azospirillum brasilense* and VAM fungi (*Glomus fasciculatum* or *G. mosseae*) significantly increased growth, plant nitrogen and phosphorus contents, tuber weight, and starch content. Infection by VAM fungi varied from 13.89% to 46.42% depending on the genotypes (21).

Studies conducted at the Division of Microbiology, Indian Agricultural Research Institute, New Delhi, India, showed that dual inoculation of pearl millet (*Pannisetum americanum*) on unsterile, phosphorus-deficient soil with *Azospirillum brasilense* and *Gigaspora margarita* and with *A. brasilense* and *Glomus fasciculatum* significantly increased the dry matter content of the shoots, root biomass, and phosphorus uptake in comparison with uninoculated control plants. Growth and phosphorus uptake of pearl millet was improved by seed inoculation with *A. brasilense* or soil inoculation with VAM fungi (*Acaulospora* sp., *Gigaspora* sp., and *G. fasciculatum*) (49).

In studies conducted at the G B Pant University of Agriculture and Technology, Pant Nagar, Uttar Pradesh, India, on two rice varieties, namely, Jay and Ratna, maximum nitrogenase activity was observed in case of dual inoculation with *Glomus caledonium* and *Azospirillum* with standard dose of NPK. The mycorrhizal infection was 41.6% and 43.2% in Jay and Ratna varieties, respectively, with dual inoculation as compared to 36.7% and 39.2%, respectively, in single inoculation with *G. caledonium* alone (47). Further studies conducted at the above University on finger millet (*Eleusine Corecana*) showed that seed inoculation with *Azospirillum brasilense* (sp. No. 7) resulted in significant root infection with the VAM fungus, *Glomus caledonium*. Dual inoculation of finger millet with *A. brasilense* and *G. caledonium*

improved dry matter production of shoots, dry weight of roots, and per cent mycorrhizal infection (75% or more) (50).

Studies conducted at the Division of Microbiology and Plant Pathology, Central Institute of Medicinal and Aromatic plants, Lucknow, India, showed, that dual inoculation of palmarosa (*Cymbopogon martinii* var. *motia*) with *Glomus aggregatum* and *Azospirillum brasilense* increased the growth, yield, and oil content significantly over VAM alone, Azosporillum alone or uninoculated controls. *A. brasilense* also stimulated the VAM colonization and increased VAM spore population in the rhizosphere soil of palmarosa. Nitrogen content of leaf tissues was higher in plants inoculated with *A. brasilense* alone while leaf phosphorus content was higher in plants inoculated with VAM alone than in dual inoculation (44).

Effect of dual inoculation with Azospirillum and VAM on physiology of host plant/ cuttings

In studies conducted at the Western Regional Research Centre, United States Department of Agriculture, Albany, USA, maize (*Zea mays*) plants grown on relatively fertile peat-based potting mixture were inoculated with either *Glomus etunicatum* or *Azospirillum brasilense* or both or were kept uninoculated and fertilized with amounts of phosphorus and nitrogen that had been found to compensate for the input of nutrients following colonization by VAM or *Azospirillum*. Maize plants colonized by *Glomus* contained more zinc, copper, and proline but less phosphorus, starch, sucrose, threonine, and alanine than corresponding phosphorus-fertilized plants. Maize plants colonized by *Azospirillum* contained less nitrogen, soluble sugars, soluble protein, leucine, and isoleucine but more leaf area, iron glutamate than corresponding nitrogen-fertilized plants. Also, roots of mycorrhizal plants contained a number of lipids [fatty acids 16 : 1 (11c); 18 : 3 (6, 9, 12); 20 : 3; 20 : 4, and 20 : 5] not found in phosphorus-fertilized controls. The physiology of the plants inoculated with *G. etunicatum* or *A. brasilense* was altered in a manner independent of host mineral status. Total plant dry weight in all treatments was statistically indistinguishable at harvest (10 weeks) (34, 35, 37).

Studies conducted at the Division of Plant Physiology, Indian Agricultural Research Institute, New Delhi, India, on wheat cv. ND 2428, inoculated with *Azospirillum brasilense* (seed inoculation) and/or *Glomus fasciculatum* (soil inoculation) showed that chlorophyll concentration, photosynthetic rate, nitrate reductase, and glutamine synthetase activities and grain yield were highest in the dual-inoculated plants. *A. brasilense* mainly increased root growth while VAM increased both root and shoot weights (40).

Studies conducted at the Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore, India, showed that mulberry cuttings dipped in a peat-based slurry of *Azospirillum brasilense* for 15 minutes, then dried in shade and planted in pot soil inoculated with 100 g of *Glomus fasciculatum* inoculum (260 spores per 100 g of soil) placed 2.5 cm below the cuttings had increased biomass and leaf weight compared with controls. Inoculation with *Azospirillum* alone or VAM alone had little effect on these parameters. Effects on plant height and root biomass were not statistically significant (32).

Studies conducted at Instituto Agronomico do Parana, Iapar, Caixa, Londrina, Brazil, showed that *Azospirillum lipoferum* isolated from cassava (*Manihot esculenta*) produced up to 130 µM of indol acetic acid (IAA) after 48 hours of inoculation, *Klebsiella* sp. accumulated about 60 µM and bacterium E 20 µM of IAA. Diazotrophic bacteria stimulated mycorrhizal root colonization of cassava by *Glomus clarum* after 30 days. The beneficial effect could be related to growth-promoting compounds able to stimulate plant susceptibility to mycorrhizal infection, spore germination or the growth of mycelium being able to increase the contact between fungal hyphae and plant root. Addition of cassava exudates stimulated growth of diazotrophic bacteria *in vitro* (3). Further studies conducted at the above place showed that co-inoculation of micro propagated cassava plants with VAM and diazotrophic bacteria gave greater root growth and nitrogen and phosphorus content (2).

Factors affecting efficiency of dual inoculation of plants with Azospirillum and VAM

Effect of fertilizers

Studies conducted at the Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK, Bangalore, India, showed that in pot trials, soil inoculation with *Glomus fasciculatum* and/or seed inoculation with *Azospirillum brasilense* increased the growth and grain yields of maize when given no fertilizers or when given 50% or 100% of the recommended doses of N, P, and K (150 + 75 + 37.5 kg per ha, respectively). The effects were more pronounced in treatments with both the inoculations. Dual inoculation of maize with VAM and *Azospirillum* in soil supplied with 50% of the recommended NPK rate gave significantly higher grain

yields than with 100% recommended NPK rate without symbiont inoculation. Inoculation of maize with *A. brasilense* increased nitrogen contents in the roots and shoots, inoculation with *G. fasciculatum* increased the phosphorus content while nitrogen and phosphorus contents were the highest in treatments with the dual inoculation (48).

In studies conducted at the Indian Agricultural Research Institute, New Delhi, India, barley (*Hordeum vulgare* L.) plants inoculated with *Glomus versiforme* alone or with *Azospirillum brasilense* were given either no fertilizers or 50 kg nitrogen per ha as ^{15}N-labelled ammonium sulphate or 30 kg P_2O_5 per ha or both nitrogen and phosphorus. Dual inoculation with *Azospirillum* and VAM increased growth and grain production compared with uninoculated control plants. Dual inoculation also caused the greatest increases in nitrogen and phosphorus uptake by roots, shoots, and grains. Inoculation with VAM alone increased grain yield only when phosphorus was applied. The amount of N_2 fixed increased with increases in the plant growth. It was highest after the dual inoculation, reaching 58.12 and 79.01 mg/plant in shoots and grains, respectively, at 120 days after sowing without applied phosphorus and 46.94 and 143.8 g/plant in shoots and grains, respectively, with applied phosphorus. Acetylene reduction rates were very low in roots of inoculated plants and were not correlated with plant nitrogen content (33).

Studies conducted at the G B Pant University of Agriculture and Technology, Pant Nagar, Uttar Pradesh, India, on finger millet (*Eleusine coracana*) showed that dual inoculation with *Azospirillum brasilense* and *Glomus caledonium* increased nitrogen, phosphorus, zinc, and iron uptake at two levels of added phosphorus (0 and 22.5 kg P_2O_5/ha) and at three levels of added nitrogen (0, 30, and 60 kg nitrogen/ha). However, there was no effect at phosphorus level of 45 kg P_2O_5/ha (50). In further studies at the above University, seeds of *E. coracana* (finger millet) treated with carrier-based inoculum of *A. brasilense* sp. No.7 ($2.9 \times 10.6 - 11.8 \times 10.6$ cells per g of inoculum) were sown in pots. *G. caledonium* was then added at the rate of 15 ml inoculum (containing 15 spores per ml of soil) per pot. Dual inoculation resulted in increase in the number of fingers per plant, length of earheads, and grain yield at 0 and 22.5 kg P_2O_5 per ha level. Yield was improved by 11–28%. While *A. brasilense* alone did not significantly increase grain yield, *G. caledonium* alone increased the grain yield by 15–25% at different levels of added nitrogen and phosphorus except at phosphorus level of 45 kg P_2O_5/ha (51).

In studies conducted at the Department of Microbiology, Indian Agricultural Research Institute, New Delhi, India, dual inoculation of pearl millet root with *Azospirillum brasilense* and *Glomus versiforme* resulted in significant increase in nitrogen (fixed), total phosphorus uptake, and grain yield over uninoculated control plants. The effect was more pronounced in the presence of 30 kg P_2O_5/ha (53).

Effect of sewage effluent

In studies conducted at the Department of Soil Sciences, King Saud University, Riyadh, Saudi Arabia, seeds of wheat cv. Yecora Rojo, were inoculated with *Azospirillum* or uninoculated and sown in pots irrigated with tap water or with a mixture of 1:1 tap water : sewage water or with undiluted sewage water (electro-conductivity 1.4 d S/m, pH 7.9, mineral nitrogen per kg 14.5 mg, soluble phosphorus per kg 19.4 mg). They were then given further subtreatments with no fertilizers; NPK fertilizer equivalent to 150 kg nitrogen + 150 kg phosphorus + 75 kg potassium per ha; soil inoculation with VAM and/or *Azospirillum*. Root, shoot, and spike dry weight; shoot and spike nitrogen; and phosphorus concentration and nitrogen activity in free soil and rhizosphere soil were greatest in undiluted sewage water and least with tap water. Shoot and spike dry weights and nitrogen and phosphorus contents were greatest with NPK but root dry weight and nitrogen activity in free soil and rhizosphere soil were greatest in dual inoculation with VAM and *Azospirillum*. Root length was greatest with undiluted sewage and was greater with dual inoculation than with VAM alone (1).

Effect of substrate

Studies conducted at the Department of Plant and Soil Biology, University of California, Berkeley, California, USA, on sorghum (*Sorghum bicolor*) grown on four different growth media with or without VAM fungal infection showed that edaphic factors other than phosphorus availability determined the host response to VAM infection. In dual inoculated plants with VAM and a strain of *Azospirillum*, the presence of *A. brasilense* in the rhizosphere increased VAM colonization and biomass while the nitrogen input due to *Azospirillum* decreased, possibly due to competition for carbohydrates. The phosphorus-fixing capacity of the soil, rather than the amount of available ($NaHCO_3$-extractable) phosphorus, influenced the balance between mutualistic and parasitic VAM-fungal growth (38).

Studies conducted at the Western Regional Research Centre, USDA–Agriculture Research Service, Albany, USA, on sorghum (*Sorghum bicolor*) grown on either a high peat or a low organic matter substrate and inoculated with *Azospirillum brasilense*

and/or *Glomus etunicatum* with controls receiving phosphorus to compensate phosphorus input by VAM, showed that total plant dry weight was statistically similar between the two treatments for each substrate at all the harvests. *Azospirillum* counts/g of root were higher in the high peat substrate compared with low organic matter substrate and were significantly higher in the mycorrhizal roots in all the harvests than in phosphorus-fertilized roots for each substrate (36).

Interaction of VA-mycorrhizal fungi with Azotobacter and Klebsiella

Effect of dual inoculation with VAM and Azotobacter/Klebsiella on plant growth

Studies conducted at the Soil Microbiology Department, Rothamsted Experimental Station, Harpenden, UK, on lettuce showed that inoculation of plants with VA-endophytes, *Glomus mosseae*, *G. caledonium*, increased yield of lettuce grown in partially sterilized, phosphorus-deficient soil but not in the same unsterile soil. *Azotobacter chroococcum* alone always increased growth of the young plants before the effects of the endophytes were observed. Dual inoculation with endophytes and Azotobacter in both sterile and unsterile soils produced longer plants than either inoculum alone. In another poorly structured unsterile soil with adequate phosphorus for plant growth, endophytes depressed the yield of lettuce. In none of the experiments did Azotobacter influence the level of the endophyte infection in the roots but the numbers of Azotobacter on the root systems were decreased in the presence of the endophytes (9).

In studies conducted at the Botany Department, Faculty of Science, Tanta University, Tanta, Egypt, Luxor tomatoes grown in unsterilized sandy soil, low in plant available nitrogen and phosphorus, were inoculated with *Glomus fasciculatum* or *Azotobacter chroococcum* or both or left uninoculated as controls. Inoculation of tomato with *A. chroococcum* enhanced root infection by *G. fasciculatum*, stimulated the plant growth, and resulted in increased shoot -N, -Ca, -Mg, and -K compared with the other treatments and root -N, -P, -Na, -Ca, and -Fe compared with uninoculated plants. Inoculation with *G. fasciculatum* recorded the highest level of phosphorus in both roots and shoots and resulted in increased -Na, -K, -Ca, -Mg, and -Fe. *A. chroococcum* displayed a synergistic effect with *G. fasciculatum* in tomato by enhancing mycorrhizal infection, plant growth, and the levels of nitrogen and phosphorus (12).

Studies conducted at the Department of Soil Science, University of Florida, Gainesville, Florida, USA, on sea oats (*Uniola paniculata*) grown in beach sand at two fertilizer (-N) levels showed that inoculation with *Klebsiella pneumoniae* and two *Azospirillum* spp. did not provide the plants with fixed atmospheric nitrogen. *K. pneumoniae*, however, increased root and shoot growth. When a sparingly soluble phosphorus source ($CaHPO_4$) was added to two sands, *K. pneumoniae* increased plant growth in sand with a high phosphorus content. The phosphorus content of shoots was not affected by the bacterial inoculation, indicating that a mechanism other than the bacterially enhanced phosphorus availability to plants was responsible for the growth increases. When sea oats were inoculated with either *K. pneumoniae* or *Acaligenes denitrificans* and a mixed *Glomus inoculum*, there was no consistent evidence of a synergistic effect on the plant growth. Nonetheless, bacterial inoculation increased root colonization by VAM fungi when the fungal inoculum consisted of colonized roots but had no effect on colonization when inoculum consisted of spores alone (59).

Studies conducted at the USDA, Forest Service, Pacific Northwest Forest and Range Experiment Station, Forestry Science Laboratory, Corvallis, USA, showed that dry weights of leaves, culms, and roots of tall fescue (*Festuca arundinacea*) inoculated with *Azotobacter chroococcum* and *Glomus mosseae* were significantly higher than plants inoculated with Azotobacter only. When molybdenum was added to dually-inoculated plants, the dry weights of tall fescue increased significantly (19).

Studies conducted at the Department of Botany, University of Allahabad, Allahabad, India, on linseed showed that the population of Azotobacter and phosphorus solubilizing microbes (PSM) in four samplings taken at seedling, vegetative, flowering, and fruiting stages of plants raised from seeds treated with single, double or triple inoculants (VAM + Azotobacter + PSM) were always higher than plants raised from uninoculated seeds. Maximum improvement in population was caused due to dual inoculation with *Azotobacter chroococcum* and PSM (*Aspergillus niger*). Inoculation with *Glomus aggregatum* caused more mycorrhization than dual or triple inoculations. Maximum improvement in shoot biomass yield, nitrogen, and phosphorus contents was obtained by triple inoculation with *A. niger, G. aggregatum*, and *A. chroococcum* though single and dual inoculations resulted in the improvement of the above parameters over plants raised from untreated seeds (22).

Studies conducted at the Department of Soil Science, Ains Shams University, Cairo, Egypt, showed that combined inoculation of wheat and maize by *Azotobacter chroococcum* (seed inoculation) and VAM (soil inoculation) in sandy soil supplied with superphosphate or rock phosphate gave improved

plant growth and increased phosphorus, nitrogen, and zinc contents in plants. Maize grew better in superphosphate treatments but in individual pots, wheat shoot growth was slightly better with rock phosphate (58).

Studies conducted at the University of Delhi, Delhi, India, on *Trigonella foenum-graecum* showed that inoculation of plants with *Glomus macrocarpum, Rhizobium meliloti*, and *Azotobacter chroococcum* resulted in maximum growth and yield when compared with dual, single inoculations or controls (without inoculation) (6).

Effect of dual inoculation with Azotobacter/ Klebsiella on growth of micropropagated plants/cuttings

Studies conducted at the EMBRAPA-Centro Nacional de Pesquisa de Biologia de Solo Seropedica, Rio de Jeneiro, Brazil, on sweet potato (*Ipomoea batatas*) cv. Princesa and Rosinha, micropropagated and transplanted from axenic conditions to fumigated soil in pots in a greenhouse showed that the greatest effects of mycorrhizal inoculation with *Glomus clarum* were observed with co-inoculation of *Azotobacter diazotrophicus* and/or mixed cultures of diazotrophs containing *A. diazotrophicus* and *Klebsiella* sp. The tuber production was dependent on mycorrhization. Nitrogen and phosphorus accumulations were increased when diazotrophs and *Glomus clarum* were inoculated together. *A. diazotrophicus* infected aerial plant parts only when inoculated together with VAM or when present within *G. clarum*. More pronounced effects on root colonization and intraradical sporulation of *G. clarum* were observed when *A. diazotrophicus* was co-inoculated. In non-fumigated soil, dual inoculation effects were, however, of lower magnitude. N^{15} analysis (in experiments where soil had been mixed with N^{15}-containing organic matter) of the aerial parts and the roots and tubers at the early growth stage (70 days) showed no statistical differences between treatments except for the VAM + *Klebsiella* sp. treatment. This indicated that the effects of *A. diazotrophicus* and other diazotrophs on sweet potato growth were caused by the enhanced mycorrhization, and consequently, a more efficient assimilation of nutrients from the soil than by N_2 fixation (42).

In another study, conducted at the above Centre, inoculation of micropropagated sweet potatoes (*Ipomoea batatas*) with *Glomus clarum* and *Azotobacter diazotrophicus* enhanced spore formation in the soil when compared with VAM inoculation alone. Plants inoculated with VAM spores containing the bacteria showed additional increases in the number of spores formed within the roots. Micropropagated sugarcane seedlings inoculated with the same VAM spores containing the diazotrophs also contained much higher numbers of *A. diazotrophicus* in the aerial parts than the seedlings inoculated *in vitro* with the bacteria alone. When grown in non-sterile soil, sugarcane seedlings again showed the greatest infection of aerial parts after inoculation with VAM spores containing diazotrophs. This treatment also increased VAM colonization and number of spores formed within the roots. Similar effects were observed in sweet sorghum except that the aerial plant parts were not infected by *A. diazotrophicus* (41).

Studies conducted at the Central Sericultural Research and Training Institute, Manandevadi, Mysore, Karnataka, India, on mulberry showed that dual inoculation of three varieties of mulberry with VAM fungi and Azotobacter improved all the parameters studied during the experiment when compared with single inoculations or controls (without inoculation) (14).

Studies conducted at the Plantation Crops Research Institute, Vittal, Karnataka, on black pepper (*Piper nigrum*) showed that rooted cuttings dipped in peat-based slurry of Azotobacter + Azospirillum + *Glomus mosseae* and planted in polythene bags gave greater plant height and shoot and root weights when compared to single inoculations. VAM treatments gave next higher yields (8).

Studies conducted at the Instituto Agronomico do Parana, Iapar, Caixa, Londrina, Brazil, on micropropagated plants of cassava (*Manihot esculenta*) showed that co-inoculation with AM fungi and diazotrophic bacteria (Bacterium E) increased shoot and root dry weights up to 50% and 105% nitrogen up to 88% and 173% and phosphorus up to 83% and 158%, respectively, when compared with plants inoculated with bacterium E alone. Co-inoculation with bacterium E and *Glomus clarum* increased mycorrhizal colonization by 40% and VAM sporulation by up to 168% when compared to inoculations with VAM alone (4).

Interaction of mycorrhizae with soil microfauna

Interactions between soil-inhabiting anthropods, earthworms, and VAM fungi may have important indirect impacts on plant community dynamics and ecosystem level processes. Microarthropods, particularly Collembolans, can negatively influence the distribution and density of VAM external hyphae through grazing, and thus, disrupt nutrient uptake by mycorrhizal fungi. Certain microarthropods detritiunes, principally sow bugs (Izopoda) and millipedes (Diplopoda), ingest and disperse infective mycorrhizal inoculum. Predatory carabid and Scarabacid beetles

have been found to contain VAM spores in their digestive system, and lumbricid earthworms and casts frequently contained VAM fungi. These animals, in turn, are prey to a number of small mammals identified as important vectors of VAM fungi (43).

Tree stumps as Collembola habitat

The Collembola generally exist in soil. Studies conducted at the Natural Resources Canada, Canadian Forest Service, Victoria, Canada, showed that in Douglas fir ecosystems, stumps provided a suitable habitat for most soil Collembola and its densities on volume basis equalled those in the soil. Well-decayed stumps contained about 850 000 individuals/m^3. A total of 216 samples collected during autumn, winter, and spring from four sites (3–8-year old regeneration site, 25–45-year old immature stands, 65–85-year old mature stands, and 200-year old stands) yielded 15 601 individuals comprising 72 species, 40 genera, and 12 families. The composition of Collembolan communities was more sensitive to the successional status of the forest than to season of sampling or to the decay status of the wood (45).

Grazing preferences by Collembola on VAM fungi

Studies conducted at the Department of Forestry, University of Dschang, Dschang, Cameroon, showed that Collembola insects, *Proistoma minuta*, grazed more heavily on *Rhizoctonia solani*, a pathogenic fungus, than the ectomycorrhizal fungi. Among ectomycorrhizal fungi, *Pisolithus tinctorius* was consumed significantly less than *Laccaria laccata, Suillus luteus* or *Thelephora terrestris* in the presence of *R. solani* (17).

Studies conducted at the Institut für Zoologie, Technishe Universitat Brawnschweig, Brawnschweig, Germany, showed that out of five Collembolan species tested for their food preferences on mycorrhizal parsley, *Onychiurus fimatus* preferred *Glomus manihotus, Folsomia candida* had a preference for *G. etunicatum, Proisotoma minuta* was found on parsley infected with *G. intraradices* or *Entrophospora schenkii*, and *Sinella coeca* demonstrated preference for *G. intraradices*. *Xenylla grisea* did not show a preference for mycorrhizal parsley (52).

In a study conducted at the University of Waterloo, Ontario, Canada, trends detected from field data and laboratory experiments suggested that: (i) mites and Collembolans prefer to graze on the litter region rather than in deeper soil layers, (ii) animals that are forced to the lower regions (due to fluctuations in temperature and moisture and interaction with animals) prefer to graze on the hyphae of conidial fungi rather than on VAM fungi, and (iii) when VAM fungal hyphae are grazed, there is a clear preference for the narrower hyphae which are those further away from roots. Thus, soil anthropods cause little reduction in the frequency of endomycorrhizal associations (23). Further studies at the above University showed that addition of soil microarthropods (mites and Collembolan species) to *Acer saccharum* seedlings was associated with decreased arbuscular (-38%) and hyphal (-30%) colonization and increased vesicular colonization (+112%) with no effect on plant biomass. The addition of decaying litter was associated with decreased arbuscular (-51%), increased hyphal (+24%) and vesicular (+117%) colonization and extraradical hyphal length (+38%), and decreased shoot (-43%) and root (-23%) biomass. However, addition of both was associated with enhanced arbuscular (+59%), vesicular (+85%) colonization, increased shoot biomass (+32%), and shoot-root biomass ratio (+25%) (24).

In studies conducted at the Durham University, USA, grazing preferences of the two *Collembolan* species, *Falsomia candida*, and *Proisotoma minuta* on 15 ectomycorrhizal fungi were tested. For *F. candida*, *Laccinum scabrum* was the most visited while *Melanogaster tuberiformis* was the least visited fungus. In pairwise comparisons with *F. candida*, and *P. minuta*, *Gautieria otthii* was preferred over *L. scabrum, Alpova olivaceotinctus*, and *M. tuberiformis*. Fungal preferences did not appear to be taxonomically based at the family level and did not appear to be related to the morphology (46).

In another study conducted at the University of Vermont, South Burlington, USA, catches of larvae of *Hapialus gracilis* obtained by using sticky traps were negatively correlated with the number of fine roots of *Picea rubens* with mycorrhizal relationships suggesting that the decline in the mycorrhizal roots was due to the larval feeding (57).

Effect of Collembola on plant growth/VAM development

Studies conducted at the University of Illinois, Department of Biological Sciences, Chicago, USA, on soyabean (*Glycine max*) showed that the number of nodules per plant in pots increased by 52% when a high density of *Falsomia candida* was added to the pots. When moderate densities of *F. candida* and *Tullbergia granulata* were added, there was 40% greater mycorrhizal root length, 5% higher leaf tissue nitrogen but no changes in leaf phosphorus, nodule number or root mass. In field, intermediate density of Collembola was associated with greater mycorrhizal root length, but not longer root length due to available high phosphorus concentration in soil. Thus, in natural habitat, intermediate Collembola density and low soil phosphorus are expected to maximize benefits of mycorrhiza to plants (31).

Studies conducted at the Department of Agricultural Microbiology, University of Agricultural Sciences, Bangalore, India, showed that casts of earthworms (*Lumbricus terrestis* L.), nests of ants (*Camponotus compressus* Fabr), and faecal pellets of the millipede (*Phyllogonostreptus nigrolabiatus* Newport) collected from the garden, harboured VAM fungal propagules. VAM propagules from earthworms survived for 12 months as determined by the inoculation of onion plants in sterilized soil while those from millipede lost their viability after four days of storage. Ants, which harboured up to 790 infective propagules/g of the nest, and earthworms can thus contribute to the dissemination of VAM fungal propagules. Samples from both mound building and subterranean termites also yielded VAM propagules but these were non-viable (15).

Studies conducted at the University of York, Heslington, UK, in both field and laboratory suggested that the yield and phosphorus uptake of VAM mycorrhizal infected plants may be influenced by populations of Collembola and that Collembolan population, in turn, is influenced by the presence of hyphae of mycorrhizal fungi. Reductions in the potential yield of mycorrhizal plants are thus related to the grazing of external mycelium by soil animals (13).

Experiments conducted at the Ohio State University, USA, on mycorrhizal perennial grass, *Panicum vergatum*, and non-mycorrhizal *Brassica nigra* showed that *P. vergatum* mass and phosphorus uptake were not affected by Collembola grazing in the absence of competition from *B. nigra* but grazing reduced tissue nitrogen concentration and root/shoot ratio. Competition with *B. nigra* plants of the same age/size (simultaneous competition) significantly reduced total root and shoot mass, nitrogen and phosphorus uptake, and increased differences in nutrient uptake (shifting competition towards *B. nigra*) in *P. vergatum* when compared to controls grown without competition. When *P. vergatum* plants were subjected to competition by *B. nigra* plants which germinated three weeks later (off-set competition) the situation was reversed (7). Further studies at the above University to determine the effect of a range of densities of Collembolan, *Falsomia candida*, on the growth, VAM infection, and phosphorus uptake by *Geranium robertianum* under greenhouse conditions showed that total and above-ground growth was greater at low Collembolan density than either at high Collembolan density or without Collembola; differences being greater on organic matter-soil mix than on sand. Root mass was not affected by Collembolan density but root length decreased with increasing Collembolan density in soil mix but not in sand. VAM-infected root length percentage was lower at any Collembolan density than without Collembola; decreasing linearly with increasing Collembolan density. Few significant differences in phosphorus uptake or tissue concentration were found. Thus, plant growth (but not phosphorus uptake) may be stimulated at low Collembolan density and inhibited at high densities (16).

In studies conducted at the University of Illinois, Chicago, USA, the Collembola, *Falsomia candida*, was associated with 40% fewer infection sites for *Glomus deserticola* on *Glycine max* when added at the planting time but no decrease in the infection sites occurred when added 15 days after planting (20).

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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.

Influence of *Casuarina equisetifolia's* plantation on soil properties raised in Arunachal Pradesh

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Introduction

Casuarina equisetifolia, an actinorhizal species having *Frankia* as an N-fixing symbiont in its root nodules (Becking 1970), is predominantly tropical and capable of growing on nutrient-poor and coastal sandy saline systems (NRC, USA 1984). The species of *Casuarina* have potential for soil conservation and rehabilitation, fuelwood production, and agroforestry systems. Root nodules not only fix and transfer nitrogen to plants but also enrich soil fertility on its decomposition (Sharma, Ambasht 1994). Therefore, it is desirable to study the influence of *Casuarina* plantations on soil fertility enrichment.

Materials and methods

The experimental sites were at the North Eastern Regional Institute of Science and Technology (NERIST) campus, Nirjuli, Itanagar, Arunachal Pradesh. The temperature ranges from a minimum 9 °C in winter to maximum of 38 °C in summer with a relative humidity of 45–90%. The rainfall is generally heavy, as much as 3000 mm precipitating annually. The soil (sand 68%, silt 21%, and clay 11%) is laterite in origin with a low fertility level.

The soil samples (0–25 cm deep) were collected from the abandoned land (without vegetal cover) and *Casuarina* plantation stand, approximately 10-years old, subsequently after the first monsoon shower. Then, these samples were brought to the laboratory and analysed for physical, chemical, and biological parameters (Jackson 1973). Vesicular–arbuscular mycorrhizal spore population was assessed by wet sieving and decanting method (Gerdeman, Nicolson 1963). The spores were identified using the manual of Schenck, Perez (1988). Root infection was assessed using the Phillips, Hayman (1970) staining technique and slide method (Giovannetti, Mosse 1980). *Frankia* population from root nodules of *Casuarina* were isolated by using the method of Baker, O'Keefe (1984).

Results and discussion

The results of physico-chemical and biological properties of the soils under *Casuarina* plantation cover and abandoned land are given in Table 1. The values ranged from 1.4% to 2.2% for organic matter, 7.05% to 14.78% for moisture content, 63.78% to 81.6% for water holding capacity, 1.17 to 1.59 g/cc for bulk density, 2.6 to 3.01 g/cc for particle density, and 55% to 47.17% for pore space, respectively, under plantation cover and abandoned land.

Table 1. Characteristics of soil under *Casuarina equsetifolia* plantation cover and abandoned land

Soil characteristics	Abandoned land	Plantation cover
Organic matter (%)	1.40	2.20
Moisture content (%)	7.05	14.78
Water holding capacity (%)	63.78	81.60
Bulk density (g/cm^3)	1.17	1.59
Particle density (g/cm^3)	2.60	3.01
Pore space (%)	55.00	47.17
pH	5.37	6.85
Organic C (%)	0.81	1.27
Available N (kg/ha)	60.00	96.00
Available P (kg/ha)	179.00	196.00
Exchangeable K (kg/ha)	683.00	786.00
Exchangeable Ca (meq%)	6.86	7.20
Exchangeable Mg (meq%)	21.60	23.90
Fungal population (10^3/g d.soil)	79.00	206.00
Bacterial population (10^6/g d.soil)	40.00	88.00
Earthworm population (no. m^3)	4.00	7.00
Nodule biomass (kg/ha)	493.00	603.00
VAM population (spores/100 g soil)	37.00	177.00
Frankia population (no. of colonies/plate)	—	7.0

The chemical properties studied were pH, organic C, available N, available P, exchangeable K, Ca, and Mg. The values ranged from 5.37 to 6.85 for pH, 0.81% to 1.27% for organic carbon, 60 to 90 kg/ha for available N, 179 to 196 kg/ha for available P, 683 to 786 kg/ha for exchangeable K, 6.86 to 7.2 milli equivalent % (meq) for exchangeable Ca and 21.6 to 23.9 meq % for exchangeable Mg, respectively, under abandoned land plantation cover.

The biological properties studied were microbial population, earthworm population, VAM spore population, *Frankia* population, and nodule biomass. The values ranged from 79 to 206 × 10^3 propagules/litre/g dry soil for fungi, 40 to 88 × 10^6 propagules/litre/g dry soil for bacteria, 4–7 m^3 for earthworm population, 37 to 177 VAM spore/100 g soil, and 493 to 603 kg/ha for nodule biomass, respectively, under abandoned land and plantation cover. *Frankia* population in root nodules of *Casuarina* was seven colonies/isolation plate. The VAM species isolated from rhizospere of *Casuarina* trees were *Gigaspora gigantia, G. margarita, Glomus mosseae, G. geosporum, Glomus fasciculatum, G. botryoides,* and *Acaulospora scrobiculata*. The root infection under *Casuarina* plantation was 73%.

From the results obtained, it is clear that soil properties increased significantly under the plantation cover as compared to the abandoned land. The data on *Frankia* population and VAM fungi associated with *Casuarina* suggest that these microsymbionts successfully enrich the soil profile and restore the soil fertility under *Casuarina* plantation which resulted in the *luxuriant* growth of the tree. On the other hand, association of VAM and *Frankia* with *Casuarina* roots decreases the pressure on phosphorus and nitrogen nutrient pools of soil. The increase in available, nutrients under *Casuarina* plantation suggests that the species is not going to affect the soil properties adversely even with the increase in age of *Casuarina*. Hence, *Frankia*-VAM-host association can be exploited for raising the plantation of *Casuarina* on poor and abandoned soils of the region.

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The role of root hairs in the mycorrhizal association of the ground orchid, *Spathoglottis plicata* blume

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Introduction

Mycorrhizae are the most prevalent and perhaps the most important of all mutualistic symbioses known to us and also one of the least understood in terms of the interaction between the symbionts, especially during the establishment of symbiosis (Bonfante-Fasolo, Perotto 1991). There are basically seven types of mycorrhizae; vesicular–arbuscular mycorrhizae (VAM), ecto, ericoid, orchid, arbutoid, monotropoid, and ectendo mycorrhizas (Peterson, Farquhar 1994). Extensive literature is available on ectomycorrhizae and VAM. However, the remaining types of mycorrhizae have been comparatively poorly studied. Among these, the orchid mycorrhizae have not received the due attention they deserve, in view of the following facts: the orchidaceae appears to exert a considerable fascination over botanists and horticulturists because of their great variability in life form and habit, in reproduction and distribution, and in the complexities of their floral structure. More than this, they are economically important on account of luxury trade in their products such as vanilla (Harley 1969). The importance of mycorrhizal infection for the germination of orchid seeds and for successful protocorm/early seedling development has been fairly well-documented in the past (Peterson, Currah 1990; Richardson, Peterson, Currah 1992). However, the subject of mycorrhizal infection in adult orchid is largely neglected (Peterson, Farquhar 1994). Infection is reported to be common in the roots of temperate species (Harley, Harley 1987) but in tropical conditions, the situation is not so clear. In view of the above facts, the present investigation was undertaken, choosing *Spathoglottis plicata*, a common ground orchid, as the study material.

Materials and method

The plants were maintained in the botanical garden of the University campus, Tiruchirappalli (10° 59' and 78.44 E and altitude 68.5 m above sea level). The place receives a rainfall of 747.8/year, the mean annual temperature keeping a maximum of 34.4 °C and a minimum of 24.4 °C. Plants were maintained in pots containing compost-rich garden soil and natural inoculum of the mycorrhizal fungus *Epulorhiza repens (=Rhizoctonia repens)*.

The roots were collected and washed thoroughly in water to remove the soil particles. They were cut into small pieces and fixed in formalin 5 ml: acetic acid 5 ml: 70% alcohol 90ml (FAA). After overnight fixation, the root bits were transferred to 70% alcohol and preserved for future use. Freehand sections (both cross and longitudinal) were stained by Toluidine blue O (Krishnamurthy 1988) for further observations. Photographs were taken using Nikon FA 35A labophot (Japan) binocular microscope.

Results and discussions

It has been observed that an entry into the host root may be through hyphae of the free mycelium or through hyphae produced by germination of sclerotia or chlamydospores. The entry was always through root hairs, with not even a single instance of direct entry through epidermis being observed. The fungal entry was always confined to that part of the root which has root hairs and no other region of the root (Burgeff 1959; Currah *et al.* 1988; Vij, Sharma 1988). The entry into root hairs may be commonly through their tips, often slightly away from the tip or rarely at the base of the root hairs. Invariably, only a single hypha enters into each root hair but occasionally two, or rarely more, may find entry into a single hair (Figure 1). After entry of the fungal hypha, the root hairs usually lose their straight cylindrical

Figure 1. Entry of fungal hyphae into the hair, X 150

nature and become crooked and distorted to various degrees as in many other infections involving rhizobia and root parasitic microbes. The distortion may be limited to the tip of the hair alone or may be seen extended variously towards the base of the hair depending upon the locus of entry of the hypha. In fact, the distorted appearance of root hairs may form one of the best indications of those hairs that were already penetrated by the fungus (Hadley, Williamson 1972; Peterson, Farquhar 1994). At the point of entry, no special, vesicular or appressorial or swollen structure could be seen in the hypha. The fungus soon enters into the cortical cells and forms hyphal balls called *pelotons* (Figure 2)

Figure 2. T S of root stained with Acridine orange and observed under UV light to show the fungal pelotons in the cortical cells. X 150
Note. The arrow indicates the pelotons

Not only are the root hairs involved as the entry points for the mycorrhizal fungus into the host root, both during primary and secondary infections, but they also serve as venues for the reproduction of the fungus as well as for their exit from the root cortex to the rhizosphere. During the actual exit process, they do not go out as hyphae but as chlamydospores and occasionally as sclerotia. Chlamydospores are formed in plenty within the root hairs in clumps or in short or long chains terminally or intercalarily (Figure 3). Such chlamydospore-containing root hairs undergo very characteristic spiral dehiscence (Figure 4) to release the spores into the rhizosphere.

Figure 3. The root hair leading to the formation of chlamydospores. X 650

Figure 4. Root hair showing chlamydospores dehiscing spirally. X 325

To the best of the author's knowledge, there is no report of chlamydospore formation in the root hairs and the dehiscence of the root hairs to release these in any orchid so far studied, although, in some parasitic species *Rhizoctonia* chlamydospores and sclerotia have been reported occasionally in the epidermal cells of the host (Sneh *et al.* 1994).

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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.

Production of arbuscular mycorrhizal fungal biomass of high purity

A novel method for the production of arbuscular mycorrhizal (AM) fungal biomass of high purity is described by Redecker D, Thierfelder H, Werner D (*Journal of Applied Botany—Angewandte Botanik* **69** (5–6): 189–191, 1995). The system consists of fine chambers separated by nylon mesh screens of different pore sizes. The two outer compartments contain *Allium schoenoprasum* precolonized with *Glomus intraradices* or the *Glomus* species in a soil substrate. Colonized roots are allowed to grow into the adjacent chambers filled with glass beads. Only the hyphae grow across the innermost compartment. Production of spores on these hyphae can be easily monitored. The material represents an infectious inoculum. Washing out spores or hyphae yields relatively pure material that can be used for DNA extraction or biochemical experiments.

Detection of ectomycorrhizal fungi during symbiotic interaction by polymerase chain reaction (PCR) amplification of their gpd genes

Degenerated oligonucleotide primers designed to bank an approximately 1.2 kb fragment of the gene encoding glyceraldehyde-3-phosphate dehydrogenase (gpd) from Ascomycetes and Basidiomycetes were used by Kreuzinger N, Podev R, Gruber F, Gobl F, Kubicek C P to amplify the corresponding gpd fragments from several species of ectomycorrhizal fungi tissue *Boletus, Amanita*, and *Lactarius* (*Applied and Environmental Microbiology* **62**(9): 3432–3438, 1996). Those from *Boletus edulis, Amanita muscaria*, and *Lactarius determinus* were cloned and sequenced. The respective nucleotide sequences of these gene fragments showed a moderate degree of similarity (72–76%) in the protein encoding region and only a low degree of similarity in the introns (56–66%). Introns, where present, occurred at conserved positions but the respective position and numbers of introns in a given taxon varied. The amplified fragment from a given taxon could be distinguished from that of others by both restriction nuclease cleavage analysis and southern hybridization.

A procedure for labelling DNA probes with fluorescein 12-dioxy uridine tri-phosphate deoxy Uridine Triphosphate (dUTP) by PCR was developed. These probes were used in a non-radioactive hybridization assay with which the gene could be detected in 2 ng of chromosomal DNA of *L. determinus* on slot blots. Taxon-specific amplification was achieved by

the design of specific oligonucleotide primers. The application of the gpd gene for the identification of mycorrhizal fungi under field conditions was demonstrated with spruce (*Picea abies*) mycorrhizal roots harvested from a Northern Alpine forest area as well as from plant breeding nursery. The interference from inhibitory substances, which sometimes occurred in the DNA extracted from the root-fungus mixture, could be overcome by using very dilute concentrations of template DNA for a first round of PCR amplification followed by second round with nested oligonucleotide primers. The authors concluded that gpd can be used to detect ectomycorrhizal fungi during symbiotic interaction.

Forthcoming events · · ·

12–16 October 1998
International Workshop on Sustainable Management of African Rainforests Today. Libreville, Gabon
For further information, please contact :
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Project FORAFRI, BP 643
Libreville
Gabon
Tel./Fax: 24 1 733 145
e-mail: FORAFRI@compuserve.com

12–16 October 1998
Forest Ecosystem and Land Use in the Mountain Areas. Seoul, Korea
For further information, please contact :
Don Lee
Seoul National University
College of Agriculture and Life Science
Department of Forest Resource
103 Seodoondong
Suwon 441 744, Korea (Rep.)
e-mail: leedk@agris.snu.ac.kr

12–23 October 1998
Biodeterioration, Conservation and Preservation of Cultural Heritage. India
The course aims to develop understanding of the biodeterioration, conservation, and preservation of cultural heritage in India. Knowledge will also be given on the fungi responsible for biodeterioration and degradation. The course cost will be Rs 3000/- for Indian participants and US$ 500/- to foreign (Asian) participants.
For further information, please contact:
Indian participants
Prof. K G Mukerji
Department of Botany,
University of Delhi
Delhi - 110 007, India
Tel.: 725 7573, 725 7725/383, 725 7502
Fax: 725 7830

Foreign (Asian) participants
Dr Jagjit Singh
Regional Director
Oscar Faber
Marlborough House
Upper Marlborough Road,
St Albans, Herts,
AL1 3UT, UK

20–29 October 1998
Workshop and Study Tour on Silviculture of Mixed Species Forests in the Subtropical Himalayas. Bhutan
For further information, please contact:
Jerry Vanclay
CIFOR, PO Box 6596
JKPWB 10065 Jakarta
Indonesia
Tel.: 62 251 326458
Fax: 62 251 326433e
e-mail: j.vanclay@cgnet.com

Recent references · · ·

Latest additions to the Network's database on mycorrhiza are published here for information of the members. The Mycorrhiza Network will be pleased to supply any of the available documents to bonafide researchers at a nominal charge.

This list consists of papers from the following journals, which are arranged in alphabetical order.

- *Canadian Journal of Microbiology*
- *Mycological Research*
- *Mycorrhiza*
- *New Phytologist*

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

Canadian Journal of Microbiology 43(11), 1997

Nurmiaho-Lassila EL, Timonen S, Haahtela K, Sen R (Department of Biosciences, Division of General Microbiology, P O Box 56 (Viikinkaari 9), 00014 University of Helsinki, Helsinki, Finland) Bacterial colonization patterns of intact *Pinus sylvestris* mycorrhizospheres in dry pine forest soil: an electron microscopy study, 1017–1035

Mycological Research 102(2), 1998

Tibbett M, Sanders FE, Cairney JWG (Department of Biology, University of Leeds, LS2 9JT, UK). The effect of temperature and inorganic phosphorus supply on growth and acid phosphatase production in arctic and temperate strains of ectomycorrhizal *Hebeloma* spp. in axenic culture, 129–135

Mycological Research 102(3), 1998

Anderson C, Chambers S M, Cairney JWG [Department of Biological Sciences, University of Western Sydney (Nepean) P O Box 10, Kingswood, NSW 2747, Australia]. Use of molecular methods to estimate the size and distribution of mycelial individuals of the ectomycorrhizal basidiomycete *Pisolithus tinctorius*, 295–300

Mycological Research 102(4), 1998

Forbes PJ, Millam S, Hooker JE, Harrier LA (Soil Biology Unit, Land Resources Department, Scottish Agricultural College, Aberdeen AB21 9TQ, Scotland, UK). Transformation of the arbuscular mycorrhiza *Gigaspora rosea* by particle bombardment, 497–501

Mycorrhiza 7(5), 1998

Berman JT, Bledsoe CS (Department of Land, Air and Water Resources, University of California, Davis, CA 95616-8627, USA). Soil transfers from valley oak (*Quercus lobata* Nee) stands increase ectomycorrhizal diversity and alter root and shoot growth on valley oak seedlings, 223–235

Jarstfer AG, Farmer-Koppenol P, Sylvia DM (Soil and Water Science Department, P O Box 110290, University of Florida, Gainesville, Fla 32611-0290, USA). Tissue magnesium and calcium affect arbuscular mycorrhiza development and fungal reproduction, 237–242

Junghans D T, Gomes E A, Guimaraes W V, Barros E G, Araújo E F (Universidade Federal de Viçosa, Departamento de Microbiologia/BIOAGRO, Viçosa, MG, 36571-000 - Brazil). Genetic diversity of the ectomycorrhizal fungus *Pisolithus tinctorius* based on RAPD-PCR analysis, 243–248

Singh Satpal, Kapoor K K (Department of Microbiology, CCS Haryana Agricultural University, Hisar, 125 004, India). Effects of inoculation of phosphate-solubilizing microorganisms and an arbuscular mycorrhizal fungus on mungbean grown under natural soil conditions, 249–253

Sharples J M, Cairney J W G (Mycorrhiza Research Group, School of Science, University of Western Sydney (Nepean), P O Box 10, Kingswood, NSW 2747, Australia). Assimilation of inorganic nitrogen by a mycobiont isolated from *Pisonia grandis* R. Br. (Nyctaginaceae) mycorrhiza, 255–260

Jumpponen A, Mattson KG, Trappe JM (Department of Forest Science, Oregon State University, Corvallis, OR 97331-7501, USA). Mycorrhizal functioning of *Phialocephala fortinii* with *Pinus contorta* on glacier forefront soil: interactions with soil nitrogen and organic matter, 261–265

Kottke I, Xiao M Qian, Pritsch K, Haug Ingeborg, Oberwinkler F (Eberhard-Karls-Universität Tübingen, Botanisches Institut, Spezielle Botanik, Mykologie und Botanischer Garten, Auf der Morgenstelle 1, D-72076 Tübingen, Germany). *Xerocomus badius—Picea abies*, an ectomycorrhiza of high activity and element storage capacity in acidic soil, 267–275

Mycorrhiza 7(6), 1998

Smith J E, Johnson K A, Cázares E. (USDA, Forest Service, Pacific Northwest Research Station, Forestry Sciences Laboratory, 3200 SW Jefferson Way, Corvallis, OR 97331, USA) Vesicular mycorrhizal colonization of seedlings of Pinaceae and Betulaceae after spore inoculation with *Glomus intraradices*, 279–285.

Massicotte H B, Melville L H, Peterson R L, Luoma D L (College of Science and Management, Faculty of Natural Resources and Environmental Studies, University of Northern British Columbia, Prince George, British Columbia, Canada). Anatomical aspects of field ectomycorrhizas on *Polygonum viviparum* (Polygonaceae) and *Kobresia bellardii* (Cyperaceae), 287–292

Siqueira J O, Saggin-Júnior O J, Flores-Aylas W W, Guimaraes P T G (Departamento de Ciência do Solo, Universidade Federal de Lavras CP 37, CEP 37.200-000 Lavras, MG-Brazil). Arbuscular mycorrhizal inoculation and superphosphate application influence plant development and yield of coffee in Brazil, 293–300

Burleigh SH, Harrison MJ (The Samuel Roberts Noble Foundation, Plant Biology Division, 2510 Sam Noble Parkway, Ardmore OK 73401, USA). A cDNA from the arbuscular mycorrhizal fungus *Glomus versiforme* with homology to a cruciform DNA-binding protein from *Ustilago maydis*, 301–306

Gaur A, Adholeya A, Mukerji KG (Tata Energy Research Institute, Darbari Seth Block, Habitat Place, Lodhi Road, New Delhi - 110 003, India). A comparison of AM fungi inoculants

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using *Capsicum* and *Polianthes* in marginal soil amended with organic matter, 307–312

Flynn D, Newton AC, Ingleby K (The Institute of Ecology and Resource Management, University of Edinburgh, Darwin Building, Mayfield Road, Edinburgh, EH9 3JU UK). Ectomycorrhizal colonisation of Sitka spruce [*Picea sitchensis* (Bong.) Carr] seedlings in a Scottish plantation forest, 313–317

Chambers SM, Sharples JM, Cairney JWG (Mycorrhiza Research Group, School of Science, University of Western Sydney (Nepean), P O Box 10, Kingswood, NSW 2747, Australia). Towards a molecular identification of the *Pisonia mycobiont*, 319–321

Comandini O, Pacioni G, Rinaldi AC (Dipartimento di Scienze Ambientali, Università dell'Aquila, Via Vetoio, I-67010 Coppito, L'Aquila, Italy). Fungi in ectomycorrhizal associations of silver fir (*Abies alba* Miller) in Central Italy, 323–328

New Phytologist 138(1), 1998

Jr Douds DD, Galvez L, Bécard G, Kapulnik Y (USDA-ARS ERRC, 600 E. Mermaid Lane, Wyndmoor, PA 19038 USA). Regulation of arbuscular mycorrhizal development by plant host and fungus species in alfalfa, 27–35

Schellenbaum L, Müller J, Boller T, Wiemken A, Schüpp H (Botanisches Institut der Universität, Hebelstrasse 1, 4056 Basel, Switzerland). Effects of drought on non-mycorrhizal and mycorrhizal maize: changes in the pools of non-structural carbohydrates, in the activities of invertase and trehalase, and in the pools of amino acids and imino acids, 59–66

Merryweather J, Fitter A (Department of Biology, PO Box 373, University of York, YO1 5YW, UK). The arbuscular mycorrhizal fungi of *Hyacinthoides non-scripta*, I Diversity of fungal taxa, 117–129

Merryweather J, Fitter A (Department of Biology, PO Box 373, University of York, YO1 5YW, UK). The arbuscular mycorrhizal fungi of *Hyacinthoides non-scripta*, II Seasonal and spatial patterns of fungal populations 131–142

Ekblad A, Wallander H, Näsholm T (Department of Forest Ecology, Swedish University of Agricultural Sciences, S-901 83 Umeå, Sweden). Chitin and ergosterol combined to measure total and living fungal biomass in ectomycorrhizas, 143–149

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-1189	*Laccaria laccata*	Quescusilex	Catalogne
2	EM-1190	*Laccaria laccata*	Hetre	Institute National de la Rechereche Agronomique (INRA), France
3	EM-1275	*Laccaria proxima*	*Picea, Pinus*	Uppsala, Sweden
4	EM-1207a	*Laccinum scaber*	Charme	—
5	EM-1279	*Lactarius lepaticus*	—	—
6	EM-1260	*Lactarius deterrimus*	Chene	Mosette
7	EM-1262	*Lactarius deterrimus*	—	—
8	EM-1206	*Lactarius tabidus*	—	—
9	EM-1199	*Lactarius deterrimus*	—	—
10	EM-1259	*Lactarius chrysorrheus*	Hetre	Vosges, France
11	EM-1205	*Lactarius subdulcis*	Chene	Mosette
12	EM-1203	*Lactarius rufus*	*Sous resineux*	Sweden
13	EM-1106	*Leccinum insigne*	Mixed hardwood forest	Houghton, USA

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