Fiber, Food, Fuel, and Fungal Symbionts

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Summary. Virtually all plants of economic importance form mycorrhizae. These absorbing organs of higher plants result from a symbiotic union of beneficial soil fungi and feeder roots. In forestry, the manipulation of fungal symbionts ecologically adapted to the planting site can increase survival and growth of forest trees, particularly on adverse sites. Vesicular-arbuscular mycorrhizae, which occur not only on many trees but also on most cultivated crops, are undoubtedly more important to world food crops. Imperatives for mycorrhizal research in forestry and agriculture are (i) the development of mass inoculum of mycorrhizal fungi, (ii) the interdisciplinary coordination with soil management, plant breeding, cultivation practices, and pest control to ensure maximum survival and development of fungal symbionts in the soil, and (iii) the institution of nursery and field tests to determine the circumstances in which mycorrhizae benefit plant growth in forestry and agri-ecosystems.

Forest and agricultural scientists are struggling to increase yields of food, fuel, and fiber which are essential to human needs and which must be produced on a fixed quantity of land. Largely as a result of research on forest trees, a new technology that attempts to use mycorrhizal fungi for the benefit of man is emerging.

Plant scientists have learned to think of a dual system—the soil and the plant. This thinking should be expanded to include a third component—the mycorrhizal fungi. Mycorrhizae (fungus roots) result from symbiotic colonization of fine roots by beneficial soil fungi. The vast majority of economically important plants form mycorrhizae (*1*). On the basis of their morphology, these associations are currently divided into two major groups: ectomycorrhizae and endomycorrhizae. Of the two, endomycorrhizae are by far the most common (*2*), but ectomycorrhizae are formed on some very important families of forest trees.

Mycorrhizae of both groups are increasingly recognized as important contributors to the cycling of soil nutrients. Many soils in the world are deficient in available major nutrients, particularly phosphorus; and plants grow poorly in areas without adequate mycorrhizal fungi. Results of recent research indicate that the growth of forest trees and certain agricultural plants can be stimulated by inoculating them with mycorrhizal fungi when such plants are growing on soils with low levels of mycorrhizal fungi and essential nutrients (*3, 4*).

Forest trees require mycorrhizae to survive and grow in the natural forest environment. In modern agriculture, crop plants have been selected and bred to give adequate yields only under luxuriant soil fertility conditions (*5*). Consequently many plant species are cultivated with minimal numbers of mycorrhizae. If fertilizers were plentiful and relatively cheap, there would be no problem. But with the cost of fertilizer increasing rapidly, we can no longer use these chemicals so lavishly. We must find ways to increase plant efficiency in nutrient uptake. The use of efficient mycorrhizal plants in conjunction with reduced fertilizer applications is a viable alternative.

Since mycorrhizal fungi can colonize a given plant species, it may be practical to improve nutrient absorption by judicious selection and management of the more efficient fungi. Such manipulation is likely to be most valuable on nutrient-deficient soils and should be considered in breeding plant varieties that can tolerate low fertility.

Ectomycorrhizae

Ectomycorrhizae are formed by fungi belonging to the higher Basidiomycetes (mushrooms and puffballs), Ascomycetes (cup fungi and truffles), and Phycomycetes in the family Endogonaceae. The host plants of these fungi are predominantly trees such as pine, hemlock, spruce, fir, oak, birch, beech, eucalyptus, willow, and poplar. Many species of fungi may be involved in the ectomycorrhizal associations of a forest, a single tree species, an individual tree seedling, or even a small segment of lateral root. As many as three species of fungi have been isolated from an individual ectomycorrhizal root cluster. Whereas a single tree species can be host to numerous species of ectomycorrhizal fungi, most fungal species can also form ectomycorrhizae with numerous tree hosts. Although some fungi are fairly host-specific, others have broad host ranges and form ectomycorrhizae with members of numerous tree genera in diverse families.

These fungal symbionts are stimulated by root exudates. Hyphae grow over the surface of feeder roots and form a fungus mantle. Hyphae then develop around root cortical cells, completely replace the middle lamella, and form the Hartig net, which is the distinguishing feature of ectomycorrhizae (Fig. 1).

Research on ectomycorrhizal associations on trees growing in natural environments has revealed that (i) any ectomycorrhizae on tree seedlings are better than none and (ii) some species of ectomycorrhizal fungi under certain environmental conditions are more beneficial than others. Appropriate methods of selecting, propagating, manipulating, and managing the most desirable fungal symbionts can lead to improvement in tree survival and growth on a variety of forest sites.

Practical use of ectomycorrhizal fungi can be of major significance in forest regeneration. Inoculation of planting stock with specific ectomycorrhizal fungi can increase survival and growth of seedlings planted on cutover lands, former treeless areas, and disturbed or adverse sites such as mining spoils.

Most work on inoculation with ectomycorrhizal fungi has been done in nurseries that produce bare-root or "containerized" tree seedlings. Another promising application, however, is inoculation of seed for broadcasting on sites that are too remote or too rough for convenient planting of seedlings.

Most ectomycorrhizal fungi produce sporophores (puffballs or mushrooms)

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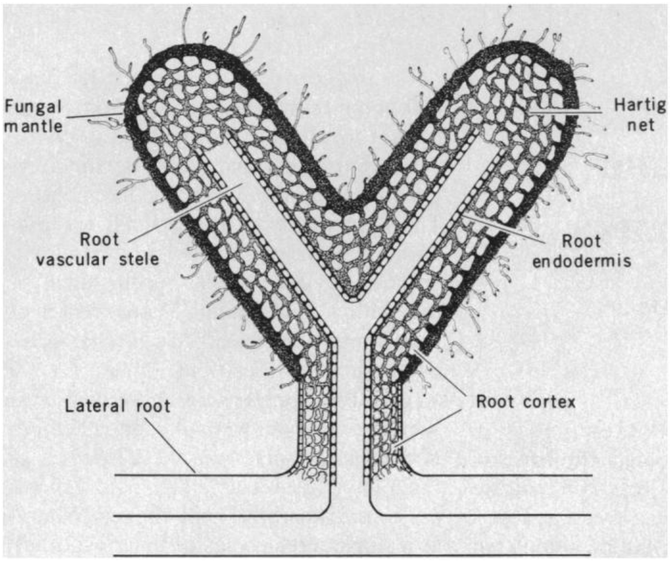


Fig. 1. Diagram of typical ectomycorrhiza including the Hartig net, fungal mantle, and external hyphae.

containing thousands of spores that can be disseminated great distances by wind, rain, insects, and small mammals. The greater the density of tree stands and the closer the proximity of the tree hosts to the seedling production areas, the greater the chances for rapid natural ectomycorrhizal development on the seedlings. If a nursery is surrounded by stands of forest trees with ectomycorrhizae, fungus spores produced in these stands can be carried by wind and thus can rapidly recolonize fumigated nursery soil. Fumigation before planting is a routine procedure to control pests. Frequently, ectomycorrhizae appear on the roots of seedlings within 6 to 8 weeks after the seedlings emerge from fumigated soil.

Although nursery soils in the southern part of the United States are rarely deficient in ectomycorrhizal fungi, the species that predominate are not always the best for reforestation. The methods used to produce seedlings of pine and oak, whether bare-root or container-grown, favor fungi that are adapted to high soil moisture and fertility. *Thelephora terrestris*, one of the most common symbionts in nurseries in the South, is an example. In the spring, this symbiont releases spores from sporocarps in forests adjacent to nurseries. The spores are windborne to fumigated nursery soil, leached by water a few centimeters, and rapidly colonize newly emerging seedling roots, often precluding colonization by other fungi that produce spores later in the year. Unfortunately, this symbiont is best adapted to fertilized and irrigated nursery soil. Its inability to function on harsh sites results in poor initial host survival and growth.

Many tree species requiring ectomycorrhizae would not reach plantable size in the nursery if they failed to develop adequate ectomycorrhizae. Ectomycorrhizal deficiencies have been experienced throughout the world in new nurseries established in areas devoid of an adequate airborne inoculum from ectomycorrhizal forests surrounding the nursery. Mycorrhizal deficiencies are also seen in nurseries that use large amounts of soluble fertilizers. Heavy fertilization, especially with nitrogen and phosphorus, changes the biochemical status of seedling roots and reduces their susceptibility to infection by mycorrhizal symbionts (*6*). In container-seedling nurseries, heavy fertilization to obtain the fastest seedling growth inhibits or eliminates mycorrhizal development.

Ectomycorrhizal fungi have been introduced into nursery soil or container-growing medium in various ways. Early workers applied inoculum in the form of duff, humus, infested soil, crushed sporophores, or excised mycorrhizal roots. Although these methods normally ensure ectomycorrhizal development, they also create problems. The inoculum may lack the most desirable fungi for the tree species and planting sites, it usually contains so much extraneous material that movement is expensive, and it often contains various harmful microorganisms and noxious weeds. In addition, sufficient quantities of sporophores or colonized roots may not be available when needed (*7*).

Mass production of ectomycorrhizal fungi in pure culture is the best solution, but it is easier said than done. Most fungi grow in pure culture only when very specific conditions are provided, and a great deal of research is required to determine the appropriate conditions. Therefore, this source of inoculum is at present limited to a small number of fungal species.

In Europe, Latin America, and the United States research results with mycelial inoculum of several symbionts have been encouraging. At our institute in Athens, Georgia, inoculation of fumigated nursery soils has improved seedling quality by lowering the cull rate of nursery seedlings and increasing root development and the overall size of seedlings (*8*). In field trials, pine seedlings inoculated with *Pisolithus tinctorius* in the nursery survive and grow better on adverse and routine reforestation sites than seedlings colonized by naturally occurring fungi (*9*).

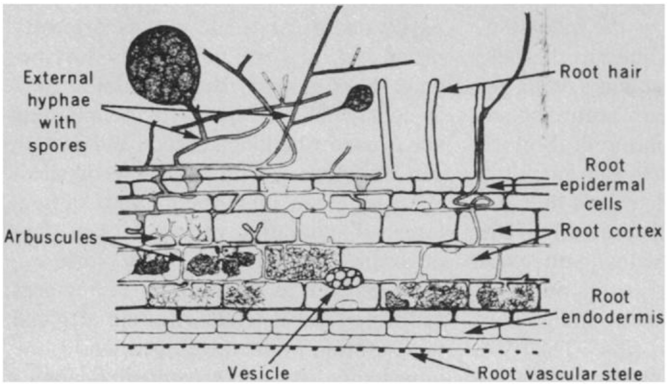
These encouraging results have created sufficient interest in the agrichemical industry to motivate Abbott Laboratories, North Chicago, Illinois, to study ways of producing a dried, vermiculite-peat moss-based inoculum of *P. tinctorius*. This material is being compared to our laboratory-produced inoculum in 46 bare-root and 11 container-tree nurseries throughout the United States. Results indicate that Abbott Laboratories' inoculum can form ectomycorrhizae on several species of pine, oak, spruce, Douglas fir, and hemlock. Thus, if tests with this material continue to be positive, forest nurseries will soon have the means to produce seedlings of these tree species with abundant ectomycorrhizae of a predetermined fungus species.

Endomycorrhizae

Vesicular-arbuscular (VA) endomycorrhizal fungi occur on most food crops throughout the world, but they are ignored by many plant scientists because they have little effect on root morphology and are difficult to detect in roots. Also, these fungi have not yet been grown in pure culture and are not detected in routine soil assays with nutrient media.

The fungi invade the cortex, but not the endodermis or stele, of feeder roots. Colonization does not alter root morphology, and the sheath of fungus mycelium common to ectomycorrhizae is lacking. Under a microscope, VA mycorrhizae are diagnosed by the presence of vesicles (terminal, spherical structures that contain oil droplets) and arbuscules (complex structures formed by repeated dichotomous branching of hyphae) in the cortical cells of differentially stained feeder roots. Mycelia emanate from the infected root to form a loose network in the rhizosphere and adjacent soil (Fig. 2).

The VA mycorrhizae are formed by certain fungal species of the family



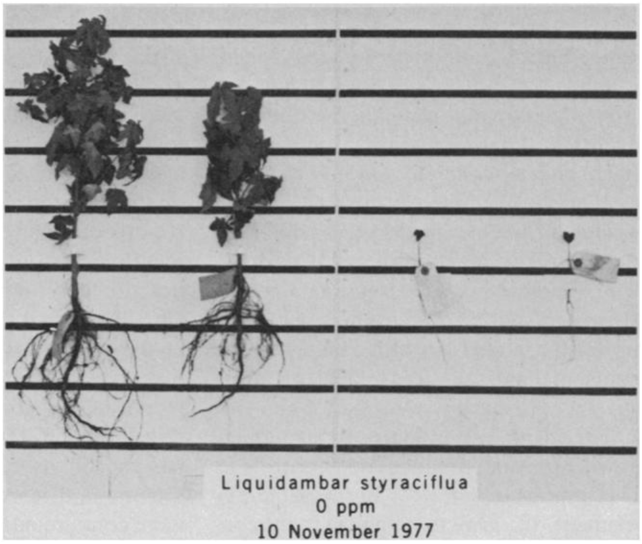


Fig. 2 (left). Diagram of typical endomycorrhiza including arbuscules, vesicles, and external hyphae with spores. [Drawing by F. E. Sanders with permission from Academic Press, London] Fig. 3 (right). Growth response of 6-month-old sweetgum seedling colonized with endomycorrhizal fungi. The pair on the left is colonized with *Glomus* spp.; the pair on right is nonmycorrhizal.

Endogonaceae. These symbiotic fungi produce large, globose to ovoid spores. The spores are grouped in sporocarps or appear singly in the soil or in plant roots. These sporocarps or spores, hypogenous beneath leaf litter or in the mineral soil, occasionally become airborne during dust storms in semiarid regions. These fungi are more commonly spread by growing from feeder root to feeder root and, at times, are disseminated by moving water, soil, insects, and animals.

The importance of VA endomycorrhizae to phosphate nutrition is indicated by recent research (*3*). The VA endomycorrhizae significantly increased growth of plants—by several hundred percent in some instances—on soils deficient in readily available phosphate. The main effect of plant response to these symbionts is increased efficiency of nutrient uptake. Thus, if plants are colonized with appropriate VA endomycorrhizal fungi, the estimate of production potential of a soil and its fertilizer requirement might change radically. If methods of producing large quantities of inocula are devised, large-scale inoculation in agriculture and forestry to increase plant yield may become feasible.

Relationships Between Symbiont and Host

Ion uptake in plants is governed by the absorbing capacity of the root and the movement of ions to the root. Plant uptake of highly mobile ions—such as nitrate, sulfate, and potassium—are largely determined by the absorbing capacity of roots. Relatively low concentrations of feeder roots through the soil profile may be adequate for uptake of such ions in soil. However, for relatively immobile ions, such as phosphate, zinc, copper, molybdenum, and sometimes ammonium, movement to the root is a limiting factor. In these cases, the distribution of root hairs and mycorrhizal fungi in the soil may determine the rate of ion uptake (*10*).

Plant species differ in their production of roots and root hairs (*11*). Many tree species have few fine feeder roots and few or no root hairs. They have far less direct contact with the soil and soil solution than grains and grasses, which have high root volumes with many fine roots and root hairs. One of the main benefits of VA endomycorrhizae is hyphal growth into soil. These structures absorb ions and transport them back to the higher plants. Therefore, plants with few feeder roots and root hairs should benefit more from mycorrhizal infection than those with numerous feeder roots (*11*).

Mycorrhizae accumulate more soluble phosphorus from soil than nonmycorrhizal roots because hyphae emanating from mycorrhizae extend beyond the zone of phosphorus depletion that develops adjacent to the root epidermis (*12*). Also, VA endomycorrhizae improve uptake of other nonmobile ions; in zinc-deficient soil in California, for example, peach seedlings with VA endomycorrhizae were able to take up much more zinc and to grow larger than nonmycorrhizal seedlings (*13*).

Mycorrhizal fungi may influence processes other than ion uptake (*14*). Some mycorrhizal fungi can grow at much lower water potential than higher plants. Hyphae extending into the soil, therefore, can increase water movement to the roots (*15*). Plants with few root hairs and feeder roots probably benefit from symbiotic infection when growing in sandy or semiarid soils. Thus, instead of irrigating to accommodate a crop, we might alter plants by genetic and mycorrhizal manipulation and tailor the crop to the existing droughty environment. In forests and grasslands, mycorrhizal associations help to conserve and cycle nutrients. Fungal hyphae readily penetrate litter and decomposing organic matter and can spatially compete with other soil microorganisms for organic and inorganic nutrients far more efficiently than nonmycorrhizal plant roots. Root destruction by soil-borne pathogens in nutrient-deficient soils will often lead to reduced plant yield. Active mycorrhizae and hyphal growth into soil from roots in other parts of the root system not infected by pathogens can compensate for a certain degree of root loss (*3*). In most instances the mycorrhizae are resistant to attack by fungal pathogens (*4*).

The size and effectiveness of the native VA endomycorrhizal fungus population in different soils varies. If the population is small, mycorrhizal infection may be sparse in the early stages of plant growth when the need for phosphate is the greatest, and the infection can inhibit seedling establishment and subsequent growth. Furthermore, the species or strains of endophyte present may not be the most efficient. Many naturally occurring species have no marked effect on growth of certain host plants. Further research is needed to ensure selection of highly efficient fungi for particular soils and crops and to develop methods for their introduction and maintenance.

The indigenous symbiont population can also be strongly affected by certain agricultural practices. For example, the use of soil fumigants or other pesticides to kill plant pathogens and nematodes also reduces populations of mycorrhizal fungi. Their populations may also decline in the absence of suitable host plants during fallow periods or during prolonged growth of nonhost plants.

In England, soil from a field of heavily mycorrhizal barley was used to inoculate a phosphorus-deficient soil in which the population of endomycorrhizal spores was low. The colonized soil was incorporated into the planting furrows, and the yield of potatoes was increased by nearly 20 percent with no additional phosphorus fertilizer (*16*). In other field experiments in Pakistan, transplants of corn, wheat, and barley that had been inoculated with VA endomycorrhizal fungi grew faster than uninoculated controls in a very infertile soil (*17*). In the same experiment, the growth response to mycorrhizae was nullified when high rates of phosphorus fertilizer were added.

Large growth responses to inoculation with VA mycorrhizae are more likely to occur in tropical than in temperate soils. Many of the arable soils of the tropics are highly leached Oxisols and Ultisols and are low in bases and relatively acid; these soils contain high amounts of exchangeable aluminum. Tropical soils are commonly deficient in phosphorus and other essential elements and they tend to immobilize added phosphorus. Rock phosphate is cheaper and more plentiful than superphosphates in tropical countries, but it is also less soluble. Particularly important, therefore, is the discovery that the interaction of rock phosphate with artificially introduced VA endomycorrhizae increases plant yields in tropical soils (*3*).

When the soil is fumigated to control disease, plants that depend heavily on VA mycorrhizae grow poorly unless the soil is inoculated with suitable fungi or high rates of phosphorus fertilizer are added. For example, some citrus cultivars are strongly dependent on mycorrhizae. They are severely stunted in fumigated nursery soil unless several hundred kilograms of phosphorus per hectare is added (*12*). Stunting can also be corrected by inoculating the soil with appropriate VA endomycorrhizal fungi. A similar relation has been observed with sweetgum trees in Georgia (*18*) (Fig. 3).

Pure culture techniques for mass producing inoculum of endomycorrhizal fungi are lacking. However, on a limited scale colonized soil or plant roots have been successfully used as the inoculum. Crops normally transplanted to the field could be grown in containers with selected fungi and their pretransplant mycorrhizal status monitored. Soil or sievings from pot cultures (an inoculum mixture of spores, hyphae, and infected root pieces) can be incorporated into the planting furrows. This technique has been successful in experiments on fumigated soils that contained no indigenous endomycorrhizal fungi (*18*). The VA mycorrhizal fungus populations can be increased by growing a heavily mycorrhizal crop in a field plot and then using the topsoil as crude inoculum (*12*). In this method, care must be taken to prevent contamination with pathogens and weeds.

If inoculum of indigenous fungal symbionts is needed, soil containing them can be fumigated to remove harmful microorganisms and noxious weeds and then seeded with a suitable cover crop to increase the residual inoculum that survived fumigation. The practical, large-scale use of specific symbionts must be produced in pot cultures in large quantities, readily transported to the field, and easily introduced into the seedling root zone. Seed inoculation might be tried as an alternative because it would require smaller amounts of inoculum and virtually no change in planting procedures (*19*).

Conclusion

Major increases in world production of food and fiber over the next few decades can result from a better understanding of the organisms and processes of the rhizosphere. The management systems that produce high yields in developed nations are often not directly transferable to developing nations. Furthermore, the environmental effects and energy costs of some of these systems are being questioned in the United States. Some treatments, such as fertilizer applications, have been formulated solely on the basis of the response of the crop plant. Until we understand the complex chemistry, physics, and biology of the rhizosphere, there remains no way of knowing whether other approaches would be cheaper or more effective.

In our work with mycorrhizal symbiosis on forest trees, results are very promising, and we have really just begun. When a mycorrhizal symbiont that is ecologically adapted to the planting site is used, seedling survival and growth can be improved on a variety of sites. When one considers the millions of hectares of potential exotic forests that might be established in Third World nations, as well as the millions of hectares of former forest lands awaiting artificial regeneration in the developed world, the importance of such treatment becomes apparent.

We have known about possible benefits of ectomycorrhizae in forestry for some time, but only in the last few years has the significance of endomycorrhizae begun to be appreciated. These structures are common not only in many species of forest trees, but also in most crop plants. We cannot help but believe that mankind will benefit from the manipulation of these symbiotic associations.

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