Chapter 10

MYCORRHIZAE

A. ECTOMYCORRHIZAE

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1. INTRODUCTION

Microorganisms are present in great numbers near the feeder roots of plants and they play vital roles in numerous physiological processes. These dynamic microbial processes involve saprophytism, pathogenicity and symbiosis. The most widespread symbiosis amongst plants is the mycorrhizal association, which involves various root inhabiting fungi and feeder roots.

The prevalence of mycorrhizal associations on plants is so common under natural soil conditions that a nonmycorrhizal plant is the exception rather than the rule. Only a few plants, such as the sedges, crucifers and certain aquatics, do not normally form mycorrhizae. Most other plants, and especially those of economic importance to man, such as forest trees and agronomic crops, form abundant mycorrhizae on their roots.

There are three classes of mycorrhizae: (1) Endomycorrhizae occur on roots of the majority of plant species of the world. This class of mycorrhizae is discussed in depth by Hayman in Chapter 10 B of this book. (2) The class of ectendomycorrhizae has the features of both ecto- and endomycorrhizae, but seems to be of lesser ecological importance than the other classes. Ectendomycorrhizae have a limited distribution in forest soils and are found on roots of normally ectomycorrhizal forest trees. Very little is known about the species of fungi involved in this association and their significance to tree growth, since only limited research and observations have been made (Mikola, 1965; Wilcox, 1971). (3) Ectomycorrhizae constitute another category of the root—fungus symbiosis and will be the subject of this sub-chapter.

Ectomycorrhizae are mainly distinguished from the other classes by the presence of an intercellular fungal network in the root cortex (Hartig net). In general the Hartig net is distributed around the primary cortical cells of the feeder roots. In most cases, the ectomycorrhizal fungi will form a sheath or a fungal mantle around the feeder root. Ectomycorrhizae are common in both Gymnosperms (Pinaceae, Cupressaceae) and Angiosperms (Salicaceae, Betulacceae, Fagaceae, Juglandaceae, Ulmaceae, Rosaceae, Leguminosae, Sapindaceae, Aceraceae, Tiliaceae, Myrtaceae, and Ericaceae) (Meyer, 1973).

Certain genera in these tree families may form all three classes of mycorrhizae on their roots. This latter condition is apparently dictated by certain soil conditions which are not understood at this time. Certain other tree species, such as *Alnus*, may form ectomycorrhizae and endomycorrhizae, as well as nitrogen-fixing nodules.

2. EXTERNAL AND INTERNAL CHARACTERISTICS OF ECTOMYCORRHIZAE

Ectomycorrhizal development on forest trees is initiated from propagules (spores or hyphae) of the fungal symbionts in the rhizosphere of the feeder roots. The propagule is stimulated by the root exudates, resulting in hyphal growth over the feeder root surface to ultimately form the mantle. Following the sheath formation, hyphae develop intercellularly in the root cortex to form the Hartig net, which may completely replace the middle lamellae between the cortical cells.

The morphology of ectomycorrhizae is determined by the interaction between the host root, the fungal symbionts and the environment. The infected root may be initially simple and non-forked and then proceed through several morphological changes until finally a complex coralloid configuration is achieved. Certain ectomycorrhizae may remain in the non-forked form while others exist as simple bifurcate structures. Branching patterns of ectomycorrhizae exhibit little or no variation between different tree species of the same genus, but striking differences can be observed between different genera. For example, *Populus* and *Salix* have monopodial and ramiform ectomycorrhizae (Fontana, 1961, 1962); *Castanea*, *Fagus* and *Quercus*, in addition to having monopodial and ramiform types of ectomycorrhizae, may have irregular pinnate forms (Fontana and Centrella, 1967); *Abies*, *Larix*, *Picea*, *Pseudotsuga*, and *Tsuga* have monopodial, ramiform and irregular, tightly pyramidal pinnate ectomycorrhizae. Pines, besides having monopodial ectomycorrhizae, appear to be one of the few groups of tree species which characteristically form the bifurcate or complex coralloid type of ectomycorrhizae

Due to the inherent difficulties in distinguishing the ectomycorrhizal fungi involved in the formation of a specific type of ectomycorrhiza on the roots of a given tree species, many authors (Melin, 1927; Dominik, 1962; Bakshi and Singh, 1968) have attempted various classification schemes to differentiate the various morphological types of ectomycorrhizae. Zak (1971a) proposed a classification scheme based on the following criteria — morphology of the mycorrhizae and the association of a specific fungus in terms of fruit bodies, rhizomorphs or hyphae with the formation of specific types of ectomycorrhizae. While the color of ectomycorrhizae may be quite variable, it is dictated by the species of the fungal symbiont involved (Zak, 1973). Ectomycorrhizae of forest trees may be brown, black, gold, red, white, yellow, or blends of these colors. Certain authors (Marks and Foster,

1973) suggest that the tannin layer present in ectomycorrhizae is the factor controlling their color. This may be relevant for those mycorrhizae having fungal symbionts with white or cream colored hyphae, but for others it is not true. *Pisolithus tinctorius* forms yellow-brown mycorrhizae, *Cenococcum graniforme* forms black, and *Poria terrestris* blue, orange or rose colored ectomycorrhizae. These are the colors of the fungal symbionts. Some morphological types of ectomycorrhizae may be transitory. This is best illustrated by *Pisolithus tinctorius* on the roots of southern pines. In the initial stages of root infection, a feeder root may remain unforked for several weeks. As the root ages and as the mycorrhizal infection continues to progress, the root may go through stages of simple bifurcation into numerous dichotomies until ultimately it is transformed into a complex coralloid. Some of these ectomycorrhizae may have as many as 36 root tips in a coralloid. Other fungi, however, may not transform a root progressively into different morphological states. An example of this is *Cenococcum graniforme*, which rarely induces dichotomy on pines and thereby preserves the monopodial configuration. *Pisolithus tinctorius*, which occurs on southern pines and many other tree species, usually induces complex coralloids by the time the ectomycorrhizae are mature. However, on *Pinus strobus* the mature mycorrhizae of *P. tinctorius* are rarely of a complex coralloid type but resemble a short-branched nodular type. This is quite consistent under a variety of soil conditions such as fertility and moisture, as well as the age of the host (Marx et al., 1976).

Within the root, the features of ectomycorrhizae are restricted to the primary cortical tissues. Mycorrhizal infection does not spread into the meristematic tissues or into the stele. Ectomycorrhizal fungi, either enzymatically and/or mechanically, penetrate the root between the epidermal cells and into the middle lamellae of the cortical tissue. The enzymatic penetration is thought to be primarily hydrolytic via pectolytic enzymes, and can progress as far as the endodermis. The degree of fungal development in the primary cortex is apparently mediated by the aggressiveness of the fungus and by the responsiveness of the host. The host response to mycorrhizal infection will be discussed briefly in the section on secondary metabolites. For details on the general structure, morphogenesis and ultrastructure of the ectomycorrhizae of forest trees, refer to Marks and Foster (1973).

3. TAXONOMY AND PHYSIOLOGY OF ECTOMYCORRHIZAL FUNGI

Numerous fungi have been identified as ectomycorrhizal. Most of these fungi are members of the higher Basidiomycetes that produce mushrooms or puffballs. Certain Ascomycetes, such as truffles, also form ectomycorrhizae (Trappe, 1971). Among the Basidiomycetes there are Hymenomycetes such as *Boletus*, *Cortinarius*, *Suillus*, *Leccinum*, *Amanita*, *Tricholoma*, *Laccaria*, and *Lactarius*. The Gasteromycetes include such examples as *Rhizopogon*, *Pisolithus* and *Scleroderma* (Smith, 1971). It is estimated that over 2100 species of these fungi can form ectomycorrhizae with forest trees in North America. According to Trappe (1971), there are three orders of ectomycorrhizal Ascomycetes — Eurotiales (*Cenococcum graniforme*), Tuberales (truffles) and the Pezizales.

Under normal forest soil conditions a single tree species, an individual tree, or even a small segment of a lateral root will have many species of fungi involved in the formation of ectomycorrhizae. As many as three different species of fungi have been isolated from an individual ectomycorrhiza. Just as the single tree species can support numerous ectomycorrhizal fungi, a single fungal species can enter into ectomycorrhizal association with numerous tree species. Some fungi, however, are apparently rather host specific, whereas others have broad host ranges and form ectomycorrhizae with tree genera in diverse families (Meyer, 1974). A good example is *Cenococcum graniforme*, which is capable of forming mycorrhizae with well over 200 species of plants throughout the world. Another example is *Pisolithus tinctorius*. Currently, this fungal symbiont is known to form ectomycorrhizae with 73 different species of forest trees, including species of *Abies*, *Betula*, *Carya*, *Eucalyptus*, *Quercus*, *Tsuga*, and *Pinus* (Marx, 1977). In all probability, as more research is conducted on ectomycorrhizae, it will become evident that host specificity within the tree families will be an exception and the rule will be that most ectomycorrhizal fungi have a broad host range.

Of the multitude of species of ectomycorrhizal fungi present in the forest ecosystems throughout the world, relatively few of these fungi have been studied in depth in vitro. Based on our current knowledge, it can be concluded that the minimum requirements for the growth of ectomycorrhizal fungi in pure culture are not highly restrictive. Both physical and chemical factors influence their growth. The optimal temperature for mycelial growth lies between 18 and 27° C for the majority of these fungi (Harley, 1969). With many fungi, growth ceases above 35° C and below 5° C (Hacskaylo et al., 1965). There are, however, exceptions to these limits. *Pisolithus tinctorius* has the capacity to grow at an optimum temperature of 28° C and will grow at 40° C (Marx et al., 1970). Certain species of mycorrhizal fungi, such as *Suillus variegatus* and *Paxillus involutus*, have been shown to grow at temperatures as low as —2 to 4° C (Slankis, 1974). Hacskaylo et al. (1965) demonstrated that the optimum vegetative growth of six ectomycorrhizal fungi was far lower than the maximum respiratory activity of those fungi. In most instances, maximum respiratory activity was measured consistently at a temperature at least 2—5° C higher than the temperature promoting optimal growth.

In general, ectomycorrhizal fungi are considered to be acidophilic. Most species tested in pure culture show optimal growth at pH 4—6 (Modess, 1941). Some fungi, such as *Suillus*, grow best at pH 3 (Hübsch, 1963). Certain strains of *Paxillus involutus* exhibit good growth at pH 2.7 and others at pH 6.4 (Laiho, 1970). Based on the limited data available, it appears that most ectomycorrhizal fungi are obligate aerobes. Melin (1923) attributed reduced growth of submerged mycelia in agar and in liquid media to lowered oxygen tensions. Indications are that the oxygen relationships in shake cultures are more advantageous in satisfying the aerobic requirement of the fungus in vitro. Mexal and Reid (1973), using various types of polyethylene glycol, showed that certain ectomycorrhizal fungi are extremely sensitive to water stress. Of the mycorrhizal fungi tested by these authors, *Cenococcum graniforme* was found to be the most tolerant to artificial drought conditions. Worley and Hacskaylo (1959) and Muttiah (1972) found that *C. graniforme* would succeed other mycorrhizal fungi on tree roots under conditions of low soil moisture availability.

Most ectomycorrhizal fungi grow best on media containing simple carbohydrates, such as glucose or mannose. Some are able to utilize more complex carbohydrates, such as the sugar alcohols mannitol and sorbitol, as well as certain disaccharides like sucrose, cellobiose, maltose, and trehalose. Ability to metabolize sucrose is not a property of all ectomycorrhizal fungi. Certain species do not grow well on sucrose as the sole source of carbon. Lamb (1974) confirmed the earlier findings of Palmer and Hacskaylo (1970) in regard to the utilization of simple carbohydrates by ectomycorrhizal fungi. Additionally, however, he was able to demonstrate that in the presence of starter glucose, many ectomycorrhizal fungi are able to metabolize carbohydrates such as cellulose, pectin and glycogen. He concluded that the starter glucose induced sufficient vegetative growth to facilitate inducible or adaptive enzymes to become effective in the hydrolysis of these more complex carbohydrates.

The nitrogen nutrition of ectomycorrhizal fungi has been reviewed by Melin (1963) and Harley (1969). In general, ammonium salts, amino acids and more complex organic sources of nitrogen are readily utilized by ectomycorrhizal fungi. Sources of nitrogen such as casein hydrolysate, malt extract and yeast extract stimulate vegetative growth. Others, such as diethylamine, proline and pyridine, as well as nitrates, are either not utilized or are toxic to many ectomycorrhizal fungi (Lundeberg, 1970).

Many ectomycorrhizal fungi require certain B vitamins for growth, thiamine and biotin being the major ones. Certain mycorrhizal fungi have been reported to be heterotrophic for inositol, nicotinic acid and pantothenic acid (Harley, 1969). Some of these vitamins in excessive concentrations can be inhibitory (Melin, 1953). Metabolic products released by ectomycorrhizal fungi into the growth medium consist of a variety of secondary metabolites. Among others, these include auxins (Slankis, 1973), gibberellins (Gogala, 1971), cytokinins (Miller, 1971), a variety of vitamins (Shemakhanova, 1962), antibiotics (Marx, 1973a), and fatty acids (Krupa and Fries, 1971).

Several investigators have studied the hydrolytic enzymes produced by ectomycorrhizal fungi. Norkrans (1950) demonstrated that *Tricholoma fumosum* could produce cellulase and utilize cellulose as a carbon source. She proposed that as long as the mycorrhizal fungus obtains glucose from the host, cellulase production by the fungus is suppressed. When the host no longer produces excess simple carbohydrates, host cellulose stimulates fungal cellulase production. Thus, she furnished a possible explanation for the formation of ectendomycorrhizae by this specific fungus. Since ectomycorrhizal fungi grow between the cortical cells, it has been suspected that they must produce pectinase in order to hydrolyze the pectin in the middle lamellae. Pectin has been found to be a good carbon source for many ectomycorrhizal fungi, at least when no other carbon source is provided (Palmer and Hacskaylo, 1970). Lundeberg (1970) confirmed the earlier reports from other workers that ectomycorrhizal fungi are inferior to litter decomposing fungi with regard to the production of cellulase, pectinase, protinase, and laccase.

4. INTERACTIONS BETWEEN FUNGAL SYMBIONTS AND TREE HOSTS

There is no doubt that the host derives several benefits from its association with ectomycorrhizal fungi. Some of these are (1) longevity of its feeder roots (Meyer, 1974); (2) increased rate of nutrient absorption from the soil (Bowen, 1973); (3) selective absorption of certain ions from the soil (Bowen, 1973); (4) resistance to feeder root pathogens (Marx, 1973a); (5) increased tolerance to soil toxins (Zak, 1971b), cold temperatures (Harley, 1969) and high temperatures (Marx et al., 1970; Marx and Bryan, 1971); and (6) tolerance to adverse soil pH, cationic and anionic concentrations, etc. (see the section on revegetation of strip-mined lands in this chapter). The ectomycorrhizal fungi not only derive the necessary nutrients for their growth from the host, but also find protection and escape from the intense microbial competition in the soil by occupying their specific niche in and on the host root.

Physiologically, mycorrhizal associations are one of the best examples in nature of balanced reciprocal parasitisms. The balanced association continues as long as there is a continuous supply of essential metabolites in the reciprocal exchange (Hackskaylo, 1971). For a detailed review of these aspects, the reader is referred to Marks and Kozlowski (1973).

Slankis et al. (1964) showed that a wide variety of organic compounds can be secreted by pine roots into the rhizosphere, including amino acids, other organic acids and carbohydrates. In these laboratory studies, the aforementioned investigators could only recover insignificant portions of the ^{14}C applied to the foliage in the root exudates (non-volatile) in question. Bowen and Theodorou (1973) reported large differences in the loss of amides and amino acids between *Pinus radiata* seedlings grown under conditions of nitrogen sufficiency and phosphorus or nitrogen deficiencies. In addition to these compounds, the metabolites designated as M-factor greatly stimulate mycelial growth of ectomycorrhizal fungi. According to Hacskaylo (1971), hyphae of ectomycorrhizal fungi in the rhizosphere probably are dependent on the root for thiamine and simple carbohydrates.

Several investigators have conducted tracer studies to determine the features related to the nutrient exchange between the two symbionts. Lewis and Harley (1965a, b, c) concluded that ectomycorrhizal fungi are capable of utilizing the sucrose in the host root and converting it into host irreversible products, such as sugar alcohols, trehalose and glycogen. Reid (1967), using foliar applications of radioactive carbon compounds, showed carbon transport from the host to the fungus.

According to Hacskaylo (1971), ectomycorrhizal fungi capable of synthesizing amino acids in vitro do not demonstrate dependency on the root for such compounds. In the nonmycorrhizal roots of certain tree species, the ornithine cycle is blocked at certain points and intermediate metabolites such as citrulline and arginine accumulate (Krupa et al., 1973a). It is unusual to find appreciable quantities of arginine, ornithine or citrulline in rapidly growing tissues, and supraoptimal amounts of these amino acids are frequently associated with mineral (such as potassium) deficiencies (Naylor, 1959). It is well known that tree seedlings with ectomycorrhizae have access to lower levels of nitrogen, phosphorus and potassium (Harley, 1969). Krupa et al. (1973a) showed that the accumulation of the ornithine cycle intermediates decreased substantially in the ectomycorrhizal roots with a simultaneous increase in the levels of glutamic acid and glutamine.

In a subsequent study, Krupa and Bränström (1974) determined the concentrations of free and bound amino acids in the fungus *Suillus variegatus*, in the nonmycorrhizal roots of *Pinus sylvestris*, and in the ectomycorrhizae. From this study, the authors suggested that while ectomycorrhizal fungi such as *Suillus variegatus* may have the ability to synthesize large amounts of ornithine cycle intermediaries like arginine in vitro, such synthesis is suppressed in vivo by the externally available free arginine pool of the host.

Marx and associates (personal communication) isolated amino acyl t-RNA from the ectomycorrhizal fungus *Pisolithus tinctorius*, from one of its hosts (*Pinus taeda*) and from the ectomycorrhizae. The kinetics of the t-RNA from the fungus and its host were different. During the mycorrhizal association, the t-RNA kinetics were more depictive of the fungal symbiont than the host. From all of these studies it is suggested that the mobilization and utilization of nitrogen initially occur in the fungus, and the host benefits only in the latter phases of the association.

Melin and Nilsson (1950) and Morrison (1957) showed the movement of phosphorus from the hyphae of ectomycorrhizal fungi to the hosts. Similar studies have also been conducted by Harley and his associates (Harley, 1969). According to many investigators (Hatch, 1937; Finn, 1942), plants with ectomycorrhizae contain more nitrogen, phosphorus and potassium than nonmycorrhizal plants. This has been subsequently confirmed by radioactive transport studies (Melin and Nilsson, 1950, 1952, 1955; Bowen and Theodorou, 1967). Phosphate transport and, perhaps, transport of other metabolites through the mantle is an active metabolic process and requires expenditure of energy. The source of energy no doubt is primarily the simple sugars or metabolites derived from carbohydrates within the roots (Hacskaylo, 1971).

Several investigators have studied the role of growth regulators in ectomycorrhizae. Fungus auxin has been proposed as a key metabolite in the establishment of mycorrhizae (Slankis, 1961). In addition to the induction of morphological changes in the feeder roots, auxin may increase the flow of soluble carbohydrates from the shoot to the root (Meyer, 1968). Unless a continuous supply of auxin is present, short roots elongate and develop root hairs (Slankis, 1967). In addition, feeder roots resume their maturing process (Fortin, 1967). Cytokinins may also be involved in these processes (Miller, 1967).

More recently, attention has been directed towards terpenoid metabolism in ectomycorrhizae. Several monoterpenoids and sesquiterpenoids have been implicated in the restriction of ectomycorrhizal fungi in the infected root and subsequent development of the balance in the reciprocal parasitism (Krupa and Fries, 1971; Melin and Krupa, 1971; Krupa et al., 1973b). For details on this subject the reader is referred to the section in this chapter on ectomycorrhizae as deterrents to root pathogens.

5. FACTORS AFFECTING ECTOMYCORRHIZAL DEVELOPMENT

This subject has been reviewed in detail by Slankis (1974) and Meyer (1974). In discussing this subject it is necessary to separate those factors which affect the host from those which affect the fungal symbionts. The main factors influencing the susceptibility of host roots to mycorrhizal infection appear to be photosynthetic potential and soil fertility. High light intensity and low to moderate soil fertility enhance the degree of mycorrhizal development. Low light intensity (below 20 percent of full sunlight) and excessively high soil fertility result in a reduction or complete elimination of mycorrhizal development. These factors appear to influence the physiological status of the feeder roots, in that they affect the levels of sugars or the formation of new feeder roots. Roots growing rapidly due to high soil fertility may actually outgrow their fungal symbionts. Recently, Marx et al. (1977) showed that high levels of nitrogen and phosphorus in the soil induced a decrease in sucrose content of feeder roots of *Pinus taeda* seedlings which in turn dramatically reduced their susceptibility to ectomycorrhizal infection by *P. tinctorius*. Generally, any soil or above-ground conditions which influence root growth also influence mycorrhizal development.

Factors which affect the fungal symbiont directly are those which regulate the survival and growth of the infective propagules or the vegetative growth of the symbionts in the rhizoplane. Extremes of soil temperatures, pH, moisture, etc., as well as the presence of antagonistic soil microorganisms, can affect the symbionts and thereby influence the mycorrhizal potential of the soil.

6. ECTOMYCORRHIZAE AS DETERRENTS TO FEEDER ROOT PATHOGENS

Several workers have observed that tree seedlings with ectomycorrhizae are more resistant to feeder root infection by fungi than are seedlings with few or no ectomycorrhizae. Wingfield (1968) observed that ectomycorrhizae formed by *Pisolithus tinctorius* on axenic seedlings of *P. taeda* enhanced their survival when growing with the root pathogen *Rhizoctonia solani*. Richard et al. (1971) found that axenic seedlings of *Picea mariana* inoculated with the ectomycorrhizal fungus *Suillus granulatus* grew well, whereas those inoculated with the root pathogen *Mycelium radicus atrovirens* were chlorotic and severely stunted. When seedlings were inoculated with *S. granulatus* and the root pathogen simultaneously, the chlorosis and stunting caused by the pathogen were eliminated. These seedlings grew as well as those with only the ectomycorrhizal fungus.

In a greenhouse study (Ross and Marx, 1972), seedlings of the Ocala race of sand pine (*Pinus clausa*) were protected against *Phytophthora cinnamomi* by the presence of ectomycorrhizae formed by *Pisolithus tinctorius*. Nonmycorrhizal pine seedlings were heavily infected by *P. cinnamomi* and exhibited massive feeder root necrosis. Only 40 percent of these seedlings survived after two months. Nonmycorrhizal roots on pine seedlings with ectomycorrhizae formed by *P. tinctorius* were also infected by the pathogen. However, 25 percent of the feeder roots were ectomycorrhizal, thus reducing the amount of susceptible root tissue exposed to the pathogen. This reduction in susceptible tissue contributed to nearly 70 percent survival of test seedlings.

In a similar study, Marx (1973b) found that shortleaf pine (*Pinus echinata*) seedlings with ectomycorrhizae were not affected by *P. cinnamomi*. Nonmycorrhizal shortleaf pine seedlings exposed to the pathogen were significantly lighter in foliar-stem and root dry weights, and had significantly fewer new lateral roots than nonmycorrhizal seedlings grown without the pathogen. *P. echinata* seedlings with ectomycorrhizae formed by either *Pisolithus tinctorius* or *Cenococcum graniforme* did not exhibit reduction in foliar-stem or root weights or development of new lateral roots in the presence of *P. cinnamomi* as did the nonmycorrhizal seedlings. Apparently the high degree of ectomycorrhizal development (70 to 89 percent) on those seedlings reduced the amount of susceptible tissue available for attack by *P. cinnamomi*, with a concurrent decrease in the pathogen inoculum density and feeder root disease development. The preceding reports show that plants with ectomycorrhizae do not exhibit reduced top growth, chlorosis, restricted root development, and eventual death, and are therefore more resistant to feeder root diseases than nonmycorrhizal plants. Obviously, with an increasing degree of ectomycorrhizal development there is a proportionate reduction in the amount of feeder roots susceptible to pathogen attack.

Recently, Stack and Sinclair (1975) reported that the addition of basidiospores of *Laccaria laccata* to nursery soil containing *Fusarium oxysporum* reduced mortality of Douglas fir seedlings during the first growing season by nearly 100 percent. The protective influence of *L. laccata* occurred before ectomycorrhizal formation.

Zak (1964) postulated several mechanisms by which ectomycorrhizae may afford disease protection to feeder roots of plants. He suggested that ectomycorrhizal fungi may (a) utilize surplus carbohydrates in the root, thereby reducing the amount of nutrients stimulatory to pathogens, (b) provide a physical barrier (i.e., the fungal mantle) to penetration by the pathogen, (c) secrete antibiotics inhibitory to pathogens, and (d) support, along with the root, a protective microbial rhizosphere population. In addition, Marx (1972) suggested that (e) metabolites produced by symbiotically infected host cortical cells may also function as inhibitors to infection and spread of pathogens in ectomycorrhizal roots.

Several workers have investigated antibiotic production by higher Basidiomycetes in pure culture. Well over 100 species of ectomycorrhizal fungi, either in pure culture or from basidiocarps, have been shown to produce antibiotics (Marx, 1972). These antibiotics are variable in their biological spectra in that they may be antifungal, antibacterial or antiviral.

Marx and Davey (1969a) extracted the antibiotics diatretyne nitrile and diatretyne-3 from ectomycorrhizae formed by *Leucopaxillus cerealis* var. *piceina* and from the rhizosphere substrate of the ectomycorrhizae on axenic shortleaf pine seedlings. Neither short roots nor the substrate adjacent to short roots on nonmycorrhizal pine seedlings contained the diatretynes. In experiments designed to determine the susceptibility or resistance of ectomycorrhizae to infection by a pathogen, these authors demonstrated that the diatretynes present in ectomycorrhizae formed by *L. cerealis* var. *piceina* were functional in the resistance of feeder roots to infection by *P. cinnamomi*. Not only were the ectomycorrhizae resistant, but nonmycorrhizal short roots adjacent to the ectomycorrhizae which contained the diatretyne antibiotics were only 25 percent susceptible to infection by zoospores of *P. cinnamomi*. Short roots on control seedlings and on seedlings with ectomycorrhizae formed by either *Laccaria laccata* or *P. tinctorius* were 100 percent susceptible to infection. It was not determined whether the diatretynes were translocated to short roots from adjacent ectomycorrhizae or simply absorbed from the rhizosphere.

The fungal mantle of ectomycorrhizae creates a mechanical obstruction to pathogens attempting root penetration. In mature ectomycorrhizae the fungal mantles are composed of tightly interwoven hyphae, often in well defined layers that usually completely cover the root meristem and cortical tissues. This hyphal network, which precludes exposure of root tissue to direct contact with the rhizosphere, usually is complete, i.e., relatively free from voids.

Marx and Davey (1969a, b) and Marx (1970) have concluded that the fungus mantles of ectomycorrhizae are formidable physical barriers to penetration by *P. cinnamomi*. This conclusion was based on histological observation of numerous pine ectomycorrhizae formed by several fungal symbionts that had been inoculated with either zoospores or vegetative mycelium of *P. cinnamomi*. Mature ectomycorrhizae with complete mantles were not infected by *P. cinnamomi*, whereas 100 percent of all nonmycorrhizal roots used for comparison were infected. Fungus mantles of ectomycorrhizae formed by nonantibiotic-producing fungal symbionts, which passively covered adjacent nonmycorrhizal root initials on pine seedlings, protected these initials from penetration by *P. cinnamomi*. Histological examination of the root initials revealed the complete absence of fungal-symbiont infection and the apparently passive but protective nature of the mantle. Short root initials not covered by fungal mantles were highly susceptible to infection. There was further evidence that the fungal mantle covering root meristems is a barrier against pathogen penetration. Meristems of ectomycorrhizae of shortleaf and loblolly pines were readily infected by *P. cinnamomi* when the mantle covering was either incompletely formed over the root tip or artificially removed. Infection, however, did not take place in the meristem tissues when the root tips were covered by a complete fungal mantle. The Hartig net surrounding the cortical cells may function as an additional physical barrier since spread of *P. cinnamomi*, originating from either infections of nonprotected meristem tissue without fungus mantle coverings or from infections through artificially excised root tips, was blocked in this region.

Evidence available at this time suggests that there is a biochemical and cytological reaction of the cortex cells to infection by ectomycorrhizal fungi. Krupa and Fries (1971) found that the fungal symbiont *Suillus variegatus* produced the fungistatic volatile compounds isobutanol and isobutyric acid in pure culture. Other volatile compounds were also identified. Volatile organic compounds were also extracted from the roots of intact seedlings of Scots pine (*Pinus sylvestris*) grown in pure culture with and without *S. variegatus*. However, infection of the roots by the fungal symbiont resulted in the production and accumulation of volatile terpenes and sesquiterpenes in concentrations up to eight times greater than that found in nonmycorrhizal roots. Many of these terpenes and sesquiterpenes are fungistatic and were considered to be produced as a nonspecific response of the host cells to symbiotic infection. These authors concluded that the nonspecific response of the host to infection by ectomycorrhizal fungi results in the increased production and accumulation of native volatile substances and nonvolatile substances (Hillis and Ishikura, 1969). These substances, when present in sufficient concentrations, may restrict the growth of ectomycorrhizal fungi within the host tissue until the symbiotic state finally results. Furthermore, volatile and nonvolatile substances could inhibit pathogens in the root, as volatile substances inhibit pathogens in the rhizosphere. Krupa and Nylund (1972) found that these monoterpenes extracted from ectomycorrhizae inhibited vegetative growth of *P. cinnamomi* and *Fomes annosus* by 50 percent when vapors from 10 microliters of the substances were used. *Phytophthora cinnamomi* was sensitive to pinene and terpinolene and *F. annosus* was sensitive to other host products. Krupa et al. (1973b) also extracted ectomycorrhizae formed in pure culture with southern pines, and found that *Pisolithus tinctorius* stimulated over a 40-fold increase and *Cenococcum graniforme* a 30-fold increase in certain fungistatic volatile monoterpenes. Each ectomycorrhizal fungus induced a shift in monoterpene synthesis in comparison to noninfected roots, suggesting that each fungal symbiont elicits a specific response by host roots. Vapors of these volatile compounds affected the vegetative growth of different ectomycorrhizal and root pathogenic fungi to different degrees in laboratory tests. Catalfomo and Trappe (1970) also found that certain ectomycorrhizal fungi from the genera *Amanita* and *Rhizopogon* and the family Boletaceae produced terpenes which, in their opinion, were involved in the protective role of mycorrhizae.

Host roots apparently respond to ectomycorrhizal infection by the production of inhibitors that also contribute to the resistance of ectomycorrhizae to pathogenic infection.

Very little research has been done on the role of different root exudates released by ectomycorrhizal and nonmycorrhizal roots and their effects on root disease. It is logical to expect differences in exudation patterns between ectomycorrhizal and nonmycorrhizal roots, since ectomycorrhizal fungi derive most if not all of their required carbohydrates, amino acids and vitamins from their intimate association with the cortex cells and the external root surface. Few root exudates could pass through the Hartig net and fungal mantle of ectomycorrhizae without some absorption and utilization by the fungal symbiont. This suggests that exudates of ectomycorrhizal roots are (a) those not utilized by the fungal symbiont, (b) metabolic by-products of the fungal symbiont, or (c) those released as a result of the metabolic interaction of the symbiotic partners. It is reasonable that these changes in root exudations should have some effect on root pathogens.

Tribunskaya (1955) found approximately 10 times as many fungi in rhizospheres of ectomycorrhizal pine seedlings as in those of nonmycorrhizal seedlings, and concluded that the fungal symbionts were responsible for the different microflora of the rhizosphere. Katznelson et al. (1962) showed that ectomycorrhizal roots of yellow birch (*Betula allegheniensis*) increased the numbers of certain physiological groups of soil bacteria and actinomycetes. Bacteria that grew in simple chemical media and total fungal numbers appeared to be reduced around the ectomycorrhizae. The types of fungi present in the various rhizospheres were different; fungal genera that contained feeder root pathogens (*Pythium*, *Fusarium*, and *Cylindrocarpon*) predominated in nonmycorrhizal rhizospheres, while the ectomycorrhizal roots supported *Mycelium radicis*, *Penicillium* spp., and other rapidly growing fungi. *Pythium* and *Fusarium* spp. were completely absent from the rhizospheres of ectomycorrhizae. These results suggest that the ectomycorrhizae had an inhibiting effect on pathogens in the root zone.

Only Ohara and Hamada (1967) have implied that the antagonistic nature of certain fungal symbionts in ectomycorrhizal association is related to the population of microorganisms in the rhizosphere. They found that bacteria, especially aerobic and heterotrophic types as well as actinomycetes, were strongly inhibited around actively growing mycelium of *Tricholoma matsutake* in forest soil or in the vicinity of its ectomycorrhizae on *Pinus densiflora*. These microorganisms were found in great abundance in adjacent soil containing neither mycelium nor ectomycorrhizae formed by this fungus; it was inferred that antibiotics were the cause of the inhibition. Rambelli (1973) has recently reviewed the various aspects of microbial rhizospheres of mycorrhizae.

Since there are differences in microbial rhizosphere populations between ectomycorrhizal and nonmycorrhizal roots, it may be logically surmised that there are differences in the competitive microbial potential near these roots. It is not known whether these differences influence root pathogen populations and subsequent development of feeder root disease.

Many plant-parasitic nematodes are found in forest soils and tree nurseries. Nematodes parasitizing roots normally limit themselves to the feeder roots, the same ones infected by ectomycorrhizal fungi. Certain nematodes feed directly on hyphae of ectomycorrhizal fungi. Riffle (1967) found an *Aphelenchoides* that fed and reproduced on mycelium of *Suillus granulatus* and caused significant reduction in linear growth of the fungus in pure culture. Sutherland and Fortin (1968) found that *Aphelenchus avenae* fed and reproduced on seven species of fungal symbionts in pure culture. One symbiont, *Rhizopogon roseolus*, apparently produced a toxin lethal to the nematode. *Aphelenchus avenae* also prevented the formation of ectomycorrhizae by *S. granulatus* on axenic red pine. Because this nematode did not enter the feeder roots, the authors concluded that it prevented ectomycorrhizal development by directly suppressing the fungus prior to symbiotic infection of the roots. Mycophagous nematodes may directly inhibit ectomycorrhizal development, but none has been reported to feed on preformed ectomycorrhizae.

Several plant-parasitic nematodes will feed on ectomycorrhizae. Ruehle (1962) found that two endoparasitic nematodes, lance (*Hoplolaimus coronatus*) and pine cystoid (*Meloidodera floridensis*), penetrated and migrated through ectomycorrhizae of pines and caused extensive damage to the cortex and vascular tissues. Ruehle and Marx (1971) reported that the lance nematode readily penetrated the fungal mantle of ectomycorrhizae formed by *Pisolithus tinctorius* and *Thelephora terrestris* on shortleaf and loblolly pine seedlings. This nematode confined its feeding to cortical tissues of lateral roots rather than short roots on nonmycorrhizal seedlings, which suggests that short roots after transformation into ectomycorrhizae were more favorable feeding sites for the nematode than nonmycorrhizal short roots.

Although none of the purported mechanisms for resistance of ectomycorrhizae to fungal attack applies to attack by nematodes, there are interactions of nematodes and fungal pathogens. Barham et al. (1974) inoculated intact ectomycorrhizae of shortleaf pine formed by *Pisolithus tinctorius* and *Thelephora terrestris* with either spiral (*Helicotylenchus dihystera*) or stunt (*Tylenchorhynchus claytoni*) nematodes and zoospores of *Phytophthora cinnamomi*. Both nematodes penetrated and migrated through the fungal mantle and Hartig net of the ectomycorrhizae. The disruption of structural integrity of the fungus mantle of certain of these ectomycorrhizae by spiral nematodes created infection courts for *P. cinnamomi*, and intracellular hyphae and vesicles of the pathogen were found in cortex cells surrounded by the Hartig net. *Phytophthora cinnamomi* did not infect ectomycorrhizae parasitized by stunt nematodes or ectomycorrhizae not inoculated with nematodes. If we assume that host inhibitors of *P. cinnamomi* were present, then perhaps the nematodes destroyed their effectiveness or the leaked inhibitors were diluted or degraded.

Many nematodes may cause root disease or act as predisposing agents to root disease. Since they can also render normally resistant ectomycorrhizae susceptible to infection by *P. cinnamomi*, they are more important in feeder root diseases of trees than once thought (Ruehle, 1973).

7. ECTOMYCORRHIZAE AND AFFORESTATION PRACTICES

Over 3000 papers have been published on mycorrhizae, and most of these relate to forest trees (Hacskaylo and Tomkins, 1973). This research has shown the complexity of mycorrhizae in forest soil ecosystems. Many forest trees, such as *Pinus*, cannot grow without ectomycorrhizae. This point has practical significance to afforestation programs with normally ectomycorrhizal trees in areas of the world where their symbiotic fungi do not occur naturally. Mikola (1969) discussed the need for a parallel introduction of the essential ectomycorrhizal fungi with these trees, particularly *Pinus*, if afforestation is to succeed. In many areas of the world, ectomycorrhizal trees and their symbiotic fungi do not occur naturally. Such areas include the high Andes of Peru (Marx, 1975), regions of Australia (Bowen et al., 1973) and Asia (Oliveros, 1932), subalpine areas of Austria (Moser, 1963), Puerto Rico (Vozzo and Hacskaylo, 1971), Africa (Gibson, 1963), former agricultural soils of Poland (Dominik, 1961), oak shelterbelts on the steppes of Russia (Imshenetskii, 1967), and former treeless areas of the United States (Hatch, 1937). Forestation attempts there were either total or near failures until ectomycorrhizae occurred on tree roots. Symbiotic root infection was ensured either by introducing soil containing ectomycorrhizal fungi or by manipulating soil containing low levels of indigenous symbiotic fungi to encourage their buildup on roots.

8. ECTOMYCORRHIZAE AND RECLAMATION OF ADVERSE SITES

In observing the physical disruption of overburden strata during strip mining operations, it becomes quite apparent that the resulting surface material does not physically, chemically or biologically resemble any other landscape in the world. Physically, the surface material is often very rocky, steeply sloped and, depending on its mineral composition, has variable weathering rates. This material may also be quite dark in color and absorbs radiant energy which causes high soil temperatures. Chemically, the surface material may contain high levels of sulfur, aluminum, manganese, etc., which can inhibit plant growth. Additionally, the material may only contain small quantities of essential elements for normal plant growth. Biologically, any macro- or microbiological system which existed in the surface soil prior to stripping may now be buried several meters beneath the stripped overburden. Some of the original topsoil may be mixed throughout the stripped profile, but even then, the surface material is nearly a biological desert in comparison to the biological status of the original profile.

The status of the mycorrhizal potential of the new surface material is of concern to us. The literature (Hutnik and Davis, 1973) is well documented with descriptions of the various physical and chemical properties of strip-mined lands. The question is, can indigenous and recolonizing mycorrhizal fungi survive these "soil" properties? Are mycorrhizae essential to all plants which may naturally or artificially revegetate this surface material? Is there significant potential for ecological selection among the vast number of species of mycorrhizal fungi to assure that adaptable ones will maintain themselves on roots of plants in these disturbed sites?

There has been only limited research on mycorrhizal associations of plants on strip-mined lands and mining wastes. Schramm (1966) published a classic piece of work on the plant colonization of anthracite wastes in

Pennsylvania. He concluded that early ectomycorrhizal development was essential for seedling establishment of *Betula lenta*, *B. populifolia*, *Pinus rigida*, *P. virginiana*, *Populus tremuloides*, *Quercus rubra*, and *Q. velutina* on this waste material. The only generally successful original plant colonists of this bare and predominantly nitrogen-deficient waste were either nitrogen-fixing plants or certain ectomycorrhizal tree species. Schramm concluded that due to their year-round effectiveness, evergreen trees, especially pines, should receive special attention. He furnished strong evidence in support of his conclusions. Seedlings from either windblown or artificially planted seed of these tree species that did not have ectomycorrhizae were chlorotic and soon dead. The majority of surviving seedlings, and especially those growing well, were heavily ectomycorrhizal. The main basidiomycetes observed by Schramm that developed near the surviving tree seedlings were *Inocybe lacera*, *Thelephora terrestris*, *Pisolithus tinctorius*, *Amanita rubescens*, and *Scleroderma aurantium* which form ectomycorrhizae on trees (Trappe, 1962).

Schramm traced the extensively developed mycelial strands formed by *P. tinctorius* from ectomycorrhizae of these various tree species through large waste volumes to the base of its basidiocarp. These mycelial strands are large and brilliant gold-yellow in color and were easily traced through the contrasting dark anthracite wastes. Some strands were traced through waste material as far as four meters from the seedlings to the basidiocarp. The ectomycorrhizae formed by *Pisolithus* were also yellow-gold in color and prolifically branched. Schramm associated *P. tinctorius* ectomycorrhizae with the most vigorously growing seedlings. In most cases, it was the first symbiont on seedling roots. The other species of ectomycorrhizal fungi appeared on roots and produced basidiocarps primarily after litter had accumulated under the seedling canopy. These observations tentatively confirmed that *P. tinctorius* was the fungal symbiont forming the gold-yellow ectomycorrhizae on these tree species. Earlier, Bryan and Zak (1961) synthesized ectomycorrhizae with *P. tinctorius* on *Pinus echinata* seedlings in aseptic culture. This pine species, however, was not encountered by Schramm in Pennsylvania.

Schramm's work strongly suggests that only a few ectomycorrhizal fungi are capable of ecologically adapting to soil conditions on the anthracite wastes. High soil temperatures could be a limiting factor in specific mycorrhizal establishment. Marx et al. (1970) found that *P. tinctorius* formed more ectomycorrhizae on *P. taeda* seedlings at a constant soil temperature of 34° C than it formed at either 14, 19, 24, or 39° C. *Thelephora terrestris* did not form ectomycorrhizae at a soil temperature of 34° and it formed more between 14 and 24° than at 29° C. *Thelephora terrestris* is one of the major ectomycorrhizal fungi on pine seedlings in nurseries throughout the United States, which probably accounts for its presence on planted coal spoils. It was on the seedling roots from the nursery prior to planting on the spoil. In a later study with these fungi, Marx and Bryan (1971) found *P. taeda* seedlings with *Pisolithus* ectomycorrhizae survived and grew as well at 40° as they did at 24°C. Seedlings with *Thelephora* ectomycorrhizae or those without ectomycorrhizae did not survive well and did not exhibit growth at 40°C. These results may explain why *Pisolithus* is the primary symbiont on young volunteer seedlings on anthracite wastes. Schramm (1966) recorded soil temperatures between 35 and 65°C in wastes at a depth of 2½ inches. Perhaps *Pisolithus* was dominant because high soil temperatures restricted earlier establishment of the other fungi.

In 1971, Marx and associates made examinations of various bituminous coal, strip-mined spoils in the eastern part of the United States. They found *Pisolithus* basidiocarps and its unique, gold-yellow ectomycorrhizae and mycelial strands to be the predominant, if not the only, ectomycorrhizal fungus on roots of *Pinus virginiana*, *P. taeda*, *P. resinosa*, and several *Betula* spp. on coal spoils in Indiana, Pennsylvania, Ohio, Virginia, West Virginia, Kentucky, Tennessee, and Alabama, as well as *P. echinata* and *P. taeda* on strip-mined kaolin spoils in Georgia. Some of these spoils had a soil reaction as low as pH 2.9, although most were between pH 3.5 and 5.5. *Pisolithus* has also been reported on coal spoils associated with *B. lenta*, *B. pendula*, *B. populifolia*, *Populus grandidenta*, *P. tremuloides*, and *Salix humilis* in West Germany (Meyer, 1973), *Pinus banksiana* in Missouri (Lampky and Peterson, 1963), and *Pinus* spp. In Indiana and Tennessee (Hile and Hennen, 1969).

The following is an example of the prevalence of *Pisolithus* on disturbed lands. On a kaolin spoil in central Georgia, basidiospores of *Pisolithus* were collected from basidiocarps under loblolly pine. In less than 12 man-hours, over 12 kg of spores were extracted from basidiocarps. There are approximately 1.1 billion spores per gram. Just to illustrate the inoculum potential of *Pisolithus* on this site, this one collection contained approximately 12.5 trillion basidiospores. Basidiospores are very functional as inoculum for ectomycorrhizal development on pines (Marx, 1976a; Marx et al., 1976).

Based on the preceding information, it appears that *Pisolithus* may be more beneficial to the establishment of *Pinus* and *Betula* species on strip-mined lands and wastes than are other species of ectomycorrhizal fungi. Assuming that *Pisolithus* ectomycorrhizae are instrumental in tree establishment and maintenance on strip-mined spoils, Marx and coworkers initiated studies to develop techniques to "tailor" seedlings in the nursery with *Pisolithus* ectomycorrhizae. The working premise was simple — why wait for natural means to establish *Pisolithus* on colonizing trees? Could tree survival and growth be improved by having *Pisolithus* ectomycorrhizae preformed on the seedlings in the nursery prior to planting on the strip-mined lands? Recently, techniques were developed for this "tree tailoring" concept (Marx and Bryan, 1975) that were effective on several species of pines in conventional tree nurseries (Marx et al., 1976). Briefly, the techniques involve the production of pure culture, vegetative mycelial inoculum of *P. tinctorius* in a vermiculite—peat moss—nutrient substrate, or the use of basidiospores mixed with a physical carrier, such as moist vermiculite. Either inoculum is used to infest artificially fumigated nursery soil. No further modifications of standard nursery practices are necessary. Effective soil fumigation (methyl bromide) done shortly before soil infestation, and the maintenance of reasonable levels of soil fertility through the growing season, appear to be the two prerequisites to successful "tailoring" of pine seedlings with *Pisolithus*. These techniques also have been used successfully with other ectomycorrhizal fungi, such as *Thelephora terrestris* and *Cenococcum graniforme*.

The introduction of *Pisolithus* into nursery soils has also significantly improved pine seedling quality in the nursery. Growth increases between 100 and 150 percent after one growing season in the nursery have been encountered on seedlings of *P. taeda*, *P. strobus*, and *P. virginiana* in the southern U.S.A. following successful soil infestation and ectomycorrhizal development by *Pisolithus* (Marx et al., 1976). After a few more years of research, ectomycorrhizal deficiencies in nurseries of many acres should be corrected by using pure cultures of highly beneficial symbionts. In the past, these deficiencies were corrected by the addition of forest litter and humus to the nursery soil. This latter practice inadvertently introduced a considerable number of pests, such as weeds and disease-causing organisms, into certain nurseries. Currently, *Pisolithus* is being tested in tree nurseries in Australia, Mexico, East Africa, Switzerland, and France, as well as in different parts of the United States. The practicability of introducing *Pisolithus* and other ectomycorrhizal fungi into the near-sterile root substrate of container-grown seedlings has also been examined. Preliminary information suggests that growth in containers and field performance of these seedlings can be improved with specific ectomycorrhizae (Marx and Barnett, 1974). This application to containerized tree stock could be as relevant as it is to standard nursery grown seedlings, since it is anticipated that in the near future container stock will account for as much as 20 percent of the seedlings used in reforestation in North America.

The performance of pine seedlings "tailored" in the nursery with *Pisolithus* ectomycorrhizae is dramatic after a few years on a variety of adverse sites (Marx, 1976b). Only a few studies will be discussed here. Table I shows growth data of Virginia and loblolly pine seedlings after two years on a severely eroded site in the Copper Basin of Tennessee, U.S.A. The soil on this site contains no organic matter nor any appreciable quantities of essential plant nutrients. On this site, *Pisolithus* ectomycorrhizae increased growth (i.e., seedling volume) by nearly 100 percent for both pine species in comparison to control seedlings with *Thelephora* ectomycorrhizae.

TABLE I

Survival and growth of Virginia and loblolly pine seedlings after two years on an eroded site in Copperhill, Tennessee. Seedlings were tailored in a nursery with *Pisolithus tinctorius* or *Thelephora terrestris* ectomycorrhizae (unpublished data)

Mycorrhizal condition at planting	Height (cm)	Stem diameter (cm)	Seedling volume (cm^3)[1]
Virginia pine			
Pisolithus	52.3*	1.46*	111.5*
Thelephora	44.8	1.15	59.3
Loblolly pine			
Pisolithus	48.7*	1.29*	81.0*
Thelephora	41.3	1.01	42.1

[1] Seedling volume (cm^3) = (stem diameter)^2 × height. *Denotes significant differences ($P = 0.05$) between means for mycorrhizal treatments of a pine species.

Thelephora is not readily adaptable to adverse soil conditions, and its pine host suffers because of it. Table II shows the dramatic effects of *Pisolithus* ectomycorrhizae on growth and foliar chemistry of pine seedlings after two years on a highly acid (pH 3.8) coal spoil in Kentucky, U.S.A. Nearly six times more growth volume on loblolly pine and nearly three-times more on shortleaf pine was induced by *Pisolithus* over the *Thelephora*-containing control seedlings. Additionally, the seedlings with *Pisolithus* ectomycorrhizae had greater amounts of foliar N, indicating a more efficient root system. Numerically these data are striking. More striking, however, are the biological implications of the findings — i.e., a specific ectomycorrhizal association (*Pisolithus*) can adapt and stimulate a host on an adverse site.

9. ECTOMYCORRHIZAE AND REFORESTATION PRACTICES

The performance of pine seedlings with *Pisolithus* ectomycorrhizae on routine (nonadverse) reforestation sites in the southern United States has also been improved. Approximately 50,000 seedlings of various pine species with specific ectomycorrhizae have been experimentally outplanted in Georgia, Florida, North Carolina, South Carolina, Louisiana, Alabama, Arkansas, Mississippi, and Oklahoma. *Pisolithus tinctorius* was the major fungus used in these tests. The oldest available results (unpublished) are two-year data from four test sites in Florida and North Carolina. All seedlings from the nurseries were graded to approximately equal heights and stem diameters before outplanting. All seedlings had approximately 65 percent of their feeder roots ectomycorrhizal at planting; however, those seedlings with *Pisolithus* had at least 75 percent of these ectomycorrhizae formed by *P. tinctorius* and the control seedlings had only naturally occurring

TABLE II

Growth and foliar nitrogen (N) and phosphorus (P) of pine seedlings with *Pisolithus* or *Thelephora* ectomycorrhizae after two years on a pH 3.8 coal spoil in Kentucky, U.S.A. (unpublished data)

Mycorrhizal condition at planting	Height (cm)	Stem diameter (cm)	Seedling volume (cm^3)[1]	Foliage	
				% N	% P
Loblolly pine					
Pisolithus	95*	2.5*	594*	1.5*	0.11
Thelephora	55	1.3	93	1.2	0.13
Shortleaf pine					
Pisolithus	55*	1.6*	141*	1.7*	0.11
Thelephora	41	1.2	59	1.4	0.12

[1] Seedling volume (cm^3) = (stem diameter)^2 × height. *Denotes significant difference between mycorrhizal conditions of a pine species ($P = 0.05$).

(primarily *Thelephora terrestris*) ectomycorrhizae. In Florida the test sites were a deep sand ridge and a palmetto flatwood. Both sites were planted to loblolly and slash pines and the Ocala variety of sand pine. The sand ridge site was considered fair to good for loblolly and slash pines and good for sand pine; the palmetto flatwood site was considered poor for loblolly and sand pines and good for slash pine in Florida. In North Carolina the sites were a clay loam hill with 25 cm of topsoil and an eroded slope of the same hill with little topsoil. The former site was considered good for loblolly, Virginia and white pines, and the latter site poor for loblolly and Virginia pines in North Carolina. White pine was only planted on the better site. After two years, seedlings with the greatest quantity of *Pisolithus* ectomycorrhizae at planting survived and grew better than seedlings with natural ectomycorrhizae. Loblolly pine seedlings with *Pisolithus* ectomycorrhizae on the good sites in North Carolina and Florida had greater volumes than seedlings with natural ectomycorrhizae, but these differences were even greater on the poor sites (Tables III and IV). The volumes of seedlings with *Pisolithus* ectomycorrhizae on the poor sites were nearly identical to those of seedlings with natural ectomycorrhizae on the good sites for loblolly pines in both states. The two sites in Florida had very strong effects on sand pine, especially on survival. Sand pine seedlings with *Pisolithus* ectomycorrhizae had a 36 percent greater volume than seedlings with natural ectomycorrhizae on the good site, and nearly a 200 percent greater volume on the poor site (Table III). Survival and growth of slash pine was significantly increased on both sites. Table IV shows the influence of *Pisolithus* ectomycorrhizae on other pine seedlings in North Carolina. As with the other pine species, *Pisolithus* ectomycorrhizae on Virginia pine caused greater differences in seedling volumes on the poor site than on the

TABLE III

Survival and growth of pine seedlings with *Pisolithus* or *Thelephora* ectomycorrhizae after two years on reforestation sites in Florida (unpublished data)

Ectomycorrhizae from nursery formed by	Survival (%)	Height (cm)	Root collar diameter (cm)	Seedling volume (cm^3)[1]
	Sand ridge site			
Loblolly pine				
Pisolithus	98	87.4	2.66	618*
Thelephora	98	78.0	2.56	511
Slash pine				
Pisolithus	95*	101.5	3.56*	1286*
Thelephora	78	93.9	3.11	908
Sand pine				
Pisolithus	43*	116.4	2.91	986*
Thelephora	16	104.7	2.63	724
	Palmetto flatwood site			
Loblolly pine				
Pisolithus	96	99.4	2.37	558*
Thelephora	96	85.4	2.01	338
Slash pine				
Pisolithus	100*	111.4*	3.45	1326*
Thelephora	93	97.2	2.47	730
Sand pine				
Pisolithus	53*	107.4*	2.07*	460*
Thelephora	27	79.4	1.41	158

[1] Seedling volume (cm^3) = (stem diameter)^2 × height. *Denotes significant differences ($P = 0.05$) between means for mycorrhizal treatments.

good site. However, volumes were similar between seedlings with *Pisolithus* ectomycorrhizae on the poor site and seedlings with natural ectomycorrhizae on the good site. Only one site was planted to white pine seedlings in North Carolina, but the results were dramatic. White pine seedlings with *Pisolithus* ectomycorrhizae had greater survival and five times greater volumes than the *Thelephora* controls.

Root evaluations for *Pisolithus* ectomycorrhizae on seedlings in these tests showed that they persisted extremely well despite competition from other ectomycorrhizal fungi that were indigenous on these sites.

The manipulation of highly beneficial ectomycorrhizal fungi in nurseries or in container programs may be the biological tool needed to improve reclamation and reforestation efforts throughout the world. At this time, only one ectomycorrhizal fungus — *Pisolithus tinctorius* — is approaching a practical application stage. However, its successful manipulation in nurseries and its subsequent impact on tree performance on a variety of sites shows the potential practical importance of these highly specialized, root-inhabiting

TABLE IV

Survival and growth of pine seedlings with *Pisolithus* or *Thelephora* ectomycorrhizae after two years on reforestation sites in North Carolina (unpublished data)

Ectomycorrhizae from nursery formed by	Survival (%)	Height (cm)	Root collar diameter (cm)	Seedling volume (cm^3)[1]
		Good site		
Loblolly pine				
Pisolithus	93	85.1	2.06	361*
Thelephora	92	79.1	1.91	289
Virginia pine				
Pisolithus	96	84.6	2.35	467*
Thelephora	92	82.5	2.15	381
White pine				
Pisolithus	94*	24.2*	0.80*	16*
Thelephora	87	14.8	0.45	3
		Poor site		
Loblolly pine				
Pisolithus	94*	87.4*	1.79	280*
Thelephora	90	74.3	1.61	193
Virginia pine				
Pisolithus	94	85.9*	2.12*	386*
Thelephora	92	75.0	1.84	254

[1] Seedling volume (cm^3) = (root collar diameter)^2 × height. *Denotes significant differences ($P = 0.05$) between mycorrhizal treatments in a pine species.

fungi in forestry. When one considers the numerous species of mycorrhizal fungi in nature that may be manipulated, it is exciting to contemplate their potential impact on forest production throughout the world.

10. CONCLUSION

It is clear that ectomycorrhizae are essential for the growth and development of tree species. The fungal symbionts mobilize and transport significant amounts of nutrients to the host plants. This is particularly true in soils with low fertility. In addition, ectomycorrhizae act as deterrents to root pathogens. More recently, artificial introduction of appropriate ectomycorrhizal fungi into nursery soils and into substrates of containerized tree seedlings has been successfully accomplished. This is particularly important to the introduction of exotic tree species into ectomycorrhizal fungus-free environments and in the establishment of man-made forests. In this chapter we have also discussed the use of ecologically adapted, ectomycorrhizal fungi on tree seedlings to enhance the establishment of trees on adverse soils, such as strip-mined lands.

As more knowledge is obtained on this complex but exciting relationship between higher plants and symbiotic fungi, more and more emphasis will be directed to the utilization of ectomycorrhizae in the biological manipulation of tree species on a variety of sites.

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