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Practical use of the mycorrhizal fungal technology in forestry, reclamation, arboriculture, agriculture, and horticulture

Abstract: Fine nonwoody roots (<2 mm) of plants, which are responsible for most mineral and water absorption, are located in the upper 20 to 30 cm of soil. The rhizosphere, the zone around these fine roots, supports diverse microorganisms in great numbers. Dynamic rhizosphere processes involve microbial saprophytic, pathogenic, and symbiotic associations with plants. Certain species of saprophytic bacteria oxidize mineral elements, like P, into soluble forms, fix atmospheric N, stimulate root growth by producing plant growth regulators, act as biological deterrents to root-disease causing organisms, and decompose man-made and natural organic chemicals in the rooting zone. The major symbiotic associations on plant roots are mycorrhizae.

There is considerable published research in the world literature proving the biological, physiological and ecological significance of ectomycorrhizae and vesicular-arbuscular mycorrhizae (VAM) to the survival, growth, development and health of many species of agricultural and horticultural plants, and of forest trees. This information is critical to our understanding of plant growth and development and their ecology. In the past, the limiting factors in the practical management of mycorrhizal fungi in plant production have been the availability of affordable and good quality inocula of the ectomycorrhizal and VAM fungi and the development of simple methods to apply these inocula either to the soil or to the roots of plants. It was only after these protocols were developed that scientists could then test under "real-world" conditions the practical and economic significance of the management of these fungi in plant productivity.

Many of the problems associated with commercial inoculum production have been eliminated allowing the production of quality products containing ectomycorrhizal and/or VAM fungal propagules to diverse plant markets. These fungal products are being commercially applied to plants in diverse green markets. The results of their application are improved survival, growth and productivity of forest trees and other woody plants on reforestation, urban and reclamation sites, horticultural crops such as those grown in woody ornamental nurseries and various agricultural crops grown in fumigated soils. This presentation is a brief review of the research and operational applications done in support of the practical use of the mycorrhizal fungal technologies in forestry, reclamation, horticulture and agriculture.

Additional key words: Ectomycorrhizae, VAM, practical treatment

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Introduction

The root systems of forest trees and other plants have impressive form, structure, and function. Roots provide plants with the water required for growth, soil minerals essential for metabolism, growth regulators to direct carbon allocation, storage of key food reserves, and physical support. In mature trees, the size of the lateral root system can exceed not only the aboveground dry weight, but also as much as four times the area of the crown. Fine nonwoody roots (<2 mm), which are responsible for most mineral and water absorption, are located in the upper 20 to 30 cm of soil. The rhizosphere, the zone around these fine roots, supports diverse microorganisms in great numbers. Dynamic rhizosphere processes involve microbial saprophytic, pathogenic, and symbiotic associations. Certain species of saprophytic bacteria oxidize mineral elements, like P, into soluble forms, fix atmospheric N, stimulate root growth by producing plant growth regulators, act as biological deterrents to root-disease causing organisms, and decompose man-made organic chemicals in the rooting zone. The major symbiotic associations on plant roots are mycorrhizae.

There is considerable published research in the world literature proving the biological, physiological and ecological significance of ectomycorrhizae and vesicular-arbuscular mycorrhizae (VAM) to the survival, growth, development and health of many species of agricultural and horticultural plants and of forest tree species (Smith and Read 1997; Quarles 1999a, b). This information is not only academically interesting but is critical to our understanding of plant growth and development and their ecology. In the past, the limiting factors in the practical management of mycorrhizae in plant production have been the availability of affordable and good quality inocula of the ectomycorrhizal and VAM fungi and the development of simple methods to apply these inocula either to the soil or to the roots of plants.

In the last decade, many of the problems associated with commercial inoculum production were eliminated allowing the production of quality products containing ectomycorrhizal and/or VAM fungal propagules to diverse plant markets. These fungal products are being commercially applied to plants in diverse green markets. The following is a brief review of the research done in support of the practical use of the mycorrhizal fungal technologies.

Forestry

The biological requirement of many species of forest trees for ectomycorrhizal associations was initially observed in the early 1900's when attempts to establish plantations of exotic pines in the tropics routinely failed until the necessary symbiotic fungi were introduced. The obligate need of pine and oak seedlings for ectomycorrhizae has also been convincingly demonstrated in the afforestation of former treeless areas, such as the grasslands of Russia and the great plains of the USA (Marx 1991).

The primary purpose for inoculating with these specialized fungi in world forestry is to provide seedlings with adequate ectomycorrhizae to improve their survival and growth after planting to create man-made forests. Such treatment has proven essential in forestation of cutover lands and other treeless areas and the introduction of exotic tree species, where native ectomycorrhizal fungi are deficient or reduced in species diversity (Byrd et al. 2000).

Most research on inoculation with ectomycorrhizal fungi has been based on two working premises. First, any amount of ectomycorrhizae formed by any fungus on roots of tree seedlings are essential to seedling survival and growth. Success in alleviating ectomycorrhizal fungal deficiencies has contributed greatly to our understanding of the importance of ectomycorrhizae to trees, especially as they relate to the establishment of plantation forests. Secondly, some species of ectomycorrhizal fungi have proven to be more beneficial to trees, under certain environmental conditions, than others. These fungal species should be deliberately managed for these applications.

The cultural procedures used to produce seedlings in bare-root or container nurseries create environmental conditions that select naturally occurring ectomycorrhizal fungi adapted to these conditions. These fungi produce mushrooms or puffballs that release many spores that are wind disseminated to the nursery soil. In most parts of the world, the fungus *Thelephora terrestris*, appears to naturally dominate the roots of most pines, oaks and spruce grown in nursery soil (Marx et al. 1984a) and in containers (Marx et al. 1982).

Most reports on inoculation techniques with ectomycorrhizal fungi developed in the US, Australia, France, Canada and the Philippines involve basidiomycetes on pines, oaks, and eucalypts (Marx 1991). Techniques were developed mainly in response to the necessity to grow tree species requiring ectomycorrhizae in tropical areas where ectomycorrhizal fungi were absent. Several types of natural and laboratory-produced inocula and several methods of application have been used over the years. Many of the techniques have proven successful while others have not.

Historically, the most widely used natural inoculum, especially in developing countries, is soil or humus collected from established pine plantations (Marx 1991). In most instances, the original soil inoculum came from mature stands on other continents and, therefore, contained a preponderance of fungi adapted to mature trees and not seedlings. The use of forest soil inoculum has major disadvantages. Species composition of ectomycorrhizal fungi in the soil inoculum is usually not known, and the inoculum may also contain harmful microorganisms and noxious weeds (Marx 1975). The use of soil inoculum, however, is consistent with the premise that any ectomycorrhizae are better than none.

Spores of various fungi have been used as inocula to form specific ectomycorrhizae on tree seedlings. Basidiospore inocula of *Pisolithus tinctorius* (Pt), *Rhizopogon vinicolor*, and *R. colossus* have been used on an experimental scale, and more recently on an operational scale, in the USA and elsewhere. Pt spores, and those of other fungi, are effective in various forms. They can be (1) mixed with a sand, clay, or vermiculite carrier before being added to soil, (2) suspended in water and drenched or irrigated, (3) dusted or sprayed, (4) pelleted and broadcast, and (5) encapsulated or coated onto seeds. (Marx et al. 1984b; Marx and Bell 1985; Castellano and Molina 1989; Marx et al. 1989). Fortunately, the spores of ectomycorrhizal fungi are small in diameter and can be washed into the root zone or onto roots which is a prerequisite to ectomycorrhizal development.

Pure mycelial or vegetative inoculum of ectomycorrhizal fungi is recommended as the most biologically sound material for inoculation. Several researchers in various parts of the world have developed procedures for culturing vegetative inocula of a variety of fungi for research purposes. Large-scale nursery applications of pure mycelial cultures has been severely hampered by the inability to produce large quantities of viable and economical inoculum (Marx et al. 1982, 1992).

Trotymow and van den Driessche (1990) reviewed the published results of outplanting trials with specific ectomycorrhizae on conifer seedlings. Of the 84 reports examined, 49 dealt with Pt ectomycorrhizae. Castellano and Molina (1990) also reviewed the world literature on outplanting performance. They found that 66 species of fungi have been used experimentally to form ectomycorrhizae on 49 tree species. Over 40 percent were with Pt on 29 different tree species. Pt has a worldwide geographic distribution and is found in forests, pecan orchards, urban settings and on adverse sites, such as severely eroded soils and mined lands. It occurs in both cold and warm climates on a broad range of tree hosts (Marx 1977). Research has shown that to obtain maximum survival and growth of southern pine seedlings by Pt ectomycorrhizae on normal reforestation sites, a threshold level of at least half of all ectomycorrhizae on seedlings at planting must be those formed by Pt (Pt index >50). On routine reforestation sites, pine seedlings with less than half of all ectomycorrhizae formed by Pt frequently may survive and grow at the same rate as seedlings with the same amount of naturally occurring *Thelephora* ectomycorrhizae (Tab. 1) that are also present on the root system.

Significant improvements in pine and oak seedling performance are also reported on other routine reforestation sites in the USA. (Tab. 2). Pt ectomycorrhizae had only minimal positive effect on survival and growth of seedlings in some cases but increased growth (plot volumes) by more than 250 percent in others. Where large differences were reported control seedlings with only naturally occurring ectomycorrhizae at planting survived and grew poorly. Where small differences were reported, the control seedlings survived and grew considerably better. These observations suggest that seedlings with abundant Pt ectomycorrhizae tolerated environmental stress factors, such as soil water deficits and high temperatures, better than control seedlings.

Similar results have been obtained on other hot and dry sites such as in the tropics for establishment of exotic pine plantations (Tab. 3). These areas have distinct wet and dry seasons, low soil fertility, and high evaporation rates. In a three-year study in Liberia (Marx et al. 1985), *P. caribaea* seedlings with Pt ectomycorrhizae grew as much during the 13.5 months of dry seasons as trees with other ectomycorrhizae grew during the 22.5 months of rainy seasons. This study demonstrated that Pt ectomycorrhizae could furnish improved drought tolerance to trees. This trait is likely mediated through the large, hyphal strand network of Pt, which exploits larger soil volumes than do fungi without this network. Other studies on different sites show similar available soil water relationships with Pt ectomycorrhizae (Walker et al. 1989; Marx and Cordell 1988; Garbaye 2000).

Table 1. Survival and growth of loblolly pine after eight years on a good quality site with different amounts (Pt indices) of *Pisolithus tinctorius* ectomycorrhizae at planting (Marx et al., 1988)

Pt Index	Survival	Height	Diameter	Volume (m^3)		Total wt (kg)	
		(m)	(cm)	Tree	Hectare	Tree	Hectare [1]
88	72a	8.1a	14.0a	0.054a	59a	75a	812a
68	65b	8.0ab	13.5b	0.054b	48b	69b	670b
46	62b	7.8bc	13.5b	0.048bc	45bc	66bc	622bc
27	62b	7.8bc	13.0c	0.045c	42c	62c	585bc
0	58b	7.7c	13.0c	0.045c	38c	61c	533c

Means in a column which are followed by a common letter are not significantly different at P = 0.05.

[1] 1 x 102

Table 2. Percent increase in survival and plot volume growth of tree seedlings with *Pisolithus tinctorius* ectomycorrhizae over controls with naturally occurring ectomycorrhizae on reforestation sites in the eastern US (see Marx et al. 1992)

Tree Species	No. Sites	Years in field	% increase over controls	
			Survival	Plot volume[1]
Pinus clausa	3	2–7	11–169	35–274
P. enchinata	3	2–4	0–39	41–141
P. elliottii var. elliotti	7	2–13	5–22	6–175
P. palustris	9	2–7	5–116	6–180
P. strobus	1	14	8	420
P. taeda	23	2–14	0–20	18–68
P. virginiana	2	2	2–4	29–55
Quercus acutissima	1	2	73	53
Q. palustris	1	2	3	39

[1] Plot volume = seedling volume (stem dia. ^{2} x height) of all surviving seedlings in plot

Table 3. Percent increase in survival and growth of pine seedlings with *Pisolithus tinctorius* ectomycorrhizae over control seedlings with other ectomycorrhizae on exotic sites (see Marx et al. 1992)

Pine species	Country	No. sites	Years In field	% Increase	
				Survival	Growth
Pinus caribaea	Congo	1	2	0	42[1]
P. caribaea	Nigeria	6	2	2–850	6–186[1]
P. caribaea	Liberia	2	3	8–14	93–111[2]
P. caribaea	Brazil	2	2	18–79	331–962[2]
P. pseudostrobus	Mexico	1	3	44	438[3]

[1] Height [2] Plot volume [3] Seedling volume

Besides Pt, other fungi have been tested for their ability to improve seedling survival and growth on diverse sites (Marx 1998). Recently, LeTacon et al. (2000) reported that ectomycorrhizae formed by *Laccaria bicolor* on containerized Douglas fir seedlings increased wood volume by 60 percent after eight years compared to trees with only naturally occurring ectomycorrhizae. This fungus persisted on tree roots for the duration of the test but did not replace any of the native ectomycorrhizal fungi indigenous on the site. It's introduction simply added to the fungal population, as did Pt in the other studies.

Vegetative inocula of *Hebeloma crustuliniforme* and *Laccaria bicolor* are being used successfully to improve seedling quality of true firs, Douglas-fir and various spruce species for establishment of Christmas tree plantations. Vegetative and spore inocula of Pt are also used to improve the quality of Virginia and eastern white pine seedlings for these plantations. Nursery bed inoculation with these specific ectomycorrhizal fungi increases the percentage of superior grade seedlings in the nursery, their overall health and their survival and growth in the plantations (Cordell 1997).

It is obvious that in the past many methods were developed to ensure the formation of ectomycorrhizae on tree seedlings used to establish man-made forests. Certain methods have advantages over others. Pure vegetative and spore inocula have the greatest biological advantages. Specific ectomycorrhizal fungi are being actively used in practical reforestation and reclamation programs in the world today (Marrs et al. 1999; Brundrett et al. 1996). Most programs involve Pt ectomycorrhizae to create tree plantations on clearcut lands and minelands. Currently about 650 million seedlings are being inoculated annually in the USA and about 150 million in Mexico with Pt spore inocula.

There has also been considerable research published on VAM fungal inoculated hardwood seedlings in both bareroot and container nurseries. Without exception, all of the researchers used research inoculum of VAM fungi and not commercial sources. A brief review of this research has been published (Marx 1996a, b). Table 4 shows an example of the response of seedlings of various hardwood trees to nursery inoculations with VAM fungi. Kormanik (1983) and Hay and Rennie (1989) showed that sweetgum and yellow poplar seedlings exhibit improved vegetative growth (Tab. 5) and reproductive responses after field planting to VAM fungal inoculation in nurseries. We are aware of a few applications of commercial VAM fungal inoculum in several forestry and reclamation projects but, other than the information

Table 4. Response of eight hardwood tree species to VAM fungal inoculation after 10 months in a fumigated bareroot nursery (Kormanik et al. 1982)

Treatment	Height (cm)	Diameter (mm)	Total wt. (g)
Prunus serotina (black cherry)			
VAM	70.0	6.9	29.3
Control	12.8	1.5	0.4
Acer negundo (boxelder)			
VAM	45.1	10.1	23.7
Control	12.8	3.2	0.7
Fraxinus pennsylvanica (*Green* ash)			
VAM	37.4	8.6	23.0
Control	6.7	2.0	0.4
Acer rubra (*Red* maple)			
VAM	35.8	6.6	10.4
Control	8.3	2.4	0.4
Acer saccharum (sugar maple)			
VAM	9.3	3.3	3.0
Control	7.1	2.5	0.8
Liquidambar styraciflua (sweetgum)			
VAM	29.6	7.0	12.1
Control	4.4	2.0	0.4
Platanus occidentalis (sycamore)			
VAM	66.6	12.9	71.3
Control	19.9	4.2	3.6
Juglans nigra (black walnut)			
VAM	24.8	7.9	85.6
Control	21.0	5.7	17.0

Table 5. Response of container-grown yellow poplar (*Liriodendron tulipifera*) to VAM fungal inoculation after 4 years on three field sites (averaged) in Tennessee (Hay and Rennie, 1989)

Treatment	Survival (%)	Height (cm)	Diameter (mm)	Plot vol. (cm^3)
VAM	66	108	22.50	363×10^4
Control	46	70	14.80	71×10^4

herein, none have been either published or otherwise made available for discussion. A comprehensive review of past research on practical application of mycorrhizal fungi in forestry was recently published (Marx 1998).

Reclamation

There is a large body of published scientific research showing the practical significance of Pt ectomycorrhizae and specific VAM fungi to revegetation of mined lands and other adverse sites in the eastern US and other parts of the world (Marx 1998; Kumar and Upadhyay 1999). Most of this field research was done on very acid coal mined lands that were also droughty with high summer soil temperatures and contained high amounts of Al, S, Mn, and Fe. Other research was done on kaolin and phosphate mines, impoverished eroded soils and on borrow pit sites. The results from all sites have been similar, consequently, only a select few are presented (Tabs. 6, 7, 8 and 9). After several years, seedlings with Pt ectomycorrhizae or with selected VAM had significantly greater survival and growth and contained less heavy metals in their foliage than seedlings with ectomycorrhizae or VAM formed by other species of naturally occurring mycorrhizal fungi (Cordell et al. 1996; Walker 1999; Walker et al. 1989).

One of the best examples of ecological adaptation by these fungi is Pt. The fruit bodies and ectomycorrhizae of this fungus have been observed to occur naturally on trees of several species growing on coal mined-lands and other adverse sites worldwide and has been credited with their survival and growth. Only a few other ectomycorrhizal fungi, such as *Scleroderma*, occasionally occur with Pt under trees on these highly stressed sites.

Table 6. Response after 3 years of live oak to *Pisolithus tinctorius* (Pt) or natural ectomycorrhizae on a pH 7.2 lignite overburden site in Texas (Davies and Call 1990)

Treatment	Survival %	Height (cm)	Plot Volume (cm^3)
Pt	96	56	263×10^2
Natural	93	35	59×10^2

Table 7. Response of 3-year-old loblolly pine to *Pisolithus tinctorius* (Pt) or natural ectomycorrhizae on a severely eroded, air pollution damaged site in Copper Basin, TN (Hatchell et al. 1985)

Treatment	% Survival	Height (cm)	Plot Vol. (cm^3)
PT	92	67	541
Natural	68	51	214
Pt + fertilizer	90	92	1,240
Natural + fertilizer	74	74	612

Table 8. Response of one-year-old forage species (from seed) to VAM on an acid coal mine spoil (Lambert and Cole, 1980)

Treatment	% Survival	Foliar wt. (g)
	Birdsfoot trefoil	
VAM	54	34
No VAM	9	3
	Crownvetch	
VAM	54	41
No VAM	7	2
	Flatpea	
VAM	44	15
No VAM	3	2

Table 9. Responses of 5-month-old sweetgum with a VAM fungal "cocktail" in nonfertilized 1:1 phosphate overburden-sand tailings mix. (Sylvia, 1988)

Treatment	Leaf Area (cm^2)	Root Length (cm)
VAM cocktail	114	960
No VAM	4	128

Unfortunately, the natural occurrence of Pt on these sites is erratic since the site must first support trees whose roots can be colonized from airborne Pt spores. The survival and growth of the trees are improved only after Pt has colonized a significant quantity of roots. Another reason for its erratic occurrence is that fruit body production in nearby forests varies by season and from year to year due to variable weather conditions.

The artificial inoculation of the tree seedlings in nurseries, either bareroot or container, with Pt spores or mycelia resolves the problem resulting from the erratic occurrence of Pt on seedling roots because, after nursery inoculation, the seedlings will have Pt ectomycorrhizae before outplanting. Table 10 shows that seedlings with established Pt ectomycorrhizae benefit immediately after outplanting from this specific ectomycorrhizal association and survive and grow better on these difficult stress sites than routine nursery-run seedlings with ectomycorrhizae formed by naturally-occurring fungi (Cordell et al. 1991; Marx 1991).

The following are two case studies showing the biological and economic significance of this mycorrhizal technology to revegetation of mined land.

Case Studies

Ohio Abandoned Mineland Program. After reviewing the successful results of field research, the Ohio Division of Mines and Reclamation established criteria in 1982 for the operational use of tree seedlings with Pt ectomycorrhizae in their coal mined land reforestation program. During the past 18 years, the goals and priorities of the reforestation program have evolved into planting tree seedlings with Pt ectomycorrhizae to provide a low-cost, low-maintenance reclamation method for abandoned minelands that contribute sediment to streams, degrade aesthetics, lack adequate ground cover for wildlife, and are not eligible for traditional reclamation techniques (major grading, resoiling and revegetating) under federal abandoned mineland guidelines. Tables 11 and 12 show some of the field results.

On Ohio mineland sites, reclamation costs have been greatly reduced by utilizing seedlings with Pt ectomycorrhizae in their reforestation project. Estimates indicated that it would cost approximately $20,000 US per hectare to reclaim strip-mined lands in Ohio using conventional methods (major grading, topsoiling, soil amendments, pH adjustments, fertilization, and revegetation). Since 1981, when they began using seedlings with Pt ectomycorrhizae, the cost of reforesting abandoned mineland has averaged only $750 per hectare. The typical site is barren, eroded with a mixture of benches and slopes. The sites are highly acidic (pH 2.9–3.4) and are not amended, i.e. addition of lime, fertilizer, or water, before planting Pt seedlings. The additional cost of using seedlings with Pt ectomycorrhizae in these plantings on a 1.5 by 1.5 meter tree spacing (4,300 trees per hectare) is about 12 percent of the total tree establishment costs.

Utah Copper Mine Site. This mine has been active for over 100 years and has disrupted more than 9,000 hectares of land. The disturbed areas have extensive erosion, sedimentation of drainages, dust hazards and little or no satisfactory vegetation. The waste rock dump slopes are highly acidic. There are numer-

Table 10. Percent increase in survival and volume growth of pine seedlings after 2 to 4 years with *Pisolithus tinctorius* ectomycorrhizae over controls with naturally occurring ectomycorrhizae on various adverse sites (see Marx et al. 1992)

Pinus species	Site	Adversity[1]	% increase in seedling	
			Survival	Volume[2]
P. resinosa	Coal mined	1.4	214	0
P. echinata	Coal mined	1.4	5	400
P. virginiana	Coal mined	1.4	87	444
P. virginiana	Coal mined	1.4	480	422
P. rigitaeda	Coal mined	1.4	0	420
P. rigida	Coal mined	1.4	57	215
P. rigida	Coal mined	1.4	8	180
P. taeda	Coal mined	1.4	20	415
P. taeda	Coal mined	1.4	14	750
P. taeda	Coal mined	1.4	41	400
P. taeda	Coal mined	1.4	96	800
P. taeda	Coal mined	1.4	16	380
P. taeda	Kaolin mined	2.4	0	1100
P. taeda	Fullers' earth	2.4	0	47
P. taeda	Eroded	3.4	0	45
P. virginiana	Eroded	3.4	0	88
P. taeda	Borrow Pit	4	17	412

[1] Adversity: 1 = pH 3.1–4.3; 2 = low fertility; 3 = eroded; 4 = droughty

[2] Seedling volume = (stem diameter)^2 × height

Table 11. Response of 6-year-old Virginia pines to *Pisolithus tinctorius* (Pt) or natural ectomycorrhizae on a pH 2.8, coal mined site in Ohio (Cordell et al. 1996)

Treatment	Survival %	Height (cm)	Plot Volume (cm^3)
Pt	98	298	74×10^4
Natural	45	160	3×10^4

Table 12. Response of pine and oak (1982–1999) to *Pisolithus tinctorius* (Pt) ectomycorrhizae on 356 coal mined sites (averaged) in Ohio. Soil pH ranged from 2.9–3.4 (Cordell et al. 2001)

Treatment	% Survival	% Replanted
Pt	85	<5
Before Pt	<50	>75

ous borrow areas with gravelly conditions and several large areas of mill tailings. There is little suitable topsoil readily available and subsoils range from poor to unsuitable quality. The high altitude-mining site has low annual precipitation and freezing winter and hot summer temperatures.

The primary reclamation objectives on these three problem soil types were to mitigate the production of acidic water, stabilize the soils, mitigate erosion and dust, establish vegetation and return the area to productive wildlife habitat use. The results to date have been very positive. Tables 13–16 show some of the results of this program. Several thousand custom seedlings have been grown in nurseries and planted on the reclaimed areas. Survival and growth rates of preinoculated trees and shrub seedlings and the grasses, flowers and shrubs inoculated at seeding in the field, are significantly better than the non-inoculated plants.

The approach taken to establish vegetation on this mining area was to use the natural systems protocol. It involved the selection of site-suitable plant species based on results from initial test plots. Site and plant species-specific mycorrhizal fungi were identified and used in conjunction with other mycorrhizal fungi to provide optimal survival and growth benefits to tree and shrub seedlings and to grasses, forbs and shrubs started from seed on site. Municipal biosolids (Marx et al. 1995b) were used as a soil organic amendment to improve the initial adverse physical, chemical and plant nutrient problems of some of the low quality soils. Unique reclamation equipment for VAM fungal

Table 13. Response of 3-year-old pine and oak to *Pisolithus tinctorius* (Pt) or natural (NI) ectomycorrhizae on a copper-mined site (pH 7.7) in Utah (unpublished, 1997)

Treatment	Height (cm)	Caliper (cm)	Root Depth (cm)	Root Spread (cm)
Ponderosa p. + Pt	30	1.58	45	75
Ponderosa p. + NI	12.5	0.95	25	25
Gambel oak + Pt	50	1.40	60	90
Gambel oak + NI	15	0.63	40	15

Table 14. Root response of pine with specific or natural ectomycorrhizae from nursery after two-years on 45 cm soil and biosolids capped copper-mined waste rock in Utah (unpublished, 1997)

Treatment	Root Depth (cm)	Root Spread (cm)	% Ecto. Roots
		Ponderosa Pine	
Pisolithus	35	40	40
Scleroderma	25	25	35
Natural	20	20	20
		Austrian Pine	
Pisolithus	30	40	35
Scleroderma	30	20	40
Natural	15	15	20

Table 15. Root response of pine with specific or natural ectomycorrhizae from nursery after two-years on 45 cm soil and biosolids capped copper-mined waste rock in Utah (unpublished, 1997)

Treatment	Root Depth (cm)	Root Spread (cm)	% Ecto. Roots
		Lodgepole pine	
Pisolithus	20	25	45
Scleroderma	35	30	75
Natural	–	Dead	–
		Jeffrey pine	
Pisolithus	25	20	30
Scleroderma	30	25	35
Natural	10	10	20

Table 16. Root response of native shrubs to VAM after two-years on 12 cm soil and biosolids mix over copper-mined waste rock (pH 2.7) in Utah (unpublished, 1997)

Treatment	Root Depth (cm)	Root Spread (cm)	% VAM
		Sagebrush	
VAM	25	40	11
No VAM	15	30	0
		Sumac	
VAM	40	90	33
No VAM	30	60	10
		Indigobush	
VAM	40	60	58
No VAM	25	45	0

inoculation, seeding and erosion mitigation was also developed. VAM fungal spores and beneficial bacteria in pelletized form were developed for practical and effective field inoculation. A container-grown tree and shrub seedling production program was established in a local tree nursery that included protocols for custom inoculation of trees and shrubs with specific ectomycorrhizal or VAM fungi and bacteria.

The ectomycorrhizal fungi included Pt and another puffball-producing fungus, *Scleroderma citrinum,* isolated from an eastern coal mine site. The VAM fungi included a *Glomus* species isolated from sagebrush growing on undisturbed native soil near the mine site and, also, a "cocktail" of several selected VAM fungal species isolated from other plant species in different physiographic locations of the US.

By using the natural systems approach to solve their vegetation establishment problems, the client has realized a reduction in costs ranging from 40 to 80 percent depending on the type of area and condition being reclaimed. Savings have occurred during the project reclamation work along with a significant reduction in long-term maintenance.

Much is known about the biological and ecological value of mycorrhizae and soil/root bacteria to survival, growth and development of plants on sites of different quality. Consistent research and field demonstration results obtained during the last two decades clearly show the ecological and economical benefits of managing specific ectomycorrhizal and VAM fungi along with specific bacteria on tree seedlings, shrubs and grasses to improve mineland vegetation establishment.

Positive results have been obtained from the environmental extremes found in the moist East to the arid West of the US. Advanced technology has also revealed the importance of a "total integrated biological package" for a successful mineland revegetation program. The package includes evaluation and consideration of site factors such as pH, available heavy metals, and soil compaction, the use of soil remediation practices such as soil ripping and soil and organic amendments, the use of unique reclamation seeding/VAM fungal inoculating equipment, the selection of site-compatible plant species, and now, the management of specific mycorrhizal fungi and bacteria on the plants. These essential physical, chemical and biological considerations comprise a natural systems approach to successful vegetation establishment on minelands.

Arboriculture

In our efforts to domesticate forest trees and other plants, we have removed them from their natural forest settings and are now growing them in commercial tree plantations, tree orchards, and in urban settings, such as roadsides, sidewalk cutouts, golf courses with tree to tree grass and other nonforest-like landscapes. Trees occur in these various urban landscapes following one of two events. Either they existed as a forest tree in the area before manmade development or they were transplanted after development. Roots of preexisting trees are routinely damaged during construction, compaction from vehicles and people, and by the ever-present “urban forest floor” of grass or concrete/asphalt instead of a forest floor of organic matter.

Large transplanted trees are routinely moved to their new urban environment with less than 10 percent of their original root system developed in the nursery but they are expected to survive and maintain a normal growth rate on their new planting site. In reality, these transplanted trees, if they survive, may need 10 years to replace the original lateral and absorbing root systems, if ever. Roots not only need large areas for proper development but they also must have soil conditions that contribute to this development.

The soils in most urban landscapes have little sustainable and recyclable high quality, native organic matter needed to drive natural soil processes. They are also compacted with poor aeration and low water storage capacity and frequently have a creeping soil pH caused by alkaline irrigation water or from liming the grass covering the roots. Many municipalities have irrigation water exceeding pH 8.5 which when repeatedly used for irrigation will eventually result in a rise in soil pH. Preferable soil pH for most trees is pH 5.0–6.0. Soil pH above this can cause harmful minor element deficiencies (even from chelated microelements) and changes in microbial populations. These trees must have the capacity to produce new functional absorbing roots. The soil must also contain infective inocula of mycorrhizal fungi to form mycorrhizae on new roots and the soil must contain the proper organic matter and associated microbes to carry out its’ essential forest soil processes. If these are absent, then the arborist is forced to maintain these trees with a row crop mentality that involves the abundant use of pesticides and inorganic fertilizers. These chemicals, when used for long periods or in excess, can inhibit or delay normal root function and forest soil processes and may increase the susceptibility of the tree to pests.

Since the mid 1990’s, the mycorrhizal fungal technology has been adopted from forestry and reclamation to accommodate arboricultural conditions. Various authors have reported increased root and mycorrhizal development following root inoculation with Pt spores of mature Northern red oak in Michigan (Marx et al. 1995a) and North Carolina (Smiley et al. 1997) and Southern live oak in South Carolina (Marx et al. 1997) growing in urban settings. Others have reported increased root and mycorrhizal development followed by improved canopy growth after backfill treatments with ectomycorrhizal fungi of large (5 to 15 cm caliper) transplanted spruce, pine and beech spp. (Marx and McCartney 1997), Northern red oak (Rao et al 2000) in Ohio and basswood (Garbaye and Curin 1996) in France. Increased root and mycorrhizal development and improved canopy growth have also been reported following VAM fungal and rhizobacterial inoculation of 3 to 4 cm diameter transplanted sugar maple in Ohio (Rao et al. 2000) and red maple, ash, crabapple and Western hackberry in Colorado (Geist 1998) in urban landscapes.

In early 1998, the staff at the Royal Botanic Garden at Kew, UK injected the root zones of 20 *Cedrus, Fagus, Platanus, Quercus,* and *Sophora* trees in serious decline at the Garden with mycorrhizal fungal and rhizobacterial inoculants. In previous years, other methods to reverse decline on these mature trees were only marginally successful and involved compressed air to reduce soil compaction, vertimulching with peat and fertilizer and mulching. Based on these previous experiences, the results of the microbial treatments exceeded all expectations of the staff. Within six months, they noticed an increase in tree canopy cover and size of buds on treated trees compared to nontreated trees. At ten months, the treated trees had recovered from pre-treatment canopy covers as low as 45 percent to post treatment high of 95 percent. The majority of the treated trees had new branch growth exceeding 15 to 25 cm compared to only 10 cm for the nontreated trees.

A knowledgeable arborist or landscaper will consider the below ground requirements acquired by trees and other woody plants from their former forest environment and design management practices to fulfill these requirements. Good quality soil organic matter, an organic mulch covering the rooting area, the largest possible volume of quality soil for maximum root development, and adequate inoculum potential of mycorrhizal fungi and beneficial soil/root bacteria are a few basic prerequisites to healthy root development and function for plants on these urban landscapes.

Agriculture

As discussed by Johnson and Pfleger (1992) and Bethlenfalvay (1992), VAM must be considered in the design of sustainable agricultural systems. Modern agricultural practices, such as crop rotation sequences, plant breeding, pesticides, tillage and fertilizer affect either the quality of infective VAM fungal propagules or the number of fungal species surviving these practices in the soil. As a general rule, the diversity of VAM fungal species decrease when natural systems are converted to agricultural systems, and diversity decreases further as the intensity of agricultural inputs increase (Siqueira et al. 1989; Schenck et al. 1989; Sieverding 1990). In order to compensate for the loss of infective propagules and VAM fungal species diversity, VAM fungi have been experimentally managed in cropping systems by artificial soil inoculations with various inocula. Increased crop production following inoculation is related to initial soil fertility (especially available P), pesticide applications, populations of indigenous VAM fungi, the infectivity of the introduced fungi and the ability of the introduced inoculant to survive in the soil before roots become available for VAM fungal colonization. Many crops that are inoculated as young seedlings in containers of near-sterile potting mixes have shown yield increases following outplanting in fumigated soils. Peppers, tomato, asparagus, cucumbers, and various melons are examples. Recently, yield increases of tomato and pepper seedlings in fumigated field soil were observed following pre-inoculation of the plant roots with a four VAM fungal species cocktail. Field crops, such as wheat, barley, corn, soybeans, potato, and onions, have also shown yield increases following field inoculations (Safir 1994). On a research scale, seed or soil treatment with the isoflavonoid formonetin has been successful in stimulating indigenous VAM fungal populations and improving yields in alfalfa, soybean, corn and potato (Nair et al. 1991; Fries et al. 1998; Safir 1994; Koide et al. 1999). Few of these published studies were large enough to be considered operational applications and fewer were done with commercial VAM fungal inoculants. These research studies were done with inoculants usually produced by the researcher in small quantities but frequently applied at high rates rather than inoculants produced in large volumes by a commercial firm and applied at more practical rates. The reader is encouraged to consult the comprehensive review of the literature on VAM and agricultural crops in the text cited in Bethlenfalvay (1992).

Horticulture

Many modern horticultural plant propagation practices discourage the natural occurrence of mycorrhizae on plant roots. Artificial potting mixes, fumigated or steamed soil, micro-propagation, high fertility and daily irrigation either eliminate the fungi or inhibit their ability to colonize roots. However, considerable basic research has been done on the response of a large variety of horticultural plants to VAM fungal inoculation. Many fruit trees (apple, avocado, banana, cherry, mango, papaya, grapevine, olive, peach, pear, pineapple and plum), berries (blackberry, raspberry, and strawberry) and pistacia and walnut have been reported to be highly responsive to VAM fungal inoculation in small research trials. Many woody ornamentals (boxwood, dogwood, hibiscus, hydrangea, hawthorne, juniperus, ligustrum, pittosporum, podocarpus, poinsettia, rose and vibernam) have also proven responsive to VAM. Grasses (bahahiagrass, bentgrass, *Bromus*, centipede, fescue, lespedeza, lucerne meadow grass, ryegrass, sea oats and switchgrass), various clovers and many flowers (aster, begonia, *Coleus*, geranium, impatiens, petunia, snapdragons, and *Vinca*) have also proven responsive. There are over 70 publications alone on the response of citrus to VAM. Recently, Hintermann and Basler (1998) in Switzerland reported a positive growth response of grape vines following inoculation of the potting soil with a commercial VAM fungal inoculant. With the exception of pecan (Sharpe and Marx 1986), little is known about the natural occurrence of ectomycorrhizae or the significance of ectomycorrhizal fungal inoculation of trees grown in horticultural production programs.

Unfortunately, space limits the hundreds of publications that could be discussed on the response of horticultural plants to mycorrhizal fungal inoculants. Considerable research has been done on the use of ectomycorrhizal and VAM fungal spore inoculants on production of various woody ornamentals. Recently, positive growth responses were observed with container-grown *Osmanthus* and *Illicium* to VAM development and to different levels of soil fertility at the Univ. of Georgia (Williams-Woodward, J., pers. comm.). Another significant observation was recently made in VA (Appleton, B., pers. comm.) indicating that composted pinebark, a widely used potting soil for ornamentals, inhibited VAM development on various plants. Apparently, the terpenes and resins in composted pinebark are functioning as fungicides limiting VAM development. Excellent VAM development was found on most ornamentals growing in inoculated potting mixes containing vermiculite, peat moss and perlite. Excessive irrigation, i.e. roots continually in saturated potting mixes, is another factor that strongly inhibits VAM development. Water roots that develop in these substrates are not very susceptible to VAM fungal colonization. Roots formed in potting mixes that are allowed to occasionally dry before another irrigation most resemble normal soil roots and are the most susceptible to VAM fungal colonization.

Conclusion

Over the past three decades a tremendous amount of research has been done on the practical use of mycorrhizal fungi demonstrating their effectiveness in improving survival and health of many plant species on diverse manmade landscapes. Most of the research was done using inocula produced by the researcher in small quantities. In most studies, inoculum was used at excessively high and impractical rates in order to guarantee rapid root colonization by the introduced fungus. Also, the application rate frequently was expressed in terms of inoculum weight without any attempt to standardize the mycorrhizal forming potential of the inoculant. It is reasonably easy to produce small quantities of good quality vegetative inocula of ectomycorrhizal fungi in pure culture, to collect large quantities of their spores from the field and to produce effective root:soil inocula of VAM fungi for research studies. However, that doesn't mean that these same techniques can be used to produce the large quantities necessary for commercial application. This issue has been discussed (Marx 1985) and effectively demonstrated with Pt inocula (Marx et al. 1984a, 1989) for forestry and mineland applications and by Gianinazzi et al. (1989) for VAM fungal inocula.

Production of good quality inocula is only the first step in the practical use of these fungi. Simple, but effective, procedures must also be developed for the correct application of the inoculants for each different plant market. One formulation of a fungal inoculant cannot be applied to all markets. A specific *delivery* system is required for each fungal product and plant situation. The basic procedures are very simple. The inoculants must be placed on the roots or applied to soil where the roots will soon be growing. Susceptible roots must be present before this inoculant can be infective, It must be applied at a rate sufficient to rapidly colonize a significant number of roots and to elicit a positive plant response.

At least seven requirements must be addressed before commercial use of mycorrhizal fungal inoculants can be seriously considered. They are:

The inoculant must be certified to contain sufficient numbers of infective propagules needed to colonize roots when applied at the rate recommended by the manufacturer for the specific application. This can only be accomplished with effective quality control and field research programs.

The inoculants, especially VAM fungal inoculants, should contain a cocktail of different fungal species adapted to different soil properties. This will allow the inoculants to be effective across a broad spectrum of ecological conditions. The number of propagules of each fungus in the inoculant should be known.

The inoculants must have a positive effect on plant survival, growth, or health. If used proactively, anticipating conditions of plant stress, treatment should maintain existing vigor and health of the plants.

The inoculants must have a significant shelf life, under realistic storage conditions, to allow for extended delays between their production and their application in the field. Spores of ectomycorrhizal and VAM fungi have greater longevity, in storage and in soil, than other fungal propagules.

The inoculants must be certified free of disease-causing organisms.

The inoculants must be compatible with other ingredients (i.e., rhizobacteria, biostimulants, surfactants and other organics) that may be part of the product formulation.

The inoculants must be economical and practical to use. The cost of the inoculants and their applications must be cost effective and, therefore, cost less than the economic loss incurred by the customer if the inoculants were not used.

The use of these commercially available products in various plant markets will change the mindset of many people in the plant industry. Establishing and growing trees, grasses, flowers, shrubs and forbes on adverse mined lands, improving the quality of seedlings for reforestation and Christmas tree plantations, returning health and vigor to mature declining urban trees, successfully establishing trees on stressed urban soils, producing high quality ornamentals with less fertilizer and irrigation, and improving yields of vegetable crops after preplant seedling inoculation are now economically feasible as a result of the commercial availability and practical application of the mycorrhizal fungi.

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