

Opportunities from Down Under — How Mycorrhizal Fungi Can Benefit Nursery Propagation and Production Systems[©]

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INTRODUCTION

Mycorrhiza means “fungus roots” which is a symbiotic association between specific fungi and the fine, young roots of higher plants. The majority of plants strictly speaking do not have roots, rather they form mycorrhiza. There are seven principle types of mycorrhiza. The two most important mycorrhizas are the endomycorrhiza and ectomycorrhiza. Endomycorrhiza typically colonize herbaceous plants, shrubs, many ornamental, fruit and nut trees, vegetables and agronomic crops and turf grasses. More than 85% of higher plants form endomycorrhizal associations. The ectomycorrhiza form associations with conifers, pines, and hardwoods such as birch, beech, eucalyptus, fir, oak, willow and magnolia. Ectomycorrhiza colonize around 10% of higher plants. Some plants, such as eucalyptus will form both endo- and ectomycorrhizal associations.

ENDOMYCORRHIZA

Endomycorrhiza are characterized by arbuscules (arbuscular mycorrhiza), and some endomycorrhiza will form both arbuscules and vesicles— these are referred to as vesicular arbuscular mycorrhiza (Fig. 1). Mycorrhizal hyphae penetrate into the host root cortical cells and extend outward into the surrounding soil, thus increasing the roots surface area. Vesicles are used for food storage, and arbuscules are involved in exchange of elements (phosphorus, magnesium, iron, etc.) which the mycorrhiza gives to the host plant. With this symbiotic association, the mycorrhiza help the host plant more efficiently absorb soil elements and soil water, while the host plant gives the fungi carbon (carbohydrates), since mycorrhiza can not photosynthesize. Arbuscular mycorrhiza produce a hydrophobic protein called glomalin, which prevents the hyphae from desiccating, and ultimately encourages soil aggregation around the root system that improves the root contact with water and mineral elements in the rhizosphere (Fig 2). This increases the plants resistance to drought and other stresses.

ECTOMYCORRHIZA

The hyphae of ectomycorrhiza form a network (Hartig net) between the root cortical cells and also extend outward into the surrounding soil (Fig. 3). Ectomycorrhiza can be seen without magnification, whereas endomycorrhiza are observed using tissue staining techniques and a compound microscope. Ectomycorrhiza can

be produced synthetically in Melin-Norkin media (Fig. 3). This allows easier production, minimizes contamination and produces very clean cultures.

Mycorrhiza are unique in that their hyphae do not penetrate the vascular stele of the host plant, unlike vascular wilt organisms such as *Verticillium*, *Fusarium*, etc. Mycorrhiza exist naturally in many commercial nursery production systems. Examples include field rose production in East Texas where rose bushes are produced in a 2-year cycles. High mycorrhizal colonization occurs in these low phosphorus, sandy-loam soil field conditions (Davies, 1987).

CHALLENGES FACING THE NURSERY INDUSTRY

There are opportunities for the utilization of mycorrhiza with the challenges facing the nursery industry and the incorporation of Best Management Practices [BMP] (Yeager et al., 1997). Industry challenges include increased production costs and greater governmental regulations. Irrigation water usage needs to be curtailed. Reducing irrigation run-off, limiting the usage of soluble fertilizers, fungicides and pesticides are being addressed. There is also a need to produce and market more stress-efficient plants that use less water and fertilizer and have greater resistance to environmental stress such as high temperature (Newman and Davies, 1988a, 1988b) and resistance to biotic stresses caused by disease and insects.

The BMP changes occurring in the nursery industry are highly conducive for mycorrhizal usage. For instance, a greater proportion of inorganic, controlled-release fertilizers are being used today than soluble fertilizers, which is beneficial for mycorrhizal associations. Under high temperature container production conditions, typical of Texas summers [49° C (120° F)], bush morning glory (*Ipomoea carnea*) grown with endomycorrhiza (AMF) plus an inorganic controlled release fertilizer (ICRF) at recommended rates did better than the ICRF alone, or organic slow release fertilizer (OSRF) alone at recommended rates, or OSRF plus AMF (Carpio et al., 2005). This is highly relevant to the nursery industry which predominately uses ICRF fertilizer.

Since there will be less pesticides available in the future, other BMPs include changing cultural practices to reduce pesticide usage, i.e. the future usage of methyl bromide is very uncertain and will likely be phased out. Water is also a key limiting environmental factor. This has led to recycling of irrigation water and other BMP programs. With these changes in cultural practices, use of ICRF fertilizers and reduction in pesticide usage, there are opportunities for utilizing mycorrhizal fungi.

BENEFITS OF MYCORRHIZA

Mycorrhiza can enhance efficiency of plant roots to absorb water and macro- and microelements from the soil or container media. This helps reduce fertility and irrigation requirements, increases drought resistance and plant resistance to pathogens (Pfleger and Linderman, 1994; Davies, et al., 1993, 1996). Mycorrhiza enhance plant health and vigor and minimize stress. Minimizing stress is particularly important because with less stress

there is greater plant resistance to pathogens and pests and a reduction in pesticide usage. In regard to pathogen resistance, there can be biotic responses with certain compounds produced by the mycorrhiza to ward-off pathogens (Pfleger and Linderman, 1994). The germination of *Fusarium* spores will trigger the germination of mycorrhizal spores. The fungal mantle of ectomycorrhiza also forms a physical barrier between roots and pathogens.

Mycorrhiza enhance the drought resistance of plants by causing changes in the host plant biochemistry (greater osmotic adjustment, lower production of abscisic acid) [Estrada-Luna et al., 2003], changes in root morphology (more new root initiation for better water absorption) [Svenson et al., 1991], greater extraradical hyphae development which allow the root system to have better contact with soil water (Davies et al., 1992). The plant is more efficient in surviving drought conditions. Other benefits include enhanced seedling growth, increased adventitious root formation of cuttings (Davies et al. 2000; Scagel, 2004) and enhanced plant transplant establishment (Davies and Call, 1990).

We have been able to demonstrate in very severe coal revegetation sites that mycorrhiza will enhance the survivability and growth of endo- and ectomycorrhizal plants (Davies and Call, 1990). Disturbed sites can also be found in the construction of houses with changes in soil structure, soil compaction and subsequent drainage and aeration problems. There are opportunities in incorporating mycorrhiza during nursery production so that when the ornamental shrub or tree is transplanted into the landscape, it has greater stress resistance, survivability and re-growth).

MYCORRHIZA AND PROPAGATION

In regards to adventitious root formation, both endo- and ectomycorrhiza can enhance adventitious root formation of cuttings (Hartmann et al., 2002). There can be enhanced root initiation and root development. The ectomycorrhiza are capable of producing auxins, gibberellins and other phytohormones. With some of the more difficult-to-root plant species, it may be useful to try combinations of mycorrhiza and auxins to stimulate better root formation. The endomycorrhiza, *Glomus intraradicis*, increased adventitious rooting of softwood cuttings and liner plant development of desert willow (*Chilopsis linearis*) [Davies et al, 2000a].

Ectomycorrhiza increased the rooting percentage or accelerated root formation of fascicular shoots of Scots pine (*Pinus sylvestris*); the ectomycorrhizal fungi *Pisolithus tinctorius* produces auxin (IAA), which can enhance rooting (Niemi et al., 2000). Endomycorrhiza plus exogenous auxin enhanced rooting of *Sciadopitys verticillata* (Douds et al., 1995), Hick's yew (Scagel et al., 2003) miniature rose cuttings (Scagel, 2004) prior to colonization (i.e. before adventitious roots formed); this suggests that there are AMF-plant signaling events occurring during the early stages of adventitious root formation.

HOW TO APPLY MYCORRHIZA

Mycorrhiza can be applied as: 1) a layer of spores, mycelium, and colonized roots banded into the container media, 2) pre-incorporated in commercial peat-based mixes, 2) placed in the dibble hole of the container prior to sticking the liner (it is ideal to let the new roots grow into the mycorrhiza), 3) gel or liquid drench system, 4) injected or mixed with backfill, or 5) incorporated with pelleted seed. The most efficient time to apply mycorrhiza is during propagation or in liner production. As an example, the Forest Keeling Nursery (<http://www.fknursery.com/>, Wayne Lovelace, personal communication) uses a commercial liquid application of mycorrhiza that is applied to germinating seed flats and at the time seedling liners are sifted up into larger containers. Monrovia Nursery (<http://www.monrovia.com/>, Jason Julian and Stewart Chandler, personal communication) pre-incorporates a commercial granular mycorrhizal mix with their propagation media. Both nurseries use a cocktail approach of mixed endo- and ecto-mycorrhiza. See Table 1 for a listing various commercial sources of mycorrhizae.

UTILIZATION OF MYCORRHIZA IN COMMERCIAL PRODUCTION SYSTEMS

Mycorrhiza can survive in nursery production systems. In a study that we conducted in a commercial nursery in Texas, four species were used as host plants [*Nandina domestica* ‘Moon Bay’, *Loropetalum chinense* variety Rubrum ‘Hinepurpleleaf’ Plumb delight[®], *Salvia gregii*, and *Photinia fraseri*] (Davies et al., 2000b). Plants were inoculated with arbuscular mycorrhizal fungi, *Glomus intraradices*, and grown in a commercial nursery in Texas. The commercial inoculum of *Glomus intraradices* only enhanced growth of *N. domestica*. However, intraradical hyphae development and colonization (total arbuscules, vesicles/endospores, hyphae) of *L. chinense*, *N. domestica*, and *S. gregii* increased at the higher fertility levels. *S. gregii* had the greatest mycorrhizal development and a 216% increase in hyphae development and colonization at the higher fertility level. This was significant for commercial nursery production, since there was not a decrease in colonization occurring at the higher fertility levels.

CHANGING OUR MENTALITY

We need to change our mentality about solely looking for greater growth and higher phosphorus uptake with mycorrhizal plants. Under high fertility conditions, it is not realistic to see growth differences with mycorrhiza. In a nursery setting, a more realistic goal is to achieve good mycorrhizal colonization so that the ornamental shrub or tree can be transplanted into a stressful landscape environment — and not only survive, but reestablish growth more quickly than a non-mycorrhizal plant. One should not be so concerned about differences in plant growth during nursery production, since water and fertility are normally not limiting factors. However, greater growth and establishment following transplanting is an important value-added asset of mycorrhizal landscape plants.

FUNGICIDES AND MYCORRHIZA

Fungicides will decrease development of mycorrhiza for a short time, depending on the persistence of the fungicide. Most foliar applications of fungicides, except those that are systemic such as Triadimefon (Bayleton) do not affect mycorrhizae because the fungicide doesn't come in contact with the mycorrhizae in soil. Soil drenches do come in contact with mycorrhiza, and may or may not have an influence depending on the mycorrhizal species and the fungicide. Fungicide can inhibit, have no effect, or actually stimulate mycorrhizal development. There are differences in mycorrhizal response, i.e. Benomyl (Benlate, Tersan-1991) will depress endomycorrhiza, but enhances ectomycorrhizal development. As a general rule, most commercial nurseries do not apply fungicides as a prophylactic treatment during propagation, but only after a disease problem occurs. For optimal mycorrhizal response it is best to delay fungicidal application during the first three weeks of colonization. Mycorrhizal colonization can occur within the first 3-5 days of treatment, depending on the inoculum source (Estrada-Luna et al., 2003). See the tables on fungicides affecting endomycorrhizae (Table 2) and ectomycorrhiza (Table 3). In these tables a fungicide was listed as inhibitory if it was reported to inhibit mycorrhizal development, regardless of other reports to the contrary. In general, pesticides and herbicides do not inhibit mycorrhizal, and are not a problem, except for a few exceptions (Table 4).

PROLIFERATION OF COMMERCIAL MYCORRHIZA SOURCE

There are a number of commercial companies now selling mycorrhiza (Table 1). It is important to determine if your host plant species is ecto- or endomycorrhizal dependent. There can be advantages to working with mixed isolates of more than one mycorrhiza that come in a "cocktail". However, there is a diminishing return to having more than three mixed mycorrhizal species in a single inoculum (J. Morton, personnel communication). It would be best to try a small test with several commercial isolates, making sure to include a non-mycorrhizal control.

COMMERCIAL ADVANTAGES OF PRODUCING MYCORRHIZAL PLANTS

Advantages of utilizing mycorrhizal fungi during propagation and production include: 1) Producing more stress resistant plants during production, which means reduced pesticide application during production; 2) more drought and nutrient tolerant plants in landscape; 3) potentially higher transplanting success & faster establishment; and 4) marketing higher value landscape plants.

WHAT MYCORRHIZAE WILL NOT DO

Mycorrhizae are not a panacea or silver bullet for solving all the nursery industry production challenges. The use of mycorrhiza along with other improved cultural practices, including BMPs, can improve propagation and production efficiency and the development of more stress resistant plants for the landscape. Mycorrhiza can enhance transplanting establishment and success. Mycorrhiza are not for all commercial plant systems, i.e. in general they work better with a more-coarse rooted, perennial plants that may be subjected to production

problems, transplanting problems, or that may be subjected to stressful landscape sites. The potential exists for marketing value-added, mycorrhizal plants that command higher prices.

INFORMATION ON MYCORRHIZA

The following web sites offer excellent information on mycorrhiza: 1) Mycorrhizal Information Exchange - Bob Augé, University of Tennessee; <http://mycorrhiza.ag.utk.edu>; 2) International Mycorrhiza Society <http://www.mycorrhizas.org/> 3) Joe Morton, INVAM— International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi; <http://invam.caf.wvu.edu>. and 4) Fred Davies, Nursery Crop Physiology Lab, Texas A&M University <http://aggie-horticulture.tamu.edu/Faculty/davies/research/mycorrhizae.html>

SUMMARY

- Mycorrhiza can be incorporated with media composed of bark, peat, sand, vermiculite, perlite, calcined clays;
- Optimum results occur when a media contains at least a 10% mineral soil component;
- The most efficient and economical time to apply is during propagation or when transplanting liners;
- Mycorrhiza works best with ICR (inorganic controlled release) fertilizers, however they can be managed in liquid fertilizer regimes;
- Avoid using fungicides within the first three weeks of mycorrhiza application;
- In general, insecticides and herbicides do not effect mycorrhiza;
- The greatest opportunity with mycorrhiza are generally with coarse-rooted perennials, shrubs and trees.

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Table 1. Partial List of Commercial Sources of Mycorrhizal Fungi in North America (2008).

COMPANY	CONTACT
AgBio Inc. (Westminster, Colorado)	Ph:877-268-2020 http://www.agbio-inc.com/
Horticultural Alliance, Inc. (Sarasota, FL)	Ph: 800-628-6373 http://www.horticulturalalliance.com/
Mikro-Tek Inc. (Timmins, Ontario, Canada)	Ph: 705-268-3536 http://www.ids-environment.com/
Mycorrhizal Applications (Grants Pass, OR)	Ph: (866) 476-7800 http://www.mycorrhiza.com/
Plant Health Care, Inc. (Pittsburgh, PA)	Ph: 1-800-421-9051 http://www.planthealthcare.com/HT/Mycorrhizal
Premier Tech (Québec, Canada)	Ph: 800-606-6926 http://www.usemyke.com/mycorise/horticulture/fhorticulture.htm
Reforestation Technologies (Salinas, CA)	Ph: 800-784-4769 http://www.reforest.com/
Roots Inc., (Independence MO)	Ph: 800-342-6173
T & J Enterprises (Spokane, WA)	Ph: 509-327-7670 http://www.tandjenterprises.com/
Terra Tech, LLC (Eugene, OR)	1-800-321-1037 http://www.terratech.net/
Tree Pro (West Lafayette, IN)	Ph: 800-875-8071 http://www.treepro.com/id80.html
Willis Orchard Company (Berlin, Georgia)	866-586-6283 http://www.willisorchards.com/

Table 2. Fungicidal Effects on Endomycorrhiza [source: D. Marx, S. Parent].

<u>No Reported Negative Effect</u>	<u>Inhibitory Effect*</u>
Carbamate (Fermate, Ferbam)	Aliette* (Fosetyl - Al)
Carbendazim (Bavistin)	Benomyl* (Benlate, Tersan-1991)
Bravo (Chlorothalonil)	Captan* (Orthocide)
Chloroneb (Tersan, Demosan)	Copper Oxychloride Sulfate* (CDCS)
Chlorothalonil (Bravo, Daconil-2787, Exotherm)	Easout* (Thiophanate methyl)
Difolatan (Sulfonimide, Difosan, Captafol)	Formalin* (Formaldehyde)
Lesan (fenaminosulf)	Metalaxyl* (Subdue, Ridomil)
Mancozeb (Dithane M-45)	PCNB* (Terraclor, Tri-PCNB)
Maneb (Dithane M-22, Manzate)	Phaltan* (Folpet)
No-Damp(Oxine benzoate) (sol)	Terrazole* (Truban, ETMT, Etridiazol)
Rovral (Chipco-26019)	Tilt* (CGA-65250)
Soufre -92 %	Thiophanate Methyl * (Cleary-3336)
Thiabendazole (Mertect)	Triadimefon (Bayleton)*
Thiram (Tersan 75, Arasan)	Vitavax* (Carboxin, DCMO)
Topsin-M (Easout, Fungo, Duosan)	
Triforine (Funginex)	

Table 3. Fungicidal Effects on Ectomycorrhiza [source: D. Marx].

<u>No Reported Negative Effect</u>	<u>Inhibitory Effect*</u>
Aliette (Fosetyl Al)	Banrot*
Benomyl (Benlate, Tersan-1991)	Chlorothalonil* (Daconil-2787, Bravo)
Captan (Orthocide)	Mancozeb* (Dithane)
Carbamate (Fermate, Ferbam)	PCNB* (Terraclor, Tri-PCNB)
Carbendazim	Triadimefon* (Bayleton)
Dexon	Zineb* (Ziram, Zerlate)
Difolatan (Sulfonimide, Difosan, Captafol)	
Fuberidazole	
Metalaxyl (Subdue, Ridomil)	
Thiophanate Methyl (Cleary 3336)	
Thiram (Arasan)	

Table 4. Insecticidal Effects on Endomycorrhiza [source: S. Parent].

<u>No Reported Negative Effect</u>	<u>Inhibitory Effect*</u>
Agribrom (bromine source)	Diazinon* (Diazinon)
Ambush 25 wp	Savon insecticide*
Ambush (Permetrin)	Malathion *(malathion)
Cygon (Dimethoate)	Oxamyl *
Kelthane (Dicofol)	
Morestan (Chinomethionat)	
Metasystox (Oxydemeton-methyl)	
Pentac (Dienochlor)	
Pirimor (Pirimicarb)	
Vendex (Fenbutatin-oxide)	
Talstar-Attain (Bifenthrin)	
Dursban (Chlorpyrifos)	
Trumpet-Dycarb (Bendiocarb)	
Enstar (Kinoprene)	
Margoson (Azateractin)	
Mavrik (Fluvinat)	
Orthene (Acephate)	
Sevin (Carbaryl)	
Avid (Abamectin)	
Citation (Cyromazine)	
Marathon (Imidacloprid)	

FIGURES

- Figure 1. (A) Schematic of endomycorrhiza [arbuscular, vesicular mycorrhiza fungi (AMF)] and related structures in a longitudinal section of a fine feeder root; (B) endospores and extrardical hyphae of *Glomus deserticola*, which can contain meters of hyphae within a thimble-size quantity of soil.
- Figure 2. Arbuscular mycorrhizal fungi can form a hydrophobic protein called glomalin, which encourages micro-aggregation of soil particles to the root system, improves soil tilth and enhances the root system to mine the rhizosphere for water and nutrients. (A) clumping of soil to root system typical of macro-aggregation of soil particles to the root system; (B) schematic of micro-aggregation; and (C) improved soil tilth that allows better drainage, better water retention and potentially less leaching.
- Figure 3. (A) Schematic of ectomycorrhizal fungi and related structures in a longitudinal section of a fine feeder root; (B) ectomycorrhizal pine root tips; (C) the ectomycorrhizal species, *Larix decidua*; (D) the ectomycorrhizal fungi, *Pisolithus tinctorius* on a Melin Norkin media, and (E) industrial, fermentation tank-size production of ectomycorrhiza which can be produced synthetically in the absence of a live plant.

Arbuscular Mycorrhiza

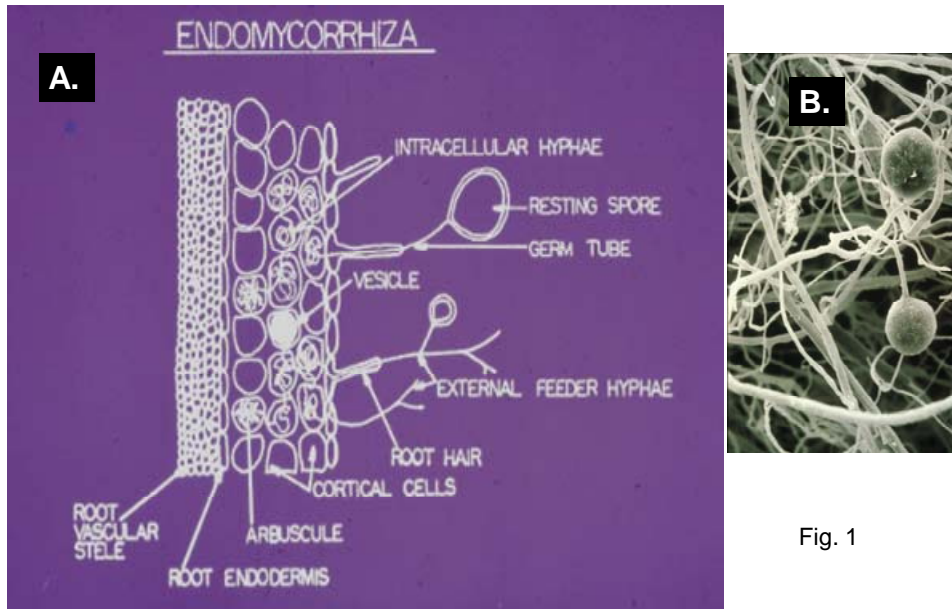


Fig. 1

AMF Plants have:

- Greater extraradical Hyphae formation
- Greater Soil Aggregation



Fig. 2



- Improved Soil Tilth
- Glomalin – hydrophobic protein

Ectomycorrhiza

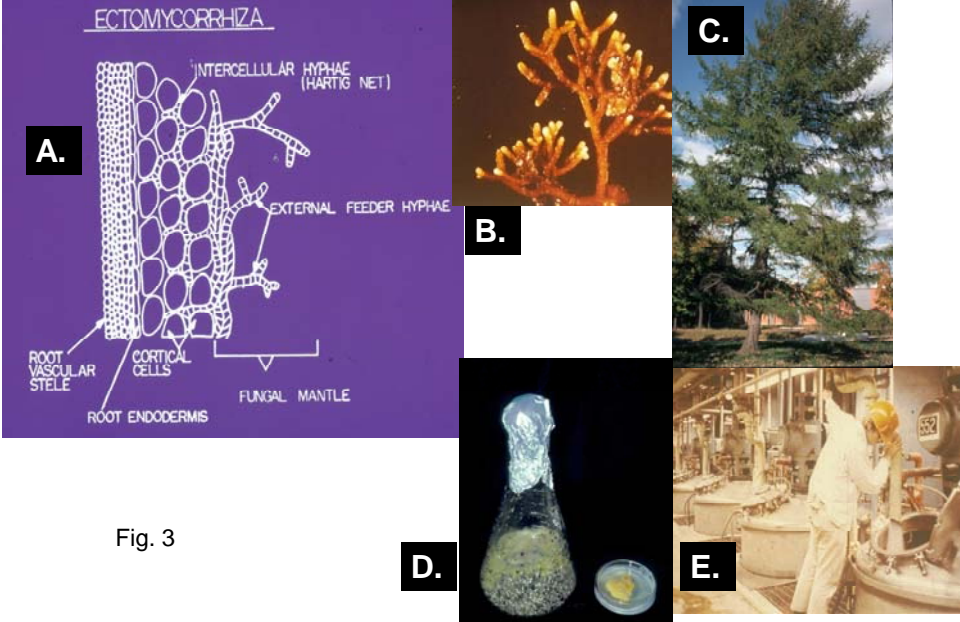


Fig. 3