Chapter 20
Mycorrhiza Management in Bareroot Nurseries

R. Molina and J. M. Trappe

Abstract
20.1 Introduction
20.2 Mycorrhizae Defined
20.2.1 Ectomycorrhizae
20.2.2 Ectendomycorrhizae
20.2.3 Vesicular-arbuscular mycorrhizae
20.2.4 Benefits of mycorrhizae
20.3 Mycorrhiza Management
20.3.1 Mycorrhiza development and occurrence in bareroot nurseries
20.3.2 The nursery soil system
20.3.3 Uses and abuses of soil fumigation and other pesticides
20.3.4 Hazards of crop rotation
20.3.5 Seedling manipulations
20.3.6 Managing VA mycorrhizal hosts
20.4 Mycorrhizal Inoculations in Bareroot Nurseries
20.4.1 Ectomycorrhizal inoculation
20.4.1.1 Soil inoculum
20.4.1.2 "Nurse" seedlings
20.4.1.3 Spores and sporocarps
20.4.1.4 Pure fungus cultures
20.4.1.5 Selection criteria
20.4.2 VA mycorrhizal inoculation
20.4.2.1 Soil and root inoculum
20.4.2.2 Pot-cultured inoculum
20.4.2.3 Application of VA inoculum
20.5 Conclusions and Recommendations
References

Abstract

Mycorrhizae, or "fungus-roots," involve the intimate association of plant roots with specialized soil fungi. Forest-tree seedlings depend upon their mycorrhizae for adequate nutrient uptake; those lacking mycorrhizae can be severely stunted and their growth in newly sown beds uneven. Nursery managers should avoid practices that cause mycorrhiza deficiency. For example, because soil fumigation destroys mycorrhizal fungus populations, alternative pest-control measures should be substituted whenever possible. Careful seedling manipulations and handling also will reduce damage to mycorrhizae. Soil disturbance should only be necessary to meet management goals so as to minimize disruption of delicate fungus-soil networks. Fertilization can both foster and inhibit mycorrhiza development; appropriate levels are best determined by experience. The integrated use of mycorrhiza-management tools with other cultural practices and the potential use of selected beneficial fungi for mycorrhizal inoculation of seedlings will help ensure the successful production of vigorous planting stock.

20.1 Introduction

Nursery managers have long recognized the importance of well-developed root systems for producing resilient planting stock. We now realize that adequate development of mycorrhizae on seedling roots is equally important—indeed, essential—for healthy seedling growth in the nursery and desired performance after outplanting.

In this chapter, we focus on the major benefits seedlings derive from mycorrhizae, how environmental factors and management practices affect mycorrhizal fungus populations and subsequent development of mycorrhizae, and methods to foster mycorrhiza development in bareroot nurseries. We also provide an update on prospects for artificially inoculating seedlings with selected, highly beneficial mycorrhizal fungi.

20.2 Mycorrhizae Defined

Mycorrhiza is a Greek word meaning "fungus-root." Nearly all land plants form some type of mycorrhiza with specialized soil fungi. Mycorrhizal associations are classic examples of mutualistic symbioses because the fungus and host plant depend on each other for survival in natural ecosystems.

The mycorrhizal fungus is best considered as a far-reaching extension of the root system. A fine network of fungus threads (hyphae) explores and extracts nutrients from a volume of soil far beyond the bounds of the roots' capabilities. Many of these nutrients are translocated through the hyphal network to the mycorrhizae, where they are released to the roots for host utilization. In exchange, the host serves as primary energy source for the fungus, providing simple sugars and possibly other compounds derived from host photosynthates.

Several different types of mycorrhizae are known, but ectomycorrhizae and vesicular-arbuscular (VA) mycorrhizae are the most common and most relevant to trees. Ectomycorrhizae are the most important to western bareroot nurseries because all members of the Pinaceae—true fir (*Abies*), larch (*Larix*), spruce (*Picea*), pine (*Pinus*), Douglas-fir (*Pseudotsuga*), and hemlock (*Tsuga*) spp.—form ectomycorrhizae. Members of the Fagaceae [e.g., beech (*Fagus*) and oak (*Quercus*) spp.] and Betulaceae [e.g., birch (*Betula*) and alder (*Alnus*) spp] as well as madrone (*Arbutus*) and basswood (*Tilia*) spp. also form ectomycorrhizae. Most other land plants form VA mycorrhizae. The Cupressaceae (cedars) and Taxodiaceae (including redwoods) are the most important in this regard in western forest nurseries; hardwoods such as sweetgum (*Liquidambar*), maple (Acer), and yellow-poplar (*Liriodendron*) spp. are important VA mycorrhizal hosts in eastern nurseries. Alder, eucalyptus

In Duryea. Mary L., and Thomas D. Landis (eds.). 1984. Forest Nursery Manual: Production of Bareroot Seedlings. Martinus Nijhoff/Dr W. Junk Publishers. The Hague/Boston/Lancaster, for Forest Research Laboratory, Oregon State University. Corvallis. 386 p.

(*Eucalyptus*), willow (*Salix*), and poplar (*Populus*) are among the genera that readily form both ectomycorrhizae and VA mycorrhizae.

20.2.1 Ectomycorrhizae

Several different forms of ectomycorrhizae are shown in Figures 1 to 6. The fungi colonize the surfaces of the short feeder roots, often forming a thick mantle around them. Ectomycorrhizae can frequently be seen with the unaided eye or a hand lens because many are white or brightly colored. Similarly, if ectomycorrhizae are abundant, a dense moldlike fungal growth is visible in the soil when seedlings are lifted. When examined microscopically in cross section (Figs. 9 and l0), the fungus is seen to enter the root, penetrating between the cortical cells to form an interconnecting network called the Hartig net. It is within this extensive hypha-root cell contact zone that nutrient exchange occurs.

The fungi also produce plant hormones that stimulate root branching and elongation, thereby increasing the root's absorptive surface. Branching patterns of ectomycorrhizae are often host determined and are therefore characteristic of the host-seedling species. For example, pine ectomycorrhizae are typically forked or dichotomously branched (Figs. 2, 4, and 21), whereas other hosts may predominantly form structures that are pinnate (Figs. 5 and 6), coralloid (Fig. 3), tuberculate, or variably branching (Fig. 1). It is important to realize that thousands of species of mushroom, puffball, and truffle fungi (higher Basidiomycetes and Ascomycetes) (Figs. 13, 14, 15, 17, 19, and 20) can form mycorrhizae on a large array of host species. Thus, the physical and physiological diversity of ectomycorrhizal forms is enormous.

20.2.2 Ectendomycorrhizae

A subtype of ectomycorrhizae is the ectendomycorrhiza. Because the mantle it forms is thin and translucent, feeder roots display the brown color of underlying epidermal cells. Ectendomycorrhizae branch like ectomycorrhizae but lack root hairs; in addition to forming a Hartig net, the fungi also penetrate scattered cortical cells (Figs. 11 and 12). Small Discomycetes (cup fungi), which form ectendomycorrhizae [3], are often common and beneficial in temperate bareroot nurseries [12].

20.2.3 Vesicular-arbuscular mycorrhizae

Vesicular-arbuscular mycorrhizae do not differentiate morphologically from nonmycorrhizal roots and are therefore not discernible by the unaided eye. Roots must be selectively stained [39] to highlight the fungus within and then examined microscopically to determine its presence and structure. In roots thus prepared (Figs. 7 and 8), hyphae of the VA mycorrhizal fungus can be seen to ramify throughout the roots and often to form the characteristic vesicles and arbuscules for which the mycorrhiza is named. Vesicles (Fig. 7) are storage organs containing carbohydrates and also serve as reproductive structures. Arbuscules (Fig. 8) are very finely branched, short-lived, intracellular structures which partake in nutrient exchange. Although these fungi are often said to "infect" the roots, they cause no apparent harm.

As with ectomycorrhizal fungi, the main portion of the VA fungus lies outside the root, exploring the surrounding soil for nutrients and translocating them to the roots. Unlike ectomycorrhizal fungi, however, VA fungi do not produce large mushroom-like reproductive structures. Instead, they produce large, mostly soil-borne, globose spores (Fig. 16). Furthermore, VA fungus spores cannot be dispersed for long distances by air movement, as can mushroom spores; spore dispersal is limited primarily to mechanical movement of soil. The consequences of this important feature on VA mycorrhizal development in nurseries will be considered later (see 20.3.4, 20.4.2).

20.2.4 Benefits of mycorrhizae

In addition to greatly enhanced uptake of nutrients, especially phosphorus, mycorrhizae confer other benefits to their hosts. They can take up water [5] and increase drought resistance of young seedlings [38]. Some mycorrhizal fungi can also detoxify certain soil toxins [53] or enable seedlings to withstand high soil temperatures [22] or extreme acidity [18]. Of practical importance to nursery management, some mycorrhizal fungi can protect roots against certain pathogens [17]. For example, the mycorrhizal fungus *Laccaria laccata* Scop. ex Fr. has been shown to protect feeder roots from Fusarium infection [44].

Historically, the absolute dependence of forest trees on their mycorrhizae was repeatedly demonstrated when ectomycorrhizal Pinaceae were introduced to the Southern Hemisphere. Only when accompanied by their associated ectomycorrhizal fungal symbionts could these exotics survive and thrive (see [32, 33]). A classic example was Monterey pine (*Pinus radiata* D. Don). Initial attempts to establish pine seedling nurseries in Australia and New Zealand failed. When mycorrhizal fungi native to pine stands were unknowingly introduced into seedling beds, however, seedlings grew vigorously and survived outplanting. Today, the pine plantations of Australia and New Zealand are among the world's more productive forests.

Similar examples of mycorrhizal dependency were evident in afforestation attempts in the treeless grasslands of the United States [29] and on the steppes of Russia [9]. More recently, Schramm [42] and Marx [18, 21] have shown the need for mycorrhizal planting stock inoculated with specifically adapted fungi for tree establishment on strip-mined and other severely disturbed sites. Thus, tree seedlings must be accompanied by their mycorrhizal fungi when planted in areas lacking suitable mycorrhizal fungi.

20.3 Mycorrhiza Management

Because each nursery is unique, each must develop its own specific mycorrhiza-management strategies through experimentation and good recordkeeping. Examples given here are general cases. In no case has research been intensive enough to provide more than fragmentary understanding of what is taking place in the soil.

20.3.1 Mycorrhiza development and occurrence in bareroot nurseries

Mycorrhiza development—or the lack of it—in bareroot nurseries is affected by several biologic and environmental factors, many of which we cannot control.

Nurseries established in forest zones or surrounded by ectomycorrhizal hosts usually produce seedlings with abundant and diverse ectomycorrhizae. If nursery beds are not fumigated, all seedlings will develop mycorrhizae early in the first growing season. Even if beds are fumigated, regular and prolific fruiting of sporocarps in neighboring forests provides abundant spore inoculum, as does fruiting of sporocarps in established nursery beds. Under these conditions, whenever spores enter the soil, ectomycorrhizae can begin developing with the first production of feeder roots 6 to 10 weeks after germination and continue developing through the growing season as fungi extend into surrounding soil and colonize roots of adjacent seedlings. By the end of the first year after fumigation, most seedlings will usually be mycorrhizal. During the second growing season, nearly all seedlings will be abundantly mycorrhizal. Thus, a rich supply of natural fungus inoculum will promote the early development of ectomycorrhizae needed to ensure uniformly healthy planting stock.

In contrast, nurseries developed away from native forests or on new ground with no history of ectomycorrhizal hosts can experience mycorrhiza deficiency, resulting in serious financial and reforestation setbacks. Such seedlings are stunted, chlorotic, and severely nutrient deficient. Mycorrhiza deficiency symptoms may persist well into the second year; even after recovery, seedling size may vary considerably within the seedbed.

Trappe and Strand [52] report a striking example of this situation in Oregon's Willamette Valley (Fig. 18). In a new nursery established on fumigated, formerly agricultural land, the first crop of Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] seedlings exhibited an unexpected, severe, phosphorus deficiency not detected by soil analysis. Only in the second year, after natural inoculation by wind-blown spores, did many seedlings recover and begin to grow; others remained stunted through the second growing season. Although other environmental and biologic factors can cause stunting or nutrient deficiency symptoms, such symptoms are characteristic of mycorrhiza deficiency. When they appear, nursery managers should carefully examine seedling roots or have them evaluated by an expert.

Different tree species vary in susceptibility to mycorrhiza deficiency. Douglas-fir and true firs appear especially mycorrhiza dependent and show symptoms of mycorrhiza deficiency more quickly than pines.

Although effects of management practices on mycorrhiza development will be discussed later in 20.3, soil fumigation as a cause of mycorrhiza deficiency deserves emphasis here. Properly applied fumigation with methyl bromide/chloropicrin gases usually eliminates mycorrhizal fungus populations along with targeted pests. Even in nurseries with large native fungus populations, fumigation can cause a lengthy delay in mycorrhiza development, resulting in substantial growth loss the first growing season. Availability of fungal spores for natural reinoculation of fumigated beds can be reduced during droughty years when mushroom production is low or when prolonged heavy rains wash spores from the air during the mushroom fruiting season [49].

Soil fumigation is particularly devastating to VA mycorrhizal fungi; we have observed that VA mycorrhizal redwoods and cedars especially suffer the consequences. Because VA fungus spores are not dispersed by air, once the population is eliminated, such spores are returned to fumigated beds only erratically through movement of spore-containing soil by machines and on shoes.

In most bareroot nurseries, root systems are dominated by a few nursery-adapted mycorrhizal fungi—in stark contrast to the hundreds of fungi common to even small areas of forest. By far the most common ectomycorrhizal fungi in bareroot nurseries are species of the genus *Thelephora*. *Thelephora terrestris* (Ehrh.) Fr. is especially common worldwide and fruits conspicuously at the base of seedlings (Fig. 20); its ectomycorrhizae are very smooth, usually a pale cream-brown (Fig. 21). The ectomycorrhizal fungi *Laccaria laccata* (Fig. 14) and *Inocybe lacera* (Fr.) Kummer (Fig. 19) and ectendomycorrhizal fungi also are common in Northwest nurseries. We have occasionally observed truffle fungi of the genus *Rhizopogon* (Fig. 15) and boletes of the genus *Suillus* (Fig. 17) to be common in a few nurseries. Nurseries surrounded by dense forest stands, such as the U.S.D.A. Forest Service Wind River Nursery in Washington, often harbor diverse ectomycorrhizae. Even in those nurseries, however, *Thelephora*, *Laccaria,* and ectendomycorrhizal species tend to predominate.

Results from the OSU Nursery Survey (see chapter 1, this volume) reaffirm many of the phenomena described above. Of the responding nurseries, about 75% report good to abundant ectomycorrhiza development on lifted seedlings. *Thelephora* and *Laccaria* species fruit most commonly, but a scattering of other species was observed. In several instances, respondents ascribed a recurring stunting problem for some tree species, particularly during the first season, to a lack of mycorrhizae. Thus, nursery managers must continue to be alert to the possibilities of mycorrhiza deficiency.

20.3.2 The nursery soil system

In general, nursery soils that are good for tree seedling growth are also good for mycorrhiza development on those seedlings. Good organic matter content, good tilth, good drainage, and adequate but not excessive nutrient levels are all associated with good mycorrhiza formation [33]. Much has been written about effects of fertilization on mycorrhiza formation, but only one conclusion can be drawn at present: because each soil is unique, levels of fertilizer which might promote mycorrhiza formation—or that might inhibit it—must be determined through experience.

It is when soil-management problems arise and when steps are taken to alleviate those problems that mycorrhizal populations are most often disrupted. This is because we often treat symptoms rather than causes of problems for lack of information on what is occurring in the soil. For example, spots of root rot may develop in a nursery because of localized poor drainage. If fungicides are applied to control the root rot, mycorrhizal fungi also may be decimated. The resulting mycorrhiza deficiency is then reflected by nutrient deficiency. If that symptom is treated by extra fertilization, mycorrhiza formation may be even further depressed. Once the nutrient-starved seedlings stop growing, their root systems are open to attack by yet other pathogens for lack of protection by mycorrhizal fungi. But the cause of the problem—poor drainage—remains uncorrected.

To minimize the chances for these kinds of deleterious chain reactions, the soil must be regarded as a system of interacting biological and physical components (see chapters 6, 7, and 9, this volume). Disrupt one component and all others are affected. Planned disruptions can be used to advantage in furthering management goals, but consistent success requires experience and care. The goals must be carefully defined because different goals may require different approaches. Managing mycorrhizae for an ultimate goal of good, uniform seedling growth in the nursery may entail different long-range plans and procedures than managing mycorrhizae for an ultimate goal of optimum survival of stock outplanted on stressful sites.

20.3.3 Uses and abuses of soil fumigation and other pesticides

As noted earlier, properly applied soil fumigation decimates beneficial organisms along with target pests. Loss of beneficial bacteria may be as serious as loss of mycorrhizal fungi. Seedlings will not grow satisfactorily until the mycorrhizal fungi, and possibly associated microorganisms, are replaced. Replacement of the VA mycorrhizal fungi required by cedars and redwoods can be slow and erratic; poor and nonuniform growth of these species on4umigated soil is common in western nurseries. Ectomycorrhizal fungi may be replaced more rapidly through aerially dispersed spores, but replacement depends both on weather favorable for spore production and on timing of the fumigation. As long as cold or dry weather does not inhibit spore production, beds fumigated in late summer or early autumn will be exposed to natural spore dispersal of mycorrhizal fungi in autumn; however, microbes antagonistic toward mycorrhizal fungi can establish concurrently in the fumigated beds. In contrast, beds fumigated in spring, just before sowing, will contain only mycorrhizal propagules that the fumigant missed.

The goal of optimum seedling growth in the nursery with minimal mycorrhiza management thus calls for minimizing fumigation. Pests should be controlled by alternative methods whenever possible (see chapter 19, this volume). When fumigation is deemed necessary, late summer is better timing than the spring in which seeds will be sown.

A more sophisticated goal than the passive approach outlined above is inoculation of planting stock with fungi selected to promote the best survival and growth in plantations. Successful inoculation can be expected to result in good, uniform growth of seedlings in the nursery as well. In this approach, soil could be fumigated to minimize populations of wild mycorrhizal fungi and microbial antagonists, preferably just before inoculation with a selected fungus; spring fumigation is preferable where weather permits. If late-summer or autumn fumigation is unavoidable, aggressive native mycorrhizal and antagonistic organisms may reinvade the soil over winter. In that case, only antagonist-resistant and highly competitive mycorrhizal fungi can be successfully inoculated. Evidence is also mounting that "helper" bacteria can be important in promoting inoculation success and that these bacteria can be cultured and inoculated along with the desired fungi [pers. commun., 14].

Selective biocides can be used instead of or in conjunction with soil fumigation. Herbicides do not generally appear to depress mycorrhiza formation and in some cases even seem to increase it, possibly by increasing exudation of sugars from roots [43, 51]. Weed control thus seems compatible with mycorrhiza management (see chapter 18, this volume). Insecticides and nematicides at field-application levels generally appear not to harm mycorrhizae or depress mycorrhiza formation. Some fungicides, on the other hand, are inhibitory, although those that inhibit ectomycorrhizal fungi do not necessarily affect VA fungi, and vice versa, at least not at concentrations occurring in soil after field applications [8, 30].

No matter how much is revealed by research about effects of pesticides on mycorrhizal fungi and mycorrhiza formation, it is important to realize that most pesticides used in nurseries are synthetic compounds that organisms have never before encountered. Moreover, a given chemical will not necessarily produce the same responses in all species of fungi or hosts or in all nurseries. Hence, first use of a chemical in a nursery should always be in trials of limited scope that include evaluation of its effects on mycorrhiza development.

Inoculating beds with mycorrhizal fungi selected for their strong protection of roots against pathogens is a potential alternative to routine fumigation or use of fungicides in some cases. A highly promising example is *Laccaria laccata*, a fungus with excellent potential for inoculation in western nurseries [34, 36]; this fungus strongly suppresses *Fusarium oxysporum* Schlecht. in nursery conditions [44].

20.3.4 Hazards of crop rotation

Switching rotations from ectomycorrhizal to VA mycorrhizal trees can produce mycorrhiza deficiency because the fungi of the two mycorrhizal types are totally different. For example, western redcedar (*Thuja plicata* D. Don), incense-cedar [*Calocedrus decurrens* (Tory.) Florin], or coastal redwood [*Sequoia sempervirens* (D. Don) Endl.], which are VA mycorrhizal, will encounter few or no propagules of VA mycorrhizal fungi in beds with a preceding crop of ectomycorrhizal Douglas-fir or pine. The deficiency will be further compounded if beds are fumigated before VA hosts are sown. Because spores of VA mycorrhizal fungi do not disperse by air, recolonization of beds can be slow and the tree crop accordingly poor. If crop rotation is deemed necessary for some reason, steps to inoculate beds with VA fungi are in order. Cover crops of VA mycorrhizal hosts may be useful in building up VA inoculum in a bed, provided that the fungi are initially present and that the cover crop is grown long enough for mycorrhizae to form on it.

The problem can occur in reverse when ectomycorrhizal hosts are sown in beds with a previous history of VA hosts (including most cover crops). If recolonization by aerially dispersed ectomycorrhizal fungi is rapid, adverse effects on seedling growth may be minimal; however, such rapid recolonization cannot be counted on. Again, inoculation of the beds with appropriate mycorrhizal fungi may prevent growth loss and unacceptable variation in seedling size within the bed.

20.3.5 Seedling manipulations

Procedures such as wrenching, undercutting, and mowing are not known to inhibit mycorrhiza formation, but they cost seedling energy. Such procedures are used either out of necessity or because their benefits to seedlings are believed to outweigh their costs. In the case of mycorrhizae, practices such as wrenching break up much of the nutrient-absorbing network of fragile hyphae that grow from the mycorrhizae into surrounding soil. These hyphae will regrow but at the cost of seedling-produced energy that would otherwise have been available to increase seedling size.

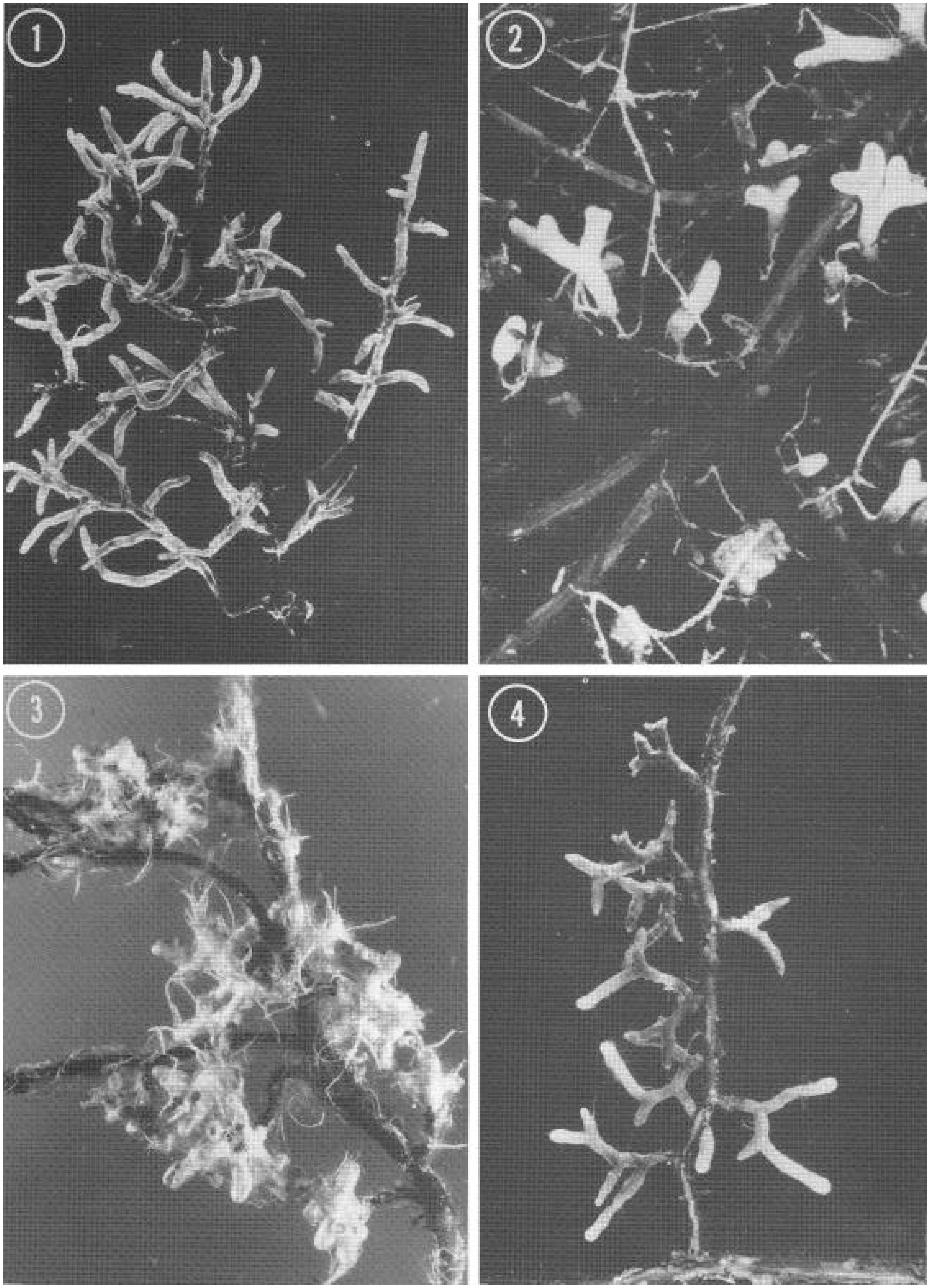
Procedures such as lifting, sorting, packing, storing, and transporting seedlings should be performed with care to minimize damage to the fine-root system. Mycorrhizae destroyed by rough handling, desiccation, or heating will have to be replaced at the planting site at a cost of seedling energy and nutrients.

20.3.6 Managing VA mycorrhizal hosts

Although most management considerations discussed for ectomycorrhizae also apply to VA mycorrhizal hosts, a somewhat different strategy is needed to foster VA mycorrhizae. For example, if certain nursery beds have been known to raise vigorous crops of cedars or if surveying indicates that the soil harbors good populations of VA fungi, nursery managers may want to use those beds exclusively for VA hosts and forego intermittent fumigation unless pathogens become a serious problem. If those beds are not to be used for a season or two to grow trees, they should be planted with a cover crop which will maintain the VA fungus populations as well as add good organic matter when plowed under. In fact, some VA mycorrhizal cover crops have been purposely planted to increase the populations of VA fungi, thus ensuring good mycorrhiza development on the next tree crop [11]. If pot-cultured VA fungus inoculum (see 20.4.2.2) is used in nursery beds to eliminate mycorrhiza deficiency or introduce more efficient fungus strains, subsequent fumigation should be avoided and intermittent cover crops planted to maintain the populations of the introduced fungi.

20.4 Mycorrhizal Inoculations in Bareroot Nurseries

Nursery managers may, choose to artificially inoculate beds with mycorrhizal fungi either to eliminate potential or current mycorrhiza deficiencies or to improve outplanting performance of seedlings. Several procedures are available for introducing either ectomycorrhizal or VA mycorrhizal fungi. In this section we discuss several general methods and strategies for inoculating, first, ectomycorrhizal hosts, then VA mycorrhizal hosts. Refer to Mikola [33], Trappe [49], Marx [20]. and Schenck [41] for detailed discussions of past and current technological advances in this field.



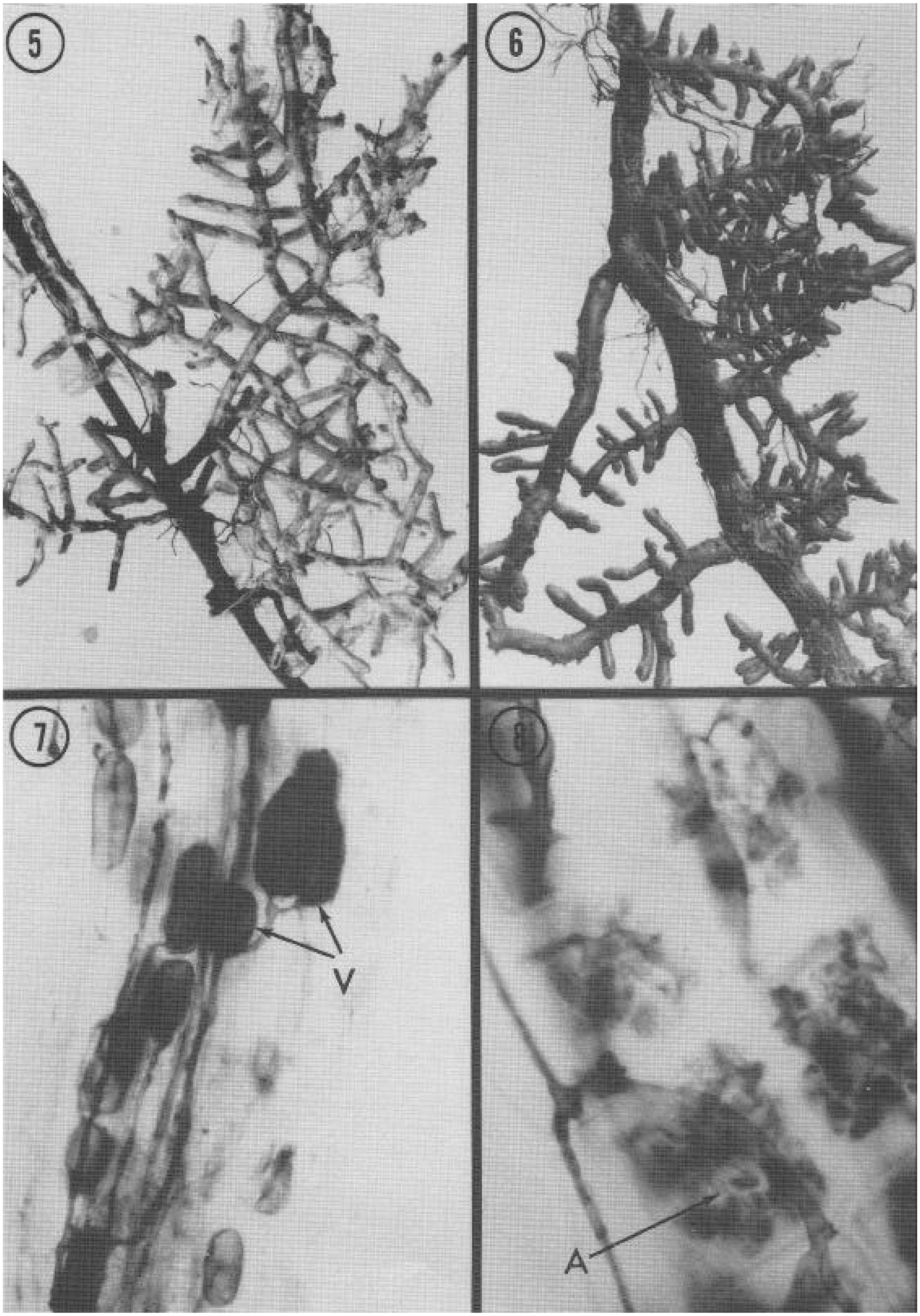
Figures 1-4. Ectomycorrhizal forms.

(1) Variably branched ectomycorrhizae formed between Sitka spruce [*Picea sitchensis* (Bong.) Carr.] and the fungus *Amanita muscaria*; 3.1 x.

(2) Ectomycorrhizae formed with pine (*in vivo*): 3.3x. Note 'he characteristic forklike dichotomous branching and colonization of the soil by fungus strands called rhizomorphs.

(3) Compact, coralloid ectomycorrhizae formed between lodgepole pine (*Pinus contorta* Dougl. ex Loud.) and *Scleroderma laeve*; 4.2x. Note the strands of mycella attached to the ectomycorrhizae (photo by B. Zak).

(4) Dichotomously branched ectomycorrhizae formed between western white pine (*Pinus monticola* Dougl. ex D. Don) and the fungus Gastroboletus subalpinus; 3.8x.

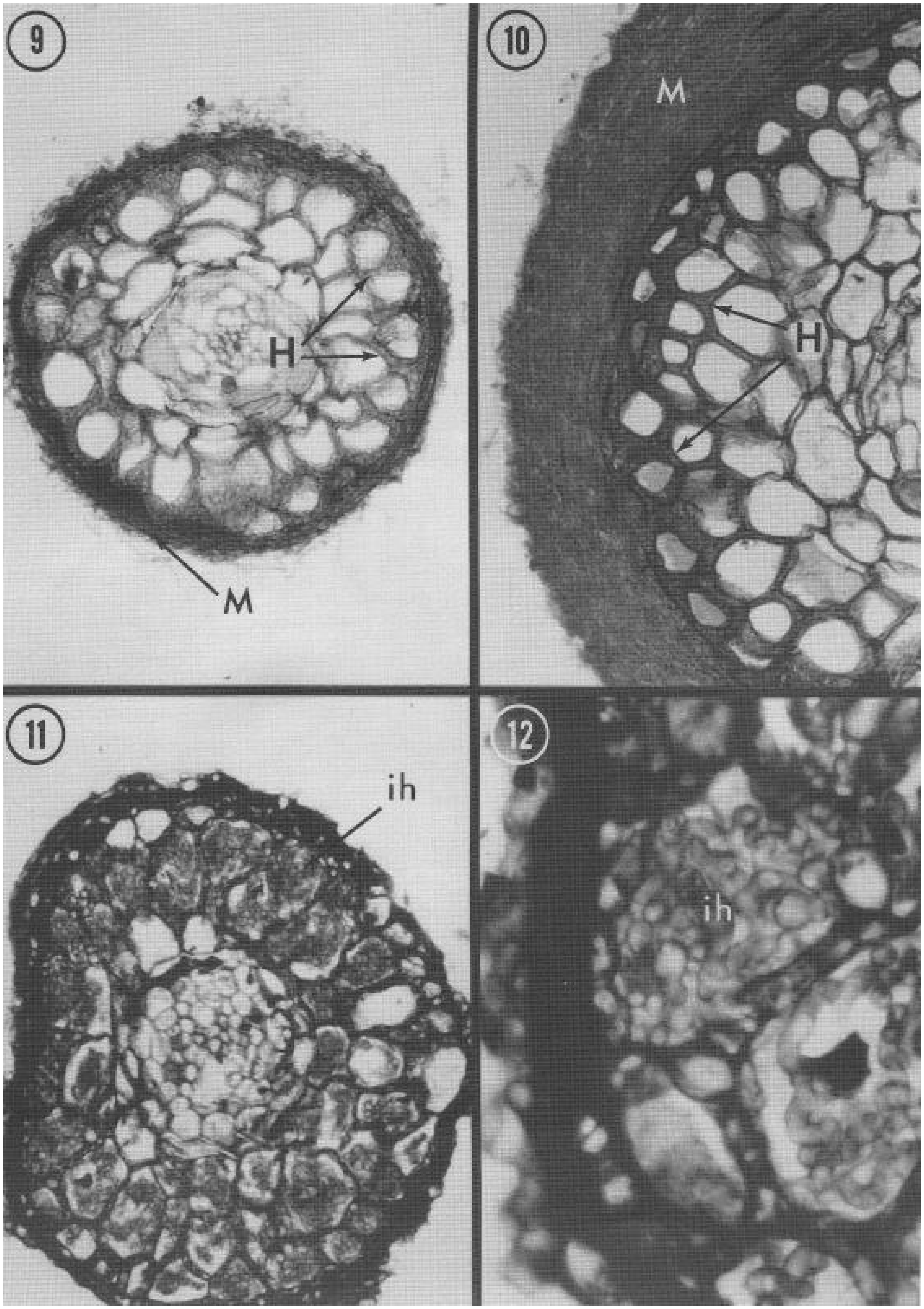


Figures 5-8. Douglas-fir ectomycorrhizae and fescue VA mycorrhizae.

(5, 6) Pinnately branched ectomycorrhizae formed between Douglas-fir and unknown fungi; 3.4x.

(7) Vesicles (V) within a selectively stained fescue root; 150x.

(8) Arbuscules (A) within a selectively stained fescue root; 600x.

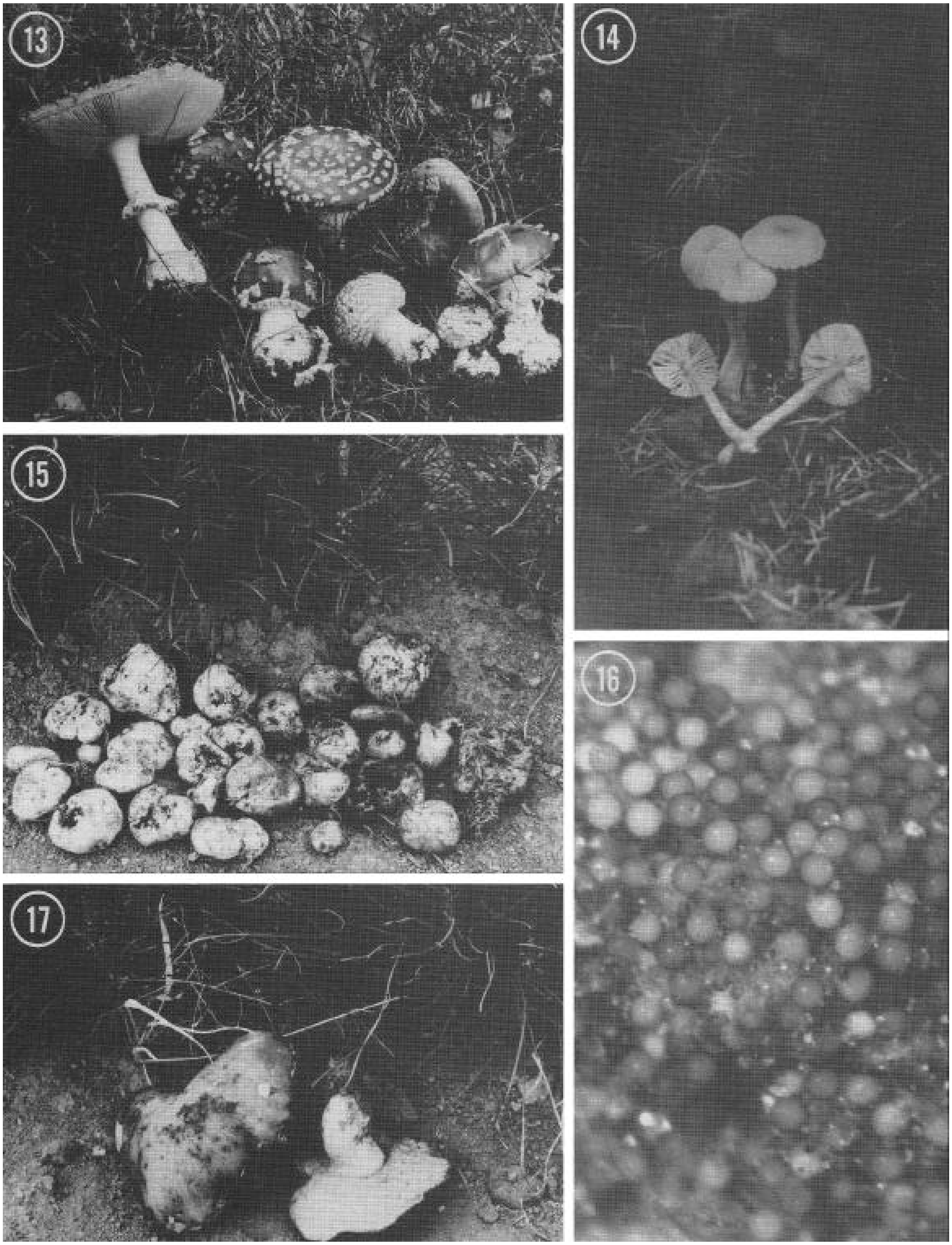


Figures 9-12. Cross sections of ectomycorrhizae and ectendomycorrhizae (H = Hartig net, M = mantle, ih = intracellular hyphae).

(9) Ectomycorrhizae formed between lodgepole pine and the fungus *Rhizopogon fuscorubens*; 50x.

(10) Ectomycorrhizae formed between western larch (*Larix occidentalis* Nutt.) and the fungus Amanita muscaria; 50x.

(11, 12) Pine ectendomycorrhizae; 160x and 630x, respectively. Note the abundant intracellular hyphae filling many cortical cells.



Figures 13-17. Ectomycorrhizal fungus fruiting bodies and VA fungus spores.

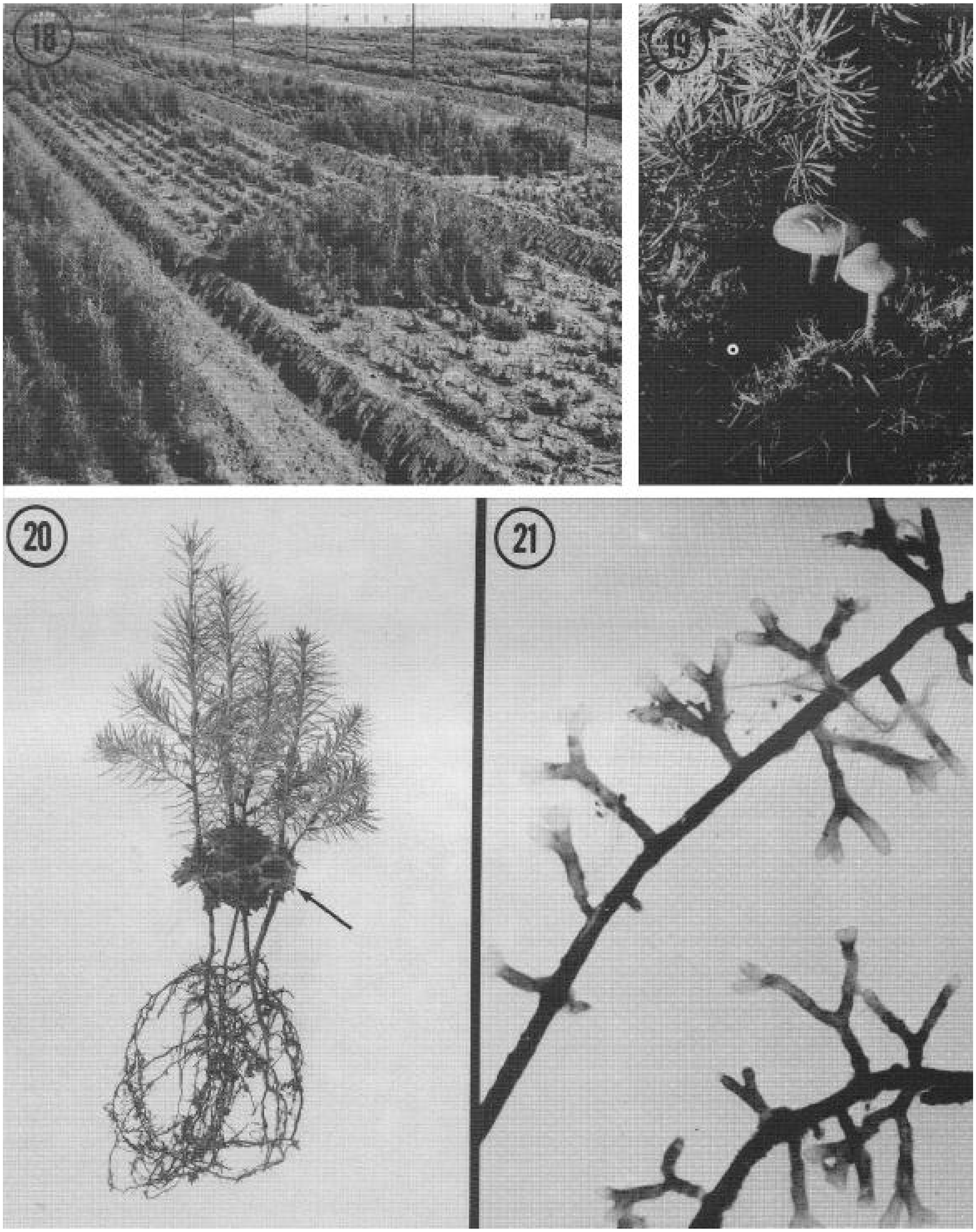
(13) ***Amanita muscaria*** **mushrooms, common throughout conifer woodlands.**

(14) ***Laccaria laccata*** **mushrooms fruiting in a Douglas-fir bed.**

(15) ***Rhizopogon vulgaris*** **truffles found fruiting in pine beds at the U.S.D.A. Forest Service Pine Nursery at Bend, Oregon.**

(16) Spores of the VA mycorrhizal fungus ***Glomus epigeaum*****; spores range from 75 to 140 wm in diameter.**

(17) Bolete mushrooms of the genus Suillus found fruiting among pine seedlings; these mushrooms are recognized by the presence of pores, rather than gills, on their underside.



Figures 18-21. Mycorrhiza deficiency in Douglas-fir beds and two common ectomycorrhizal fungi in Northwest nurseries.

(18) Apparent mycorrhiza deficiency in 2+0 Douglas-fir beds. Scattered clumps of tall seedlings are mycorrhizal, whereas nonmycorrhizal seedlings remain severely stunted (see [52] for greater detail).

(19) Sporocarps of the ectomycorrhizal fungus *Inocybe lacera* found fruiting in the clumps of mycorrhizal Douglas-fir seedlings in Figure 18.

(20) Crustlike sporocarp of the very common ectomycorrhizal fungus *Thelephora terrestris* on the stems of Douglas-fir seedlings.

(21) Ectomycorrhizae formed between pine and *Thelephora;* 3.2x.

20.4.1 Ectomycorrhizal inoculation

Four primary sources of ectomycorrhizal inoculum are available: soil inoculum, mycorrhizal "nurse" seedlings interplanted in beds, spores and sporocarps, and pure fungus cultures. Each has advantages and disadvantages, so nursery managers should carefully weigh each option before selecting which approach best suits their needs.

20.4.1.1 Soil inoculum

The most commonly used and probably the most reliable inoculum is forest soil taken from beneath ectomycorrhizal hosts. About 10% by volume of soil inoculum is incorporated into approximately the top 10 cm of nursery-bed soil before sowing or transplanting [33]. Inoculation of new or fumigated beds by soil taken from established beds (beds previously supporting seedlings with good mycorrhiza development) is also feasible. The major drawback is the logistics of collecting and transporting the large quantities of soil needed. Unfortunately, weed seeds, rhizomes, and potential pathogens may be introduced along with the beneficial fungi. Nonetheless, soil inoculation continues to be regularly and successfully used in many areas of the world to promote healthy mycorrhiza development [33].

20.4.1.2 "Nurse" seedlings

Planting mycorrhizal "nurse" seedlings (mycorrhizal seedlings from which the fungus can spread and colonize new. seedlings) or incorporating chopped roots of ectomycorrhizal hosts into nursery beds can provide a source of ectomycorrhizal fungus inoculum. However, mycorrhizal colonization may spread slowly and unevenly, the large "nurse" seedlings can interfere with cultural practices, and the risk of introducing unwanted pests remains.

20.4.1.3 Spores and sporocarps

Spores and chopped sporocarps (mushrooms, puffballs, and truffles) of some ectomycorrhizal fungi provide an excellent source of natural inoculum. The Gasteromycetes (puffballs and related fungi) with abundant spore masses offer better sources of large numbers of spores than the gilled fungi. Several recent studies have shown spores of the puffball fungus *Pisolithus tinctorius* Coker and Couch to be effective inoculum for southern pines [19, 20, 28]. Large quantities of spores are easily collected, and a variety of application methods, including dusting, spraying, coating seeds, and applying in a hydromulch, have been effective. Marx [20] reports acceptable levels of mycorrhiza formation, improved seedling growth in the nursery, and improved outplanting success following inoculation with *P. tinctorius* spores. *P. tinctorius* also fruits abundantly in many areas of the Northwest: however, our experimental nursery inoculations with its spores have produced erratic results [1]. Further research is needed before this plentiful source of natural inoculum can be recommended.

Good inoculation success has also been noted when seeds coated with dried *Rhizopogon* spores [45, 46, 47] or pulverized *Rhizopogon* sporocarps [4] have been introduced into nursery beds. In recent experiments, Castellano and Trappe [unpubl. data, 2] found fresh and dried spore suspensions of *Rhizopogon vinicolor* Smith and R. colossus Smith to be effective in inoculating bareroot and container-grown Douglas-fir. Success with *R. vinicolor* is particularly promising; Pilz [40] and Parke et al. [38] have shown that fungus to be an important mycorrhizal symbiont of newly outplanted Douglas-fir seedlings. *R. vinicolor* also improves drought resistance of inoculated seedlings [38], an important consideration for hot, dry sites.

Unfortunately, it can be difficult to collect large enough quantities of spores of most fungi for large-scale nursery inoculations. Application methods and rates for effective inoculation as well as methods of spore storage need further research before spore inoculation can be operational.

20.4.1.4 Pure fungus cultures

The final inoculum source is pure cultures of specially selected, beneficial ectomycorrhizal fungi; intense research is currently in progress worldwide for developing this promising source. A pure culture of a specific fungus is first isolated, usually from a sporocarp or, occasionally, directly from its ectomycorrhiza (see [37]). The nutritional and growth requirements of such a fungus and its ability to form ectomycorrhizae, stimulate growth, or offer other benefits, such as disease protection or drought resistance, to its hosts can then be evaluated. This background information is vital for selecting the best isolates for attaining specific nursery goals.

20.4.1.5 Selection criteria

The thousands of ectomycorrhizal fungi are characterized by tremendous physiological diversity, including ease of isolation, growth in pure culture, effectiveness as mycelial inoculum, and benefit to the host. Consequently, criteria have been developed for selecting the most promising fungi for small- and large-scale testing so that, ultimately, nursery goals can be met. The major selection criteria are summarized by Molina ([35]; see [20] and [49] for greater detail):

- **Good growth in culture:** Most ectomycorrhizal fungi grow slowly; relatively fast-growing isolates are preferred.
- **Effectiveness in forming mycorrhizae:** Many fungi can easily be grown in culture for inoculum production, but only some of these consistently perform well as vegetative inoculum.
- **Special ecological adaptations:** For example, the common ectomycorrhizal fungus *Cenococcum geophilum* Fr. is well known for its drought resistance and is also an important symbiont of trees growing at timberline. Simi-larly, some fungi are more effective than others in produc-ing enzymes important for nutrient absorption.
- **Competitive ability:** Marx [20] emphasizes that the introduced fungus must compete well against the resident mycorrhizal fungi and dominate the root systems of inoculated stock. Our preliminary studies also point to a need for the introduced fungus to resist antagonistic soil microorganisms that can build up over winter after autumn soil fumigation. The isolate should also protect roots against pathogens such as *Phytophthora* or *Fusarium* spp.
- **Host range:** Many fungi can form mycorrhizae with most ectomycorrhizal hosts, whereas others will form ectomycorrhizae only with specific hosts such as Douglas-fir or pines. Because modern nurseries often raise many tree species, it is important that ectomycorrhizal hosts and fungi be compatible.
- **Improved seedling performance in plantations:** This is the ultimate criterion to be met before an isolate can be recommended for wide-scale nursery inoculation.

Marx and Kenny [27] review past and recent research developments on production of ectomycorrhizal fungus inoculum. Basically, Marx and Bryan [23] refined a system to grow pure cultures of specific fungi in a vermiculite substrate moistened with nutrient solution. After about 3 months' incubation, the vegetative inoculum is washed, dried, and refrigerated until used. just before sowing, the inoculum is worked into the rooting zone of nursery beds where it remains quiescent until planted seeds germinate and seedlings produce feeder roots, a period of about 6 to 8 weeks. The fungus is sheltered within the vermiculite particles during this period.

Limited success has been achieved with *Pisolithus tinctorius* by Donald Marx and coworkers at the U.S.D.A. Forest Service Institute for Mycorrhiza Research and Development (Athens, Georgia). Inoculation of nurseries in the southern United States has yielded excellent establishment of *P. tinctorius* on seedling root systems. As a result, seedling growth in the nursery has significantly increased, at times doubling that of noninoculated controls [24]. More importantly, *P. tinctorius* inoculation has significantly increased survival and growth of outplanted inoculated seedlings on extremely disturbed sites such as mine spoils [18, 21], as well as on routine regeneration sites [25]. Experimentation is continuing, to render this technology operational.

Such results prompted efforts to produce *P. tinctorius* inoculum for large-scale nursery inoculations. From 1977 through 1980, Marx et al. [26] conducted complex nationwide tests of *P. tinctorius* vegetative inoculum (Mycorrhiz®) produced by Abbott Laboratories (Chicago, Illinois) in 30 conventional bareroot nurseries located in 25 states. Final results indicated that one isolate of *P. tinctorius* could be produced in large industrial fermentors for use in bareroot nurseries. A broadcast rate of approximately 1 liter inoculum per square meter of soil surface gave the best results. Large tractor-drawn seeders have been modified to rapidly incorporate such inoculum into the rooting zone when seed is sown [16]; unfortunately for western nurseries, inoculation was satisfactory only on pine species grown in southern and southeastern nurseries, the region from which the single P. tinctorius strain originated. That this strain did poorly in northwestern nurseries reinforces the premise that fungus strains adapted to particular regions and habitats should primarily be selected for use in those regions.

Encouraged by the commitment of industrial representatives and interest of nursery managers and foresters, several groups of mycorrhiza researchers are now collecting, selecting, and testing promising species and strains of ectomycorrhizal fungi for nursery inoculations. In the Northwest, we have had encouraging results in ongoing studies with the ectomycorrhizal fungus *Laccaria laccata*. It has performed well on container-grown seedlings [34, 36] and in bareroot nurseries [unpubl. data, 7]. The inoculum was produced for experimental use by Sylvan Spawn Laboratory of Butler County Mushroom Farms (Worthington, Pennsylvania); this firm can produce small to large amounts of vegetative inoculum of diverse ectomycorrhizal fungi. At the Pacific Northwest Forest and Range Experiment Station, long-range research plans include continued work to select and test new and promising fungus strains with the hope of finding dependable strains to meet both nursery production and reforestation goals. Nursery managers are thus encouraged to remain alert to future developments in this field.

20.4.2 VA mycorrhizal inoculation

Unlike ectomycorrhizal fungi, VA mycorrhizal fungi have not yet been isolated and grown in pure culture because they must be attached symbiotically to their hosts to grow and reproduce. This presents a major obstacle to aseptic mass production of VA mycorrhizal fungi for large-scale nursery inoculations. Methods are available, however, to circumvent these difficulties and ensure VA mycorrhizal colonization of nursery stock.

20.4.2.1 Soil and root inoculum

As with ectomycorrhizal inoculation, the easiest method is to incorporate soil (plus root fragments) taken from under VA mycorrhizal hosts. Fortunately, VA mycorrhizal fungi show little or no host specificity; those associated with grasses, legumes, and several herbs and shrubs can form VA mycorrhizae with cedars, redwoods, sweetgums, and maples. Thus, locating soil with VA mycorrhizal fungi is relatively easy. The same drawbacks noted for soil inoculation of ectomycorrhizal hosts (see 20.4.1.1) apply here: the risk of introducing pests is ever present, and the need to move large quantities of soil can be impractical.

20.4.2.2 Pot-cultured inoculum

Refined techniques to multiply and introduce selected VA fungi are becoming available through intense research efforts in pot culturing [6]. In this technique, soil-borne spores which are very large are first sieved from the soil, examined microscopically, and identified to species (see [50]). Spores are then surface sterilized and mixed with sterilized soil in which a host plant such as sorghum is greenhouse grown. As host roots penetrate the inoculated substrate, the spores germinate and colonize the roots to form mycorrhizae. After about 4 to 6 months, the fungus has established its hyphae-soil network and has produced more spores. Once such pot cultures are established, the soil containing spores, mycelium, and colonized root fragments can be used to inoculate nursery or field crops or start new pot cultures, thus multiplying available inoculum for future use.

Pot culturing also affords the opportunity to select species, strains, or mixtures thereof that offer the greatest benefit to the targeted host species. As with ectomycorrhizal fungi, research is underway to produce commercial quantities of dependable VA fungus inoculum for large-scale nursery and field inoculations. Fortunately for forest-tree nurseries, the gains made on research directed towards VA inoculation of agricultural crops provide information directly applicable to forest-tree seedling inoculations.

20.4.2.3 Application of VA inoculum

Given the availability of the above inoculum source, Menge and Timmer [31] list several field-inoculation procedures. VA fungus inoculum can be broadcast and rototilled into seedbeds, a method that has worked well with citrus seedlings [48]; however, a major disadvantage is that large amounts of inoculum are needed to obtain rapid root colonization. VA fungus inoculum can also be banded or side dressed next to seeds or seedlings. This is particularly effective when inoculum quantities are limited [31]; for best results, bands should be placed in an area of root proliferation, usually about 5 to 15 cm from seedlings or seeds. Placing inoculum in layers or pads directly beneath seeds where developing roots will penetrate the inoculum is the most effective. Layering of inoculum has been successful for peach [13] and citrus [10]. If enough inoculum is available, it can be applied with commercial tractor-drawn seeders or fertilizer banders [31].

Seed has been pelleted with VA fungus inoculum, but success of the technique has been erratic so far [31]. Optimum placement of inoculum for rapid root colonization is a problem yet to be solved.

As with ectomycorrhizal inoculations, two major questions must be addressed before,, large-scale VA inoculations are feasible. First, what specific fungus species or mixture of species is best for particular hosts grown under various nursery conditions? Second, how much inoculum is needed to provide adequate mycorrhiza development and ensure healthy seedling growth? The second question is crucial for establishing the cost effectiveness of mycorrhizal inoculation. Fortunately, much of the current practical application of VA mycorrhiza research is focusing on these questions as well as on methods of producing mass inoculum.

20.5 Conclusions and Recommendations

Tree seedlings have evolved a beneficial, mutual dependency upon mycorrhizal fungi for normal root functions. Recognition, utilization, and management of mycorrhizae are part of the skillful production of resilient planting stock. In developing mycorrhiza-management tools, nursery managers and staff must learn to recognize the presence—and absence—of various mycorrhizal types and understand how mycorrhizal fungus populations are affected by nursery operations.

We recommend books on mycorrhizae by Marks and Kozlowski [15] and Schenck [41] as excellent references for nursery staff. Nursery managers are urged to keep abreast of current mycorrhiza research aimed toward practical use in nursery production and reforestation. Mycorrhiza research has truly "mushroomed" over the last decade, and knowledgeable mycorrhiza specialists are available nationwide to assist. The continuing interest and research support we have received from several nurseries convince us that the time is right for garnering the full benefits of mycorrhiza management.

Specific recommendations

- Include mycorrhiza management into the entire nursery management scheme.
- Become familiar with the various types of mycorrhizae and groups of fungi involved in mycorrhizal associations.
- Regularly examine seedling roots to monitor and record mycorrhiza development throughout the nursery.
- Observe and record the effects of new or experimental management practices on mycorrhiza development as well as on other seedling characteristics.
- Be alert to and avoid practices that cause mycorrhiza deficiency.
- Recognize that fumigation destroys mycorrhizal fungus populations in addition to pathogens and weed seed. Consider alternative, selective biocides to eliminate specific pests.
- If mycorrhiza deficiency becomes a problem with newly planted seedlings or if newly cultivated or fumigated ground presents a high risk for developing mycorrhiza deficiency, consider one of the mycorrhizal inoculation options discussed in this chapter.
- Remain alert to research developments on mycorrhizal inoculation of nursery seedlings with pure cultures of fungi proven effective and beneficial.
- Obtain the assistance of a mycorrhiza specialist to help optimize mycorrhiza-management practices.

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