Target Seedling Symposium

Chapter 6
Mycorrhizae and Realistic Nursery Management

C.B. Davey, Carl Alwin Schenck Professor of Forestry, Soil Science and Plant Pathology, North Carolina State University, Raleigh, North Carolina

●

ABSTRACT

In the Pacific Northwest, when producing target seedlings for reforestation of sites that have not been drastically disturbed, there will likely be adequate mycorrhiza development on the seedlings without the necessity of soil inoculation in the nursery. This represents the majority of seedlings being produced. However, problems in the nursery can occur through excessive use of certain pesticides, especially soil fumigants and fungicides. These may require re-establishment of mycorrhizal fungi in the nursery soil. When growing seedlings for planting on drastically disturbed or inhospitable sites, or for planting on natural grasslands, nursery inoculation may represent the difference between success or failure. For the future, there exists ample opportunity to significantly increase forest productivity through matching tree genotypes with mycorrhizal fungus genotypes. At present, however, our knowledge base is inadequate for taking full advantage of these possibilities.

6.1 Introduction

The fossil records clearly show that the early land plants appeared before roots had evolved. These plants were equipped with rhizomes, the precursors of roots. Of interest to this discussion is the fact that the rhizomes of these early land plants were generally associated with fungi in an arrangement similar to mycorrhizae. Thus when Gymnosperms, and later Angiosperms, evolved the root-fungus association was ready and waiting. The point is that mycorrhizae appear to be as old as the plants with which we are dealing. They are not of modern origin and certainly not of man's design.

The mycorrhizal condition was first described by a German forest pathologist (Frank 1885). This was followed by a 30- to 40-year period of open warfare over whether this was a pathological or beneficial relationship. As late as 1918, the pathological nature was still assumed (Rankin 1918, 82-84): "A short account of (mycorrhizas) is, however, of interest since the structures are now generally considered to represent a diseased condition of the roots and not a true type of symbiosis or mutual-advantage relation, as was previously believed by many."

For the purpose of this discussion of the implications of mycorrhizae to the production of "target seedlings," we need to learn what the practical nursery manager should know and/or do about the mycorrhizae on the seedlings being produced. More than 40 years ago, Wilde (1944) concluded that 99 percent of all practicing foresters will not have to lose any sleep over the problem of mycorrhizal inoculation. That turned out to be a bit optimistic. However, in areas where native tree seedlings are produced for outplanting on sites that are not drastically disturbed, inoculation is generally not necessary. However, the term native species must be taken carefully. In 1953, I worked with Wilde in Wisconsin, and we had to inoculate an entire nursery which was built to produce native (to Wisconsin) tree species. The problem was that the area selected for the nursery was on prairie soil and thus ectomycorrhizal fungi were entirely absent.

On a more global basis, Mikola (1980) wrote a review of the movement of mycorrhiza inoculum across international boundaries as foresters sought to plant exotics in various parts of the world. Such movements of inoculum have been absolutely essential for the successful establishment of such species in new places. Once both a tree species and its associated mycorrhizal fungus (or fungi) have been established in a favorable soil and climate, the inoculum remains viable for a long time. This is true both in bare root nurseries and field plantings. The exception is in containerized nurseries where inoculation is necessary with every crop.

6.2 Essentiality and Physiology of Mycorrhizae

The essentiality of roots is hardly in question. There are some difficult questions concerning roots, however, that warrant at least some consideration. These include: Why don't roots die? Why do roots die? And what effects do mycorrhizae have on the answers to the first two questions? Finally, then, are mycorrhizae important to seedling survival and growth? Are there quantitative and qualitative differences among mycorrhizae formed by different fungi? And if there are, what should the practical nursery manager do about the situation, if anything? Hopefully, we will address all of these questions as we progress through this discussion.

Let's start with a brief summary of the effects that have been attributed to mycorrhizae. Discussion of these will come later:

1) Uptake of poorly mobile or immobile nutrients.

2) Absorption of water and of mobile ions as soil dries and diffusion rates decrease.

3) Countering of toxicities caused by high exchangeable aluminum, strong acidity, strong alkalinity, high salts, and heavy metals.

4) Tolerance of high soil temperature.

5) Improved soil aggregation and thus protection from wind or water erosion.

6) Nutrient conservation on the planting site through very efficient recycling of nutrients that become available.

7) Root protection from disease through antibiosis, physical barrier to pathogen penetration of host tissue, improved tree nutrition which can quickly compensate for the loss of feeding roots, and avoidance of infection through enhanced suberization of fine roots.

The typical planting site is less than optimal in some respects. Thus, the problems associated with yield improvement are at least involved with improving stress tolerance (Jones 1985). There is ample literature to show that seedlings with proper mycorrhizae have increased tolerance to stresses imposed by low soil fertility, low available moisture, and root pathogens. Not all mycorrhizal fungi impart all of these benefits, but each benefit has been demonstrated as attributable to the mycorrhizal condition.

6.3 Natural Status of PNW Soils

This statement can be fairly short and to the point. There is no shortage of fungi capable of forming mycorrhizae on the roots of the native tree species in the PNW (Trappe 1977). Just as there is a decided change in the tree species east and west of the Cascade crest, so there is a change in the mycorrhizal fungi associated with those species and on those different soils. Somewhat less dramatic changes also occur east and west of the crest of the Coast Range.

6.4 Ecology of Roots, Including Mycorrhizae

6.4.1 Root and mycorrhiza physiology

There are three main parts to any tree root system. These include: 1) the large structural roots, 2) the long exploratory fine (1 -2 mm in diameter) roots, and 3) the short, fine roots. It is these short, fine roots that become infected with certain specific fungi and produce the mycorrhizae. In certain tree species, it is only the ultimate short roots that become infected and form ectomycorrhizae. In all other tree species, the vescicular-arbuscular mycorrhizal (VAM) fungi infect both types of non-suberized fine roots. Typically, the fine roots average about 5 percent of the root mass but about 90 percent of the root length (Bowen 1985). The mycorrhizal hyphae greatly extend this length.

The amount of photosynthate going to roots is usually underestimated. The standing root biomass, at any given moment, is about 20-30 percent of the total tree biomass. Carbon lost from root respiration, root exudates, sloughed cells, and fine root turn-over are missed. Estimates of total photosynthate going to roots of PNW tree species have varied from a low of 8 percent to a high of 66 percent (Bowen 1984). The mycorrhizal fungi on Pacific silver fir have been estimated as using about 15 percent, of the total photosynthate (Vogt et al. 1982). In a very detailed study of 6-month-old loblolly pine seedlings, over a 12 week period, Vongkaluang (1978) showed that 30 percent of the photosynthate went to the root system while 25 percent of the seedling weight was in the root system. Details of the energy partitioning are shown in Table 6.1.

The energy and carbon cost of maintaining the root system is decreased by the sloughing-off of fine roots during times when conditions are not conducive to growth. This turnover of fine roots usually exceeds the annual turn-over of foliage (Sanchez et al. 1989). Rapid production and turn-over of fine roots and mycorrhizae give the perennial plant great plasticity in dealing with environmental stress. It also suggests one reason why nursery inoculation of seedlings with mycorrhizal fungi may not last long in normal forest soil. On the other hand, in severely disturbed locations or in places where the tree species is introduced as an exotic, the fungus may perpet-

Table 6.1—*Energy partition by root systems of 6-month-old loblolly pine seedlings over a 75 day period.*

	Energy partition by root system (small calories/day)			
Day	Root tissue	Respiration	Sloughings	Exudates
0	60	15	15	30
25	100	25	25	40
50	140	70	60	70
75	560	150	90	80

This table is adapted from Vongkaluang 1978.

uate itself through many generations of mycorrhizae (see Section 6.6).

At this point, it is appropriate to address the two related questions of why roots *don't* die and why roots *do* die. There are various cycles in the plant, but the place to start this discussion is at a point where the seedling has a small excess of foliage in relation to the fine roots. At that point, the foliage produces more carbohydrate than the top can use since the roots are inadequate for providing a commensurate supply of mineral nutrients and water. The result is that the excess carbohydrate is translocated to the root system. This allows the formation of new fine roots in locations in the soil where the nutrients have not been excessively utilized. The new fine roots both lose organic exudates to the rhizosphere and become invaded by mycorrhizal fungi. The exudates tend to feed a large population of saprophytic microbes in the rhizosphere and these in turn discourage the invasion of the roots by pathogenic organisms. In some cases, the mycorrhizal fungi also protect the roots from certain pathogens (Marx and Davey 1969). These new mycorrhizal roots are very efficient in the uptake of nutrients from the soil in their vicinity. They supply the foliage which then begins to form more structural material in the top. As the network of mycorrhizal fungi expands in the soil, they require increasing supplies of carbohydrate from the top to sustain their respiring biomass. At some point, the cost to the plant in carbohydrate exceeds the value in returned nutrients. At that point, the plant either reduces the carbohydrate being translocated to the fine roots or it forms an abscission layer at the base of the fine root. In either case, the fine root can no longer supply the mycorrhizal fungi or the rhizosphere saprophytes with the needed carbohydrate, and the fine root either dies from starvation or it is invaded by some weak pathogen which kills it. After this has happened to enough fine roots, we are back to the initial step and the process repeats. This is actually a very efficient strategy for the plant since new mycorrhizal roots in nonexplored soil are much more energy efficient than larger, older ones in exploited soil. It also helps explain the high rate of turnover of the fine roots. Non-mycorrhizal fine roots have much higher carbon demand per unit of nutrients taken up and thus they pass the break-even point much sooner than mycorrhizal roots. Con-
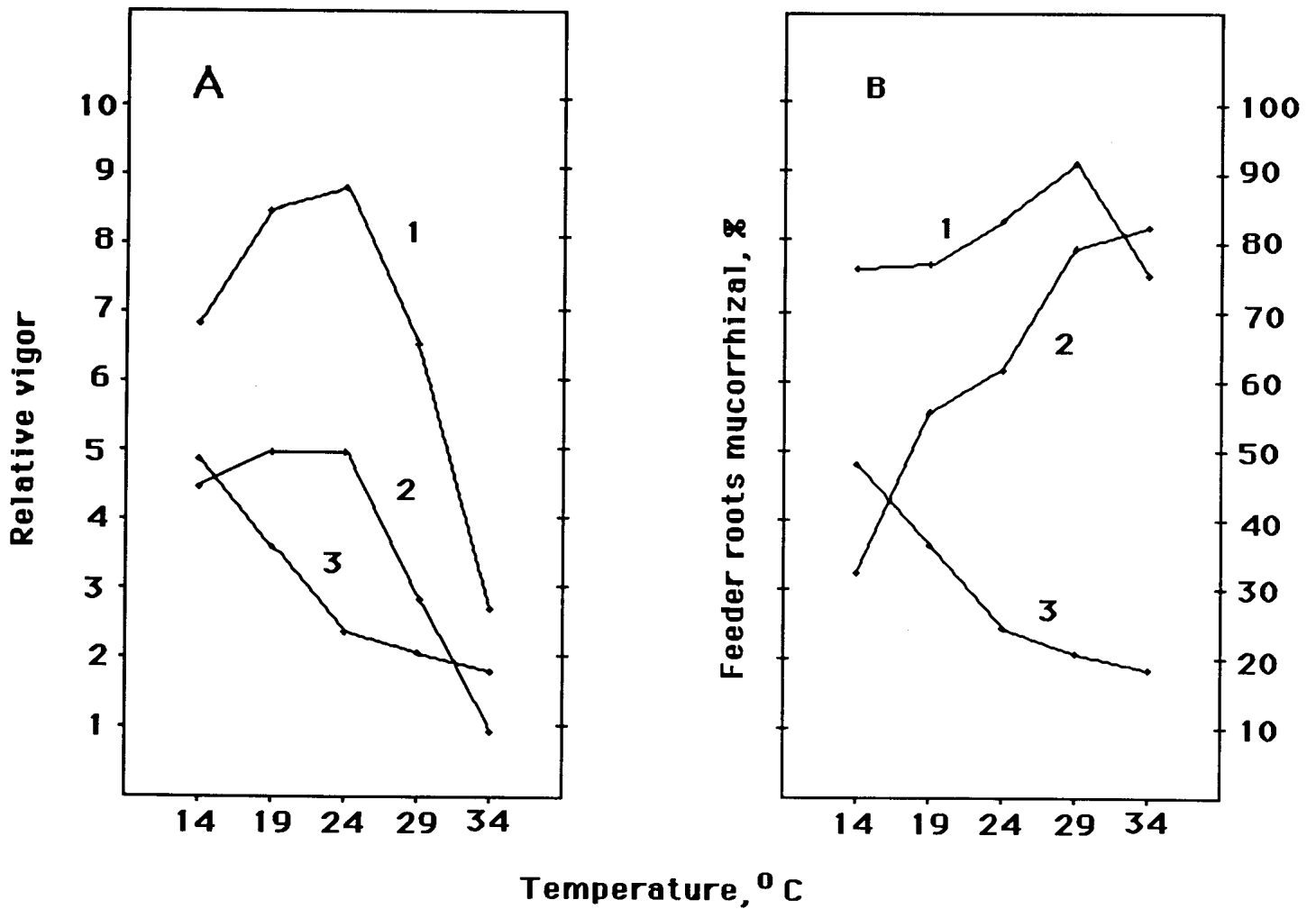
Figure 6.1—*Relation between ectomycorrhizal development (2) an loblolly pine seedlings by Thelephora terrestris (A) and Pisolithus tinctorius (B) and relative seedling vigor (1) as influenced by soil temperature. Control line (3) represents relative vigor of nonmycorrhizal seedlings (from Marx et al. 1970).*

sequently, the mycorrhizal condition actually extends the life-expectancy of the short, fine roots. Mycorrhizae have been reported to absorb P and Zn at about the same rate per unit of surface area as non-mycorrhizal, non-suber-ized roots (Bowen 1985). Bowen (1985) also reported that the mycorrhizal hyphae length-to-weight ratio is at least 500 times that of the fine, non-suberized roots. Thus we may conclude that the uptake of P and Zn are at least 500 times greater per unit weight of carbon devoted to the mycorrhizal hyphae than to that devoted to the fine roots. The pattern of uptake of poorly mobile nutrients, such as P, is first an intense depletion of a narrow band around the root. This is delineated by the length of the root hairs on plants where they exist. Then there is a limited deple-tion beyond this, depending on both the ion and the soil colloids involved. Then, with development of mycor-rhizae, soil further out is explored, often intensely. This is because the hyphae not only extend much farther than the root hairs but they also branch and by virtue of their small diameter they can enter much smaller pores than

root hairs. Finally, mycorrhizal hyphae produce phos-phatases and phytases that permit them to obtain P direct-ly from the humus. Roots frequently possess similar enzymes but they seldom have access to the humus layer.

Both added P and mycorrhizae caused considerable rate increases in photosynthesis and dry matter accumulation (Rousseau and Reid 1990). At low and medium levels of mycorrhizal development, net photosynthetic response was due to enhanced P uptake. At high levels of mycor-rhizal development, as much as 1 7 percent of the growth increase was attributed to "mechanisms other than enhanced phosphorus nutrition" (Rousseau and Reid 1990). All inoculation was done at a very low (0.1 ppm) solution P level. The other mechanisms are usually assumed to be hormonal in nature.

Generally, mycorrhizal hyphae will be more important in the uptake of ammonium (NH_4^+) than nitrate (NO_3^-) since nitrate is highly mobile and will get to the roots via

mass flow (soil solution movement to roots) when transpiration is active, and by diffusion when it is not (Bowen and Smith 1981). Ammonium, being a cation, is more tightly bound to soil particles on the cation exchange (CEC) sites and thus does not move to the roots. The foraging mycorrhizal hyphae are effective at accumulating ammonium from the CEC sites and transporting it to the host plant. Also, there is some evidence that the mycorrhizal hyphae are able to accumulate simple organic nitrogen compounds directly from the humus layer. In fact, this has been used as an argument as to why the humus accumulates on some sites . The mycorrhizal hyphae appear to be more efficient at getting the nitrogen from the humifying material than the soil saprophytes. This leaves the carbonaceous residue deficient in nitrogen and hence its rate of decomposition is reduced and the thickness of the humus layer increases.

There has been considerable discussion in the literature regarding the ability of forest trees to use nitrate nitrogen. In order for any plant to utilize nitrate, it must contain an enzyme known as nitrate reductase. This enzyme converts nitrate to a form that the plant can use for growth. One possible mode of action would be for the mycorrhizal fungi to take up and reduce the nitrate and pass the reduced N on to the host plant. An investigation of nitrate reductase activity by nonmycorrhizal fine roots of Douglas-fir and by seven ectomycorrhizal fungi showed that the roots possessed more nitrate reductase than any of the fungi by at least a factor of six (Ho and Trappe 1980). However, the Douglas-fir roots do not have what would be considered high nitrate reductase activity. In comparison with a plant like wheat which does efficiently utilize nitrate, the Douglas-fir roots were only one-eighth to one-fourth as active. And the mycorrhizal fungi were as little as 1 percent as active. This research shows that for Douglas-fir at least, while nitrate may be used, it is not an efficient nitrogen source, and while the mycorrhizal fungi may help some in nitrate accumulation, they do not enhance its reduction prior to utilization by the plant.

Progeny testing in tree improvement programs has given indirect evidence to support the idea that at least one of the causes of poor performing progeny is poor roots. This allows us to speculate that either 1) genetic improvement in trees may reflect better roots and thus reduce our concern over mycorrhizae or 2) if a poor performer possesses a very desirable trait (wood property, disease resistance, etc.) we may be able to improve its performance through inoculation with appropriate mycorrhizal fungi.

6.4.2 Natural selection
In several studies, involving different techniques, it has been found that inoculum collected from beneath stands of various species of trees will often result in mycorrhiza formation on a given species that is being tested. The most successful inoculum, however, is almost always from beneath the species being inoculated. This suggests that there has already been considerable natural screening of candidate fungi by nature.

6.4.3 Interactions
The influence of soil temperature on mycorrhiza formation has been investigated under conditions of both low (Amaranthus and Perry 1989) and high (Marx et al. 1970) temperature. In soil temperature incubators, Marx et al. (1970) investigated aseptic synthesis of mycorrhizae on loblolly pine from a soil temperature of 14° to 34° C with the mycorrhizal fungi Thelephora terrestris (Tt) and Pisolithus tinctorius (Pt). With Tt, mycorrhiza abundance increased from 14° to 24°. It then decreased rapidly from 24° to 29° and was zero by 34° (Figure 6.1). With Pt, mycorrhiza development continued to increase all the way to 34° while seedling development peaked at 29°. In a second study, Marx and Bryan (1971), investigated the effects of an extreme soil temperature (4O°) for 5 weeks on the survival of loblolly pine seedlings that were mycorrhizal with Tt or Pt, or were non-mycorrhizal. Survival of non-mycorrhizal seedlings was 45 percent, of Tt-mycorrhizal seedlings it was 70 percent, and of pt-mycorrhizal seedlings it was 95 percent. These results showed that there was as much difference between mycorrhizal fungi (95 - 70 = 25 percent) as there was between mycorrhizal and non-mycorrhizal seedlings (70 - 45 = 25 percent). Subsequent work by numerous investigators in various climates and soils has confirmed that Pt offers a real benefit where the planting site is likely to be hot, but is of no particular value on cold sites (e.g., Riffle 1989).

In a detailed study of the effect of temperature on the growth of various mycorrhizal fungi in pure culture and on mycorrhiza formation with radiata pine, Theodorou and Bowen (1971) found very strong relationships. Generally, they found 25° C to be optimum for both growth of the fungi in culture and mycorrhiza formation. Growth was almost nil at 15° and very low at 30°. In a sandy soil, after 14 weeks, 36 percent of seedling roots were mycorrhizal at 25° while only 6 percent were at 15°. There were differences among the fungi tested. The authors concluded that there is a need to select fungi for mycorrhizal inoculation on the basis of the soil temperatures appropriate to the season and site, as well as to their ability to stimulate seedling growth.

Different plant responses to both ectomycorrhizal and VAM fungi may be related to their relative ease and speed of infection, differences in their rate of spread throughout the root system, and the growth pattern of the hyphae in the soil. Hyphal growth in the soil is difficult to study, but several fungi have been traced for at least 12 cm (5 inches) from the root surface. On the other hand, soil compaction has been shown to be quite adverse to hyphal penetration. Compaction from bulk density 1.2 to 1.6, which is not at all uncommon, reduced hyphal penetra-

tion by 90 percent (Bowen 1980). This would be expected to seriously reduce nutrient uptake and subsequent tree growth or even survival. This has occurred frequently on skid trails and decks and unfortunately also in nurseries. Often, ripping or ripping plus discing is all that is needed to restore the productivity of such compacted areas in the field or nursery.

When one is investigating the value of a plant being mycorrhizal, it is not correct to compare the performance of a large mycorrhizal plant with a small non-mycorrhizal one (Bowen 1980). Rather the correct comparison is between the mycorrhizal plant and a non-mycorrhizal plant that has been fertilized sufficiently to reach the same size as the mycorrhizal one. Then their behavior can be fairly compared and also their cost of production can be evaluated. This restriction should also be used when comparing plants that are mycorrhizal with differing fungi. Only then can truly equitable comparisons be made. It has been noted that mycorrhizal plants have a lower root/shoot ratio than non-mycorrhizal ones. This disappears when the non-mycorrhizal plant is fertilized up to the same size as the mycorrhizal one, and suggests that the root/shoot ratio is really a function of plant nutrition and not something peculiar to the mycorrhizal condition (Bowen 1980).

Growth depression as a consequence of a seedling being mycorrhizal, while rare, is possible. One circumstance in which this occurs is during the time that the mycorrhizal association is forming. The plant must invest much photosynthate to the association before it realizes any benefit from it. This is probably common but of such short duration as to be of little consequence. In highly fertile soil, where the mycorrhizae are of reduced value to the seedling but where their formation is not significantly inhibited, the seedling invests more than it receives in return. In some estimates, the mycorrhizal fungus accounts for up to 17 percent of the total root system weight, a significant investment if not needed or if not needed in such abundance. Imbalanced nutrition can occur and result in growth suppression. In soils with very high P levels, the mycorrhizae tend to overload the seedling with P and this upsets nutrient balances within the plant and actually depresses growth. One of the most common problems in nursery fertilization is excessive P application. Thus this type of growth suppression is probably more common than we realize. Unfortunately, the common response to less-than-desired growth is to apply more fertilizer and in some cases this may be exactly the wrong response. It is certainly counter productive.

6.5 Mycorrhizae and Nursery Practice

6.5.1 Fumigation
Fumigation of the nursery soil is the single most important

operation in nursery soil management, as far as the mycorrhizal fungi are concerned (Danielson and Davey 1969). Methyl bromide and chloropicrin are both general poisons with chloropicrin more toxic to fungi than methyl bromide. Soil fumigation has been conducted safely in our nurseries for about 30 years. There have been some mistakes made, occasionally, because of improper metering of the fumigant or intentional high rates of application. Soil temperature, moisture content, and organic matter content all affect the results of any given fumigation. The damage done to mycorrhizal fungi differs between the ecto- and endomycorrhizal fungi. With a nursery that is surrounded by ectomycorrhizal trees, there is a delay in mycorrhiza formation, following an overdose of the fumigant, but the ectomycorrhizal fungus spores are air-borne and will naturally re-inoculate the soil over the first growing season. The spores of the endomycorrhizal fungi are soil-borne and do not blow around.

Consequently the adverse effects of an overdose of fumigant will persist considerably longer where endomycorrhizal seedlings are grown compared with where ectomycorrhizal seedlings are grown. An interesting example of the effect on an endomycorrhizal species was seen in the D.H. Phipps Nursery (Elkton, Oregon) several years ago. Because of high soil moisture and low soil temperature at the time of fumigation, the fumigant action was concentrated more in the top few inches than usual. The endomycorrhizal fungi were essentially eliminated. During bed shaping, a little non-fumigated soil was dragged into the first few feet of each bed from the road. The crop planted was western redcedar and except in those first few feet near the road, where those seedlings were mycorrhizal, the crop was a failure.

6.5.2 Fertilization
There has been concern that inoculum that is successful in stimulating seedling growth in the rich environment of the nursery soil may not be particularly effective in the much less fertile soil in the field. Lamar and Davey (1988) found that VAM fungi they isolated from green ash *(Fraxinus pennsylvanica)* from a low P field location was highly effective in stimulating seedling growth in a high P nursery soil. They concluded that there are at least some VAM fungi that are effective in both fertile and infertile soil in stimulating the growth of seedlings and young outplants.

In soil with less than 15 ppm available P, roots of citrus seedlings infected with the pathogen *Phytophthora parasitica* and the VAM fungus *Glomus fasciculatus* were healthier and weighed more than roots infected by the pathogen alone (Davis and Menge 1980). Above 56 ppm P in the soil, the beneficial effect of the mycorrhizal fungus was lost. The evidence strongly suggests that the tolerance to infection by the pathogen in trees with the mycorrhizal fungus is associated with improved plant

Table 6.2—*Influence of <u>Glomus fasciculatus, Phytophthora parasitica</u> and soil phosphorus on root health and root infection by <u>Glomus fasciculatus.</u>*

	Healthy roots (%) Soil P (pm)			Root tissue infected with Glomus fasciculatus (%) Soil P (ppm)		
Inoculation	6	56	600	6	56	600
Non-inoculated	100z	100z	100z	0a	0a	0a
Phytophthora parasitica (Pp)	33v	74xy	86y	0a	0a	0a
Glomus Fasciculatus (Gf)	100z	100z	100z	54d	38c	12b
Pp plus (Gf)	50w	72x	79xy	27c	30c	8b

This table is adapted from Davis and Menge 1980. Values within a comparison (Healthy roots and Root tissue infected with *G. fasciculatus)* followed by the same letter are not significantly different ($p = 0.05$) according to Duncan's Multiple Range Test.

nutrition, especially P. Although the disease is significantly reduced in low P soil, there is no apparent direct effect of the mycorrhizal fungus on the pathogen. Rather, the effect is indirect through improved tree nutrition (Table 6.2).

6.5.3 Irrigation
Irrigation, as long as it is adequate for the seedlings and the water is of acceptable quality, is seldom a determinant of mycorrhiza formation or function. In general, the tree seedlings will suffer from improper (inadequate or excessive) irrigation or low quality water sooner and more severely than the mycorrhizal fungi.

6.5.4 Root pruning
It seems strange that we increase the number of roots by removing roots from the seedling. However, that is exactly the outcome of proper root pruning, both undercutting and lateral pruning. The removal of apical meristems of the initial roots allows several new roots to be formed in their place. These new roots appear in the zone where some mycorrhizal activity has already occurred and they become mycorrhizal and active relatively soon. The number of times any crop should be undercut has been the subject of several investigations. We can summarize them by saying that the greatest effect is caused by the first undercutting. The second one still produces significant changes. The third and subsequent undercuttings produce progressively smaller changes. There is a species factor in these results and probably soil and climatic ones as well, but in general, the effect continually decreases in intensity as the number of undercuttings increases but the fibrosity and mycorrhizal intensity do increase. The nursery manager should be able to decide when enough is enough.

6.5.5 Fungicides and other pesticides
Other than the general poisons, such as methyl bromide and chloropicrin, pesticides are more-or-less restricted to a narrow range of target organisms. In general, we can say that herbicides have little if any effect on mycorrhizal fungi. Insecticides and bacteriocides have slightly more effect. The fungicides have the most effect and the effect varies with the specific chemical. In the South, the systemic fungicide Bayleton is routinely used for control of fusiform rust on southern pine seedlings. This fungicide must be used carefully, however, since it is also toxic to several fungi that form mycorrhizae on the same tree species that are being protected from the disease. However, with proper usage, the only noticeable effect is a slight delay in the initiation of mycorrhiza formation. There is no loss in seedling quality and there is great reduction in the disease. Some fungicides, such as Subdue, have no apparent adverse effect on mycorrhiza formation.

6.6 Seedling Performance in the Field
If one is to select mycorrhizal fungi for inoculation in the nursery on the basis of improved nutrient uptake, it is better to select on the basis of uptake of nutrients from low concentrations rather than on the basis of a high rate of uptake. Most mycorrhizal fungi will be able to take up nutrients fast enough for optimum growth, but the ability to be effective in very low nutrient concentrations, which are common in forest soils, is very important (Lamar and Davey 1988). Some mycorrhizal plants appear to increase the availability of nutrients in the rhizosphere, by producing organic acids, phosphatases, and iron siderophores. Selection for these traits may also be important. The forester has both the tree and mycorrhizal fungus genomes from which to select in order to enhance tree growth and wood production (Bowen 1985). How to take advantage of these possibilities is still mostly a mystery. When one considers that approximately 2,000 individual fungal species will form mycorrhizal associations with Douglas-fir (Trappe 1977), we certainly have plenty to select from. The problem lies in the fact that we know very little about the behavior of nearly all of them. This makes realistic selection all but impossible. Making the problem even more difficult is the fact that there are large strain differences within individual genera and species. For example, there are strains of *Pisolithus tinctorius* that will form ectomycorrhizae on pines but not on eucalypts. There are other strains that will form mycorrhizae on eucalypts but not on pines. Finally, there are strains that will form mycorrhizae on both pines and eucalypts. These basic differences must be accounted for even before we consider any traits that are related to nutrient uptake or other properties.

Another confounding situation is the fact that just as there is a succession of plants on an area, so there is a success-

sion of fungi that form mycorrhizae on roots as trees age. This fact discourages some people from even considering trying to influence the mycorrhizal fungi that will dominate the roots of their seedlings. This is quite short-sighted, however. If proper fungi are established on seedling roots and this results in improved survival and early growth of those seedlings, we will have improved the stocking of the new stand. That in itself is a major gain in productivity in most locations. Then if the early height advantage is merely maintained through the growth of the stand the wood production will be again increased. I have heard foresters say, with some scorn, "What difference does an extra five feet of non-merchantable top make?" The fact is that they are looking at the wrong end of the tree. The five extra feet are in the butt log, not in the top, and it makes a great deal of difference. The bottom line on this point is that the succession of mycorrhizal fungi which inhabit tree roots in the field should not diminish our appreciation for the positive effects that are potentially possible from nursery inoculation.

In site prepared forest soil, a mycorrhizal fungus species that infects and responds quickly is likely to give seedlings a competitive advantage over other plants on the site. They will capture the site and become the dominant vegetation in the least amount of time. Then the photosynthate that is produced on the site will be deposited as wood in the crop trees rather than in some of the competition. This can have significant economic consequences since it will either shorten the rotation or increase the wood produced.

In a recent study, it was reported that rapid new root and mycorrhiza formation occurred in Douglas-fir outplants in cold planting sites in the Klamath Mountains in response to adding soil from a good Douglas-fir plantation to the planting hole (Amaranthus and Perry 1989). Inoculated seedling survival rate was 36 percent while non-inoculated survival rate was only 11 percent. Such a technique may be useful in the reforestation of difficult sites. Possibly the transferred soil contained mycorrhizal fungi that represent different successional stages of mycorrhizae. The authors concluded, however, that more study would be needed to identify the specific microorganisms involved and their effects and interactions in the transfer soil.

Places where the succession of mycorrhizal fungi does not occur, or occurs very slowly, include drastically disturbed lands (e.g., mine spoils) and areas where the tree is being planted as an exotic. As an example, I have been working in a vast grassland in Venezuela called the Ilanos. There are no native ectomycorrhizal plants on the Ilanos. In this project, about 100,000 acres (40,000 ha) of *Pinus caribaea* have been planted annually for about 20 years. The original 54 seedlings that were brought from Trinidad were mycorrhizal with *Thelephora terrestris*

(Tt). All seedlings in the Venezuelan project were inoculated from those or subsequent trees until 1982, when *Pisolithus tinctorius* (Pt) was introduced. There are four nurseries on the project and each is inoculated annually with Pt spores. The surrounding plantations supply plenty of air-borne Tt spores. A detailed assessment of both the plantations and the nurseries was made this past February. All trees and seedlings inspected were mycorrhizal and, regardless of age, Tt mycorrhizae dominated. In the younger plantations, Pt mycorrhizae formed a small percent of the total mycorrhizae. There has been no succession of the mycorrhizal fungi in the stands over the 20 year period simply because the only alternative was for the trees to be non-mycorrhizal and any that may have tried that route are no longer around to be counted.

Since an abundance of mycorrhizae requires a considerable investment in carbon and energy by the plant, the question could be asked as to how many or how much mycorrhizae is optimum. This question was investigated by Last et al. (1990) with Sitka spruce *(Picea sitchensis)* for two years in four different field soils (two peats and two mineral soils). They inoculated the seedlings with either *Laccaria proxima* (one isolate) or *Paxillus involutus* (two isolates) and found that with either fungus, the height growth was positively related to the total numbers of mycorrhizae per plant. They concluded that, irrespective of treatment, seedlings with similar numbers of mycorrhizae tended to be of similar height (Figure 6.2).

Other findings in the study by Last et al. (1 990) were that the *Laccaria* isolate increased mycorrhizal numbers per plant more than either of the *Paxillus* isolates; fewer mycorrhizae were formed in peat than in mineral soil; at the end of the first year, most of the mycorrhizae in the peats came from the inoculum while there was a considerable range in the mineral soils (from 7 percent in one of the *Paxillus* isolates to 1 00 percent with the *Laccaria* isolate); by the end of the second year, *Laccaria* still accounted for 77 percent of the mycorrhizae but the two *Paxillus* isolates each accounted for less than 5 percent; and irrespective of treatment, seedlings with similar numbers of total mycorrhizae tended to be the same size. The authors' final conclusion was that an increase in numbers of mycorrhizae per plant from very few to some (e.g., 10^1 to 10^2) is hardly noticeable while an increase from many to a great many (e.g., 10^3 to 10^4) causes really discernable differences in yield.

Interactions between tree species and mycorrhizal fungi have been shown to influence field survival of outplanted seedlings. Richter and Bruhn (1989) inoculated 8-week-old red and jack pines with four different fungi and grew them for an additional 26 weeks, after which they were outplanted. Survival was checked after one and two growing seasons in the field (Table 6.3). Red pine inoculated with *Laccaria* bicolor survived about 20 percent better in
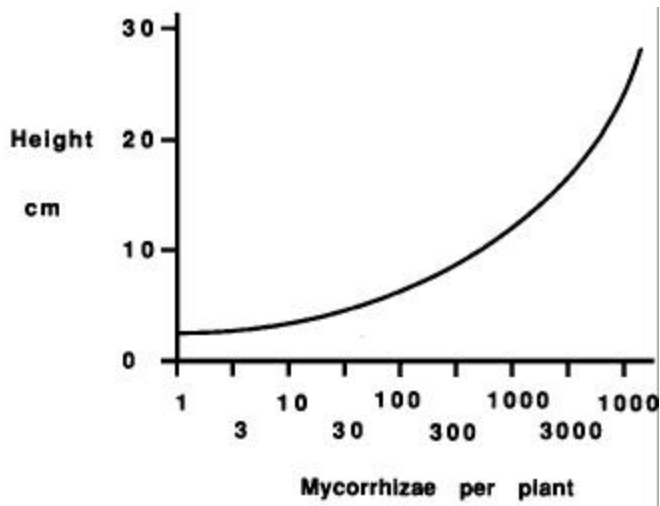
Figure 6.2—*Height of two-year-old Sitka spruce seedlings as affected by the number of mycorrhizae per plant (adapted from Last et al. 1990).*

both years than the *Thelephora terrestris* controls. Other treatments did not significantly affect survival. Data show that seedlings inoculated with *Laccaria bicolor* had significantly more mycorrhizae per plant than with other inocula. Jack pine exhibited a nonsignificant 10 percent increase in survival.

In a comparison of ponderosa, scots, and Austrian pines that were either inoculated with *Pisolithus tinctorius* or

Table 6.3—*First and second year survival of container-grown red and jack pine seedlings outplanted on an excessively drained sandy soil in Baraga County, Michigan.*

Pine species	Year	Control[1]	Laccaria bicolor	Scleroderma citrinum	Isolate W31-2B2
				----%	
Red	1	52.7	74.0**	60.7	62.0
	2	35.3	54.0*	35.3	40.0
Jack	1	67.0	77.0	70.0	68.0
	2	59.0	70.0	60.0	59.0

This table adapted from Richter and Bruhn, 1989. The ** and * represent significant difference from the control treatment at the p = 0.005 and 0.05 level, respectively.

[1]The control seedlings were naturally mycorrhizal with *Thelephora terrestris.*

[2]The W31-2B isolate was *a* confirmed mycorrhiza former but it had not been identified.

were allowed to become mycorrhizal with indigenous fungi and outplanted in prairie soils in both Kansas and Nebraska, survival and growth were monitored for 5 years (Riffle 1989). While the Pt remained viable on all three pine species, it did not increase either survival or growth when compared with mycorrhizal, but not inoculated, stock. Possible reasons for the lack of response included mostly non-acidic soils (pH 6.8 to 7.1 in Kansas and pH 5.5 to 8.0 in Nebraska) which result in induced Fe deficiency and thus reduced carbohydrate for the mycorrhizal fungi, replacement of Pt by other mycorrhizal fungi brought to the planting site from the nursery, insufficient inoculum used in the nursery to produce seedlings with a very high percent Pt mycorrhizae, and little high temperature stress. It has been noted above that Pt is particularly valuable where there is a high soil temperature. Inadequate inoculation with Pt spores was recently reported by Marx (personal communication) to be responsible for low Pt mycorrhization on *Pinus caribaea* in Venezuela in competition with *Thelephora terrestris.* In that case, Marx suggested that the inoculation rate be increased from 20 g to 6 kg/ha (0.25 oz. to 5.5 lbs/acre).

It has been proposed that mycorrhizae may increase the tolerance of trees to contaminated soil, especially where heavy metals are concerned. Since paper birch has shown the ability to grow relatively near the large nickel smelter in Sudbury, Ontario, seedlings were inoculated with four different mycorrhizal fungi and grown at two levels (high and very high) of either nickel or copper (Jones and Hutchinson 1986). The authors did find that seedlings that were mycorrhizal with *Scleroderma flavidum* were more nickel tolerant than seedlings inoculated with the other three fungi. The authors proposed both a passive and an active mechanism for the nickel tolerance. None of the fungi increased copper tolerance. Thus, this effect appears to be highly specific.

6.7 Alternative Futures

As we look from the present to the future and ever better target seedlings, what can we discern from this review of what is known, and what are the potentials of mycorrhiza management? For the present, the practical nursery manager must remember that the mycorrhizal fungi are in the soil and should not be overly abused. This abuse may arise from over-fumigation; improper use of certain pesticides, especially some fungicides; and over fertilization, especially with phosphorus. This last point does not really represent actual abuse of the mycorrhizal fungi but it may significantly impair the ability of the tree seedling and the fungus from entering into this beneficial (essential) association.

Eventually, it may be important to know both the genotype of tree seed being planted *and* the genotype of the mycorrhizal fungus being involved in the synthesis of the

mycorrhizae. In fact, although we seldom have to consider it at present, we are not raising a single species—we are raising at least a dual species (tree+fungus) and usually a multiple species (tree+fungi). The exact combination of tree and fungus can have major consequences in silvicultural decisions. As pointed out by Bowen (1980), "Infection with an appropriate mycorrhizal fungus can radically change the estimate of the production potential of a soil and its fertilizer requirement." In other words, Site Index estimates, as determined by tree measurements, can be drastically different, depending on the mycorrhizal fungus infecting the roots. Bowen (1980) further stated, "Almost no nutritional studies in either tropical or temperate plants have ensured adequate mycorrhizal infection was present." Even that will not be the ultimate in intensive forest management. Rather than just "adequate" infection, we will need to know by what genotype of which fungus is infection caused. I mentioned this possibility to the director of our regional forest nutrition cooperative and his only comment was that he hoped he would be retired before such precision in our research were required. I suspect he will be, but that does not diminish the fact that the possibility exists and we should not be surprised when it eventually becomes the norm, rather than some researcher's dream. Certainly we will need to learn a great deal more about the fungi themselves before we can take even the first steps in the direction of this possible future. However, I would not have fulfilled my responsibility if failed to at least mention the possibilities involved.

In some distant future, we may be able to combine our knowledge of tree genetics with a vastly improved understanding of the ecology of the mycorrhizal fungi and some of the newer information on tree nutrition such as that now coming out of the laboratory of Torsten Ingestad in Sweden (Ingestad et al. 1981). Some day we may be able to spend less money, do less environmental damage, produce much more wood of excellent quality in less time on less area, and give the spotted owl rest while offending no one—neither the preservationist, the conservationist, the ecologist, the forester, the logger, nor the mill manager. Sounds nice, doesn't it? Let's work toward that future.

LITERATURE CITED

Amaranthus, M.P.; Perry, D.A. 1989. Rapid root tip and mycorrhiza formation and increased survival of Douglas-fir seedlings after soil transfer. New For. 3:259-264.

Bowen, G.D. 1980. Mycorrhizal roles in tropical plants and ecosystems. In: Tropical Mycorrhiza Research:165-190. Mikola P. Clarendon Press, Cambridge.

Bowen, G.D. 1984. Tree roots and the use of soil nutrients. In: Bowen, G.D.; Nambiar E.K.S. eds. Nutrition of Plantation Forests:147-179. Academic Press.

Bowen G.D. 1985. Roots as a component of tree productivity. In: Cannell, M.R.; Jackson, J.E. eds. Attributes of Trees as Crop Plants:303-315. Institute of Terrestrial Ecology.

Bowen G.D.; Smith, S.E. 1981. The effects of mycorrhizas on nitrogen uptake by plants. In: Clark, F.E.; Rosswall, T. eds. Terrestrial Nitrogen Cycles. Ecol. Bull. (Stockholm) 33:237-247.

Danielson, R.M.; Davey, C.B. 1969. Microbial recolonization of a fumigated nursery soil. For. Sci. 15:368-380.

Davis R.M.; Menge, J.A. 1980. Influence of *Glomus fasciculatus* and soil phosphorus on Phytophthora root rot of citrus. Phytopathology 70:447-452.

Frank, A.B. 1885. Ueber die auf Wurzelsymbiose beruhende Ernhnung gewisser Bume durch Unterirdische Pilze. Ber. dtsch. bot. Ges. 3:128-1 45.

Ho, I.; Trappe, J.M. 1980. Nitrate reductase activity of nonmycorrhizal Douglas-fir rootlets and some associated mycorrhizal fungi. Plant Soil 54:395-398.

Ingestad, T.; Aronsson, A.; Gren, G. 1981. Nutrient flux density model of mineral nutrition in conifer ecosystems. Studia For. Suecia 1 60:61-71.

Jones, H.G. 1985. Strategies for optimizing the yield of tree crops in suboptimal environments. In: Cannell, M.G.R.; Jackson, J.E. eds. Attributes of Trees as Crop Plants:68-79. Institute of Terrestrial Ecology.

Jones M.D.; Hutchinson, T.C. 1986. The effect of mycorrhizal infection on the response of *Betula papyrifera* to nickel and copper. New Phytol 102:429-442.

Lamar R.T.; Davey, C.B. 1988. Comparative effectivity of three *Fraxinus pennsylvanica* Marsh. vesicular-arbuscular mycorrhizal fungi in a high-phosphorus nursery soil. New Phytol. 109:1 71-181.

Last F.T.; Wilson, J.; Mason, P.A. 1990. Numbers of mycorrhizas and seedling growth of *Picea sitchensis*—what is the relationship? Agric. Ecosys. Environ. 28:293-298.

Marx, D.H.; Bryan, W.C. 1971. Influence of ectomycorrhizae on survival and growth of aseptic seedlings of loblolly pine at high temperature. For. Sci. 17:37-41.

Marx, D.H.; Bryan, W.C.; Davey, C.B. 1970. Influence of temperature on aseptic synthesis of ectomycorrhizae by *Thelephora terrestria* and *Pisolithus tinctorius* on loblolly pine. For. Sci. 16:424-431.

Marx, D.H.; Davey, C.B. 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. IV. Resistance of naturally occurring mycorrhizae to infections by *Phytophthora cinnamomi* Rands. Phytopathology 59:559-565.

Mikola, P. 1980. Mycorrhizae across the frontiers. In: Mikola, P. ed. Tropical Mycorrhiza Research:3-10. Clarendon Press, Cambridge.

Rankin, W.H. 1918. Manual of Tree Diseases:398. The Macmillan Co., New York.

Richter, D.L.; Bruhn, J.N. 1989. Field survival of containerized red and jack pine seedlings inoculated with mycelial slurries of ectomycorrhizal fungi. New For. 3:247-258.

Riffle, J.W. 1989. Field performance on ponderosa, scots, and Austrian pines with *Pisolithus tinctorius* in prairie soils. For. Sci. 35:935-945.

Rousseau, J.V.D.; Reid, C.P.P. 1990. Effects of phosphorus and ectomycorrhizas on the carbon balance of loblolly pine seedlings. For. Sci. 36:101 -112.

Sanchez, P.A. et al. 1989. Organic input management in tropical agroecosystems. In: Coleman, D.C. et al. eds. Dynamics of Soil Organic Matter in Tropical Ecosystems:125-152. University of Hawaii Press, Honolulu, Hawaii.

Theodorou, C.; Bowen, G.D. 1971. Influence of temperature on the mycorrhizal associations of *Pinus radiata* D. Don. Austral. J. Bot. 19:13-20.

Trappe, J. 1977. Selection of fungi for ectomycorrhizal inoculation in nurseries. Ann. Rev. Phytopath. 15:203-222.

Vogt, K.A. et al. 1982. Mycorrhizal role in net primary production and nutrient cycling in *Abies amabilis* (Dougl.) Forbes ecosystems in western Washington. Ecology 63 :370-380.

Vongkaluang, I. 1978. Allocation of photosynthate to shoots and roots of *Pinus taeda* L. seedlings:37. Ph.D. Dissertation, North Carolina State University.

Wilde, S.A. 1944. Mycorrhizae and silviculture. J. For. 42:290-291.