The Container Tree Nursery Manual

Volume Five
The Biological Component: Nursery Pests and Mycorrhizae

Chapter 2—Mycorrhizae

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Contents

5.2.1 Introduction

A major advantage in rearing container seedlings is the production of large, robust seedlings within a single growing season. This contrasts with the 2- to 3-year cycle of producing plantable seedlings of desired size in bareroot seedling nurseries in the western and northern United States. Most current criteria of seedling quality are limited to the condition and size of the seedling stem and foliage. Less attention is paid to the quality of roots on nursery seedlings, even though we are well aware of the paramount importance of roots in providing structural support and nutrient and water uptake. Thus, to completely evaluate the "health" of a seedling and predict its survival potential, we must increase our awareness of root quality.

To develop criteria for evaluating seedling root quality in the nursery, we must incorporate knowledge of the root dynamics of wild seedlings. This knowledge is critically important because once seedlings are removed from the nursery and planted into soil, the roots must function under soil conditions as mediated by complex and uncontrolled environmental and biotic factors. These soil conditions will differ drastically from the well-watered, well-fertilized nursery growing media.

In natural soils, all forest trees form symbiotic, mutually beneficial associations between their roots and specialized soil fungi. The fungus-root organ is called a **mycorrhiza (mycorrhizae** is plural). Mycorrhizae provide many benefits to the seedling and adult tree, especially in enhancing water and nutrient uptake. Indeed, seedlings strongly depend upon mycorrhizae for growth and survival as evidenced by the failure of nonmycorrhizal seedlings to survive when planted into soil lacking mycorrhizal fungi (Trappe 1977). Thus, the presence and abundance of mycorrhizae must be a major consideration for evaluating root system health and predicting outplanting performance.

Although considerable research is currently in progress on the role of mycorrhizae in plant nutrition and practical uses in forestry, an abundance of information and concepts are available for immediate use in tree seedling nurseries. Our objectives for this chapter are fourfold:

1. Describe the different types of mycorrhizae common to forest tree seedlings grown in container nurseries.

2. Define the benefits imparted by mycorrhizae to seedling nutrition, growth, and survival.

3. Document the occurrence of mycorrhizae on container grown seedlings and describe how routine nursery practices affect the development of mycorrhizae.

4. Recommend ways for nursery managers to incorporate mycorrhizal management into their cultural regimes and offer management strategies to enhance mycorrhizal development and subsequent seedling survival and growth after outplanting.

5.2.1.1 What are mycorrhizae?

The word **mycorrhizae** literally means "fungus roots" and defines the intimate associations between plant roots and specialized soil fungi, the mycorrhizal fungi. Nearly all the world's land plants form some type of mycorrhiza, and with few exceptions, all major forest tree species form mycorrhizae. Two major mycorrhizal types prevail among forest trees: **ectomycorrhizae,** which are formed with the important coniferous species of the Pinaceae and hardwoods in the Fagaceae and Betulaceae; and **vesicular-arbuscular (VA) mycorrhizae,** which are common on other hardwoods, particularly in the maples, sweetgums, cedars, and redwoods. Although similar in overall function and benefit to the host plant, these two types of mycorrhizae differ strongly in regard to the fungi involved, their morphology, and potential applications in forest tree nurseries. Table 5.2.1 lists the major genera of forest trees raised in nurseries of temperate North America along with the types of mycorrhizae they form.

First we will describe each major type and how to identify them and then outline the major benefits.

Table 5.2.1—*Types of mycorrhizae formed by major genera of forest trees raised in nurseries in temperate North America*

Ectomycorrhizae
birch (*Betula*)
Douglas-fir (*Pseudotsuga*)
fir (*Abies*)
hemlock (*Tsuga*)
larch (*Larix*)
oak (*Quercus*)
pine (*Pinus*)
spruce (*Picea*)
Ectomycorrhizae and vesicular–arbuscular mycorrhizae
eucalyptus (*Eucalytpus*)
juniper (*Juniperus*)
poplar (*Populus*)
walnut (*Juglans*)
Vesicular–arbuscular mycorrhizae
ash (*Fraxinus*)
cherry/plum (*Prunus*)
maple (*Acer*)
redwood (*Sequoia*)
sweetgum (*Liquidambar*)
sycamore (*Platanus*)
thuja "cedar" (*Thuja*)
yellow-poplar (*Liriodendron*)

5.2.1.2 Types of mycorrhizae

Ectomycorrhizae. Ectomycorrhizae develop on the short, feeder roots, as opposed to the longer, woody, structural lateral roots. In fact, once a root develops a lateral meristem and starts forming woody tissue, mycorrhizae can no longer form. Ectomycorrhizae can be easily recognized by the characteristic fungal sheath or mantle tissue that envelopes the feeder roots; often the fungal mycelium, or thread-like mold growth, can be seen emanating directly from the mantle and colonizing the soil or rooting substrate (fig. 5.2.1 and 5.2.2). When an ectomycorrhiza is sectioned and its internal anatomy is examined under a microscope, we can see the second major characteristic of ectomycorrhizae: the intercellular growth of the fungus between the epidermal and cortical cells that forms the **Hartig net** (fig. 5.2.3). It is within this extensive zone of fungus-root cell contact that nutrients and water are exchanged between fungus and host; the fungus brings in and releases to the host nutrients and water and in return receives plant-made sugars and other products of photosynthesis.

The fungi that form ectomycorrhizae are primarily Basidiomycotina and Ascomycotina (table 5.2.2), including many of the common forest mushrooms (fig. 5.2.4 and 5.2.5) and puffballs (fig. 5.2.6), as well as the hypogeous (below-ground) fruiting fungi called truffles (fig. 5.2.7-5.2.9). Well-known fungal genera that form ectomycorrhizae include *Amanita, Boletus*, *Hebeloma, Laccaria, Lactarius, Pisolithus, Rhizopogon, Russula, Scleroderma, Suillus, and Tricholoma (all Basidiomycotina), and Cenococcum* and *Tuber* (Ascomycotina) (see Miller (1982) for a complete listing of ectomycorrhizal fungus genera). Another common ectomycorrhizal fungus in seedling nurseries is *Thelephora terrestris* (and closely related species in the same genus). *Thelephora* **fruiting bodies (or sporocarps)** commonly occur as leathery, erect brown sheets or mats on the bases of seedling stems (fig. 5.2.10) or on and around the drainage holes of individual containers or bottoms of Styroblocks® (fig. 5.2.11 and 5.2.12). *Thelephora* species are the most common ectomycorrhizal fungi in container nurseries; we will discuss their occurrence and importance in later sections.

Table 5.2.2—*Comparison of some of the fungi forming the three different types of mycorrhizae and some of the forest tree genera involved*

Mycorrhiza type	Fungi involved		Common forest tree associates
	Class	Representative genera	
Ectomycorrhizae	Basidiomycotina	*Boletus, Suillus, Leccinum, Cortinarius, Tricholoma, Russula, Rhizopogon, Amanita, Hymenogaster, Gautieria, Hysterangium, Lactarius, Paxillus, Gastroboletus, Martellia, Scleroderma*	Beech, birch, Douglas-fir, eucalyptus, hazel, hemlock, larch, oak, pine, poplar, spruce, true fir, willow
	Ascomycotina	*Tuber, Genea, Elaphomyces, Hydnotrya, Geopora, Balsamia, Sphaerosporella, Cenococcum*	Beech, birch, Douglas-fir, eucalyptus, hazel, hemlock, larch, oak, pine, poplar, spruce, true fir, willow
	Zygomycotina	*Endogone*	Douglas-fir
Ectendomycorrhizae	Ascomycotina	*Phialophora, Chloridium,* "E-strain"	Birch, pine, spruce
Vesicular-arbuscular (Endomycorrhizae)	Zygomycotina	*Acaulospora, Endogone, Entrophospora, Gigaspora, Glomus, Sclerocystis, Scutellospora*	Ash, baldcypress, basswood, "cedar" (*Chamaecyparis, Libocedrus, Thuja*), cypress, eucalyptus, giant sequoia, maple, redwood, sweetgum, sycamore, yellow-poplar

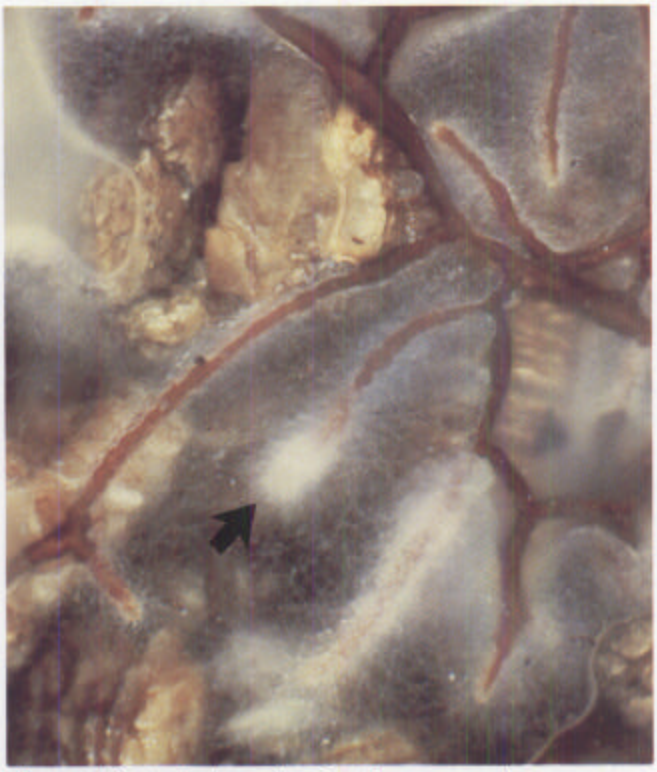

Figure 5.2.1—*Lodgepole pine–*Hymenogaster *sp. ectomycorrhizae. Note mycorrhizal root tip (arrow).*

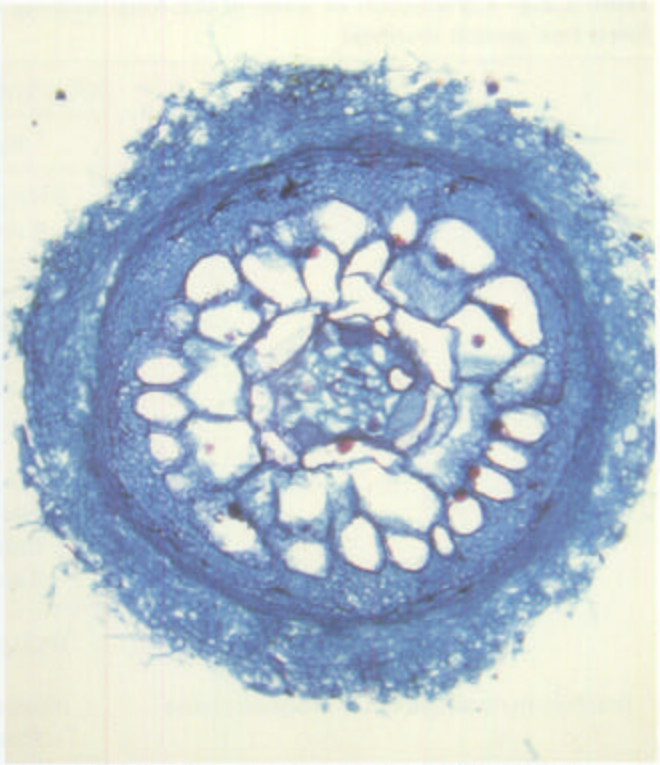

Figure 5.2.2—*Ectomycorrhizae of ponderosa pine–*Hebeloma crustuliniforme.

Figure 5.2.3—*Cross-section of lodgepole pine–*Martellia medlockii *ectomycorrhizae.*

Figure 5.2.4—Amanita muscaria *mushrooms, common under most Pinaceae.*

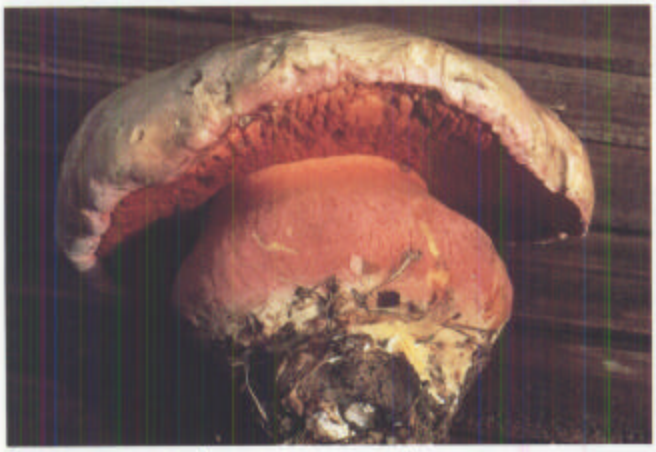

Figure 5.2.5—Boletus satanus *mushrooms, common under oaks (courtesy of D. Luoma, Corvallis, OR).*

Figure 5.2.6—*The puffball fungus* Scleroderma cepa, *common under hardwoods, especially oaks and hazel.*

Figure 5.2.8—*A false-truffle fungus,* Rhizopogon occidentalis, *common under pines in western North America (courtesy of E. Trueblood, Nampa, ID).*

Figure 5.2.7.—*A truffle-like fungus related to* Boletus, *Gastroboletus turbinatus (courtesy of H. Saylor, Hayward, CA).*

Figure 5.2.9—Rhizopogon smithii, *common under pines in western North America (courtesy of D. Luoma, Corvallis, OR).*

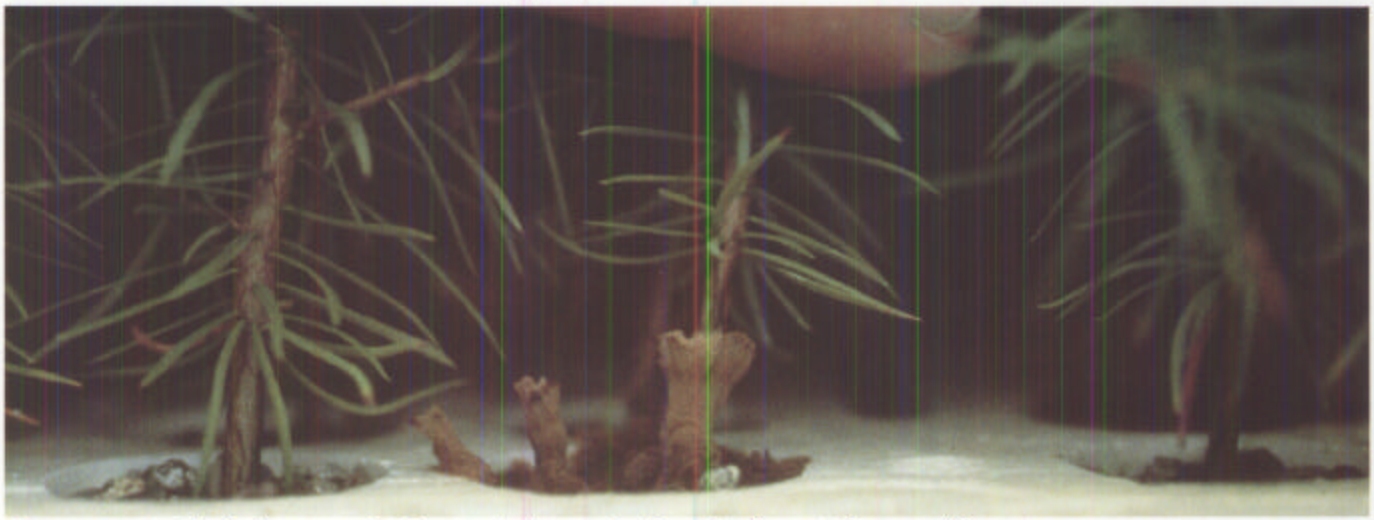

Figure 5.2.10—Thelephora *sp. fruiting at the base of a Douglas-fir container seedling.*

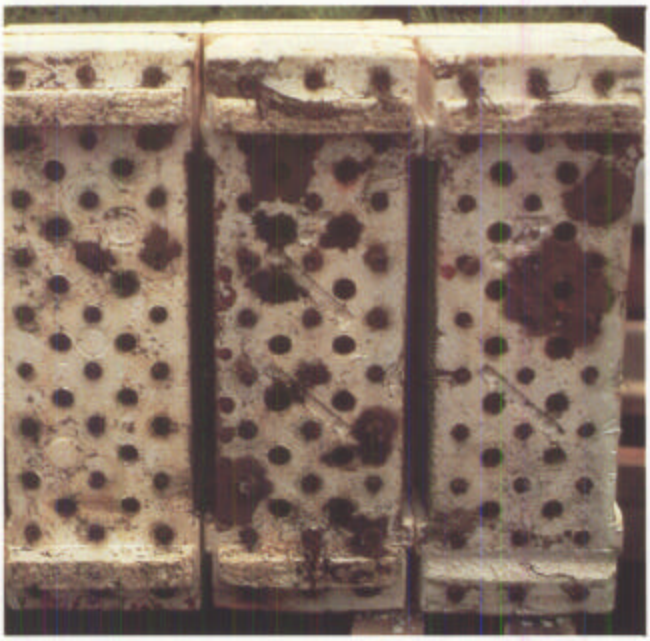

Figure 5.2.11—*Felt-like fruiting bodies of* Thelephora *sp. attached to the bottom of Styroblocks®.*

Figure 5.2.12—*Close up of* Thelephora *sp. fruiting body appressed to Styroblock®.*

Because most container tree seedling nurseries use artificial growing media, which lack ectomycorrhizal fungi, it is important to understand how container seedlings can become ectomycorrhizal, either naturally or by controlled methods. Many ectomycorrhizal fungi produce spores that are disseminated by the wind, allowing long-distance dispersal from forests to nurseries for inoculation of seedlings. However, the farther a nursery is located from ectomycorrhizal forests or large tracts of ectomycorrhizal trees, the less will be its chance of receiving natural wind-dispersed spore inoculum. Within the nursery, fruiting bodies produced on ectomycorrhizal seedlings offer a reliable source of spores to inoculate neighboring seedlings and future crops. The practical implications of spores as sources of inoculation will be discussed in detail in section 5.2.6.2.

The structural appearance of ectomycorrhizae is a function of both fungus and host plant. Thousands of different fungi form ectomycorrhizae, many with more than one host plant, so the overall appearance of different fungus-host combinations can vary tremendously. Figures 5.2.13 through 5.2.16 illustrate ectomycorrhizal form and diversity. Ectomycorrhizal morphology is often characteristic for a particular host genus. For example, the root tips of ectomycorrhizae in pines often branch dichotomously into complex structures (fig. 5.2.17). Other ectomycorrhizal forms range from simple cylinders to complex, pinnate, coralloid, or even compact tubercle forms (fig. 5.2.18). The amount of mycelium emanating from an ectomycorrhiza is another important diagnostic character. External mycelium (or **hyphae)** can range from sparse, nearly invisible threads to prolific wefts and root-like strands of hyphae **(rhizornorphs)** that transport nutrients and water (fig. 5.2.15 and 5.2.18).

Ectendomycorrhizae. Ectendomycorrhizae represent a second type of mycorrhiza, which can be abundant on nursery stock, particularly pines and spruces. Ectendomycorrhizae look like ectomycorrhizae in general form but usually lack the thick, often colorful mantle and abundant visible external hyphae usual for ectomycorrhizae (fig. 5.2.19). In cross section, the fungus can be seen penetrating into cortical cells as well as forming a Hartig net between them (fig. 5.2.20). Although we know little of the ecology of ectendomycorrhizal fungi or their effects on seedling nutrition, growth, and survival, ectendomycorrhizae have been shown to be beneficial in some instances (LoBuglio and Wilcox 1987, Wilcox and Ganmore-Neumann 1974). The fungi are Ascomycotina and mostly lack mushroom-like fruiting structures, although some form small cup-shaped fruiting bodies on the surface of the growing medium.

***Vesicular-arbuscular mycorrhizae*.** Vesicular-arbuscular (VA) mycorrhizae appear strikingly different from ectomycorrhizae: they do not modify root morphology and the fungal component is invisible to the unaided eye. Roots must be differentially stained and observed under the microscope to satisfactorily discern the fungal structures and degree of root colonization (fig. 5.2.21). As implied in the name, two structures characterize the VA mycorrhiza-vesicles and arbuscules. Vesicles are balloon-shaped structures, usually filled with lipids (oil droplets), that serve both as energy storage organs and as reproductive structures (fig. 5.2.22). Arbuscules are finely branched, intracellular, short-lived structures that serve as nutrient exchange sites between fungus and host (fig. 5.2.23). VA mycorrhizae also have abundant fungal mycelium that ramifies through the root cortex and extends out into the soil.

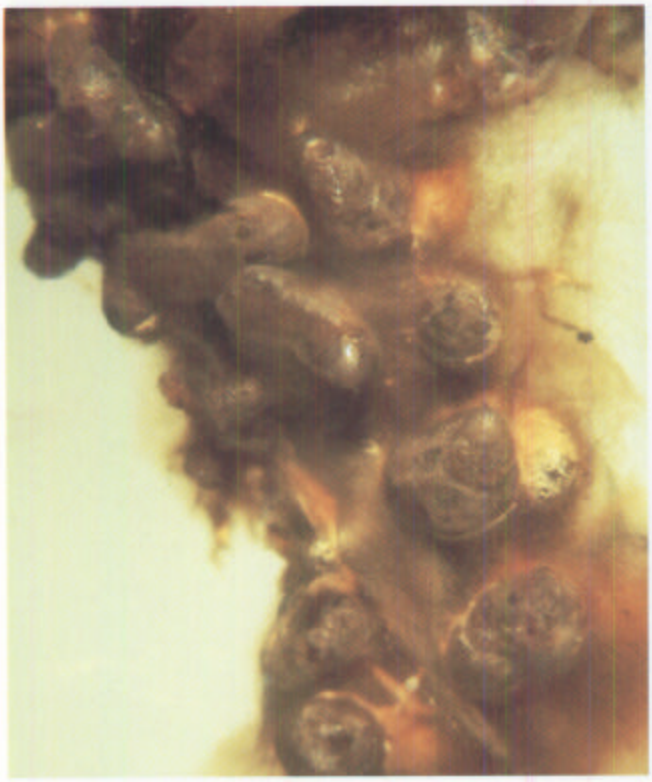

Figure 5.2.13—Rhizopogon *ectomycorrhizae of lodgepole pine.*

Figure 5.2.14—*Unidentified western hemlock ectomycorrhizae found within rotten wood.*

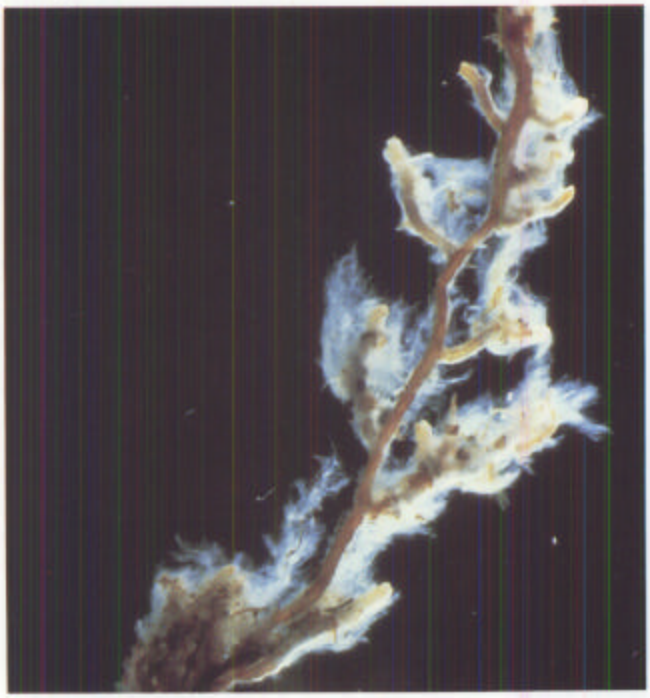

Figure 5.2.15—*Douglas-fir*–Hebeloma crustuliniforme *ectomycorrhizae.*

Figure 5.2.16—*Golden yellow lodgepole pine*–Alpova trappei *ectomycorrhizae.*

Figure 5.2.17—*Ponderosa pine*–Laccaria laccata *ectomycorrhizae.*

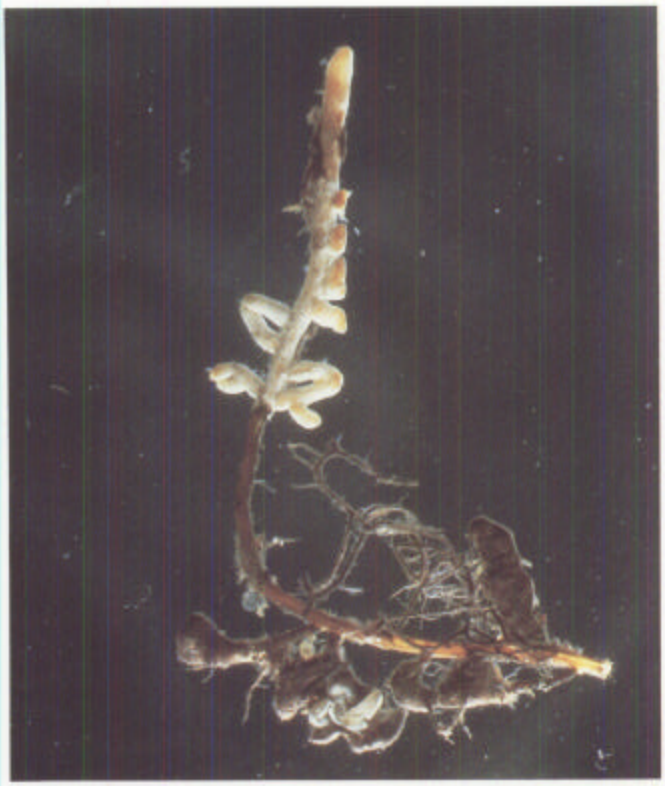

Figure 5.2.18—*Douglas-fir*–Rhizopogon vinicolor *ectomycorrhizae.*

Figure 5.2.19—*The ectendomycorrhizal E-strain fungus–Engelmann spruce (courtesy of G. Hunt, Balco, Kamloops, BC).*

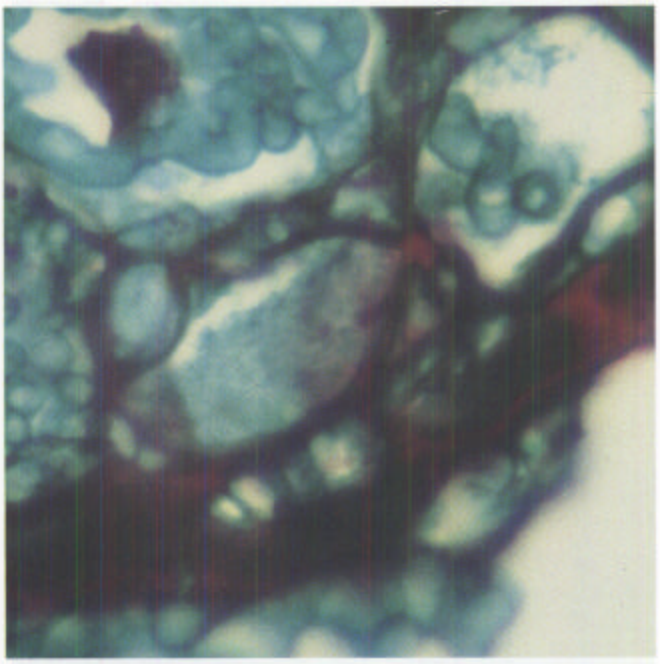

Figure 5.2.20—*Cross-section of an ectendomycorrhiza showing the intercellular and intracellular penetration of the root cells.*

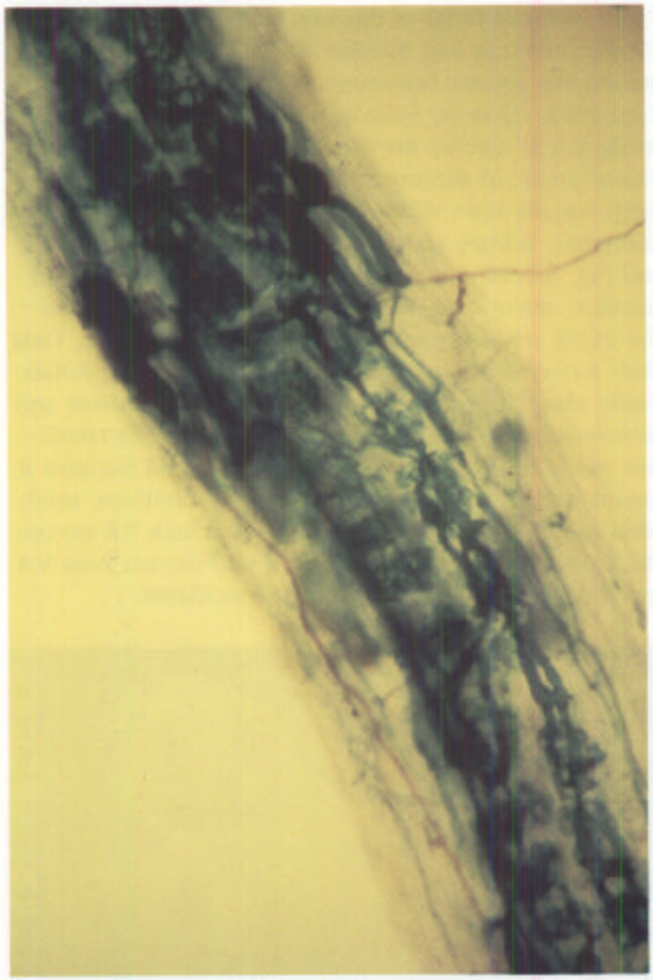

Figure 5.2.21—*Typical vesicular-arbuscular (VA) mycorrhizae.*

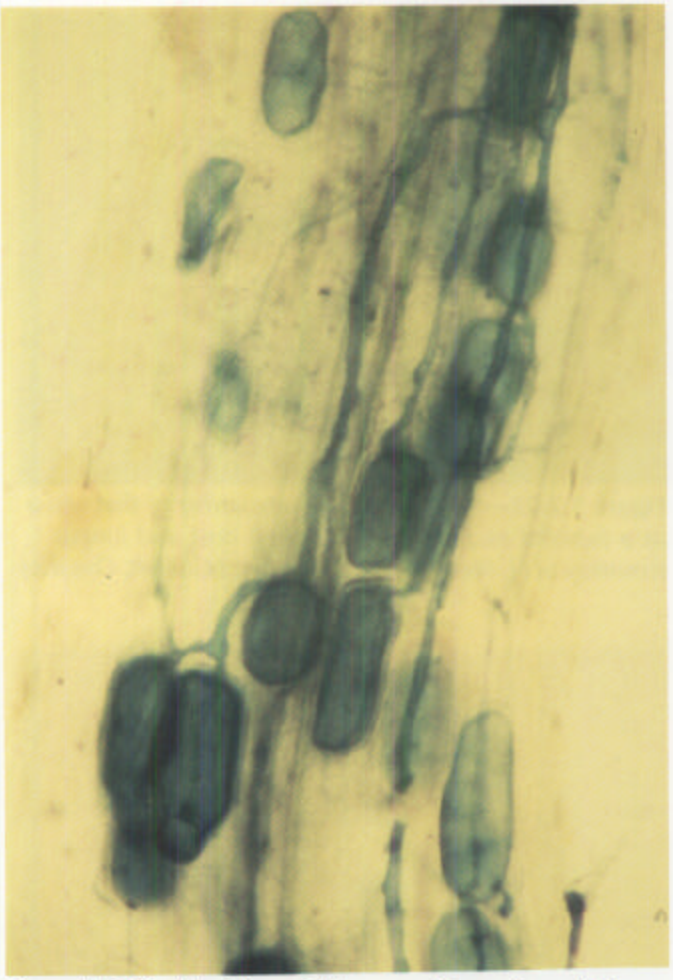

Figure 5.2.22—*Vesicles of VA mycorrhizae thought to function in energy storage and as asexual spores.*

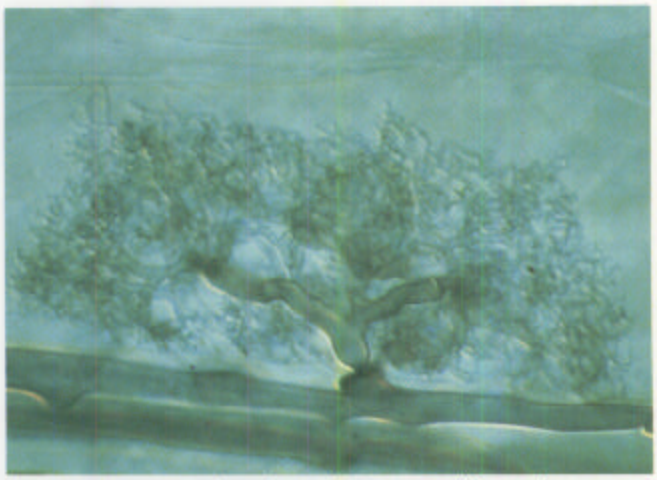

Figure 5.2.23—*Arbuscles of VA mycorrhizae that serve as a nutrient exchange site between host and fungus (courtesy of H. Massicotte, University of Guelph, Ontario).*

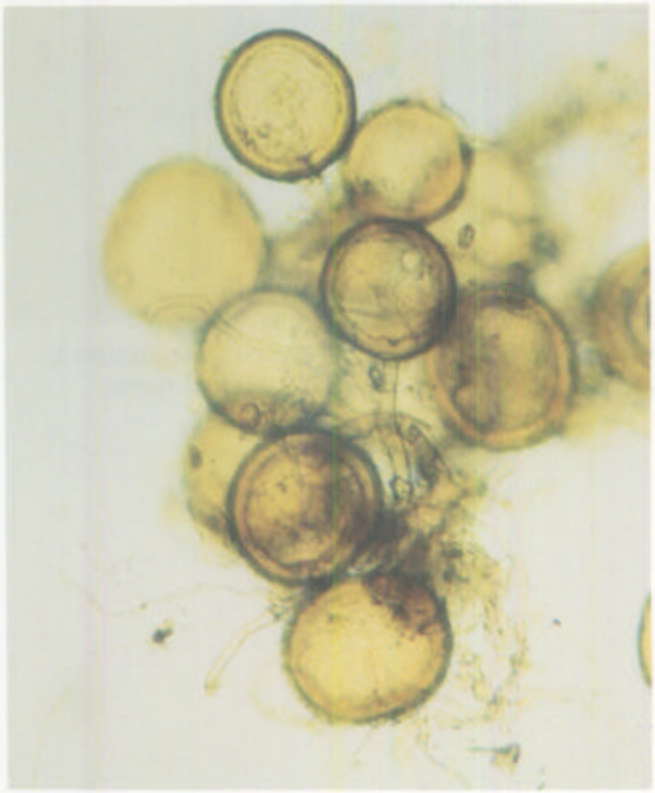

Figure 5.2.24—*Spores of the VA mycorrhizal fungus* Glomus fasciculatum.

Zygomycotous fungi in the family Endogonaceae form VA mycorrhizae and number a few hundred species among the genera *Acaulospora, Entrophospora, Gigaspora, Glomus, Sclerocystis*, and *Scutellospora* (table 5.2.2). Unlike the mushrooms and puffballs characteristic of ectomycorrhizal fungi, VA mycorrhizal fungi usually form relatively large (30 to 900 gm in diameter), solitary spores or clumps of spores in the soil (fig. 5.2.24 and 5.2.25). Because of their size and location, these spores are not wind disseminated like the much ,smaller spores of ectomycorrhizal fungi. Thus their movement is primarily by processes of soil movement; small animals and insects may also eat them and disseminate the spores in fecal droppings. This restrictive spore dispersal mechanism is significant because it greatly reduces their ability to colonize container seedlings growing in artificial media, which lack VA mycorrhizal fungi. In section 5.2.6.4 we will discuss how VA mycorrhiza-forming plants can be inoculated.

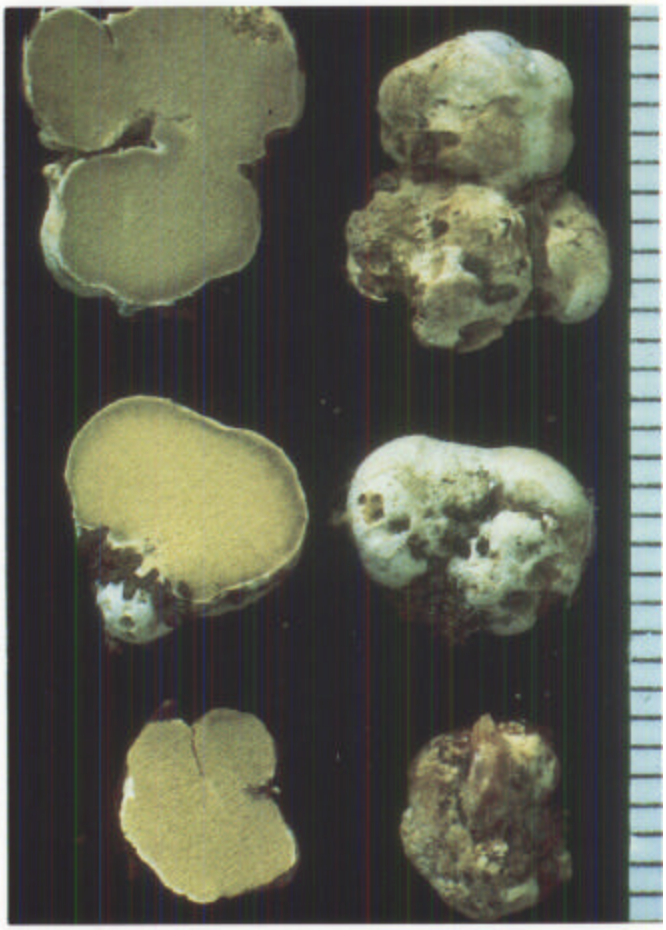

Figure 5.2.25—*Fruiting bodies of the VA mycorrhizal fungus* Glomus microcarpum.

5.2.1.3 Major benefits of mycorrhizae

Mycorrhizae benefit plant nutrition, growth, and survival in many ways; the best known benefits are enhanced uptake of water and mineral nutrients, especially phosphorus and nitrogen (Bowen 1973). These benefits are due in part to the exploration of soil for nutrients and water by hyphae to an extent far beyond the capabilities of roots alone. Some researchers estimate that mycorrhizal fungus hyphae can explore volumes of soil hundreds to thousands of times greater than can roots. Ectomycorrhizal fungi also produce growth regulators that stimulate feeder root elongation and branching, thus increasing the total number of feeder roots produced. Such root branching also benefits absorption of nutrients by increasing root surface area. Some ectomycorrhizal fungi produce dense mycelial mats in the soil for capturing nutrients, while others also produce rhizomorphs--large strands of parallel hyphae--that act as conduits for the flow of nutrients to and from the ectomycorrhizae (fig. 5.2.26). Ectomycorrhizae also reduce root respiration, which would increase root longevity (Marshall and Perry 1987). Although VA mycorrhizal fungi do not alter gross root morphology, they too explore great volumes of soil with their external mycelia and thus return nutrients and water from a soil zone beyond the limits of root hairs. Readers are referred to the texts by Harley and Smith (1983), Marks and Kozlowski (1973), and Schenck (1982) for more detailed information concerning mycorrhizal effects on plant mineral nutrition.

Figure 5.2.26—*Douglas-fir–Rhizopogon vinicolor ectomycorrhizae. Note large strands of parallel hyphae–rhizomorphs (arrows), which are connected to the mycorrhizal feeder root.*

Mycorrhizal fungi can protect roots against pathogens in several ways (Marx 1972). The fungus mantle of ectomycorrhizae provides a direct barrier against pathogen entry. Moreover, many ectomycorrhizal fungi produce antibiotics antagonistic to some root pathogens (Wilkins and Harris 1944, Wilkins and Partridge 1950). For example, Marx (1969a, 1969b, 1970) reported strong antibiotic production by the ectomycorrhizal fungus *Leucopaxillus cerealis* against *Phytophthora cinnamomi.* In nursery studies, *Laccaria laccata* suppressed *Fusarium oxysporum* on Douglas-fir seedlings (Sylvia 1983; Sylvia and Sinclair 1983a, 1983b). Unfortunately, much of the exploratory research of ectomycorrhizal control of pathogens has been done with pure cultures of fungi or in small, isolated studies. Use of mycorrhizae for biological control of root pathogens is lagging behind other applications and needs serious research attention.

Nursery managers should be aware of one other aspect of mycorrhiza--disease interactions: mycorrhizae indirectly protect plants against many types of pathogens (Schenck 1981) by benefiting plant growth. Healthy plants with well-balanced nutrition resist disease better than plants with poor nutrition. Mycorrhizae contribute vitally to adequate plant nutrition: they thereby contribute indirectly to the plant's resistance to disease. Because timing may be critical for resistance, the sooner the mycorrhizal fungus is present in the substrate, the greater the potential for pathogen control. By ensuring that mycorrhizae develop on seedlings, ery managers also provide some degree of protect against pathogens.

Other benefits of mycorrhizae include enhanced ro ing of cuttings (Linderman and Call 1977, Navratil Rochon 1981), increased root regeneration, increasE salt tolerance, and reduced drought stress (Parke et 1983a). Some of these beneficial attributes may be important in nursery management for mycorrhizae, whereas others are important for seedling survival an growth after outplanting.

5.2.2 Current Status of Mycorrhizae in Container Nurseries

5.2.2.1 Results of nursery survey

To our knowledge, there has never been a systematic survey of the types of mycorrhizae found in container tree seedling nurseries. To this end, we sent a questionnaire to container tree seedling nurseries across the United States and Canada, and 78 nursery managers responded (table 5.2.3). Although many believe that it is important to inoculate, only 6% of these nurseries have fungal inoculation programs. Seventy-seven percent of the nursery managers believe that mycorrhizae are important; less than half of them think that mycorrhizae are important during nursery culture. However, most believe that mycorrhizae are most important after the seedlings are outplanted. Eighty percent of the managers indicate they can recognize mycorrhizae on their seedlings. About two thirds of them survey their stock for mycorrhizae but report only low to moderate levels of mycorrhizal development. Our observations of some of their stock indicate that they likely underestimate the amount of mycorrhizae (table 5.2.4). Many managers find fruiting bodies in their nurseries but usually cannot identify them. When fruiting bodies have been identified by or for the nursery manager,

Table 5.2.3—*Responses of 78 container tree nursery managers to mycorrhiza survey*

Survey question	Percentage of respondents		
	Yes	Undecided	No
Nurseries with an inoculation program	6		94
Are mycorrhizae important?	77	18	5
in nursery?	42	6	53
upon outplanting?	80	12	8
Is inoculation necessary?	21	17	62
Can nursery staff recognize mycorrhizae?	80	3	17
Is stock surveyed for mycorrhizae?	66		34
When stock is surveyed how much mycorrhizae are observed?			
none	6		
low	40		
moderate	33		
abundant	21		
Are sporocarps found in your nursery?	56		44
When sporocarps are found, what are their identities?			
Thelephora terrestris	18		
Pisolithus tinctorius	6		
Laccaria laccata	3		
Endogone lactiflua	3		
Unknown	71		

Table 5.2.4—*Types of mycorrhizae identified on seedlings sent to us from container tree nurseries from across the United States and Canada*

Host	Age (mon)	*Thelephora* species		Ectendo-mycorrhiza		*Rhizopogon* type		Other	
		% of seedlings	root system rating*	% of seedlings	root system rating*	% of seedlings	root system rating*	% of seedlings	root system rating*
Betula									
yellow birch	ND							66	2.0**
Larix									
western larch	7	100	4.3			20	1.0		
western larch	12	100	3.1			40	1.9		
Picea									
Engelmann spruce	12	100	5.0						
Engelmann spruce	24	100	5.0						
white spruce	4	18	2.0						
white spruce	8	58	3.1						— †
white spruce	10	28	1.5	100	4.2				
white spruce	ND	100	5.0						— †
white spruce	ND	100	5.0						
black spruce	4	36	1.8						
black spruce	4	40	2.3						
black spruce	4	100	5.0						
black spruce	8	100	2.9						
black spruce	8	100	5.0						— †
black spruce	ND	100	1.0						
black spruce	ND	100	2.2						
black spruce	ND	100	5.0						
blue spruce	7	0	0.0						— ‡
red spruce	5	88	3.0						
red spruce	8	100	5.0						— †

ND = Not determined

* Rating for percentage of the seedling root system with a particular type of ectomycorrhiza, 0 = none, 5 = 100%.

** *Lactarius*-type ectomycorrhizae (green mantle)

† Poor root system, few feeder roots, no "water roots."

‡ Poor root system, few feeder roots, many "water roots."

§ Unidentified bright yellow ectomycorrhizae.

|| Unidentified buff ectomycorrhizae.

¶ *Cenococcum geophilum* ectomycorrhizae (black mantle).

Table 5.2.4 (continued)—*Types of mycorrhizae identified on seedlings sent to us from container tree nurseries from across the United States and Canada*

Host	Age (mon)	*Thelephora* species: % of seedlings	*Thelephora* species: root system rating*	Ectendomycorrhiza: % of seedlings	Ectendomycorrhiza: root system rating*	*Rhizopogon* type: % of seedlings	*Rhizopogon* type: root system rating*	Other: % of seedlings	Other: root system rating*
Pinus									
jack pine	4	100	4.2	8	1.5				
jack pine	ND	55	2.1	45	1.6				
lodgepole pine	8	100	5.0						— †
western white pine	12	100	3.9	70	1.2	20	4.0		
Austrian pine	20			100	5.0	4	2.5		
longleaf pine	4			10	4.0	5	3.0		
longleaf pine	6			30	3.0				
ponderosa pine	8	100	5.0						
ponderosa pine	ND			100	5.0				
ponderosa pine	ND			100	5.0				
red pine	4	30	1.7						
red pine	5	44	3.1	22	2.9				
red pine	ND	100	5.0						— †
red pine	ND	0	0.0						
eastern white pine	8			2	4.0	0.5	2.0	16	1.0 §
Scotch pine	16			100	5.0				
Pseudotsuga									
Douglas-fir	7	0	0.0						— ‡
Douglas-fir	1+0	0	0.0						
Douglas-fir	ND	34	2.8						— ‡
Quercus									
laurel oak	2							50	2.0 ||
Tsuga									
western hemlock	ND	66	2.1					0.5	1.0 ¶

ND = Not determined

* Rating for percentage of the seedling root system with a particular type of ectomycorrhiza, 0 = none, 5 = 100%.

** *Lactarius*-type ectomycorrhizae (green mantle)

† Poor root system, few feeder roots, no "water roots."

‡ Poor root system, few feeder roots, many "water roots."

§ Unidentified bright yellow ectomycorrhizae.

|| Unidentified buff ectomycorrhizae.

¶ *Cenococcum geophilum* ectomycorrhizae (black mantle).

Figure 5.2.27—Pisolithus tinctorius *fruiting body.*

Figure 5.2.29—*Distinctive pale green ectomycorrhizae of yellow birch container seedlings.*

Figure 5.2.28—Endogone lactiflua *fruiting bodies.*

Pisolithus tinctorius (fig. 5.2.27), *Laccaria laccata*, *Thelephora terrestris,* and *Endogone lactiflua* (fig. 5.2.28) prove to be the most common.

In addition to the survey questionnaire, we asked the nursery managers to send a representative sample of their seedlings to our laboratory for evaluation. We examined up to 50 seedlings of 19 different tree species from 18 nurseries (table 5.2.3). Ectomycorrhizal colonization was estimated by type per seedling for each nursery. Most seedlings had some ectomycorrhizae; many were totally colonized. Thelephora spp. formed the majority of ectomycorrhizae among the 19 tree species, especially on larch, spruce, and some species of pine. Ectendomycorrhizae of undeterminable identity were abundant on several pines, especially jack, western white, Austrian, longleaf, ponderosa, and Scotch pines. Ectomycorrhizae formed by laurel oak and yellow birch were unlike any noted on the conifers (fig. 5.2.29).

5.2.2.2 Observations in Pacific Northwest nurseries

We have monitored ectomycorrhizal development on container seedlings in several Pacific Northwest nurseries for 15 years and found that it varies between nurseries and years. However, we have noted several consistencies. We see an abundance of conifer seedling ectomycorrhizae formed with *Thelephora* spp. *Thelephora spp.* are well adapted to nursery conditions in which abundant water and soluble nutrients stimulate rapid colonization of the growing media by the fungus, closely followed by development of fruiting bodies (fig. 5.2.10-5.2.12). Another reason for their prominence in container nurseries is that Thelephora fruiting bodies form early in summer and become a source of spore inoculum for the rest of the nursery. Ponderosa pine seedlings often have a high degree of colonization by ectendomycorrhizae in addition to *Thelephora ectomycorrhizae*. Engelmann and white spruce are typically heavily colonized by *Thelephora* and *Laccaria laccata* at high rates of soluble and slow-release fertilizer. However, when slow-release fertilizer is eliminated or reduced, *Thelephora* mycorrhizae are readily replaced by E-strain (an unidentified Ascomycota that forms ectendomycorrhizae), *Amphinema byssoides* (fig. 5.2.30), and occasionally *Cenococcum geophilum* mycorrhizae (Hunt 1987). We have also observed the ectendomycorrhiza-forming Ascomycotina *Sphaerosporella brunnea* on pines, as have others in Canada (Danielson 1984). Other trees such as true firs, Douglas-fir, and western hemlock often form few or no mycorrhizae despite the same exposure to *Thelephora* and *Sphaerosporella* spores.

Seedlings in Northwest nurseries frequently have **water roots,** the thick, fleshy, opaque **nonmycorrhizal** roots lacking roothairs (fig. 5.2.31) that develop in saturated growing media. We urge nursery managers to check seedlings regularly for water roots when assessing root quality. Water roots are not colonized by mycorrhizal fungi and may even become infected by fungal pathogens (see chapter 1). Water roots are discussed in more detail in section 5.2.8.3.

Figure 5.2.30—Amphinema byssoides *on Engelmann spruce, a common naturally occurring fungus when slow-release fertilizer is not used (courtesy of G. Hunt, Balco, Kamloops, BC).*

Figure 5.2.31—*Water root formation on Douglas-fir container seedlings. Normal root formation on right, abnormal on left. (Courtesy of C. Hunt, Balco, Kamloops, BC.)*

5.2.2.3 Mycorrhizae: why some seedlings are mycorrhizal and others are not

Although most container nurseries will have some seedlings (especially Douglas-fir and pines) that are ectomycorrhizal with one fungus or another, these seedlings (except for seedlings ectomycorrhizal with *Thelephora*) are erratically distributed within the nursery. This erratic distribution is caused by the inability of ectomycorrhizal fungi to spread vegetatively (that is, with their mycelia) from container to container. Each seedling must therefore have fungus spores land on and wash into its growing medium, find a susceptible feeder root, germinate, and form ectomycorrhizae. *Thelephora* spores do just that with amazing efficiency. *Thelephora* spp. grow rapidly after germination, form abundant ectomycorrhizae, and produce fruiting bodies midway through the growing season. Such adaptations *by Thelephora* spp. make them the dominant type in container nurseries. Most other ectomycorrhizal fungi may not produce fruiting bodies, do not distribute their spores through the air, or often grow slowly. Such fungi spread even more erratically throughout the nursery than *Thelephora*.

5.2.3 How to Check Seedlings for Mycorrhizae

Seedlings should be assessed after they have been hardened off. During the seedlings' juvenile and rapid growth phases, mineral nutrition, especially that of nitrogen, is extremely high. This high availability of soluble fertilizer will inhibit most fungi to some extent. It is not uncommon to observe proliferation of mycelium and mycorrhiza formation as the first stage of seedling hardening begins. The timing of mycorrhizal assessment will greatly influence the amount of mycorrhizae observed.

Mycorrhizae can be distinguished from pathogenic fungi by the presence of visible mycelia surrounding the root and the lack of decay.

To assess ectomycorrhizal development, first remove the seedling from the container and gently wash the roots free of growing media. Suspend the root system in a dish (1 to 2 inches deep) that is partially filled with tap water and gently spread the root system so that feeder roots are clearly visible. Mycorrhizae are then assessed by viewing the immersed roots under a stereomicroscope at 5 to 15 times magnification.

5.2.3.1 Ectomycorrhizae

Ectomycorrhizae may be difficult to recognize at first, but with a little practice nursery staff can soon distinguish between ectomycorrhizal (fig. 5.2.32 and 5.2.33) and nonmycorrhizal (fig. 5.2.34) feeder roots. Ectomycorrhizae of hardwoods are not as easily discernible as those of conifers. The following key characters will guide recognition:

1. Ectomycorrhizae are typically swollen and lack root hairs.

2. The fungus mantle or sheath is usually a different color than the feeder roots; some mantles are brightly colored or pure white (fig. 5.2.32 and 5.2.33).

3. Fungus mycelium or hyphal strands often grow out from the mantle tissue, giving a cottony appearance (fig. 5.2.32).

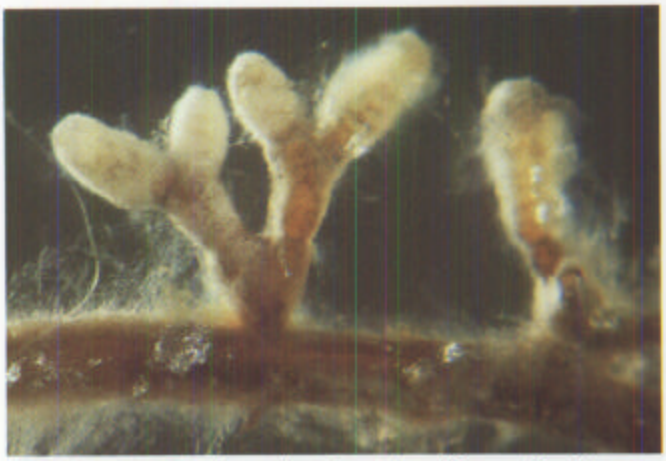

Figure 5.2.32—*Lodgepole pine–Martellia medlockii ectomycorrhizae.*

Figure 5.2.33—*Typical pinnate ectomycorrhizae of Douglas-fir–Lactarius rubrilacteus.*

Figure 5.2.34—*Nonmycorrhizal lodgepole pine feeder root with abundant root hairs.*

4. Mature ectomycorrhizae typically branch several times in regular or irregular patterns (fig. 5.2.32 and 5.2.33).

5. Nonmycorrhizal feeder roots are not swollen, are usually covered with root hairs, and for many conifer species, are unbranched (fig. 5.2.34).

Careful examination of the ectomycorrhizae shown in figures 5.2.13-5.2.18, 5.2.32 and 5.2.33 will help nursery staff recognize these characters.

5.2.3.2 Ectendomycorrhizae

Ectendomycorrhizae are more difficult to recognize. They can appear nonmycorrhizal because the fungal mantle can be sparse and thin. Ectendomycorrhizae usually lack root hairs, however, and are usually not significantly swollen. Absolute assessment of ectendomycorrhizae or verification of young ectomycorrhizae involves examining the roots with a compound light microscope for Hartig net formation or intracellular fungus growth. Although with training this, too, is easy, nursery staff can usually consult a specialist if necessary. Readers are referred to Wilcox (1982) for a more detailed description.

Counting total feeder roots during mycorrhizal evaluation is time consuming and usually unnecessary. Once you recognize ectomycorrhizae, you can easily estimate proportions of the root system with ectomycorrhiza colonization by major categories. Colonization categories of 1 = none, 2 = low (1 to 25%), 3 = medium (26 to 75%), and 4 = high (76 to 100%) will provide functional groupings (Grand and Harvey 1982) to evaluate the ectomycorrhizal component of seedling root quality (Benson and Iyer 1978).

5.2.3.3 Vesicular-arbuscular mycorrhizae

The assessment techniques mentioned in section 5.2.3.2 will not work for VA mycorrhizae because roots must be stained and observed under a compound light microscope to discern the mycorrhizal structures. If nurseries use completely artificial growing media (that is, no soil) to grow VA mycorrhizal hosts (maple, sycamore, sweetgum, redwood, western redcedar, juniper), few or no plants will have mycorrhizae unless inoculated. Remember that VA mycorrhizal fungal spores are usually not disseminated through the air. Nursery managers are referred to the detailed procedures described by Kormanik and McGraw (1982) for staining and assessing VA mycorrhizal roots. Their procedures are within a longer text on mycorrhizal methods and principles published by the American Phytopathological Society (Schenck 1982). We recommend this reference text for nurseries developing either ectomycorrhizal or VA mycorrhizal inoculation programs.

5.2.4 Mycorrhizal Fungi That Fruit in Container Nurseries

Overall ectomycorrhizal diversity is low in container nurseries compared to natural conditions. For reasons already mentioned, *Thelephora* spp. are the most common ectomycorrhizal fungi that fruit in both container and bareroot nurseries. Fruiting bodies of *Laccaria laccata* (fig. 5.2.35), *Inocybe lacera* (fig. 5.2.36), Hebeloma crustuhniforme (Castellano and Trappe 1987) and H. arenosa (Burdsall and others 1986) (fig. 5.2.37) are next in frequency of occurrence, particularly with pines or Douglas-fir. E-strain, *Amphinema byssoides,* and *Mycelium radicus atrovirens* are common on Engelmann spruce grown without slow-release fertilizer (Hunt 1987). Occasionally, seedlings (usually hemlocks) are colonized by *Cenococcum geophilum*, which forms a characteristic black ectomycorrhizae (fig. 5.2.38). Other fungi have been observed but with very low frequency. For example, fruiting bodies of *Rhizopogon rubescens* have been found in ornamental stock that was first grown in bareroot beds, then transferred to large pots (fig. 5.2.39).

Figure 5.2.35—Laccaria laccata *fruiting with ponderosa pine container seedling.*

Figure 5.2.36—Inocybe lacera *fruiting with Douglas-fir bareroot seedlings.*

Figure 5.2.37—Hebeloma arenosa *fruiting with Colorado blue spruce container seedlings.*

Figure 5.2.38—*Western hemlock–*Cenococcum geophilum *ectomycorrhizae.*

Figure 5.2.39—Rhizopogon rubescens *fruiting on the substrate surface of Austrian pine container seedlings.*

5.2.5 Determining the Need for Mycorrhizal Inoculation

Tree species in the Pinaceae and Fagaceae, which include the major coniferous forest species and the oaks, require ectomycorrhizae for survival and growth in natural ecosystems. This has been convincingly demonstrated in attempts at afforestation in the treeless grasslands of the Soviet Union and the United States (Mikola 1970). Ectomycorrhizal inoculation has proven beneficial in a wide variety of instances, for reclamation of adverse sites, reforestation of clearcut areas, reforestation after wildfire, and introduction of exotic species (Marx 1980).

5.2.5.1 In-nursery benefits

Nonmycorrhizal seedlings usually grow well in artificial growing media if water and soluble nutrients are supplied. Nonmycorrhizal feeder roots of these same seedlings will not properly take up water and nutrients from soil after outplanting until they form mycorrhizae. The old working premise that "any ectomycorrhizae on seedlings are better than none" is under close scrutiny. Some ectomycorrhizal fungi are better than others for selected applications. We have seen a lag in growth, and a reduction in survival, of nonmycorrhizal seedlings and those ectomycorrhizal with "nursery-adapted" fungi when outplanted on sites demanding quick establishment for survival, for example, droughty, south facing steep slopes in the Siskiyou Mountains of southwest Oregon. The time needed for seedling root systems to replace nursery-adapted fungi with fungi better adapted to site conditions leads to increased mortality and reduced initial seedling growth. An effective inoculation program requires mycorrhizal fungi that function efficiently in the seedling's growing environment, be it the nursery or in the field. The nursery inoculation program must have clear objectives:

1. Reduction in cull percentage in the nursery.
2. Increased stem caliper or leader growth in the nursery and/or field.
3. Protection against pathogens.
4. Rapid mycorrhizal colonization to alleviate stunting.
5. Increased outplanting survival.

Nursery managers and foresters should use mycorrhizal inoculation as another tool in their effort to grow seedlings and reforest land. The effectiveness of inoculation techniques varies by host and fungus, so flexibility is paramount to success. One fungus (or ecotypic isolate) may accomplish one to many objectives for one or many host species (or even seed sources). A flexible inoculation program would be able to meet some objectives for one portion of the stock and other objectives for other portions of the stock. **No one fungal species, isolate, or ecotype will meet the objectives of all nurseries.** The technology is currently available to tailor an inoculation program for each nursery, but fine-tuning the program to individual nurseries is probably a 2- to 3-year process.

5.2.5.2 Outplanting benefits

The critical test of benefit from mycorrhizal inoculation is seedling performance after it is planted in the field (Marx 1980). Regardless of how mycorrhizal inoculation affects growth in the nursery, the seedling must establish and grow once it is outplanted. Mycorrhizal inoculation may indeed produce no increase in seedling growth in the nursery but will give seedlings a better chance to survive or grow better once outplanted (Castellano 1987).

A significant increase in survival, stem caliper, or stem height can justify the expense of inoculation. Outplanting response to inoculation will differ for various habitat types as well as seedling host and fungal species (Dixon 1986). On sites that are extremely difficult to regenerate (ones that have been planted numerous times without seedling survival), seedling survival is of paramount importance (fig. 5.2.40 and 5.2.41). A successful nursery inoculation program begins with the careful evaluation of the need for inoculation by the forester, and his/her linking with an experienced nursery manager and mycorrhizal specialist to produce appropriately inoculated seedlings (Kidd 1982).

Much of the published work on practical application of ectomycorrhizal inoculation is concerned with *Pisolithus tinctorius* inoculation. Dr. Donald Marx and colleagues at the USDA Forest Service Institute for Mycorrhizal Research and Development, Athens, Georgia, demonstrated the first wide-scale application of ectomycorrhizal inoculation for improving seedling field performance. Numerous studies have shown the benefit of *P. tinctorius* ectomycorrhizae to seedling outplanting performance (Beckjord and McIntosh 1984; Berry 1982; Dixon et al. 1981; Dixon et al. 1984b; Kais et al. 1981; Marx and Hatchell 1986; Navratil et al. 1981; Parker et al. 1986; Riffle and Tinus 1982; Ruehle 1981, 1982; Ruehle et al. 1981b; Valdes 1986).

Figure 5.2.40—*Enhanced survival of Douglas-fir container seedling inoculated with* Rhizopogon vinicolor.

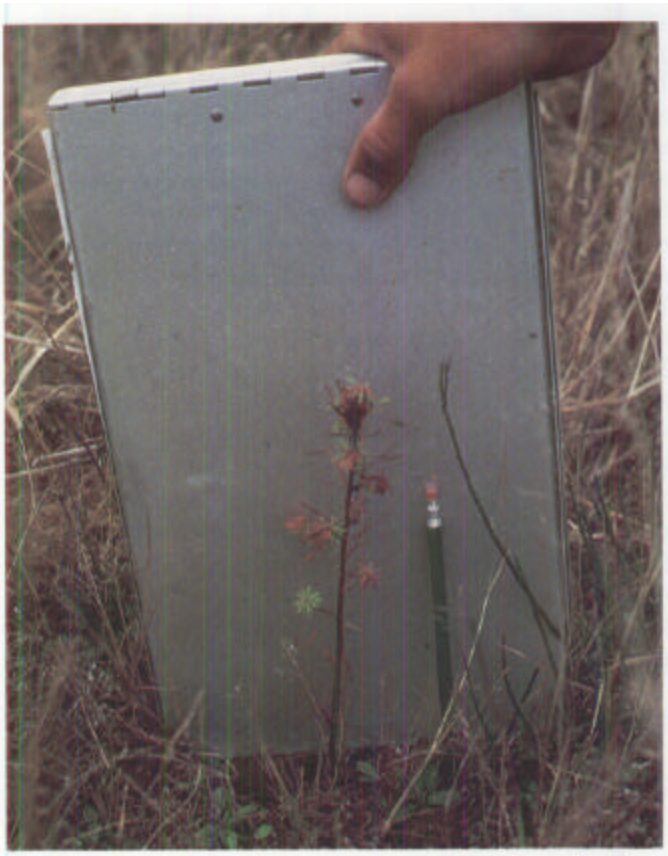

Figure 5.2.41—*Mortality of noninoculated Douglas-fir container seedling outplanted on a stressful site in southwest Oregon.*

Studies in other regions have shown P. tinctorius to be of no benefit (Alvarez and Trappe 1983a, Grossnickle and Reid 1982, Pilz and Znerold 1986, Ruehle 1983). **Clearly no one fungus will work well in all situations.**

Other fungi have also increased field performance of various conifer seedlings, including *Cenococcum geophilum* (Kropp et al. 1985, Riffle and Tinus 1982), *Laccaria laccata* (Thomas and Jackson 1983), *Suillus bovinus* (Ekwebelam and Odeyinde 1985), *Suillus luteus* (Ekwebelam and Odeyinde 1985), *Rhizopogon luteolus* (Ekwebelam and Odeyinde 1985), *Rhizopogon roseolus* (Riffle and Tinus 1982), *Rhizopogon vinicolor* (Castellano and Trappe 1985), and *Thelephora terrestris* (Riffle and Tinus 1982, Thomas and Jackson 1983).

Some inoculated fungi do not persist on seedling roots after outplanting and thus do not impart any advantage as originally designed. For example, in some habitats, Pisolithus tinctorius ectomycorrhizae are aggressively displaced from the feeder roots of inoculated seedlings by native mycorrhizal fungi after outplanting (Dixon et al. 1981, Dixon et al. 1984b, Hung and Trappe 1987, McAfee and Fortin 1986, Ruehle 1983). In the Pacific Northwest, researchers commonly observe the displacement of Pisolithus tinctorius and other inoculated fungi *(Thelephora terrestris, Laccaria laccata*, and *Hebeloma crustuliniforme*) after outplanting (fig. 5.2.42), most commonly by a *Rhizopogon*-type (Bledsoe et al. 1982, Castellano and Trappe 1987). However, some fungi have been shown to persist for several years on inoculated seedlings (Castellano and Trappe 1985, Danielson 1985). Persistence of the inoculated mycorrhizal fungus is an important criterion when selecting mycorrhizal fungi for inoculation.

Figure 5.2.42—*Replacement of* Thelephora *sp. ectomycorrhizae by* Rhizopogon vinicolor *on Douglas-fir container seedling.*

5.2.6 Sources of Inoculum and Inoculation Techniques

Soil, spores, and vegetative mycelium are the three primary sources of ectomycorrhizal and VA mycorrhizal inoculum for container seedlings. Each has advantages and disadvantages in relation to the objectives and economics of the inoculation program. We will discuss ectomycorrhizal fungus inoculum first and then VA mycorrhizal fungus inoculum.

5.2.6.1 Soil inoculum

Historically, soil inocula taken from beneath ectomycorrhizal host trees have been used extensively, especially in developing countries (Mikola 1970). In bareroot nurseries, up to 10% by volume of soil inoculum is incorporated into the soil (top 10 cm of beds) before sowing (fig. 5.2.43). Goodwin (1976) used 2 ounces of screened pine straw as inoculum for loblolly pine container seedlings and found a significant increase in height growth after 3 years in North Carolina. Parke et al. (1983b) reported enhanced growth of Douglas-fir container seedlings inoculated with litter and humus taken from beneath Douglas-fir trees. This method requires large quantities of soil on an annual basis. One of the most serious disadvantages of soil inoculum is that weed seeds, rhizomes, and potential pathogens may also be inadvertently transported into the nursery with the soil. Another disadvantage is the inconsistency of the inoculum quality due to varying times and sources of soil collection. We do not recommend this method unless other forms of inoculum are not available.

Figure 5.2.43—*Incorporation of forest soil into the soil at a bareroot nursery.*

5.2.6.2 Spore inoculum

Spores or macerated fruiting bodies of some ectomycorrhizal mushrooms, puffballs, or truffles (and false truffles) provide good inoculum. Truffles (Ascomycotina) and false truffles (Basidiomycotina), from now on together referred to as truffles, are uniquely suited for this because their fruiting body tissue consists mostly of spore-bearing tissue (fig. 5.2.44-5.2.46), and the fruiting bodies can be quite large (fig. 5.2.47).

To prepare the spore inoculum, freshly collected fruiting bodies are rinsed with tap water to remove adhering soil or organic matter, then cut into pieces (1 to 3 cm^3) and blended with tap water at high speed for 2 to 3 minutes, until all pieces are thoroughly blended (fig. 5.2.48 and 5.2.49). The final consistency is similar to thick chocolate milk (fig. 5.2.50). We have found it unnecessary to purify spore suspensions. Li and Castellano (1987) and Li (1987) have found beneficial microorganisms within and on the surface of mature fruiting bodies of various ectomycorrhizal fungi; these organisms should be encouraged, not excluded (Garbaye and Bowen 1987, Linderman 1988, Schroth and Weinhold 1986).

Spore concentrations within the resulting suspension are determined with a hemacytometer (blood cell counter) and stored in the dark under refrigeration (up to 5 °C or 41 °F) until used. We recommend using fresh spores whenever possible, but have stored spore suspensions of various *Rhizopogon* species up to 3 years without a significant reduction in inoculum effectiveness (Castellano 1987).

Figure 5.2.44—*Cross-sections of fruiting bodies of* Rhizopogon ochraceisporus.

Figure 5.2.45—Rhizopogon truncatus *fruiting bodies associated with rotten wood (courtesy of H. Saylor, Hayward, CA).*

Figure 5.2.46—*Cross-section of* Chamonixia caespitosa *fruiting body.*

Figure 5.2.47—*Examples of large* Tuber gibbosum *fruiting bodies.*

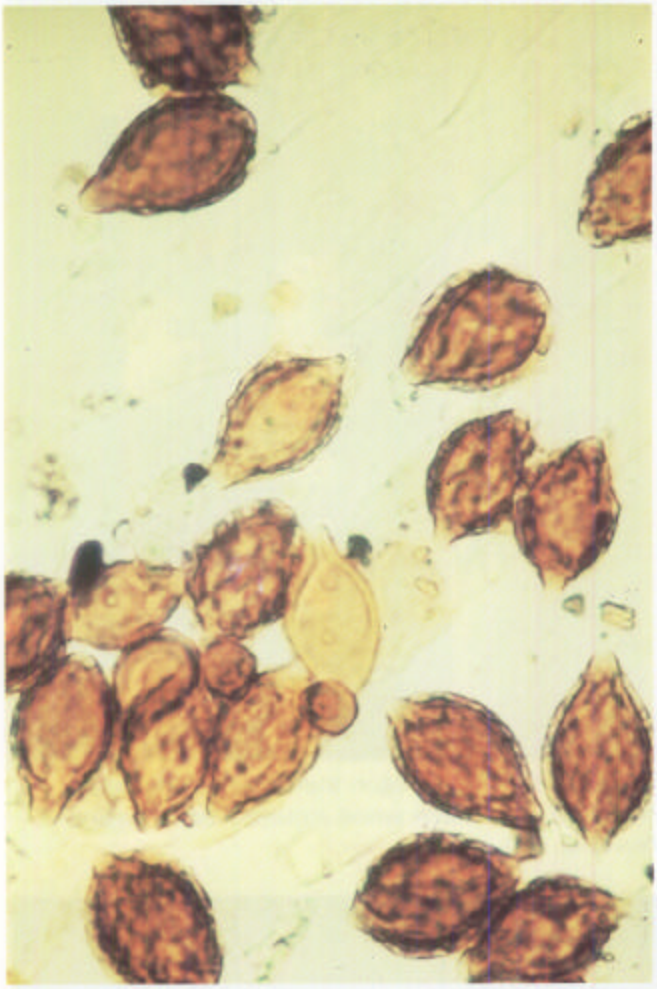

Figure 5.2.48—Hymenogaster *sp. spores floating free within the spore suspension.*

Figure 5.2.49—*Spores and asci of* Tuber gibbosum *in spore suspension.*

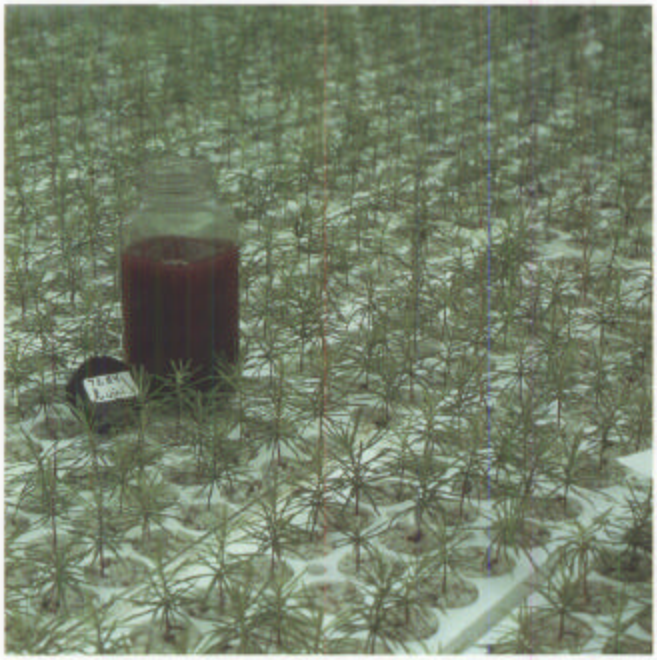

Figure 5.2.50—Rhizopogon vinicolor *spore suspension ready for dilution.*

Spores are applied 6 to 12 weeks after sowing, either with a standard watering can (fig. 5.2.51) or through the existing irrigation system (fig. 5.2.52). Most truffle spores are less than 50 µm in diameter and will pass freely through most filters and nozzle tips. The desired amount of spores is mixed into a watering can containing sufficient water to cover a certain number or area of seedlings (Styroblocks® or racks of plastic tubes). Applying spores twice, 2 to 3 weeks apart, works best to assure even distribution (fig. 5.2.53), especially when using the irrigation system instead of watering cans.

Alternatively, spores can be applied to the seed before sowing (Marx and Bell 1985, Marx et al. 1984, Theodorou 1984, Theodorou and Benson 1983, Theodorou and Bowen 1973). Although we have not tried this method, it may prove more effective than the watering can method in inoculating each seedling. Seed treatment would also allow finer control in matching ecotypes of fungi to specific seed sources.

Figure 5.2.51—*Douglas-fir container seedlings being inoculated 8 weeks after germination with a spore suspension of* Rhizopogon vinicolor *with a watering can.*

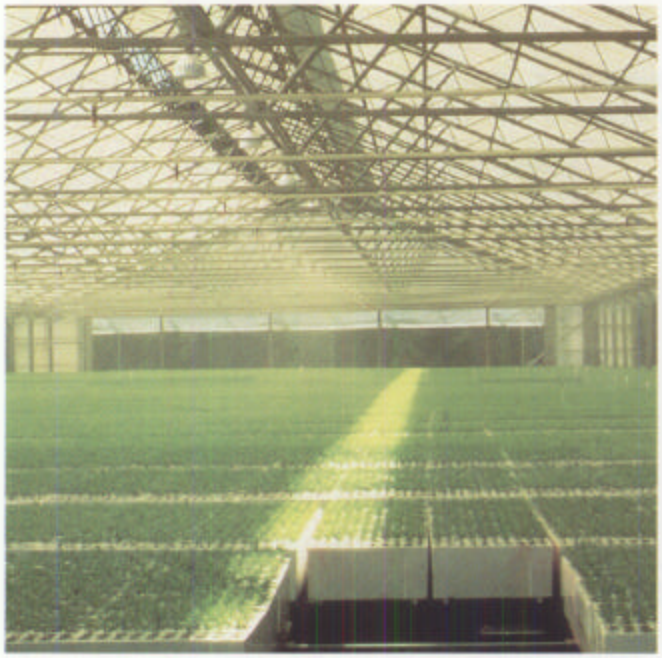

Figure 5.2.52—*Douglas-fir container seedlings being inoculated 8 weeks after germination with a spore suspension of* Rhizopogon vinicolor *with the overhead irrigation system.*

Figure 5.2.53—*Four Douglas-fir seedlings (inoculated with* Rhizopogon vinicolor *spores) taken from the same Styroblock®.*

Table 5.2.5—*Container conifer seedlings successfully inoculated with various* Rhizopogon *species in Oregon.*

Conifer host	*Rhizopogon* species
Douglas-fir	*R. parksii* *R. truncatus* *R. vinicolor* *R. villosulus*
ponderosa pine	*R. fuscorubens* *R. subgelatinosus* *R. ochraceorubens* *R. evadens*
Engelmann spruce	*R. subgelatinosus*

Source: Castellano (1987).

In our recent operational trials, Castellano (1987) has successfully inoculated seven million Douglas-fir container seedlings each of the last 2 years by adding a spore suspension of *Rhizopogon vinicolor* into the fertilizer injector system. Using the overhead irrigation system, a known quantity of spores was applied to blocks of 250,000 8-week-old seedlings in 5 minutes or less. The treatment consisted of a 1-minute prewetting of the growing media, a 2-minute spore application, and an additional 2-minute wetting of the growing media to leach the spores downward into each cavity. The additional 2-minute wetting period also serves to rinse the water lines in case other fungal isolates or species are to be used for different stock.

We have tested many different fungal species using the spore suspension method for inoculation; species in the genus *Rhizopogon* succeed the best (table 5.2.5). For (Douglas-fir we have focused on *Rhizopogon vinicolor*, which is host-specific to Douglas-fir and has been successfully inoculated as basidiospores onto seedlings grown in both bareroot and container nurseries (Parke et al. 1983b, Castellano and Trappe 1985, Castellano et al. 1985). This fungus-host combination produces mycorrhizae with prolific rhizomorphs that likely function in water transport (Duddridge et al. 1980). Parke et al. (1983a) demonstrated that Douglas-fir seedlings inoculated with *R. vinicolor* withstood and recovered from drought better than noninoculated seedlings or those inoculated with Pisolithus tinctorius, Laccaria laccata, or an unidentified native forest fungus. Most importantly, Douglas-fir seedlings inoculated with *R. vinicolor* survived and grew significantly better than noninoculated nursery run seedlings (with abundant *Thelephora* ectomycorrhizae) on routine sites (Castellano and Trappe 1985) and difficult reforestation sites (Castellano unpublished data) in southwestern Oregon.

In the southern hemisphere, spores of another *Rhizopogon* species, *R. luteolus*, have been successfully used to inoculate and stimulate growth of pines in Australia (Theodorou 1971; Theodorou and Bowen 1970, 1973) and South Africa (Donald 1975).

Marx (1976, 1980) and others (Ruehle 1980b) have had similar success with inoculating *Pisolithus tinctorius* onto assorted pine species in the southeastern United States. *Pisolithus tinctorius* has stimulated the growth of oak and pine seedlings both in the nursery and upon outplanting, particularly on mine tailing or highly eroded sites. Although *P. tinctorius* occurs in limited habitats in the Pacific Northwest, it has not performed well in nursery inoculations or outplanting trials (Alvarez and Trappe 1983a, 1983b; Castellano and Trappe unpublished data).

Spore suspensions of various fungi are available for commercial distribution, especially in the Pacific Northwest, from Forest Mycorrhizal Applications (PO Box 385, Murphy, OR 97533).* They recently began collecting and distributing spore suspensions of various species of *Rhizopogon, Suillus,* and other ectomycorrhizal fungi.

As with vegetative inoculum, not all fungi can be inoculated effectively with this method. This inoculum is not free of other organisms, but, in the 7 years of our experience with this type of inoculum, we have never encountered any harmful effects to the seedlings we have treated. The fruiting bodies used for preparing the suspension are only seasonally available and, unlike vegetative inoculum, the genetic make-up of the spores will vary from year to year and place to place.

5.2.6.3 Mycelial inoculum

Over the past few years, much research has concentrated on the production and utilization of pure culture inoculum of selected ectomycorrhizal fungi. Molina and Palmer (1982) detail isolation and maintenance of ectomycorrhizal pure cultures. Marx and Kenney (1982) elaborate on production of ectomycorrhizal inoculum. Basically, a pure culture of a particular fungus is obtained by isolating fungal material (spore germination or vegetative tissue explant) onto special media (fig. 5.2.54), that is then grown under aseptic conditions to produce inoculum. The bulk inoculum, usually produced in a peat-vermiculite carrier moistened with nutrient solution, is mixed into container growing media prior to filling containers and sowing seed.

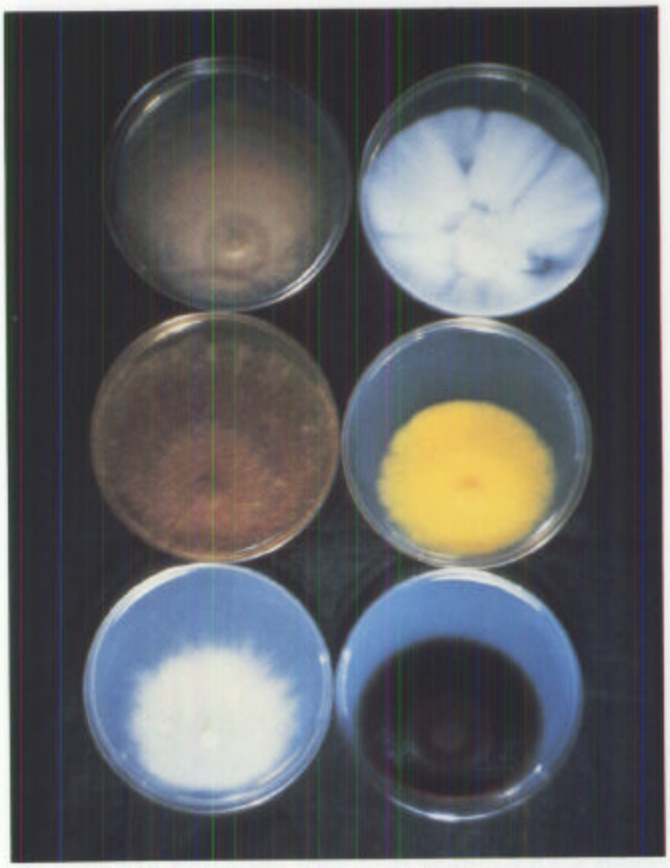

Figure 5.2.54—*Various ectomycorrhizal fungi growing on synthetic medium in petri plates.*

Vegetative inoculum of some fungal species is commercially available from Mycorr Tech Inc. (440 William Pitt Way, Pittsburgh, PA 15238). Their product comes in 7- to 10-liter bags (fig. 5.2.55), is effective (Hung and Molina 1986a, 1986b; Hung and Trappe 1987); and has a reasonable shelf life (Hung and Molina 1986a). Currently, Pisolithus tinctorius, Hebeloma crustuhniforme, and Laccaria laccata are readily available (Maul 1985); other ectomycorrhizal fungi may be produced as demand warrants.

* The sources of mycorrhizal inocula listed in this chapter are currently (1988) the only suppliers of mycorrhizal inocula known to exist. It is not the intention of the U.S. Department of Agriculture, or the Forest Service, to recommend the products of these companies over any others that may be developed in the future.

Figure 5.2.55—*Filling bags with vegetative inoculum in a peat–vermiculite carrier under aseptic conditions (courtesy S. Maul, Mycorr Tech, Pittsburgh, PA).*

In another type of noncommercial mycelial inoculum, fungal sclerotia are embedded in a liquid or gel base Boyle et al. 1985, Boyle et al. 1987, Danielson et al. 1984b, Grenville et al. 1985, LeTacon et al. 1983, Mauperin et al. 1987).

Vegetative inoculation has a higher initial cost and requires more labor than the spore inoculation method. As with spore inoculation, different fungal species also differ in their effectiveness in vegetative inoculation. For Example, *Rhizopogon vinicolor* grows well on artificial media but is not effective in colonizing feeder roots when used as a vegetative inoculum (Molina 1980).

Marx et al. (1982) used *Pisolithus tinctorius* in the first wide-scale application of ectomycorrhizal inoculum in container nurseries. They compared the effectiveness of vegetative inoculum produced in their research laboratory against that of a commercially produced inoculum on 10 pine species, Douglas-fir, western hemlock, and bur oak. Both inoculum sources were effective. Inoculum was most effective after leaching with water to remove excess nutrients. No other inoculum characteristic or treatment significantly affected inoculation success, except that a captan drench after seeding improved the effectiveness of the inoculum.

In the Pacific Northwest, other promising fungi such as *Hebeloma crustuhniforme* and *Laccaria laccata* are easily isolated, grow well in pure culture, and when developed on a peat-vermiculite carrier are effective inocula for Douglas-fir container seedlings. Low levels of vegetative inoculum are effective even under normal operational conditions of abundant water and soluble fertilizer (Hung and Molina 1986b). In addition, vegetative inoculum of both fungi has a shelf life of up to 6 months (Hung and Molina 1986a).

Unfortunately, we have not demonstrated survival or growth enhancement, either in the nursery or in plantations, to justify the cost of vegetative inoculum of either *H. crustuliniforme* or *L. laccata* (Molina 1982, Castellano 1987). Under nursery (Hung and Trappe 1987) and field conditions (Castellano 1987) in the Pacific Northwest, *Laccaria laccata* and to a lesser degree *Hebeloma crustuliniforme* ectomycorrhizae are quickly replaced after transplanting by indigenous fungi (what appears to be a *Rhizopogon*-type).

5.2.6.4 Vesicular-arbuscular inoculum

Two major features of VA mycorrhizal fungi greatly influence both natural and artificial means of seedling inoculation. First, as noted in section 5.2.1.2, VA mycorrhizal fungus spores are not typically wind dispersed like many ectomycorrhizal fungus spores; VA mycorrhizal fungus spores will not blow in from outside the nursery, or from within the nursery, to naturally inoculate seedling. Thus, VA mycorrhizal host plants grown in artificial growing media or sterilized soil will not form mycorrhizae. Second, since VA mycorrhizal fungi cannot be grown in pure culture (that is, in absence of a host), bulk vegetative (mycelial) inoculum, as currently produced for ectomycorrhizal fungi, is not available. Nonetheless, other inoculum sources and techniques are available for VA mycorrhizal fungi and in many ways parallel those used for ectomycorrhizal fungi.

Taking soil from beneath VA mycorrhizal hosts in nature and incorporating it into the container substrate is a simple inoculation method. Parke et al. (1983b) reported enhanced growth of western redcedar container seedlings inoculated with litter and humus taken from beneath Douglas-fir trees. However, as for ectomycorrhizal inoculation, we discourage this technique because of the potential of introducing unwanted pests into the nursery and the large quantity of soil needed.

Although we cannot, as yet, produce pure vegetative cultures of VA mycorrhizal fungi, we can still mass produce fungus inoculum by allowing a known VA fungus to grow in association with a host and then use the soil and roots as inoculum. This procedure is called "pot culturing." In general, spores of a particular VA mycorrhizal fungus are first retrieved from natural soil by various separation techniques (see Ferguson and Woodhead 1982), identified, surface sterilized, and then added into a sterile growing medium in which a host like sorghum or clover is then grown. As the plant grows, it forms VA mycorrhizae with the desired fungus; the fungus then spreads through the growing medium and produces abundant spores. These spores can then be retrieved from the growing medium for use as inoculum (fig. 5.2.56), or, more commonly, the entire growing medium with the mycelium, spores, and roots (chopped) it contains can be used as inoculum.

Figure 5.2.56—*Bulk inoculum of VA mycorrhizal spores (arrows) on inert clay carrier (courtesy of T. Wood, NPI, Salt Lake City, UT).*

VA mycorrhizal fungus pot cultured inoculum is usually added to the growing media in one of two methods (see Menge and Timmer 1982 for additional information). In the first method, the inoculum is mixed evenly throughout the growing media prior to filling the cavities. In the second method, the inoculum is banded about 3 to 5 cm (1 .5 to 2 inches) below the surface of the growing medium. Although the banding method may be labor intensive, it assures rapid contact between the roots and fungus as the roots grow down through the inoculum band. Information is variable as to how much inoculum is needed to ensure successful inoculation, but, from our experience, inoculating with approximately 200 to 500 spores per seedling is a good beginning for testing an inoculum's effectiveness in the nursery. For example Kough et al. (1985) used 20 ml (0.7 ounces) of pot cultured inoculum (spores + soil + chopped roots) to successfully inoculate cedar and redwood seedlings growing in 160-cm^3 containers; the 20 ml of inoculum contained 400 to 770 spores and 30 to 68% of the root pieces were colonized. VA mycorrhizal fungi are sensitive to high levels of fertilizer, as are many ectomycorrhizal fungi. Thus, careful monitoring of mycorrhizal development under various management practices will be needed to develop compatible regimes.

Figure 5.2.57—Mahonia aquifolium *noninoculated and inoculated with VA mycorrhizal fungi from NPI (courtesy of T. Wood, NPI, Salt Lake City, UT).*

Figure 5.2.58—Ilex *sp. noninoculated and inoculated with VA mycorrhizal fungi from NPI (courtesy of T. Wood, NPI, Salt Lake City, UT).*

Pot cultured inoculum provides the best source of VA mycorrhizal fungi for several reasons. If the pot cultured inoculum is grown properly, there is little risk of introducing unwanted pests or pathogens. The inoculum is usually reliable, efficient, and easily introduced into growing media. Most importantly, pot culturing allows the use of selected highly beneficial fungus strains to provide maximum enhancement of seedling growth and survival (fig. 5.2.57 and 5.2.58). Considerable research has been conducted and is currently in progress on selection of beneficial VA mycorrhizal fungi for plant inoculation. Although the majority of this research has been done with agricultural crops, information is also available for VA mycorrhizal forest tree species (see Brown et al. 1981; Kormanik 1985; Kormanik et al. 1977, 1981, 1982; and Kough et al. 1985).

A commercial source of VA mycorrhizal fungi is now available; others continue to be developed. One promising source of inoculum is being developed and marketed by NPI (417 Wakara Way, Salt Lake City, Utah 84108). They are able to produce inoculum of several VA mycorrhizal fungi (fig. 5.2.59) and are developing methods for bulk production of axenically grown inoculum free of pathogens (Wood 1987). NPI is also involved in site reclamation, so their experience with incorporating microbial inoculants into plant rearing programs will be an added source of consultation for nurseries wanting to begin VA mycorrhizal inoculation programs.

Figure 5.2.59—*Nutri-link, VA mycorrhizal inoculum available from NPI (courtesy of T. Wood, NPI, Salt Lake City, UT).*

As with implementing an ectomycorrhizal inoculation program, nursery managers should have clear objectives for VA mycorrhizal inoculations. VA mycorrhizal inoculation can improve growth in the nursery and reduce fertilizer costs; inoculated stock can also perform better than noninoculated stock, especially when planted into environmentally stressful habitats or where native VA mycorrhizal fungi are lacking (Johnson 1987). Whatever your objectives, working with knowledgeable specialists to aid in selection of VA mycorrhizal fungi, techniques of inoculation, and evaluation of inoculation success is strongly recommended.

5.2.6.5 Isolate selection and ecotypic variation

Tables 5.2.6 (by fungus) and 5.2.7 (by host) list the many different fungus--host combinations that have been successfully inoculated onto container seedlings. The response of the seedling host can vary considerably. Of the 118 successful fungus-host combinations listed, only 105 combinations have growth characteristics reported for comparison. Over one-third of the fungus--host combinations stimulated seedling growth, whereas nearly a fourth reduced seedling growth. Six percent increased **and** decreased growth of the same seedling host in different trials. For the most part, growth of hardwoods (especially oaks) was consistently stimulated by fungal inoculation, whereas growth of pines, spruces, firs, and Douglas-fir seedlings was more often not affected or suppressed rather than stimulated. Growth of larch seedlings was unaffected by inoculation. *Hebeloma crustuliniforme*, and *Laccaria laccata* reduced seedling growth more often than it increased it. *Pisolithus tinctorius* stimulated a majority of the responsive hosts. Although many of these symbionts had little or no effect on container seedling growth in the nursery, some of these symbionts stimulated increased seedling field performance (Thomas and Jackson 1983). The nursery manager with advice from a mycorrhizal specialist can select fungus-host combinations that have promise to meet objectives for a particular host species.

Mycorrhizal fungi constantly compete with other mycorrhizal fungi and microorganisms for living space in the seedling rhizosphere. just as some mycorrhizal fungi can antagonize pathogens, so can some mycorrhizal fungi antagonize other mycorrhizal fungi. In pure culture some *Rhizopogon* species produce chemicals that inhibit such fungi as *Cenococcum geophilum, Hebeloma crustuliniforme, Laccaria laccata, Pisolithus tinctorius*, and *Thelephora terrestris* (Castellano 1987). Understanding competitive interactions between mycorrhizal fungi will allow us to select fungal species or isolates for their ability to dominate root systems upon inoculation and continue to provide selected benefits to the inoculated seedlings when outplanted.

Table 5.2.6—*Successful fungus–host inoculations (by fungus) and effects on growth of container seedlings*

Fungus	Host*	Growth†			Source
		Height	Stem caliper	Weight	
Amanita muscaria	Sitka spruce	0	0	0	Shaw et al. 1982
Astraeus hygrometricus	jack pine	0	nr	0, –	Danielson et al. 1984a
Cenococcum geophilum	tamarack	nr	nr	nr	Zhu & Navratil 1987
	western larch	0	0	0	Molina 1980
	white spruce	0	0	0	Shaw et al. 1982
	Sitka spruce	+	nr	nr	Shaw et al. 1987
	jack pine	+	0	0	Langlois & Fortin 1982
	jack pine	0	nr	0	Danielson et al. 1984a
	lodgepole pine	0	0	0	Molina 1980
	western white pine	0	–	0	Kidd et al. 1983
	ponderosa pine	0	0	–	Molina 1980
	Taiwan red pine	0	–	–	Hung 1983
	Douglas-fir	+	0	0	Molina 1980
	Douglas-fir	0	0	0	Graham & Linderman 1981
	English oak	+	+	+	Dixon et al. 1984a
	northern red oak	0	0	+	Marx 1979b
	western hemlock	0	nr	nr	Kropp 1981
	western hemlock	0	0	–	Molina 1980
Endogone lactiflua	Monterey pine	+	nr	nr	Chu–Chou 1985
Hebeloma crustuliniforme	white spruce	0	0	0	Shaw et al. 1982
	Sitka spruce	0	–	–	Shaw et al. 1982
	Sitka spruce	–	nr	nr	Shaw et al. 1987
	jack pine	0	–	–	Langlois & Fortin 1982
	western white pine	0	0, –	0	Kidd et al. 1983
	Monterey pine	+	nr	nr	Chu–Chou 1985
	Taiwan red pine	–	–	–	Hung 1983
H. cylindrosporum	black spruce	nr	nr	0	Gagnon et al. 1987
H. sinapizans	maritime pine	nr	nr	nr	Branzanti & Zambonelli 1987
Hydnangium carneum	river redgum eucalyptus	nr	nr	0	Malajczuk & Hartney 1986
Laccaria bicolor	black spruce	nr	nr	0	Gagnon et al. 1987

* Host species are listed alphabetically by their generic and specific epithets.
† Weight refers to dry and/or fresh weight of shoot and/or root, nr = data not reported, 0 = not significantly different compared to control, – = significantly decreased compared to the control, + = significantly increased compared to control.

Table 5.2.6 (continued)—*Successful fungus–host inoculations (by fungus) and effects on growth of container seedlings*

Fungus	Host*	Growth†			Source
		Height	Stem caliper	Weight	
L. laccata	river redgum eucalyptus	nr	nr	0	Malajczuk & Hartney 1986
	tamarack	nr	nr	nr	Zhu & Navratil 1987
	western larch	0	0	0	Molina 1980
	Sitka spruce	–	–	0, –	Shaw et al. 1982
	Sitka spruce	0, +	nr	–, +	Thomas & Jackson 1983
	jack pine	0	0	0	Langlois & Fortin 1982
	lodgepole pine	0	–	–	Molina 1980
	western white pine	0	0, –	0	Kidd et al. 1983
	maritime pine	nr	nr	nr	Branzanti & Zambonelli 1987
	ponderosa pine	0	–	–	Molina 1980
	ponderosa pine	0	0	0	Hung & Molina 1986b
	Monterey pine	+	nr	nr	Chu–Chou 1985
	Taiwan red pine	–	–	–	Hung 1983
	Douglas-fir	0, –	0, –	–	Molina 1982
	western hemlock	0	0	–	Molina 1980
L. proxima	jack pine	nr	nr	0	Danielson et al. 1984a
	jack pine	0	nr	0	Danielson et al. 1984a
L. paradoxus	jack pine	0	nr	0	Danielson et al. 1984a
Paxillus involutus	maritime pine	nr	nr	nr	Branzanti & Zambonelli 1987
Pezizella ericae	Chapman rhododendron	0	0	0	Barnes & Johnson 1986
Pisolithus tinctorius	European alder	+	+	+	Walker et al. 1982
	yellow birch	nr	nr	nr	Maronek & Hendrix 1980
	sweet birch	+	+	+	Walker et al. 1982
	pecan	nr	nr	+	Sharpe & Marx 1986
	Atlas cedar	0	0	0	Ruehle et al. 1981a
	river redgum eucalyptus	nr	nr	0	Malajczuk & Hartney 1986
	tamarack	nr	nr	nr	Zhu & Navratil 1987
	Norway spruce	+	0	nr	Maronek & Hendrix 1980
	Engelmann spruce	+	+	+	France and Cline 1987
	jack pine	+	0, +	0, +	Navratil et al. 1981
	jack pine	+	0, +	0, +	Navratil et al. 1981
	jack pine	0	nr	0	Danielson et al. 1984a
	jack pine	0	0	–	Langlois & Fortin 1982
	Caribbean pine	0, –	0, +	0, –	Marx et al. 1984
	Caribbean pine	0, –	nr	nr	Ivory & Munga 1983
	sand pine	0, +	nr	0, +	Marx et al. 1982
	lodgepole pine	0, +	nr	0, +	Cline & Reid 1982
	lodgepole pine	0	0	0	France and Cline 1987
	shortleaf pine	+, –	+	+, –	Barnett 1982

*Host species are listed alphabetically by generic and specific epithets.
†Weight refers to dry and/or fresh weight of shoot and/or root, nr = data not reported, 0 = not significantly different compared to control, – = significantly decreased compared to control, + = significantly increased compared to control.

Table 5.2.6 (continued)—*Successful fungus–host inoculations (by fungus) and effects on growth of container seedlings*

Fungus	Host*	Growth†			Source
		Height	Stem caliper	Weight	
	shortleaf pine	0	0	0,+	Marx et al. 1984
	slash pine	0,–	0	0,–	Marx et al. 1984
	limber pine	0	0	0	France and Cline 1987
	Aleppo pine	0	0	+	Ruehle et al. 1981a
	western white pine	0	–	0,+	Kidd et al. 1983
	Austrian pine	0	–	nr	Maronek & Hendrix 1980
	oocarpa pine	0	0	0	Marx et al. 1984
	longleaf pine	0	0,–	+,–	Barnett 1982
	maritime pine	0	0	0	Ruehle et al. 1981a
	ponderosa pine	+	+	nr	Landis & Gillman 1976
	ponderosa pine	0	0	0	Riffle & Tinus 1982
	eastern white pine	nr	nr	nr	Ruehle 1985b
	Scotch pine	0	0	0	Riffle & Tinus 1982
	loblolly pine	0	0	0	Ruehle & Marx 1977
	Taiwan red pine	0,–	–	0,–	Hung 1983
	Virginia pine	0	0	0	Marx et al. 1984
	Populus sp.	0,+	nr	0,+	Navratil & Rochon 1981
	Douglas-fir	0	0	0	Molina 1979
	Douglas-fir	+	+	+	France and Cline 1987
	sawtooth oak	nr	nr	nr	Beckjord et al. 1986
	white oak	0,+	+	0,+	Dixon et al. 1984a
	English oak	+	+	+	Dixon et al. 1984a
	northern red oak	0	0	+	Marx 1979b
	black oak	+	+	+	Dixon et al. 1984a
Pisolithus tinctorius	black oak	0,–,+	0,–	0,–	Baser et al. 1987
	bur oak	0,+	0	0,+	Marx et al. 1982
	eastern hemlock	0	0	nr	Maronek & Hendrix 1980
	western hemlock	0,+	0,+	0,+	Marx et al. 1982
Rhizopogon colossus	Douglas-fir	0,+	0,+	0,+,–	Castellano et al. 1985
R. luteolus	Caribbean pine	0	nr	nr	Ivory & Munga 1983
	lodgepole pine	0	nr	0	Cline & Reid 1982
	ponderosa pine	0	nr	0	Cline & Reid 1982
	Monterey pine	0,+	nr	nr	Theodorou 1984
R. nigrescens	Caribbean pine	0,–	nr	nr	Ivory & Munga 1983
R. roseolus	ponderosa pine	0	0	0	Riffle & Tinus 1982
R. rubescens	Monterey pine	+	nr	nr	Chu–Chou 1985
R. vinicolor	Douglas-fir	0,–	0,–	0,–	Castellano et al. 1985

*Host species are listed alphabetically by generic and specific epithets.
†Weight refers to dry and/or fresh weight of shoot and/or root, nr = data not reported, 0 = not significantly different compared to control, – = significantly decreased compared to control, + = significantly increased compared to control.

Table 5.2.6 (continued)—*Successful fungus–host inoculations (by fungus) and effects on growth of container seedlings*

Fungus	Host*	Growth†			Source
		Height	Stem caliper	Weight	
Scleroderma auranteum	northern red oak	nr	nr	nr	Beckjord et al. 1985
S. bovista	Caribbean pine	0	nr	nr	Ivory & Munga 1983
S. citrinum	sawtooth oak	nr	nr	nr	Beckjord et al. 1986
S. verrucosum	river redgum eucalyptus	nr	nr	0	Malajczuk & Hartney 1986
S. paradoxum	river redgum eucalyptus	nr	nr	0	Malajczuk & Hartney 1986
S. texense	Caribbean pine	0, –	nr	nr	Ivory & Munga 1983
Sphaerosporella brunnea	jack pine	nr	nr	0, –	Danielson 1984
	jack pine	0	nr	–	Danielson et al. 1984b
Suillus granulatus	lodgepole pine	0	nr	0	Cline & Reid 1982
	maritime pine	nr	nr	nr	Branzanti & Zambonelli 1987
	ponderosa pine	0	nr	0	Cline & Reid 1982
	ponderosa pine	0	0	0	Riffle & Tinus 1982
	Scotch pine	0	0	0	Riffle & Tinus 1982
	white oak	0	+	0	Dixon et al. 1984a
	English oak	+	+	0	Dixon et al. 1984a
	black oak	+	+	+	Dixon et al. 1984a
S. luteus	white oak	0	+	0	Dixon et al. 1984a
	English oak	+	+	+	Dixon et al. 1984a
	black oak	+	+	+	Dixon et al. 1984a
S. tomentosus	jack pine	+	0	0	Langlois & Fortin 1982
Thelephora terrestris	bearberry	nr	nr	nr	Linderman & Call 1977
	Sitka spruce	0	nr	0	Shaw et al. 1982
	Sitka spruce	0, +	nr	–, +	Thomas & Jackson 1983
	jack pine	0	–	–	Langlois & Fortin 1982
	jack pine	nr	nr	0	Danielson et al. 1984a
	Caribbean pine	0	nr	nr	Ivory & Munga 1983
	ponderosa pine	0	0	0	Riffle & Tinus 1982
	Scotch pine	0	0	0	Riffle & Tinus 1982
	white oak	0	+	0	Dixon et al. 1984a
	English oak	+	+	0, +	Dixon et al. 1984a
	black oak	+	+	0, +	Dixon et al. 1984a
Tuber sp.	Monterey pine	+	nr	nr	Chu-Chou 1985

* Host species are listed alphabetically by generic and specific epithets.
† Weight refers to dry and/or fresh weight of shoot and/or root, nr = data not reported, 0 = not significantly different compared to control, – = significantly decreased compared to control, + = significantly increased compared to control.

Table 5.2.7—*Successful fungus–host inoculations (by host) and effects on growth of container seedlings*

Host	Fungus	Growth*			Source
		Height	Stem caliper	Weight	
Alnus					
European alder	*Pisolithus tinctorius*	+	+	+	Walker et al. 1982
Arctostaphylos					
bearberry	*Thelephora terrestris*	nr	nr	nr	Linderman & Call 1977
Betula					
yellow birch	*Pisolithus tinctorius*	nr	nr	nr	Maronek & Hendrix 1980
sweet birch	*Pisolithus tinctorius*	+	+	+	Walker et al. 1982
Carya					
pecan	*Pisolithus tinctorius*	nr	nr	+	Sharpe & Marx 1986
Cedrus					
Atlas cedar	*Pisolithus tinctorius*	0	0	0	Ruehle et al. 1981a
Eucalyptus					
river redgum eucalyptus	*Hydnangium carneum*	nr	nr	0	Malajczuk & Hartney 1986
	Laccaria laccata	nr	nr	0	Malajczuk & Hartney 1986
	Pisolithus tinctorius	nr	nr	0	Malajczuk & Hartney 1986
	Scleroderma verrucosum	nr	nr	0	Malajczuk & Hartney 1986
	S. paradoxum	nr	nr	0	Malajczuk & Hartney 1986
Larix					
tamarack	*Cenococcum geophilum*	nr	nr	nr	Zhu & Navratil 1987
	Laccaria laccata	nr	nr	nr	Zhu & Navratil 1987
	Pisolithus tinctorius	nr	nr	nr	Zhu & Navratil 1987
western larch	*Cenococcum geophilum*	0	0	0	Molina 1980
	Laccaria laccata	0	0	0	Molina 1980
Picea					
Norway spruce	*Pisolithus tinctorius*	+	0	nr	Maronek & Hendrix 1980
Engelmann spruce	*Pisolithus tinctorius*	+	+	+	France and Cline 1987
white spruce	*Cenococcum geophilum*	0	0	0	Shaw et al. 1982
	Hebeloma crustuliniforme	0	0	0	Shaw et al. 1982
black spruce	*H. cylindrosporum*	nr	nr	0	Gagnon et al. 1987
	Laccaria bicolor	nr	nr	0	Gagnon et al. 1987
Sitka spruce	*Amanita muscaria*	0	0	0	Shaw et al. 1982
	Cenococcum geophilum	+	nr	nr	Shaw et al. 1987
	Hebeloma crustuliniforme	0	–	–	Shaw et al. 1982
	H. crustuliniforme	–	nr	nr	Shaw et al. 1987
	Laccaria laccata	–	–	0, –	Shaw et al. 1982
	Thelephora terrestris	0	nr	0	Shaw et al. 1982

*Weight refers to dry and/or fresh weight of shoot and/or root, nr = data not reported, 0 = not significantly different compared to control, – = significantly decreased compared to control, + = significantly increased compared to control.

Table 5.2.7 (continued)—*Successful fungus–host inoculations (by host) and effects on growth of container seedlings*

Host	Fungus	Growth*			Source
		Height	Stem caliper	Weight	
Pinus					
jack pine	*Astraeus hygrometricus*	0	nr	0, –	Danielson et al. 1984a
	Cenococcum geophilum	+	0	0	Langlois & Fortin 1982
	C. geophilum	0	nr	0	Danielson et al. 1984a
	Hebeloma crustuliniforme	0	–	–	Langlois & Fortin 1982
	Laccaria laccata	0	0	0	Langlois & Fortin 1982
	L. proxima	nr	nr	0	Danielson et al. 1984a
	L. proxima	0	nr	0	Danielson et al. 1984a
	Lactarius paradoxus	0	nr	0	Danielson et al. 1984a
	Pisolithus tinctorius	+	0, +	0, +	Navratil et al. 1981
	P. tinctorius	+	0, +	0, +	Navratil et al. 1981
	P. tinctorius	0	nr	0	Danielson et al. 1984a
	P. tinctorius	0	0	–	Langlois & Fortin 1982
	Sphaerosporella brunnea	nr	nr	0, –	Danielson 1984
	S. brunnea	0	nr	–	Danielson et al. 1984b
	Suillus tomentosus	+	0	0	Langlois & Fortin 1982
	Thelephora terrestris	0	–	–	Langlois & Fortin 1982
	T. terrestris	nr	nr	0	Danielson et al. 1984a
Caribbean pine	*Pisolithus tinctorius*	0, –	0, +	0	Marx et al. 1984
	P. tinctorius	0, –	nr	nr	Ivory & Munga 1983
	Rhizopogon luteolus	0	nr	nr	Ivory & Munga 1983
	R. nigrescens	0, –	nr	nr	Ivory & Munga 1983
	Scleroderma bovista	0	nr	nr	Ivory & Manga 1983
	S. texense	0, –	nr	nr	Ivory & Munga 1983
	Thelephora terrestris	0	nr	nr	Ivory & Munga 1983
sand pine	*Pisolithus tinctorius*	0, +	nr	0, +	Marx et al. 1982
lodgepole pine	*Cenococcum geophilum*	0	0	0	Molina 1980
	Laccaria laccata	0	–	–	Molina 1980
	Pisolithus tinctorius	0, +	nr	0, +	Cline & Reid 1982
	P. tinctorius	0	0	0	France and Cline 1987
	Rhizopogon luteolus	0	nr	0	Cline & Reid 1982
	Suillus granulatus	0	nr	0	Cline & Reid 1982
shortleaf pine	*Pisolithus tinctorius*	+, –	+	+, –	Barnett 1982
	P. tinctorius	0	0	0, +	Marx et al. 1984
slash pine	*P. tinctorius*	0, –	0	0, –	Marx et al. 1984
limber pine	*P. tinctorius*	0	0	0	France and Cline 1987
Aleppo pine	*P. tinctorius*	0	0	+	Ruehle et al. 1981a
western white pine	*Cenococcum geophilum*	0	–	0	Kidd et al. 1983
	Hebeloma crustuliniforme	0	0, –	0	Kidd et al. 1983
	Laccaria laccata	0	0, –	0	Kidd et al. 1983
	Pisolithus tinctorius	0	–	0, +	Kidd et al. 1983
Austrian pine	*P. tinctorius*	0	–	nr	Maronek & Hendrix 1980
oocarpa pine	*P. tinctorius*	0	0	0	Marx et al. 1984

*Weight refers to dry and/or fresh weight of shoot and/or root, nr = data not reported, 0 = not significantly different compared to control, – = significantly decreased compared to control, + = significantly increased compared to control.

Table 5.2.7 (continued)—*Successful fungus–host inoculations (by host) and effects on growth of container seedlings*

Host	Fungus	Growth*			Source
		Height	Stem caliper	Weight	
longleaf pine	*P. tinctorius*	0	0, –	+, –	Barnett 1982
maritime pine	*Hebeloma sinapizans*	nr	nr	nr	Branzanti & Zambonelli 1987
	Laccaria laccata	nr	nr	nr	Branzanti & Zambonelli 1987
	Paxillus involutus	nr	nr	nr	Branzanti & Zambonelli 1987
	Pisolithus tinctorius	0	0	0	Ruehle et al. 1981a
	Suillus granulatus	nr	nr	nr	Branzanti & Zambonelli 1987
ponderosa pine	*Cenococcum geophilum*	0	0	–	Molina 1980
	Laccaria laccata	0	–	–	Molina 1980
	L. laccata	0	0	0	Hung & Molina 1986b
	Pisolithus tinctorius	+	+	nr	Landis & Gillman 1976
	P. tinctorius	0	0	0	Riffle & Tinus 1982
	Rhizopogon luteolus	0	nr	0	Cline & Reid 1982
	R. roseolus	0	0	0	Riffle & Tinus 1982
	Suillus granulatus	0	nr	0	Cline & Reid 1982
	S. granulatus	0	0	0	Riffle & Tinus 1982
	Thelephora terrestris	0	0	0	Riffle & Tinus 1982
Monterey pine	*Endogone lactiflua*	+	nr	nr	Chu-Chou 1985
	Hebeloma crustuliniforme	+	nr	nr	Chu-Chou 1985
	Laccaria laccata	+	nr	nr	Chu-Chou 1985
	Rhizopogon luteolus	0, +	nr	nr	Theodorou 1984
	R. rubescens	+	nr	nr	Chu-Chu 1985
	Tuber sp.	+	nr	nr	Chu-Chou 1985
eastern white pine	*Pisolithus tinctorius*	nr	nr	nr	Ruehle 1985b
Scotch pine	*P. tinctorius*	0	0	0	Riffle & Tinus 1982
	Suillus granulatus	0	0	0	Riffle & Tinus 1982
	Thelephora terrestris	0	0	0	Riffle & Tinus 1982
loblolly pine	*Pisolithus tinctorius*	0	0	0	Ruehle & Marx 1977
Taiwan red pine	*Cenococcum geophilum*	0	–	–	Hung 1983
	Hebeloma crustuliniforme	–	–	–	Hung 1983
	Laccaria laccata	–	–	–	Hung 1983
	Pisolithus tinctorius	0, –	–	0, –	Hung 1983
Virginia pine	*P. tinctorius*	0	0	0	Marx et al. 1984
Populus sp.	*P. tinctorius*	0, +	nr	0, +	Navratil & Rochon 1981
Pseudotsuga					
Douglas-fir	*Cenococcum geophilum*	+	0	0	Molina 1980
	C. geophilum	0	0	0	Graham & Linderman 1981
	Laccaria laccata	0, –	0, –	–	Molina 1982
	Pisolithus tinctorius	0	0	0	Molina 1979
	P. tinctorius	+	+	+	France and Cline 1987
	Rhizopogon colossus	0, +	0, +	0, +, –	Castellano et al. 1985
	R. vinicolor	0, –	0, –	0, –	Castellano et al. 1985

*Weight refers to dry and/or fresh weight of shoot and/or root, nr = data not reported, 0 = not significantly different compared to control, – = significantly decreased compared to control, + = significantly increased compared to control.

Table 5.2.7 (continued)—*Successful fungus–host inoculations (by host) and effects on growth of container seedlings*

Host	Fungus	Growth*			Source
		Height	Stem caliper	Weight	
Quercus					
sawtooth oak	*Pisolithus tinctorius*	nr	nr	nr	Beckjord et al. 1986
	Scleroderma citrinum	nr	nr	nr	Beckjord et al. 1986
white oak	*Pisolithus tinctorius*	0, +	+	0, +	Dixon et al. 1984a
	Suillus granulatus	0	+	0	Dixon et al. 1984a
	S. luteus	0	+	0	Dixon et al. 1984a
	Thelephora terrestris	0	+	0	Dixon et al. 1984a
bur oak	*Pisolithus tinctorius*	0, +	0	0, +	Marx et al. 1982
English oak	*Cenococcum geophilum*	+	+	+	Dixon et al. 1984a
	C. geophilum	0	0	+	Marx 1979b
	Pisolithus tinctorius	0	0	+	Marx 1979b
	P. tinctorius	+	+	+	Dixon et al. 1984a
	Scleroderma auranteum	nr	nr	nr	Beckjord et al. 1985
	Suillus granulatus	+	+	0	Dixon et al. 1984a
	S. luteus	+	+	+	Dixon et al. 1984a
	Thelephora terrestris	+	+	0, +	Dixon et al. 1984a
black oak	*Pisolithus tinctorius*	+	+	+	Dixon et al. 1984a
	P. tinctorius	0, −, +	0, −	0, −	Baser et al. 1987
	Suillus granulatus	+	+	+	Dixon et al. 1984a
	S. luteus	+	+	+	Dixon et al. 1984a
	Thelephora terrestris	+	+	0, +	Dixon et al. 1984a
Rhododendron					
Chapman rhododendron	*Pezizella ericae*	0	0	0	Barnes & Johnson 1986
Tsuga					
eastern hemlock	*Pisolithus tinctorius*	0	0	nr	Maronek & Hendrix 1980
western hemlock	*Cenococcum geophilum*	0	nr	nr	Kropp 1981
	C. geophilum	0	0	−	Molina 1980
	Laccaria laccata	0	0	−	Molina 1980
	Pisolithus tinctorius	0, +	0, +	0, +	Marx et al. 1982

*Weight refers to dry and/or fresh weight of shoot and/or root, nr = data not reported, 0 = not significantly different compared to control, − = significantly decreased compared to control, + = significantly increased compared to control.

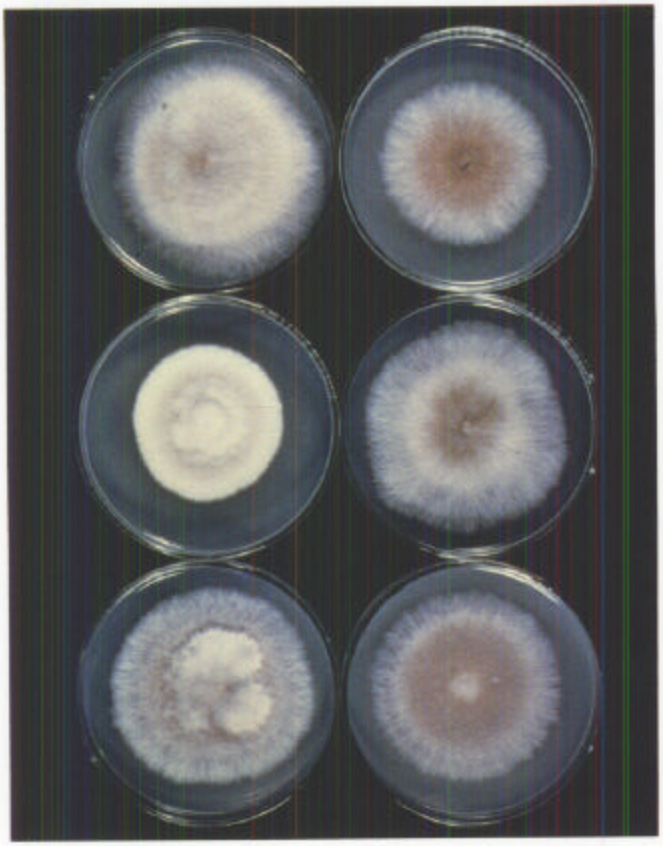

Figure 5.2.60—*Six different isolates of* Rhizopogon vinicolor *showing the diversity of macroscopic morphology within species.*

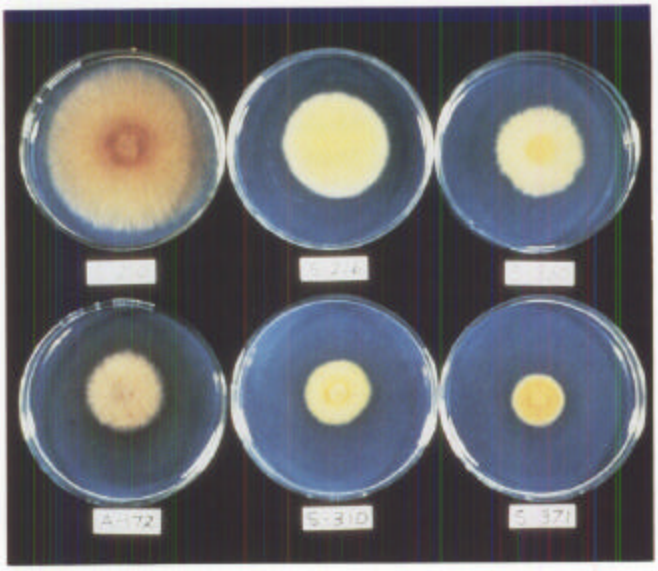

Figure 5.2.61—*Six different isolates of* Pisolithus tinctorius *showing a wider diversity of macroscopic morphology within species than* Rhizopogon vinicolor.

The influence of fungal genetic composition on the ability of a fungal species to form mycorrhizae with hosts from different seed sources has not been studied. Even within a fungal species, isolates from different habitats have different morphological characters (fig. 5.2.60 and 5.2.61). The applicability of inoculating a specific seed source of seedling host with an ecotype of a particular fungus has the potential of matching fungi and seedling host to habitat. Different genotypes of Scotch pine (Lundeberg 1968), lodgepole and ponderosa pine (Cline and Reid 1982), Sitka spruce Walker et al. 1986), European larch (Zhu and Navratil 1987), and Douglas-fir (Wright and Ching 1962) formed significantly differing amounts of ectomycorrhizae when inoculated with the same fungal isolate and grown under common conditions. Growth response of the seedling host can also differ (Cline and Reid 1982, Zhu and Navratil 1987). *Pisolithus tinctorius* (Dixon et al. 1984a, Marx 1981, Molina 1979), *Suillus granulatus* (Dixon et al. 1984a) and *Hebeloma crustuliniforme* (Molina 1987) exhibit the same varying pattern of response (host growth or ectomycorrhizal formation) when the same seed source but different fungal isolates are used for inoculation, but *Laccaria laccata* does not (Molina 1982). The mycorrhizal fungus and tree host have co-evolved to some degree within their geographic cal (ecotypic) realm. Mycorrhizal research programs are currently investigating the importance of matching ecologic adaptations of trees and fungi for wide-scale application.

5.2.7 Evaluating Inoculation Success

Given the diversity in crop species, growing conditions, and management techniques in container nurseries, mycorrhizal inoculations that work well in one nursery may not work well in others. **We urge each nursery to test recommendations for mycorrhizal management in their nurseries on a small scale before trying to inoculate the entire nursery**. A few thousand seedlings are more than enough for first inoculation attempts. Be sure to incorporate some variation from the standard inoculation procedure into test programs, for example, vary spore rates, timing of application, fertilizer levels, and types of fertilizer so that you can learn more about how your management practices interact with inoculation success. Also, try to work with a scientist or statistician who can help develop a simple experimental design to facilitate the analysis of results.

5.2.7.1 Rating mycorrhizal formation

Nursery managers or growers should ideally have some basic training in identification of mycorrhizal types. This experience can be through one-on-one training with an expert or at a workshop. Even after training, nursery managers and growers should send some of their treated seedlings to a recognized expert in the field to corroborate their findings. Helpful hints are supplied in section 5.2.3.

5.2.7.2 Designing outplanting trials

The true test of a nursery inoculation program may not be apparent until after the seedlings are outplanted. Because seedling size is a major factor in outplanting success, measure seedlings before outplanting to assure that you are comparing seedlings of similar size in the field (fig. 5.2.62). In one study (Barnett 1982), differences in initial seedling size were more closely related to field performance than amount of Pisolithus tinctorius ectomycorrhiza. Also realize that rough handling of seedlings during any phase of the planting process can be detrimental to ectomycorrhizae (Tabbush 1986).

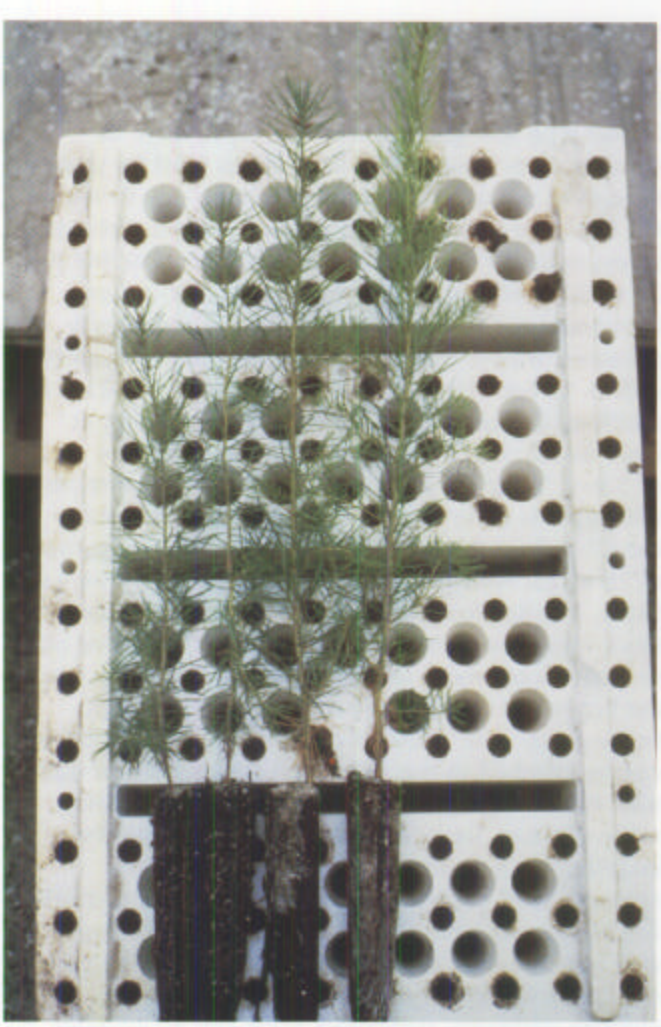

Figure 5.2.62—*Variation in height of Douglas-fir container seedlings correlated with abundance of* Rhizopogon vinicolor *ectomycorrhizae.*

The outplanting trial design should be simple and straightforward. A randomized block design with 3 to 5 blocks spread across a representative area of a specific habitat type will generate enough information to extrapolate to similar sites. The blocks must be small enough to reduce within-block variation due to micro-site but large enough to provide meaningful replication. When in doubt consult with a statistician. We find that 20 to 50 seedlings of each treatment combination (noninoculated or inoculated) per block are usually adequate. Blocks should be separated from one another with 3- to 6-m (10- to 20-foot) buffer strips. Spacing of seedlings can be critical. We have used spacing as close as 1.2 x 1.2 m (4 x 4 feet) but prefer 2.4 x 2.4 or 3.6 x 3.6 m (8 x 8 or 12 x 12 feet) to correlate with what is done operationally. Within the blocks, seedlings from the same treatment are planted in rows of 10 to 25, with row location randomized within each block. It is helpful in subsequent years to have the block corners marked with 1.2-m (4-foot) metal or plastic stakes (not wood, which breaks easily) and to mark the beginning and end of each seedling row with heavy-gauge wire stakes. Treatment codes on metal tags can be attached to the stakes at the beginning of each row. Protecting the seedling is critical (fig 5.2.63): many vigorous inoculated seedlings are lost to deer browsing because they are more palatable than noninoculated control seedlings. Measurements of seedling height and stem caliper at time of outplanting provide baseline data to calculate future annual increment of growth. Measurements are taken anytime during seedling dormancy depending upon site accessibility.

We find that at the beginning of the second year we have a more accurate comparison of inoculation treatments than the first year because first year seedling growth will be influenced by nursery practices (that is, fertilization and watering regimens) of the previous year. Typically, measurements are taken for the first 5 years; evaluation of these data determines if monitoring should continue beyond that. Although not routine, we encourage excavation of seedling root systems to allow observation of the persistence of inoculated fungi on old feeder roots and their growth onto new feeder roots. Five to ten seedlings per inoculation treatment per year is adequate. Techniques for examining mycorrhizae on root systems from the field are similar to those discussed in section 5.2.3.

5.2.7.3 Economic considerations

Cost effectiveness is difficult to generalize because it depends on type of inoculation, individual nursery management costs, and scale of inoculation. Most importantly, these calculations are compounded by the specific inoculation objectives and definition of effectiveness. For example, a nursery trying to increase stern caliper and seedling uniformity or reduce culls will judge effectiveness (and its costs) differently than a nursery whose objective is to improve seedling field performance. The former deals with immediate benefit and costs while the latter deals with an effectiveness concept tied to the future. Nursery managers need to calculate specifics of cost-effectiveness as they develop individual inoculation programs. This is another reason to keep the scale of first inoculations small.

A new company is now preparing spore suspensions of various fungi for commercial distribution, especially in the Pacific Northwest. Forest Mycorrhizal Applications (1032 Starlite, Grants Pass, OR 97526) has recently begun collecting and distributing spore suspensions of various species of *Rhizopogon, Suillus,* and other ectomycorrhizal fungi. The *1988* cost of the inoculum was from *0.25* to *0.95¢* per thousand seedlings; application is additional (see section *5.2.6.2).*

Mycorr Tech Inc. (440 William Pitt Way, Pittsburgh, P~ 15238) is presently supplying commercial vegetative inoculum. In 1988, their product cost approximately $1.00 to 2.00 per thousand seedlings; application is additional. Tests have shown their product to be reliable, reproducible, relatively quickly available, and uncontaminated (see section 5.2.6.3).

Commercial sources of VA mycorrhizal fungi are now available and continue to be developed. NPI (417 Wakara Way, Salt Lake City, Utah 84108) produces inoculum of several VA mycorrhizal fungi. In 1988, their product costs approximately $2.00 to 5.00 per thousand seedlings, depending on inoculation procedure. We have noted that product costs have steadily decreased during the last 2 years (see section 5.2.6.4).

Figure 5.2.63—*Protection of an experimental trial of inoculated Douglas-fir seedlings with plastic tubing on a site in southwest Oregon.*

5.2.8 Factors Affecting Mycorrhizal Development

5.2.8.1 Root development

The major lateral roots of conifers grown in containers typically grow out to the container wall and then downward parallel to the container side for their first 10 to 15 cm. This growth form discourages initiation of secondary laterals; many downward trained roots continue in this fashion after outplanting. On the outplanting site, the upper portion [10 to 15 cm (4 to 6 inches)] of the soil profile usually has high oxygen, moisture, and nutrient availability and thus is conducive to high rates of microbiological activity (Harvey et al. 1987). To insure seedling establishment after outplanting, exploration of the upper soil layers by feeder roots and ectomycorrhizae is desirable.

Nursery techniques to manipulate root form of container seedlings and enhance root growth potential of seedlings after outplanting are relatively new. One involves coating the inside of the container with a root pruning chemical contained within a latex paint. After the paint dries the containers are filled with growing media and sown in normal fashion (Romero et al. 1986). Various concentrations of three different chemicals have been tried. Trifluralin (a herbicide) at all concentrations tested (0.56 to 70.88 g/l of paint) adversely affected ponderosa pine seedlings (McDonald et al. 1981). A 5-g/l concentration of indolebutyric acid (IBA) applied to the container wall increased ponderosa pine seedling growth somewhat, but growth was weak and erratic compared to container wall treatment with 50 g/l concentration of cupric carbonate ($CuCO_3$) (McDonald et al. 1984). Seedlings grown in containers treated with $CuCO_3$ and then transplanted and grown for an additional 5 weeks had 27% of their new roots as side roots, while the untreated seedlings produced only 8%. Seedlings treated with 100 g/l of $CuCO_3$ had significantly higher shoot and root dry weight and larger stem height than seedlings treated with 0.1 g/l, and also had one fourth (3.7 vs. 12.2) as many roots deflected down the container wall (McDonald et al. 1981). Unfortunately, some latex paint carriers can be phytotoxic, with the detrimental effects overcome only at the higher $CuCO_3$ concentrations. Other potential carriers need to be tested.

The effects of $CuCO_3$ and IBA on ectomycorrhizal fungus inoculation have been determined for ponderosa, lodgepole (McDonald et al. 1981), loblolly, longleaf, shortleaf, and eastern white pine (Ruehle 1985a). In all cases, 50 g of $CuCO_3$ /liter of latex paint was used. Treated ponderosa and lodgepole pine seedlings inoculated with Suillus granulatus or Pisolithus tinctorius had somewhat larger stem height and caliper and significantly reduced root deflections compared to nontreated seedlings. $CuCO_3$ treatment of remaining pine species had little effect on seedling growth, except that feeder root formation was usually stimulated. Formation of ectomycorrhizae was either not affected (loblolly and shortleaf pine), stimulated (longleaf pine), or depressed (eastern white pine) (Ruehle 1985a). In a follow-up outplanting trial, P. tinctorius-inoculated loblolly and longleaf pine grown in containers treated with $CuCO_3$ survived and grew better than untreated P. tinctorius-inoculated seedlings on a routine reforestation site in the southeast United States (Ruehle 1987).

Copper sulfide has also been used to prevent root spiraling in Chinese pine seedlings grown in polyethylene-coated kraft paper containers (Dong and Burdett 1986). Unfortunately the effects of the chemical on ectomycorrhizal inoculation were not explored.

Nursery managers may want to try some of these feeder root enhancement techniques on a small scale and carefully monitor the effects on root growth and mycorrhizal development before wide-scale application.

5.2.8.2 Fertilizer

Mycorrhizae and mycorrhizal fungi are extensions of a plant's root system; they extract nutrients and water from soil and translocate them to the host. Plants respond to mycorrhizal formation most strongly in soils of low fertility. It follows that most mycorrhizal fungi are adapted to the infertile conditions of forest soils. Many mycorrhizal fungi do not grow well in artificial growing media that are frequently drenched with high levels of soluble fertilizer or amended with slow-release fertilizer. Inhibition of mycorrhizae by high levels of fertilization plus the lack of mycorrhizal fungus propagules in artificial growing media pose the greatest challenge to mycorrhiza management programs.

Because various species of mycorrhizal fungi respond differently to fertilization, fungi adapted to nursery fertility conditions can be used or fertilizer application can be modified to promote colonization by a desired, but fertilizer-sensitive fungus. For example, high levels of soluble NPK fertilizer reduce ectomycorrhizal formation by *Pisolithus tinctorius* (Crowley et al. 1986, Danielson et al. 1984a, Dixon et al. 1985, Ekwebelam and Reid 1983, Maronek et al. 1981, Maronek et al. 1982, Marx et al. 1982, Pope and Chaney 1984, Ruehle 1980a, Ruehle and Wells 1984, Rupp and Mudge 1985). Reducing fertility levels by half may double ectomycorrhizal colonization for some hosts (see Marx et al. 1982). On the other hand, some fungi such as *Laccaria laccata* and *Rhizopogon vinicolor* are little affected by high levels of soluble fertilizer. Inoculation with these fungi in commercial nurseries has been successful **without** altering the routine fertilization regime (Castellano et al. 1985, Danielson et al. 1984a, Hung and Molina 1986a, Molina and Chamard 1983, Tyminska et al. 1986).

Vesicular-arbuscular mycorrhizal formation of container yellow-poplar (Verkade and Hamilton 1985) and southern magnolia (Maronek and Hendrix 1978) seedlings has been encouraged by certain fertilization regimes.

Fertilizer type can also affect mycorrhiza development. The two common types of fertilizer, soluble and slow-release, have been shown to affect the successful outcome of ectomycorrhizal inoculation (Castellano et al. 1985, Maronek et al. 1982). Castellano et al. (1985) found that the inoculation success of *Rhizopogon vinicolor* spore application on Douglas-fir container seedlings was reduced by slow-release fertilizer but not by soluble fertilizer. As recommended in volume four of this series, we advise against the use of slow-release fertilizer due to the unknown aspect of what the seedlings are actually exposed to by way of fertilizer nutrients.

Although foliar application of NPK is not routinely used in container nurseries, black oak seedlings receiving foliar NPK had significantly greater *Pisolithus tinctorius* ectomycorrhizae and fructose content of feeder roots compared to the soluble NPK drench treatment (Dixon et al. 1981).

Fertilizer form is also important; compared to nitrate-N, ammonium-N is usually better utilized by a variety of mycorrhizal fungi (Bledsoe and Zasoski 1983, Littke et al. 1984, Harley and Smith 1983). Ammonium-N fertilization decreases the pH of the growing media whereas nitrate-N fertilization will increase the pH of the growing media. As we will see later, many ectomycorrhizal fungi prefer acidic growing conditions, so fertilization with nitrate-N will adversely affect inoculation of alkaline-sensitive fungi.

Given variable responses to fertilizers by different mycorrhizal fungi, we cannot recommend specific levels, types, or forms of fertilization to promote mycorrhizal development on container seedlings. Optimum fertilization levels must be determined by each nursery manager, depending on whether the objective is promoting mycorrhizal development of naturally occurring fungi or ensuring inoculation with a particular fungus. Nursery managers should also realize that mycorrhizal fungi may provide seedling growth stimulus equal or similar to high levels of fertilization and thus result in a fertilizer cost saving. If enhancing outplanting performance via mycorrhizal inoculation is a goal, close control over the fertility and how it is applied is essential.

Mycorrhizal management should be considered as part of the overall container tree seedling culture. Be open minded about modifying fertilization levels, application schedules, and fertilizer forms to meet mycorrhizal management objectives. Nursery managers and staff are highly skilled in developing the optimum cultural practices to produce vigorous planting stock; encouraging mycorrhizal development on container stock requires these same skills.

5.2.8.3 Water

Either too much or too little water reduces feeder root formation (Ruark et al. 1982), especially in Douglas-fir and spruce. Many nurseries water their seedlings to growing medium saturation every day (Matthews 1983). One symptom of over-irrigation is the formation of water roots-thick fleshy, opaque nonmycorrhizal roots that lack root-hairs (fig. 5.2.64). These water roots act as giant sponges that readily absorb water and soluble nutrients. They lack the feeder roots needed for mycorrhizal formation (Castellano 1987, Dixon et al. 1985) and are essentially nonfunctional in water and nutrient uptake upon outplanting (Castellano 1987, Dixon et al. 1983). Water roots have been observed to die and decompose soon after outplanting (G. Hunt 1987). These water roots are sometimes seen in extreme situations, usually compacted growing media. Heavy irrigation with good porous growing media will not cause problems. Peat quality is critical: poor peat with a high percentage of "fines" will cause growing media to drain poorly. Also, xylem-girdling insects can cause water roots by restricting water flow to the shoot. From our experience, some inoculation experiments have failed because the fungus did not have the opportunity to form ectomycorrhizae due to excessive water roots. Root dry weight is not a good indication of root quality; a root system with large water roots may have the same dry biomass as one with many small feeder roots.

Seedlings that are somewhat overwatered (but not to the point of having excessively swollen roots) develop many unbranched or poorly branched laterals near the surface of the container walls and at the container bottom. In these seedlings, optimum development of feeder roots and thus mycorrhizae occurs only in the inside portion and near the top of the plug where aeration is best. These seedlings have extremely poor root regeneration potential upon outplanting.

Figure 5.2.64—*Various degrees of water root formation on Douglas-fir container seedlings. Normal root formation at right, abnormal at middle and left. (Courtesy of G. Hunt, Balco, Kamloops, BC.)*

To avoid water roots, and thus encourage good development of feeder roots and ectomycorrhizae, nursery managers must regularly examine root systems and modify watering regimes as appropriate. As emphasized before, this must become a regular practice when assessing root and overall seedling quality throughout the growing season.

5.2.8.4 Growing media

The physical and chemical makeup of the growing media will influence success of mycorrhizal inoculation programs. Pore size and distribution and pH (optima and tolerance) will directly affect not only feeder root formation (Ruark et al. 1982) and distribution (fig. 5.2.65) but also ectomycorrhizal development. Compacted growing media not only inhibit feeder root initiation but also inhibit lateral and feeder root extension. The high percentage of peat moss in most artificial growing media affects their physical and chemical properties, that is, pH. Our field observations infer that some ectomycorrhizal fungi prefer soils with high organic matter contents (for example, decomposed logs, with pH = 4), whereas others grow well in mineral soils with greatly reduced amounts of organic matter (for example, recently burned areas, with pH = 7). Ectomycorrhizal fungi have various pH optima for growth in pure culture as well (Hung and Trappe 1983). Some fungi grow equally well over a relatively wide pH range, whereas others are less tolerant (Hung and Trappe 1983). For example, *Pisolithus tinctorius* formed more ectomycorrhizae at pH 5.5 than at 6.5 when inoculated onto pecan seedlings (Sharpe and Marx 1986).

Growing medium compaction does not seem to eliminate fungal growth, but it greatly reduces formation of feeder roots needed for ectomycorrhizal colonization. The container growing media should provide adequate pore space for oxygen exchange to promote vigorous growth by both roots and fungi. We recommend selecting fungi that grow well over a wide range of growing media pH for nursery inoculation.

Figure 5.2.65—*Poor Douglas-fir feeder root distribution within the container. Abnormal distribution on the left, normal on right. (Courtesy of G. Hunt, Balco, Kamloops, BC.)*

5.2.8.5 Temperature

As with pH, ectomycorrhizal fungi have tolerance ranges and optima for temperature (Hacskaylo et al. **1965, Marx and Bryan** 1971, Marx et al. 1970, Samson and Fortin 1986). Growing medium temperatures in containers can vary widely, from cold [0 °C (32 °F)] in winter or during preplanting storage to hot [38 °C (100 °F)] during summer. Some mycorrhizal fungi will tolerate this wide temperature fluctuation during seedling production, others will not. For example, aseptic loblolly pine seedlings inoculated with *Thelephora terrestris* or *Pisolithus tinctorius* grew well and formed abundant ectomycorrhizae at 25 °C (77 °F). When these same seedlings were transferred to a room with 40 °C (104 °F) soil temperatures, the *T. terrestris*--inoculated seedlings died or declined while P. tinctorius-inoculated seedlings thrived (Marx and Bryan 1971).

Ectomycorrhizal feeder roots also differ in ability to withstand cold. In many nurseries, preplanting cold storage of seedlings is common. *P. tinctorius* ectomycorrhizae survived cold storage on shortleaf pine (Marx 1979a) but not on ponderosa pine (Alvarez and Linderman 1983) or Douglas-fir (Castellano unpublished data). Cold storage of *P. tinctorius* vegetative inoculum decreases its effectiveness, while inoculum of *Laccaria laccata* and *Hebeloma crustuliniforme* formed abundant ectomycorrhizae after cold storage (Hung and Molina 1986a).

Knowledge of the temperature tolerance of various fungi must be used in selecting a fungus for your inoculation program.

5.2.8.6 Pesticides

Pesticides cause a multitude of complex reactions on target and nontarget organisms. Generalizations about reactions to pesticides must be approached with caution. For example, pesticides that affect mycorrhizal fungi or mycorrhiza development can affect seedling growth response either for better or for worse. Trappe et al. (1984) review effects of pesticides on mycorrhizal fungi and mycorrhiza development. Tables 5.2.8 to 5.2.11 are condensed from Trappe et al. (1984) for reference to container nurseries.

Sterilants. Artificial growing media are generally considered to be "essentially sterile" and therefore, sterilants are not normally used in container nurseries. Because of recent problems with root diseases, however, some nursery managers are beginning to sterilize their growing media and containers (table 5.2.8). Methyl bromide--chloropicrin mixes are effective sterilants, and under optimum conditions of application they nearly eliminate both beneficial and pathogenic soil organisms from the treated growing media. Optimum conditions are rare, however, so soil organisms are rarely completely eliminated. Methyl bromide fumigation is used in bareroot seedling mycorrhiza inoculation programs to reduce competition by wild fungi with the inoculated fungus. For the artificial growing media (for example, milled bark) in container nurseries, steam pasteurization serves the same purpose effectively.

Fungicides. Most fungicides are selective for certain groups of fungi (table 5.2.9). The thiazoles (benomyl, carbendazim, and fuberidizole) will suppress Zygomycotina but are less detrimental or even stimulatory (Pawuk and Barnett 1981, Pawuk et al. 1980) to most Basidiomycotina or Ascomycotina. Since VA mycorrhizal fungi are Zygomycotina, special attention needs to be paid to the application of this group of fungicides in nurseries where VA mycorrhizal hosts are grown. The thiazoles would be the fungicides of choice for nurseries growing ectomycorrhizal hosts (Pinaceae). The dithiocarbamates (ferbam, mancozeb, zineb, and ziram) and substituted aromatics tend to inhibit mycorrhizal fungi of both groups. The dicarboximides (captafol and captan) are usually not inhibitory at low application rates (see table 5.2.8) but can be at higher application rates (Pawuk et al. 1980) or can even be stimulatory to both groups of mycorrhizal fungi (Owston et al. 1986).

Table 5.2.8—*Sterilants that decrease ectomycorrhizal development*

Active ingredient	Trade name
allyl alcohol + ethylene dibromide	allyl alcohol + ethylene dibromide
dazomet	Dazomet
formaldehyde	formalin*
metam sodium	Carbam*
sodium azide	Smite
di-trapex	Vorlex

*Sterilants that had no effect at low application rates tended to decrease ectomycorrhizae at higher rates.

Table 5.2.9—*Fungicides that decrease ectomycorrhizal development*

Active ingredient	Trade name
banrot	Banrot
triadimefon	Bayleton
benodanil	Benodanil*
chlorothalonil	Bravo*
captan	Captan*
chloroneb	Chloroneb
etridiazol	Etridiazol
fenaminosulf	Lesan
maneb	Maneb
mancozeb	Mansate
olpisan	Olpisan
quintozene	PCNB
folpet	Phaltan
sulfuric acid	sulfuric acid
thiram	Thiram
zinc white	zinc oxide
zineb	Zineb*
ziram	Ziram

*Fungicides that had no effect at low application rates tended to decrease ectomycorrhizae at higher rates.

The importance of choosing chemicals for pest control carefully is illustrated by programs to control fusiform rust on southern pine seedlings inoculated with *Pisolithus tinctorius*. Ferbam has been used to control fusiform rust in southern forest nurseries, but it requires repeated applications to be effective. Recently, bayleton has proven effective in fusiform rust control and is applied only a few times during the growing season. Although bayleton costs more than ferbam, the fewer applications reduce labor for a significant savings over use of ferbam. Unfortunately, bayleton selectively inhibits formation of *Pisolithus tinctorius* ectomycorrhizae compared to naturally occurring ectomycorrhizal fungi (Kelley 1987, Marx and Cordell 1984, Rowan 1984). Hence it works against *Pisolithus* inoculation success.

Seed treatment with fungicides appears not to affect ectomycorrhizal development following germination, unless the seeds are coated with ectomycorrhizal fungus spores (Theodorou and Skinner 1976). Fungicidal treatment of seeds of VA mycorrhizal hosts can negatively affect VA mycorrhizal development following germination, however Ualali and Domsch 1975).

Herbicides. Interpreting results from herbicide trials is difficult because effects on the host plant can indirectly affect the mycorrhizal fungus. Usually, herbicide concentrations that significantly affect mycorrhizal fungi are considerably higher than recommended application rates (table 5.2.10).

Some herbicides, like simazine, actually stimulate growth of mycorrhizal fungi in axenic culture as well as under field conditions.

Insecticides and nematicides. Generally, high concentrations of insecticides or nematicides inhibit fungal growth in pure culture (table 5.2.11). Relatively little information is available on effects of these compounds on mycorrhizal fungi, however, so we cannot provide firm recommendations.

General pesticide recommendations. The literature on interaction of pesticides with mycorrhizal fungi and subsequent mycorrhizal development is confusing and incomplete. Much work is needed to understand why one host-fungus combination is affected in certain conditions and another is not. Careful observation and recordkeeping by the nursery manager is important for integrating mycorrhizal management into the total nursery operation. Growers must ascertain what and how much pesticide will affect their various crops under specific growing conditions. The literature provides a guide to some of the potential incompatibilities between pesticide, substrate, host, environment, and mycorrhizal fungus.

Table 5.2.10—*Herbicides that decrease ectomycorrhizal development*

Active ingredient	Trade name
allyl alcohol	allyl alcohol
amitrole	Amitrole*
ammonium sulfamate	Ammate
atrazine	atrazine
2,4–D	2,4–D
dalapon	Dalapon*
paraquat	Paraquat*
tetrafluor-propionic acid	Tomilon*
trifluralin	Trifluralin

*Herbicides that had no effect at low application rates tended to decrease ectomycorrhizae at higher rates.

Table 5.2.11—*Insecticides and nematicides that decrease ectomycorrhizal development*

Active ingredient	Trade name
aldrin	Aldrin*
BHC	BHC*
chlordane	Chlordane*
nemafene	D–D
toxaphene	Toxaphene*

*Insecticides and nematicides that had no effect at low application rates tended to decrease ectomycorrhizae at higher rates.

5.2.9 Conclusions and Recommendations

We cannot overemphasize that mycorrhizae must be included in any assessment of root development and seedling quality. Trees have co-evolved with and become dependent upon their mycorrhizal associations for survival and healthy growth in all forestry settings. Foresters and nursery managers are well aware of the critical stress period that seedlings experience at transplanting. Thus, it is of the utmost priority that nurseries grow and send to the reforestation site seedlings with abundant mycorrhizae on their root systems. Seedlings without mycorrhizae will have to form them before the seedlings can begin to actively take up water and nutrients from the soil. Thus, seedlings with mycorrhizae are better prepared to immediately begin soil exploration and so stand a better chance for survival and early growth than nonmycorrhizal seedlings.

Considerable research on mycorrhizal applications in forestry is now in progress. A primary focus continues to be the selection of fungi for nursery inoculation based on specific ecological benefits, for example, providing drought tolerance. Another research direction concentrates on how much natural fungus inoculum is left on variously disturbed reforestation sites. This latter direction is extremely important because it will help foresters predict which reforestation sites may be suffering from a natural mycorrhizal fungus deficiency and thus need inoculated nursery stock. In the future, both research directions will provide nursery and forest management tools to enhance tree regeneration programs worldwide.

To reach these goals we offer the following recommendations to aid nursery managers in incorporating mycorrhiza management practices into their overall seedling production programs. The nursery staff should:

- As a first step, learn the basic biology of mycorrhizae, understand why they are important, and be aware of the major benefits they provide plants.
- Learn to recognize mycorrhizae, identify different types, and quantify the amount of mycorrhizae on a seedling root system.
- Understand that nursery practices, especially watering, fertilization, and pesticide application, affect mycorrhizal development in order to avoid negative impacts.
- Regularly examine and keep careful records of feeder root and mycorrhizal development of different stock throughout the nursery. Correlate this information with records of other nursery practices to become familiar with how one influences the other.
- Explore the various options for inoculation that are available when the need for an inoculation program develops, and seek the advice of a mycorrhizal specialist for actual implementation.
- Experiment wisely with inoculations, beginning on a small scale and with well-designed studies that include controls.
- Keep abreast of current progress in mycorrhizal technology through reading, attending workshops, or consult with a mycorrhizal specialist periodically.
- Obtain the reference text, *Methods and Principles of Mycorrhiza Research,* published by the American Phytopathological Society (Schenck 1982).
- Include some measure of mycorrhizal development in assessing the overall quality of your seedlings.
- Finally, let your customers know about your inoculation program and its benefits, because good mycorrhizal development is an additional selling point to the commercial market.

5.2.10 References

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