# Mycorrhizal Biotechnology for Reclamation of Oil Sand Composite Tailings and Tailings Land in Alberta

**A.M. Quoreshi[1], D.P. Khasa[2], G. Bois[2], J.L. Jany[2], E. Begrand[1], D. McCurdy[3], M. Fung[4]**

[1] Symbiotech Research Inc. # 201, 509-11 Avenue, Nisku, AB, T9E 7N5
[2] Forest Biology Research Centre, University of Laval, Quebec, G1K 7P4
[3] Bonnyville Forest Nursery, 5110-55th Avenue, Bonnyville, Alberta, T9N 2M9
[4] Environmental Affairs, Syncrude Canada, Fort McMurray, Alberta, T9H 3L1

## Introduction

Reclamation of disturbed land in Alberta with tailings sand (TS) and composite tailings (CT) created by the Canadian oil sands industry is a high priority agenda. Surface mining of oil sands by the Canadian tar sands industry produces huge areas of degraded land in Alberta with composite tailings sand (CT) and tailing sand (TS) that requires reclamation (Fung and Macyk 2000). Over the last 30 years, it is estimated that oil sands extraction has produced over 3 x $10^8$ $m^3$ tailings. Composite tailings are one of the most challenging materials for land reclamation and are generally known to be nutrient poor, with high alkalinity and salinity, extremely low in organic matter, and lack necessary biological activity. Because of prevailing poor soil conditions and lack of biological activity in disturbed land, establishment of vegetation has been challenging, and often requires repeated plantings to offset huge mortality. Mycorrhizal fungi are a crucial component in plant communities and play an essential role in ecosystem establishment and productivity of plant species (van der Heijden et al. 1998, Jonsson et al. 2001).

Improved reclamation of adverse sites has been achieved by inoculating conifer and hardwood tree species with beneficial mycorrhizal fungi (Marx 1991, Malajczuk et al. 1994). It is now an established fact that the presence of microorganisms, particularly mycorrhizal fungi, benefits the vegetation by increasing a plant's ability to survive in difficult sites. Therefore, the presence of mycorrhizae with seedling root system before outplanting must be a major consideration for successful reclamation of disturbed land.

In this summary report, we briefly describe the different types of mycorrhizae, which play an essential role in converting disturbed lands into productive lands. We will also describe several experiments on mycorrhizal inoculation techniques and procedures under commercial forest nursery conditions, different field trials conducted on oil sands tailings, and selection of appropriate species of mycorrhizal fungi for use in reclamation of tailings sands sites. A study on mycorrhizal inoculum potential of reclamation site is also briefly presented. Results are discussed in the context of application of mycorrhizal biotechnology to the revegetation of oil sand areas.

### Benefits of Mycorrhizae on Mined Lands Sites

Mycorrhizas are symbiotic associations between plant roots and beneficial fungi, which greatly increase the efficiency of nutrient and water uptake of plants in exchange for carbohydrates (Smith and Read 1997). Other benefits include enhance resistance to pathogens (Duchesne et al. 1988, Krop and Langlois 1990), the ability to buffer plant species against several environmental stresses (Malajczuk et al. 1994), drought resistance, and they can also reduce transplanting shock (Molina et al. 1992; Smith and Read 1997). Mycorrhizal fungi can also improve plant growth and survival in soils contaminated by heavy metals (Jones and Hutchinson 1988) and salt (Azcón and El-Atrash 1997).

Mycorrhizal fungi are considered a necessary component of a self-sustaining ecosystem. The symbiotic association between plant-fungi sometime reduces growth of non-host weeds. Mycorrhizal associations are also known to increase soil biota by stimulating other beneficial microorganisms associated with the rhizosphere. Some mycorrhiza produce a substance called "glomalin" that acts as a soil aggregation agent. Furthermore, mycorrhizal fungi contribute toward carbon sequestration. Up to 30% of soil biomass is a result of mycorrhizal activity. The fundamental importance of the mycorrhizal association in restoration and to improve revegetation is well recognized but the use of inoculation technique in plantation forestry is not extensive in Canada. The primary reason could be the lack of availability of high quality commercial inoculum, improved technology for inoculation in commercial nursery setting, and lack of knowledge on mycorrhizal biotechnology.

In general, native mycorrhizal fungi are present in most healthy ecosystems, but are destroyed by different disturbances. When soil is disturbed, the populations of mycorrhizal fungi and other microorganisms that help plant roots in acquiring water and nutrients can be destroyed. The lack of mycorrhizal fungi on any disturbed areas is the basis of inoculation of seedlings. Furthermore, it is not just to make individual plants mycorrhizal, but it is important to establish the mycorrhizal network in soil that mediate ecosystem function. Therefore, re-establishment of symbiotic fungi should be considered as an essential constituent of any degraded land reclamation program.

### Types of Mycorrhizas

Smith and Read (1997) recognize seven types of mycorrhizas depending on the plant and fungus involved. However, four main types of mycorrhizas; arbascular mycorrhizas (AM), ectomycorrhizas (ECM), ectoendomycorrhizas, and ericoid mycorrhizas are important for altering disturbed lands into productive agricultural and forested lands. This report is mainly directed toward ECM and AM (Fig. 1). The ECM, which are generally found on pines, spruces, firs, hemlocks, larches, poplars, beeches, eucalyptus, alders, hickories, and oaks, and formed by basidiomycetes, ascomycetes, also several species of zygomycetes comprising some 5,000 to 6,000 species (Brundrett et al. 1996). Ectomycorrhizas are characterized by the presence of hyphae between root cortical cells producing a net like structure called Hartig net and easily recognized by the presence of mantle or sheath of fungal mycelium that may cover the fine, short feeder roots.

The Arbuscular mycorrhizas (AM) are formed by a small group of fungi in the new phylum Glomeromycota and class Glomeromycetes (Schüβler et al. 2001) of eight known genera and characterized by the development of finely branched hyphae called arbuscules within root cortical cells, where the metabolic exchanges between the fungus and the host plant take place. Arbuscular mycorrhizas do not modify root morphology and the fungal part is invisible without a microscope. All AM fungal species are obligate biotrophs, meaning they depend entirely on the host plant for carbon. This means, unlike ECM fungi, AM fungi cannot be cultured in the absence of plants or artificial nutrient media. AM fungi are more common in agricultural crops, grasses, and some hardwoods (poplars, walnuts, maples, cedars, elms, willows, sycamore, redwoods, ashes, cherries, locusts, alders, oleasters, etc.).

## Canadian Oil Sands Industry

Oil sands are naturally occurring deposits of bituminous sand -- a mixture of bitumen, which is a thick sticky form of crude oil, sand, clay and water (Fig. 2). Oil sands deposits are found in several locations around the world. In Canada, oil sands deposits are located in northern Alberta near Fort McMurray about 450 km north of Edmonton city (Fig. 3). Canada has ¾ of the world's oil sands deposits, which comprises 1/3 of the world's known oil reserves. It is estimated that 1.7 trillion barrels of oil in Alberta oil sands deposits and provides approximately 20% of Canada's oil needs.

Three large companies, such as Syncrude Canada Ltd., Suncor Energy Inc., and Albion Sands Energy Inc. are involved in commercially productive operations in Alberta's oil extraction process from oil sands deposits. The mining activity produced vast areas of land

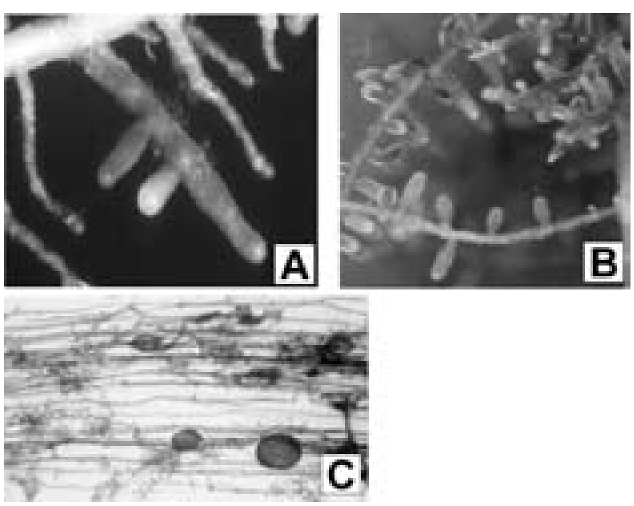


***Figure 1.*** *Examples of morphological features of typical ectomycorrhizas (A, B) and Arbuscular mycorrhizas (C) formation.*

***Figure 2.*** *Oil sand is a mixture of bitumen, sand, water and clay.*

unproductive with little or no biological activity due to production of composite tailings (CT) and tailings sand (TS) as byproducts of extraction process of the oil sands industry. These are a combination of course sand, fine silt and clay, some residual bitumen and water. Tailing sands are alkaline in nature, low in nitrogen and phosphorous, and very low in organic matter content. Development of oil sand industry has a major impact in the natural environment. Therefore, reclamation of these challenging materials is needed.

Briefly the processes in the oil sands industry include, mining the areas, removal of Muskeg and Overburden layers, extraction of raw oil (Bitumen), upgrading of bitumen into sweet light crude oil, returning tailings and overburden to areas, and finally land reclamation.

## What is Reclamation?

Reclamation is the process of reconverting disturbed land to its former or any other productive uses. The main objective of reclamation is to reclaim disturbed land to a stable, biologically self-sustaining state as soon as possible. This means creating a landscape with productive capability similar, if not more so, to that before it was disturbed. The re-installation of microbiological activities to mining sites is known to enhance revegetation and reclamation success.


***Figure 3**. Oil sands deposits are found in Northern Alberta near Fort McMurray, Alberta, Canada.*

## Inoculum Production

Effective utilization of mycorrhizal biotechnology in forest plantations and revegetation practices depends on the availability of high quality inoculum, improved technology for application of inoculum and an understanding of benefits of using mycorrhizal inoculum for plant development. Any mycorrhizal inoculation program cannot become a regular practice if adequate quantities of efficient inoculum are not available. Several methods of inoculation can be used in practice. Three types of inoculum (Fig. 4) are currently being used in nurseries to inoculate seedlings: (1) vermiculite-peat based solid-substrate inocula, (2) liquid/mycelial slurry inocula, and (3) spore inocula.

Pure vegetative inoculum of selected fungi is recommended as the most biologically effective materials for inoculation since harmful organisms are excluded (Marx and Kenny 1982). ECM fungi are generally grown in the laboratory using a fermentor for large-scale inoculum production. AM fungi cannot be grown on culture media. AM fungi must be cultured with a host plant. AM inoculum typically consists of root fragments, spores, fungal hyphae, and growth medium from the open pot culture method of inoculum production.

### Solid-substrate inocula

It is easy to produce sufficient quantities in bag culture either for large trials or research purposes. We used Glucose Yeast Malt Extract (GYME) medium, excellent in producing huge fungal cultures using a fermentor. In

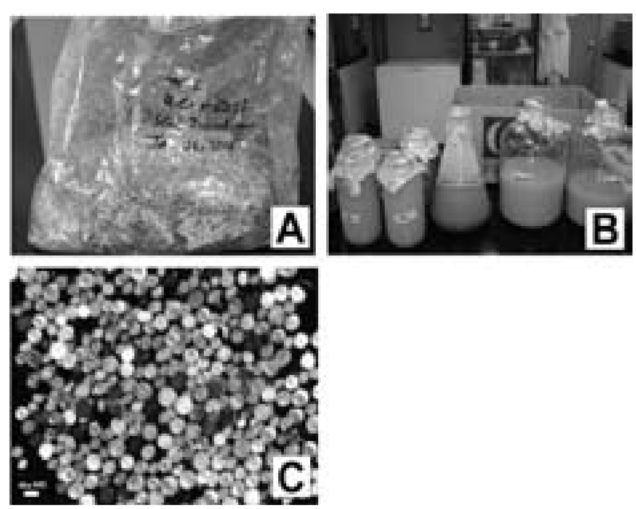


***Figure 4**. Different types of mycorrhizal inoculum can be used for inoculating nursery seedlings. Vermiculite-peat based solid vegetative inoculum in bag culture (A), liquid/mycelial slurry inoculum (B), and spore inoculum (C).*

this process, vermiculite and peat moss moistened with a nutrient solution is inoculated with liquid mycelial culture, produced by either a shake flask or in a fermentor and incubated in the dark for a certain period. The colonized solid substrate (vermiculite-peat) subsequently used as pure vegetative solid inocula mixed with a growing substrate.

### Liquid inocula

The liquid inocula are grown from selected fungi on suitable liquid media using shake flask or fermentor. The mycelial suspension was continuously agitated on a mechanical shaker or in fermentor. Before using as liquid inoculum the mycelial suspension needs to be homogenized with a Waring blender. The suspension usually diluted before inoculation with water to obtain desired concentration of propagules/ml in the mycelial slurrey. The liquid inoculum can be mixed with growing substrate at sowing seeds.

### The criteria of effective inoculum

The effectiveness of an inoculum is depends on its characteristics as a source of propagules. Fungal biomass should be produce under sterile conditions and must have an appropriate physiological state for high viability and storage capacity.

Mycelia carrier materials should be uniformly coupled with fungal propagules (biomass) and have the capacity to protect the fungal component during the production and handling process. Furthermore, inoculum should be in a form for which no problem in delivery in growing substrate or soil. Finally, a form of inoculum, which is practical and compatible to large-scale production and use, low in volume, easy to ship and handle, and cost effective.

## Inoculation of Nursery Seedlings

Inoculation of nursery seedlings is required if the plants are mycorrhizal host species, and the seedlings need to be planted into soil with inadequate mycorrhizal inoculum potential, most importantly, revegetate any disturbed area caused by mining or other disturbances.

In order to obtain a better colonization of ectomycorrhizal fungi in nursery seedling, a slight modification of generous fertilization schedule practiced by commercial nursery is needed. Hunt (1992) recommended that nursery managers should limit application of soluble fertilizers with an upper limit of 80-100 ppm N and 30-35 ppm P.

Currently, inoculation of jack pine and white spruce is being done by our group using both liquid and solid vegetative inocula at Bonnyville Forest Nursery, Alberta. The fungal isolates were isolated from Canadian forests. Several techniques are available to apply inocula during seedling production. However, we found that the most convenient and practical way of inoculating seedlings under commercial nursery seedlings are mixing inocula either solid vegetative or liquid mycelial slurry, with growing substrate at the time of sowing the seeds. Our inoculation program showed successful ectomycorrhizal formation under commercial nursery conditions (Fig. 5). Our results showed that inoculation of jack pine seedlings with different inoculum types and different fungal species resulted in adequate ectomycorrhizal formation between 51 and 90% of short roots.

## Application of Microbial Biotechnology in Land Reclamation

Several peer-reviewed research efforts focused on CT phytotoxicity, plant tolerance to saline conditions and establishment of soil microbial activity, and

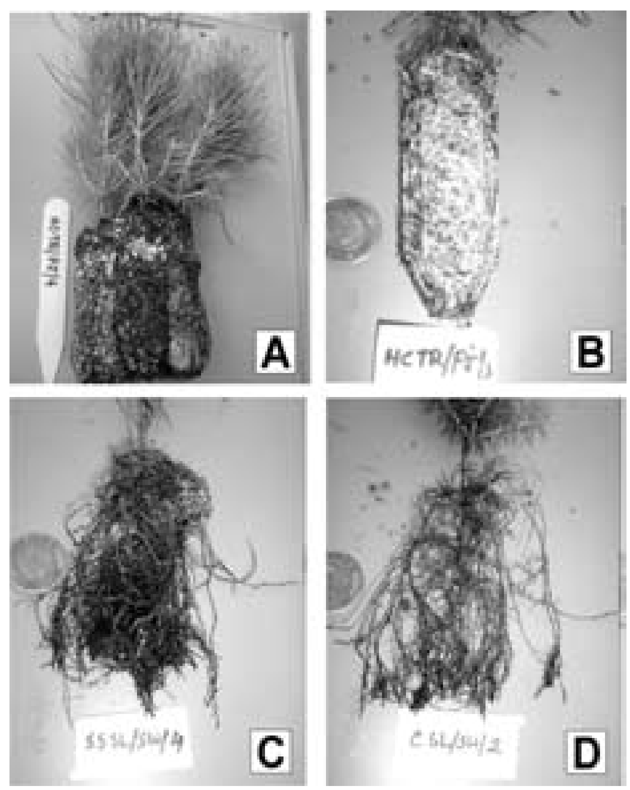


***Figure 5.*** *Containerized jack pine seedlings successfully inoculated with Hebeloma crustuliniforme (A, B). Note white mass of fungal mycelia covered the root plug. Compare the root system of inoculated (C) with Suillus sp. and non-inoculated (D) white spruce seedlings.*

emphasized the use of mycorrhizal fungi to enhance plant establishment.

As an application of mycorrhizal biotechnology in reclamation of Canadian oil sands areas, our group has involved in several nursery and field trials. One of our efforts was to evaluate mycorrhizal inoculum (ECM and AM) potentials of reclamation materials and tailing sands from Canadian oil sand areas (source: Bois et al. 2005). In this study, jack pine, hybrid poplar and red clover plants were used in a greenhouse bioassay to evaluate the mycorrhizal inoculum potential of composite tailing sands (CT. The inoculum potential was also compared with three other reclamation materials, such as common tailing sands (TS), deep overburden (OB), and muskeg peat (MK) and with three sites reclaimed in different years. Results of this study indicated that CT was completely devoid of mycorrhizal propagules, while all other materials showed some level of inoculum potential. CT & TS were also devoid of ECM propagules. Controlled inoculation of seedlings in the nursery with selected strains could compensate for low natural inoculum potential and improve outplanting performance. Details of this study can be obtained from Bois et al. 2005.

In another experiment, in vitro selection of the most promising ectomycorrhizal fungi was obtained for use in the reclamation of saline-alkaline habitats (source: Kernaghan et al. 2002). The main objective was to identify appropriate ECM species for tolerance and use in saline-alkaline composite tailings (CT) sites. Pure cultures of several fungal species indigenous to the Canadian boreal forest were grown on media containing different levels of $CaCl_2$, $CaSO_4$, NaCl, $Na_2SO_4$, and on media containing CT release water. Among the fungal isolates tested, members of the Boletales, mainly *Suillus brevipes, Rhizopogon rubescens* and *Paxillus involutus*, and *Amphinema byssoides* (Aphyllophorales) were most sensitive to alkalinity and their growth was completely inhibited by CT release water. However, *Laccaria* and *Hebeloma* spp. showed tolerance to alkalinity and survived on the medium with CT release water. Calcium chloride proved to be the most toxic of the salt tested in this experiment. This study recommends inoculating seedlings with a combination of fungal species; each with its own beneficial characteristics is suitable for CT site. *Laccaria bicolor* is recommended for its rapid growth and overall salt tolerance, *Hebeloma crustuliniforme* is recommended for its excellent tolerance to the CT release water, as well as *Wilcoxina mikolae* for its tolerance to $CaCl_2$ .

Syncrude Canada Inc. has been using several amending materials to amend the disturbed areas and reclamation. These include LFH, fresh peat, and stockpiled peat. One study by our group is in progress to evaluate the inoculum potential and ECM diversity of these three different amending materials. The aim of this experiment is to assess both quantitatively and qualitatively, and to draw an inventory of the ECM species present in these materials. The result would give us a clear picture of each site quality in terms of mycorrhizal status of amending materials. One-year-old jack pine and white spruce seedlings were used in bait plants experiment. Preliminary results indicate that at least 3-5 different ECM morphotypes were present in all three amending materials. Molecular analyses of root samples are in progress. However, based on presence of ECM morphotypes, it appears that fresh peat is the most suitable material for better amendment of the areas followed by LFH and stockpiled peat. The trial will be monitored over the next years to let plants grow further to see the stability of inoculum potential and diversity of reclamation materials as well as growth and survival of planted trees.

Another progress has been made by our group toward the application of mycorrhizal biotechnology in reclamation or revegetation work is the development of mycorrhizal DNA fingerprints. Microsatellite SSR markers were developed for *Hebeloma* species for the detection of introduced strains and molecular ecology applications (Source: Jany et al. 2003). This potent marker can be used as an efficient tool for monitoring the persistence of this fungus into the field.

## Outplanting of inoculated seedlings

The success of any inoculation program depends upon sufficient demonstration of the advantages of inoculation in nurseries. In our recent field trials program established in spring 2005 at Syncrude oil sand areas, we intend to test the field performance of ECM inoculated jack pine and white spruce seedlings planted on highly disturbed saline alkaline sites. In this field experiment, we have selected one of the difficult sites that composed of mainly overburden materials, which is highly alkaline (pH > 8). We will monitor the field experiments for next three years to evaluate the growth and survival of inoculated seedlings as well as persistence of introduced mycorrhizal fungi in the field.

## Conclusions

The potential benefits of the application of mycorrhizal biotechnology in revegetating Alberta's oil sand areas are promising. Mycorrhizal inoculation and secure development of mycorrhizas during seedling production in nurseries are particularly important for revetation of disturbed lands. Although there may be some sporadic mycorrhizal formation occurred in nurseries with nursery adapted fungal species, controlled mycorrhizal inoculation of tree seedlings assures a more rapid, efficient, and even mycorrhizal formation of whole nursery with targeted fungal species. In our studies, we found that tailing sand (TS) and composite tailing (TS) is devoid of active ECM propagules. All other amending materials tested have shown very low levels of mycorrhizal inoculum potential. The results suggest that pre-inoculation of nursery seedlings with appropriate mycorrhizal fungi would benefit revegetation of disturbed mined lands. In this process, not only improve survival and growth of individual plant but also we can re-establish the network of fungal mycelium in soil system, which is a particular goal of habitat restoration.

We have shown successful use of fermentor and effective method of large amounts of inoculum production and have demonstrated effective inoculation of forest tree seedlings under commercial nursery settings. We suggest that selection of appropriate plant genotype and their specific site tolerant microsymbionts is likely to maximize the success of land reclamation.

## Acknowledgements

The research is supported by the Alberta Ingenuity Fund, Syncrude Canada Ltd., Bonnyville Forest Nursery, and Laval University, Quebec

## References

Azcón, R., and El-Atrash, F. 1997. Influence of arbuscular mycorrhizae and phosphorus fertilization on growth, nodulation and $N_2$ fixation ($^{15}N$) in Medicago sativa at four salinity levels. Biology and Fertility of Soils 24: 81-86.

Bois, G.Y., Piché, Fung, M.Y.P., and Khasa. D.P. 2005. Mycorrhizal inoculum potentials of pure reclamation materials and revegetated tailing sands from the Canadian oil sand industry. 15(3): 149-158.

Brundrett, M., Bougher, N., Dell, B., Grove, T., and Malajczuk. N. 1996. Working with mycorrhizas in forestry and agriculture. ACIAR, Canbera, Australia.

Duchesne, L.C., Peterson, L.R., and Ellis, B.E. 1988. Interaction between ectomycorrhizal fungus Paxillus *invulutus* and *Pinus resinosa* induces resistance to *Fusarium oxyporum*. Can. J. Bot. 66: 558-562.

Fung, M.Y.P., and Macyk, T.M. 2000. Reclamation of oil sand mining areas. *In* Reclamation of drastically disturbed lands. *Edited by* R.I. Barnhisel, R.G. Darmody, and W.L. Daniels, American Society of Agronomy monographs, 2nd edn. 41: 755-744.

Hunt, G.A. 1992. Effect of mycorrhizal fungi on quality of nursery stock and plantation performance in the southern interior of British Columbia. For.Can. and Min. For. FRDA Report.

Jany, J.L., J. Bousquet and D. P. Khasa. 2003. Microsatellite markers for *Hebeloma* species developed from expressed sequence tags in the ectomycorrhizal fungus *Hebeloma cylindrosporum. Molecular Ecology Notes* 3: 659-661.

Jones, M.D., and Hutchinson, T.C. 1988. Nickel toxicity in mycorrhizal birch seedlings infected with *Lacterius ruffs* or *Scleroderma flaidum.* I. Effects on growth, photosynthesis, respiration and transpiration. New Phytol. 108: 451-459.

Jonsson, L.M., Nilsson, M-C., Wradle, D.A., and Zackrisson, O. 2001. Context dependent effects of ectomycorrhizal species richness on tree seedling productivity. Okios. 93: 353-364.

Kernaghan, G., B. Hambling, M. Fung and D. P. Khasa. 2002. *In vitro* selection of boreal ectomycorrhizal fungi for use in reclamation of saline-alkaline habitats. *Rest. Ecol.* 10: 43-51.

Kropp BR, Langlois CG (1990) Ectomycorrhizae in reforestation. Can. J. For. Res. 20: 438-451.

Leake, J.R. 2001. Is diversity of ectomycorrhizal fungi important for ecosystem function? New Phytol. 152: 1-8.

Malajczuk, N., Reddell, P., and Brundrett, M. 1994. Role of ectomycorrhizal fungi in mine site reclamation. *In* Mycorrhizae and Plant Health. *Edited by* F.L. Pfleger and R.G. Linderman. APS Press, St. Paul, MN. pp. 83-100.

Marx, D.H. 1991. The practical significance of ectomycorrhizae in forest establishment. *In* Ecophysiology of Ectomycorrhizae of Forest Trees. The Marcus Wallenberg Foundation. Symposia Proceedings No. 7, Stockholm, Sweden. pp. 54-90.

Marx, D.H., and Kenney, D.S. 1982. Production of ectomycorrhizal inoculum. *In* Methods and principals of mycorrhizal research. *Edited by* N.C. Scenck, Americal Phytopathological Society. St. Paul, MN. pp. 131-146.

Molina, R., H. Massicotte, and J.M. Trappe. 1992. Specificity phenomena in mycorrhizal symbioses: community-ecological consequences and practical implications. *In* Mycorrhizal Functioning: An Integrative Plant-Fungal Process. *Edited by* M.J. Allen. Chapman & Hall, New York, pp. 357-423.

Schüβler, A., Schwarzott, D., and Walker, C. 2001. A new fungal phylum, the Glomeromycota: phylogeny and evolution. Mycol. Res. 105: 1413-1421.

Smith, S.E. and D. Read. 1997. Mycorrhizal symbiosis. 2nd ed. Academic Press, London.

Van der Heijden, M.G.A., Klironomos, J.N., Ursic, M., Moutoglis, P., Streiwolf-Engel, R., Boller, T., Wiemken, A., and Sanders, I.R. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature (London). 396: 69-72.