

ROLE OF MYCORRHIZAE IN FORESTATION OF SURFACE MINES¹

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Abstract.--A brief introduction to ecto- and endomycorrhizae and their importance to plants is presented. Recent findings confirm the significance of ectomycorrhizae, particularly those formed by *Pisolithus tinctorius* in nurseries, to survival and growth of pine seedlings on strip-mined lands. Commercial inoculum of this fungus may be available in 1981. Recent research shows that endomycorrhizal fungi affect growth of grasses and certain hardwood trees on coal spoils.

INTRODUCTION

Mycorrhiza (fungus-root) is a symbiotic association between the fine feeder roots of green plants and highly specialized root-inhabiting fungi. Each partner in the mycorrhizal association depends on the other to one degree or another for existence. In natural forest soils, which are low in fertility in comparison to artificially fertilized agricultural soils, mycorrhizal associations are essential to forest plants. These associations increase (1) nutrient and water absorption and, thus, nutrient cycling, (2) feeder root health and longevity, and (3) tolerance to drought, high soil temperatures, soil toxins (inorganic and organic), and extremes of soil pH. Most trees must have mycorrhizae to survive; other plants may only need them for maximum growth in natural soils.

With the exception of aquatics, some halophytes and a few other plants, the majority of flowering plants in the world form either ecto- or endomycorrhizae in natural soils (Mollock and others 1980).

Ectomycorrhizae occur naturally on many important forest tree species around the world. All members of the gymnosperm family *Pinaceae* (pine, spruce, fir, larch, hemlock, etc.) as

well as certain angiosperms (willow, poplar, aspen, hickory, pecan, oak, birch, beech, eucalypt, etc.) normally form ectomycorrhizae. Some of these trees can be either ectomycorrhizal or endomycorrhizal, depending on soil conditions. Ectomycorrhizal fungal infection is initiated from spores or hyphae (propagules) of the fungal symbionts inhabiting the rhizosphere of the feeder roots. Propagules are stimulated by root exudates and grow over the feeder root surface and form a fungus mantle. Then hyphae develop around root cortical cells and form what is called the Hartig net. The Hartig net is the main distinguishing feature of ectomycorrhizae. Ectomycorrhizal roots may be unforked, bifurcate, multiforked (coralloid), nodular, or in other shapes. The color of an ectomycorrhiza is usually determined by the color of the hyphae of the fungal symbiont and may be brown, black, white, red, yellow, or blends of these colors. Individual hypha, strands of hyphae, or rhizomorphs may radiate from the fungus mantles into the soil and to the base of the fruit bodies of the fungi.

Most fungi which form ectomycorrhizae with forest trees are Basidiomycetes that produce mushrooms or puffballs (fruit bodies). Certain Ascomycetes such as truffles also form ectomycorrhizae. One genus of mushroom-producing fungi, *Cortinarius*, is composed of over 2000 species distributed throughout the forests of the world; all species form ectomycorrhizae. If other genera are considered, over 5000 ectomycorrhizal fungus species probably exist--including 2100 in North America. The fruit bodies of these fungi produce billions of spores that are widely disseminated by wind and water. Most ectomycorrhizal fungi depend on their hosts for simple carbohydrates, amino acids, vitamins, etc. necessary to complete their life cycles. Ectomycorrhizal development,

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therefore, is a prerequisite for fruit body production by these fungi. Not all fungi which form mushrooms and puffballs are ectomycorrhizal; many are litter decomposers and a few are pathogenic on trees.

Endomycorrhizae are formed on most economically important agronomic and forage crops, woody perennials, ornamentals, fruit and most nut trees, as well as maple, elm, green ash, sweetgum, sycamore, black walnut, and other important forest trees. Alder can form both endo- and ectomycorrhizae as well as symbiotic N-fixing nodules--three different symbiotic root associations at one time. Endomycorrhizal fungi form a loose network of hyphae on feeder root surfaces and do not develop the dense fungus mantle of ectomycorrhizae. These fungi often form large, conspicuous, thick-walled spores both on the root surfaces and in the rhizosphere, and sometimes in feeder root tissues. Hyphae of endomycorrhizal fungi penetrate the cell walls and progress into the cortical cells of the root. These infective hyphae may develop specialized absorbing or nutrient-exchanging structures (haustoria) called arbuscules in the cytoplasm of the cortical cells. Thin-walled, spherical to ovate vesicles may also be produced in cortical cells by these fungi. The term "vesicular-arbuscular" mycorrhizae has been coined to denote this type of endomycorrhizae. As in ectomycorrhizae, endomycorrhizal fungus infection rarely occurs in meristematic or vascular tissues. Endomycorrhizal infection, however, does not cause major morphological changes in roots. Endomycorrhizae, therefore, cannot be detected with the unaided eye; they must be assessed microscopically.

The fungi which form endomycorrhizae with trees are mainly Phycomycetes. They do not produce large, above-ground fruit bodies or wind-disseminated spores as do most ectomycorrhizal fungi, but some of them produce large spores on or in roots. Some species also produce large sporocarps (5 to 10 mm diameter) containing many spores on roots. These fungi spread through the soil by growing from feeder root to feeder root; they are also disseminated by moving water, soil, insects, or animals. They are so widespread that it is nearly impossible to find natural soils anywhere in the world that do not contain them. Spores of these fungi are able to survive in soil for many years without a plant host. Based on the limited research done on endomycorrhizal fungi, most species appear to have very broad host ranges. For example, *Glomus mosseae* is known to form endomycorrhizae with grasses, cotton, corn, pepper, tomato, peas, soybean, and sorghum, as well as sycamore, black walnut, sweetgum, citrus, peach, black locust, green ash, black cherry, boxelder, sugar maple, and red maple. It undoubtedly can infect numerous other plant

species as well. Since endomycorrhizal fungi cannot be grown routinely in the laboratory, production of inoculum is difficult. Usually a fast growing plant, such as sorghum, is used to culture these fungi. Roots and soil from these plants containing the introduced fungus are harvested and used as inoculum.

Many factors affect mycorrhizal development. Some factors affect the tree and others affect the fungal symbionts. Generally, any soil or above-ground condition which influences root growth also influences mycorrhizal development. A susceptible feeder root must be formed by the tree before mycorrhizal infection can occur. The main factors influencing susceptibility of tree roots to mycorrhizal infection appear to be photosynthetic potential and soil fertility. High light intensity and low to moderate soil fertility enhance mycorrhizal development; the other extremes of these conditions (light intensity below 20 percent of full sunlight and excessively high soil fertility) reduce, or may even prevent, mycorrhizal development. Light intensity and fertility appear to influence either the biochemical status of feeder roots, such as controlling levels of simple sugars, or the synthesis of new feeder roots, both of which are prerequisites to symbiotic infection. Roots growing rapidly because of very high soil fertility contain few simple sugars and they are not highly susceptible to ectomycorrhizal fungus infection (Marx and others 1977).

The factors that affect the fungal symbionts directly are those which regulate survival of the fungus in the soil or its growth on roots. Extremes of soil temperature, pH, moisture, etc., and the presence of antagonistic soil microorganisms can affect the survival of symbionts and thereby influence the mycorrhizal potential of the soil. Mycorrhizal fungi cannot grow and reproduce in soil unless they are in symbiotic association with plant roots. These fungi are capable, however, of surviving in a dormant condition for several years without a plant host.

There are several excellent reviews available on ectomycorrhizae (Malloch and others 1980, Marx and Krupa 1978, Marks and Kozlowski 1973) and endomycorrhizae (Malloch and others 1980, Sanders and others 1978, Hayman 1978).

Mycorrhizae and forestation of undisturbed soils

Failures in artificial regeneration programs have shown that many forest trees will not survive long after transplanting if the seedlings do not have an adequate complement of mycorrhizae from the nursery. Most reports deal with trees such as pines, spruces, oaks, and eucalypts and their establishment in areas of the world void of naturally occurring ecto-

mycorrhizal fungi. Mikola (1969) and, more recently, Marx (1980) discussed the need for a parallel introduction of ectomycorrhizal fungi into these areas. This introduction is especially critical in the successful establishment of exotic pine plantations in the tropics. These various reports have conclusively shown that (1) ectomycorrhizae formed by any fungus on roots of seedlings is better for the seedling than no ectomycorrhizae at all, and (2) ectomycorrhizae formed by certain species of fungi are more beneficial to seedlings on certain sites than ectomycorrhizae formed by other fungi. The value of specific ectomycorrhizae, such as that formed by *Pisolithus tinctorius*, to the improved survival and rapid early growth of pine seedlings has been demonstrated on various reforestation sites in the Southern United States (Marx 1980). Little work has been reported on the significance of endomycorrhizae to the artificial regeneration of hardwoods. It is known, however, that endomycorrhizae improve the quality of hardwood seedlings in the nursery (Kormanik and others 1977) which should also improve their field performance.

Ectomycorrhizae and forestation of surface mines

Marx (1975, 1976, 1977a) has reviewed the significance of specific ectomycorrhizae to survival and growth of pines on various spoils left after surface mining for coal and kaolin. Only a few key reports are mentioned here.

Schramm (1966) published a comprehensive report on plant colonization of anthracite wastes in Pennsylvania. He found that early ectomycorrhizal development was essential for the establishment of *Betula*, *Pinus*, *Populus*, and *Quercus* spp. seedlings which developed from seed on the wastes. Fruit bodies of the major fungi that developed near the surviving seedlings were the mushroom-forming fungi *Inocybe lacera*, *Amanita muscaria*, and *Thelephora terrestris*, and the puffball-producing fungi *Scleroderma aurantium* and *P. tinctorius*. The latter fungus appeared to be dominant and Schramm was able to trace its unique gold-yellow hyphal strands from similarly colored ectomycorrhizae to the base of the puffball. He associated this specific fungus with the most vigorously growing seedlings. Since Schramm's original work, numerous other reports on the occurrence of *P. tinctorius* on coal spoils and other adverse sites have been published.

Based on these observations and the conclusion that the natural occurrences of *P. tinctorius* ectomycorrhizae on seedlings growing on adverse sites were instrumental in their establishment and growth, we developed techniques to "tailor" bareroot and containerized seedlings in nurseries with *P. tinctorius*

ectomycorrhizae. Briefly, the techniques involve growing mycelium of *P. tinctorius* in pure culture in the laboratory in vermiculite-peat moss-nutrient substrate. After 2 to 3 months growth, the substrate is leached with water, broadcast onto fumigated nursery soil, and mixed thoroughly into the soil. The bed is then seeded. By the end of the growing season, abundant *P. tinctorius* ectomycorrhizae are developed on seedling roots. The seedlings with *P. tinctorius* ectomycorrhizae also have various quantities of wild-type ectomycorrhizae formed by naturally occurring fungi whose spores are wind disseminated into the fumigated nursery soil. The most prevalent wild-type fungus encountered is *Thelephora terrestris*. *T. terrestris* ectomycorrhizae are the most commonly found ectomycorrhizae on seedlings used for reforestation throughout the United States and in many other parts of the world. This fungus is ecologically adapted to good soil conditions such as those found in nurseries. It is highly beneficial to seedlings planted on routine sites, but is not well adapted to the harsh soil conditions normally encountered on strip-mined lands.

In earlier reviews (Marx 1975, 1976, 1977a), preliminary data showed the advantage of *P. tinctorius* ectomycorrhizae to survival and growth of pine seedlings on adverse sites as compared to seedlings with *T. terrestris* ectomycorrhizae. Very little work has been done on the significance of endomycorrhizae to hardwoods since the earlier reports. The following is the state of the art since the last review on the significance of mycorrhizae to forestation of surface-mined lands.

Ectomycorrhizae and forestation of strip-mined lands

Harris and Jurgensen (1977) observed that cuttings of willow and hybrid poplar grew poorly on copper tailings in Michigan. Ectomycorrhizae were not present even on cuttings inoculated with a forest soil extract supposedly containing ectomycorrhizal fungi. Copper tailings may have been toxic to indigenous or introduced inoculum of ectomycorrhizal fungi, or the physiological conditions of the rooted cuttings may have been inadequate for root infection to occur. A similar study was installed on iron tailings. Seedlings of both hardwood species grew well and formed abundant ectomycorrhizae from indigenous fungi and from those contained in the forest soil inoculum. Better seedling growth and development of ectomycorrhizae on the iron tailings could have been due to better soil fertility or fewer toxic chemicals.

Marx and Artman (1979) reported that bare-root loblolly pine seedlings with abundant *P. tinctorius* ectomycorrhizae had a 400 percent

greater plot volume after 3 years on an acid coal spoil (pH 4.1) in Kentucky than seedlings with *T. terrestris* ectomycorrhizae. On plots where fertilizer starter tablets were used volumes for *P. tinctorius* seedlings were nearly 250 percent greater. In the same test, shortleaf pine seedlings with *P. tinctorius* ectomycorrhizae were also over 400 percent larger without fertilizer tablets and over 100 percent larger with fertilizer tablets than seedlings with *T. terrestris* ectomycorrhizae. Seedlings with *P. tinctorius* ectomycorrhizae also contained more foliar N and less foliar S, Fe, Mn, and Al than seedlings with *T. terrestris* ectomycorrhizae. The fertilizer starter tablets stimulated seedling growth for the first and second growing seasons, but once the nutrients in the tablets were depleted growth increment slowed and N deficiency symptoms appeared on foliage. This deficiency was less striking on *P. tinctorius* seedlings than on seedlings with *T. terrestris*. In this same report, plot volumes for loblolly pine with *P. tinctorius* ectomycorrhizae were over 180 percent greater than those for seedlings with *T. terrestris* ectomycorrhizae after 4 years on an acid coal spoil (pH 3.4) in Virginia.

In two other experiments (C. R. Berry, unpublished data), container grown (root medium with 20 percent sewage sludge) loblolly, pitch, and loblolly x pitch pine hybrids with *P. tinctorius* or *T. terrestris* ectomycorrhizae were outplanted on acid coal spoils in Tennessee and Alabama. All loblolly pines were from a single parent and pitch pine were from two parent trees. After two and one-half growing seasons the results were striking (Table 1). On the Tennessee spoil, *P. tinctorius* ectomycorrhizae increased loblolly pine plot volumes by 585 percent and pitch pine plot volumes by 12 percent for one parent and 125 percent for the other. *P. tinctorius* ectomycorrhizae increased hybrid plot volumes by 120 to 575 percent. Overall, volumes on plots with *P. tinctorius* ectomycorrhizae were over 200 percent greater than plot volumes with *T. terrestris* ectomycorrhizae. On the Alabama spoil, loblolly pine plots with *P. tinctorius* ectomycorrhizae had nearly 300 percent greater volumes than those with *T. terrestris* ectomycorrhizae. The advantage in plot volumes of *P. tinctorius* over *T. terrestris* ectomycorrhizae was 150 and 240 percent for the pitch parents. The pitch pine parent that benefited relatively little on the Tennessee spoil (parent #78) was strongly stimulated by *P. tinctorius* ectomycorrhizae on the Alabama spoil. The reason for this is being investigated. The response of the hybrids to *P. tinctorius* ectomycorrhizae on the Alabama spoil was between 400 and 1400 percent. The overall increase in plot volume attributable to *P. tinctorius* on this spoil was over 350 per-

cent. Regardless of genotype, seedlings with *P. tinctorius* ectomycorrhizae had more N and less Ca, Mn, Zn, Cu, and Al in foliage than seedlings with *T. terrestris* ectomycorrhizae.

Many construction projects, such as dams, highways, and buildings, require extensive earth fill to meet design criteria. When insufficient soil fill is available on site it becomes necessary to "borrow" soil from another location. Generally, the resulting borrow pits are stripped excavations from which all the A and B soil horizons have been removed which present special problems in revegetation. Unlike coal or other surface mining spoils, borrow pits usually do not have toxic levels of heavy metals, extremes of soil acidity, or extremely high soil temperatures. Frequently, however, soil in borrow pits is highly compacted with poor internal drainage and have low levels of available plant nutrients and organic matter.

Two studies were installed on a borrow pit in Aiken, South Carolina, in which soil amelioration techniques and specific ectomycorrhizae were studied to determine their effects on the establishment of pines and grasses. The entire borrow pit was subsoiled to a depth of 0.9 m on 1.2 m centers in two directions. The first study (Berry and Marx 1980) measured effects of applying processed sewage sludge or fertilizer (560 kg/ha of 10-10-10) and dolomitic limestone (2240 kg/ha) with and without bark and/or bottom furnace ash on growth of loblolly pine and fescue grass. In the fall of 1975, sludge, bark, and ash were applied 1.3 cm deep (125 m³/ha), and all plots were double disked. The following winter, loblolly pine seedlings with either *P. tinctorius* or *T. terrestris* ectomycorrhizae formed in a bareroot nursery were planted. By the end of the first growing season, root evaluation revealed that indigenous *P. tinctorius* on the borrow pit formed ectomycorrhizae on *T. terrestris* seedlings and, thus, negated the effects of "tailoring" seedlings with *P. tinctorius* in the nursery. However, sludge with or without the other organic amendments increased pine seedling volumes by 2800 percent and biomass of fescue grass by 500 percent. Soil amended with sewage sludge also contained more N, P, and organic matter, as well as a higher cation exchange capacity, than soil from non-sludge plots. Seedling foliage contained more N and less Ca in sludge plots. In the second test (Ruehle 1980), sludge or fertilizer + lime were similarly applied in the fall of 1975, but the container-grown loblolly pine seedlings with either *P. tinctorius*, *T. terrestris*, or no ectomycorrhizae (produced in a special growth room) were not planted until the fall of 1977. The interim between sludge application and seedling planting significantly decreased the indigenous levels of *P. tinctorius*

Table 1.--Survival and growth of loblolly and pitch pines and their hybrids with *Pisolithus tinctorius* ectomycorrhizae after 2-1/2 growing seasons on coal spoils in Tennessee and Alabama (C. R. Berry, unpublished data).

Pine line	Ectomycorrhizae at planting	Survival %	Height cm	Stem caliper cm	PVI ^a (x 10 ²)
Tennessee Spoil					
Loblolly 23	<i>P. tinctorius</i>	38	70	2.5	48
	Natural	48	40	1.3	7
Pitch 62	<i>P. tinctorius</i>	88	62	2.8	81
	Natural	93	47	2.0	36
Pitch 78	<i>P. tinctorius</i>	85	46	2.2	36
	Natural	94	42	1.8	32
62 x 11-9	<i>P. tinctorius</i>	88	89	3.3	169
	Natural	74	49	1.8	25
62 x 23	<i>P. tinctorius</i>	89	82	3.4	156
	Natural	76	55	2.0	41
62 x 11-20	<i>P. tinctorius</i>	90	84	3.2	156
	Natural	95	64	2.4	71
78 x 23	<i>P. tinctorius</i>	91	86	3.4	175
	Natural	81	54	2.1	48
58 x 11-20	<i>P. tinctorius</i>	90	87	3.1	162
	Natural	83	56	2.1	51
62 x 11-10	<i>P. tinctorius</i>	100	100	3.3	219
	Natural	85	66	2.2	78
Overall mean (TN)	<i>P. tinctorius</i>	85	79	3.0	133
	Natural	81	53	2.0	43
Alabama Spoil					
Loblolly 23	<i>P. tinctorius</i>	79	76	2.9	126
	Natural	73	51	1.7	32
Pitch 62	<i>P. tinctorius</i>	41	57	3.0	41
	Natural	46	38	1.7	16
Pitch 78	<i>P. tinctorius</i>	69	52	2.6	48
	Natural	44	37	1.7	14
62 x 11-9	<i>P. tinctorius</i>	70	67	2.9	101
	Natural	59	46	1.6	19
62 x 23	<i>P. tinctorius</i>	63	69	3.2	106
	Natural	44	36	1.3	7
62 x 11-20	<i>P. tinctorius</i>	76	80	3.6	154
	Natural	68	50	1.7	30
Overall mean (AL)	<i>P. tinctorius</i>	66	67	3.0	96
	Natural	56	43	1.6	20

^aPlot volume index (PVI) computed by (stem caliper, cm)² x height, cm x number of surviving seedlings per plot.

ectomycorrhizae in the soil, since the integrity of the ectomycorrhizal treatments on seedlings was reasonably maintained for the duration of the study. The effects of sludge and specific ectomycorrhizae were significant. After 2 years in sludge plots,

seedlings with *P. tinctorius* ectomycorrhizae had 265 and 528 percent greater plot volumes than seedlings with *T. terrestris* or no ectomycorrhizae at planting. In the fertilizer + lime plots, the initially nonmycorrhizal seedlings had plot volumes 158 percent less

than the seedlings with either of the two ectomycorrhizal treatments. As a group, seedlings on sludge plots had 900 percent greater plot volumes than those on fertilizer plots. Differences in soil and foliar analyses and grass biomass were similar to those obtained in the other borrow pit study. After 2 years, the initially nonmycorrhizal seedlings had abundant ectomycorrhizae; *P. tinctorius* accounted for about 10 percent of these.

There appears to be little doubt that pine seedlings with *P. tinctorius* ectomycorrhizae survive and grow faster on adverse sites than routine nursery seedlings with naturally occurring ectomycorrhizae. These results indicate that seedlings for planting on adverse sites should be tailored in the nursery with *P. tinctorius* ectomycorrhizae. Until now, the only inoculum of *P. tinctorius* available was produced in small quantities on highly defined growth medium under rigidly controlled conditions in research laboratories. For commercial use, large volumes of highly functional inoculum of *P. tinctorius* are required. In 1976, the Institute for Mycorrhizal Research and Development, Athens, Georgia, joined with Abbott Laboratories¹ to devise means of producing vermiculite-based vegetative inoculum of this fungus in large fermentors. After 4 years of testing different formulations of inoculum in over 40 nurseries in 33 States and Canada, adequate procedures for producing functional inoculum have been accomplished. Preliminary results from our 1980 bareroot and container nursery tests in five Southern States are excellent. In 1980, a modified seeder was used for the first time to apply inoculum. This machine broadcasts inoculum, incorporates the inoculum into the root zone, levels the soil, and sows the pine seed in one pass over the nursery bed. This machine greatly simplifies application of vegetative inoculum. If the final results from the 1980 tests are as good as the preliminary results, inoculum will be available from Abbott Laboratories by early 1981.

The use of basidiospores of *P. tinctorius* as inoculum instead of the vermiculite-based inoculum also shows promise. There are certain advantages to the use of basidiospores: (1) no germ-free growth phase is required in fermentors, (2) they are easy to apply via hydromulch to nursery soil (Marx and others 1979), (3) after drying they store for years under refrigeration, and (4) they can be collected directly from adverse sites by the user. Unfortunately, basidiospores also have

certain disadvantages: (1) there are good and poor years for production of *P. tinctorius* fruit bodies in the field, (2) basidiospore collections are often contaminated with other microorganisms and insects which may or may not be harmful to nursery seedlings, (3) viability of basidiospores cannot be determined prior to use since successful germination procedures have not been developed, and (4) by far the most important disadvantage, basidiospores are usually not as effective in forming abundant *P. tinctorius* ectomycorrhizae early in the growing season on nursery seedlings as is properly produced vegetative inoculum. This latter point is very important since research results show that in order for seedlings to obtain maximum benefit from this fungus, at least half of all the ectomycorrhizae on the seedling roots must be formed by *P. tinctorius* (Ruehle and others²). For the past 3 years, adding basidiospores of *P. tinctorius* to encapsulated pine seed has also been studied; results look very promising. Since *P. tinctorius* forms ectomycorrhizae with numerous tree species (Marx 1979), namely, species of *Pinus*, *Quercus*, *Betula*, *Picea*, *Tsuga*, *Carya*, *Salix*, *Populus*, *Abies*, and *Eucalyptus*, its use could significantly improve reclamation efforts of a variety of strip-mined lands.

Endomycorrhizae and forestation of strip-mined lands

With the exception of the outplanted sycamore and sweetgum studies on kaolin spoils reported earlier (Marx 1977a), there are no published works on the effects of endomycorrhizae on growth of hardwoods or other plants tested directly on strip-mined lands. Since the last review, field observations and greenhouse studies have been reported on various plant species.

In Wyoming, Miller (1979) collected root samples from plants growing on a 2-year-old revegetated strip-mined spoil (previously segregated and stored topsoil replaced to a depth of 1 foot) and an adjacent undisturbed site. He found no endomycorrhizae on plant roots growing on the disturbed site but, with few exceptions, abundant endomycorrhizae were found on the diverse plant species growing on the adjacent undisturbed site. Inoculum of endomycorrhizal fungi was present in the disturbed soil, but was not infective. Miller speculated that the absence of infectivity by

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the inoculum may be due to the presence of *Halogeton*, a herbaceous plant with suspected allelopathic capacity. He further speculated that disturbed areas are first colonized by pioneer plant species (*Halogeton* and other species) which are nonmycorrhizal and possibly allelopathic. Miller concluded that succession to mycorrhizal-dependent plant species should follow the elimination of *Halogeton*. Reeves and others (1979) found that 99 percent of the plant cover on a natural, undisturbed sage community in Colorado were endomycorrhizal, and that less than 1 percent of the plant cover on a disturbed area (subsoiled roadbed) were endomycorrhizal. In their opinion, the reestablishment and maintenance of the mycorrhizal fungus component is vital in producing stable plant ecosystems on disturbed areas. Using soils from these same sites in an endomycorrhizal bioassay, Moorman and Reeves (1979) found that the disturbed soil produced only 1/40 as much endomycorrhizal infection on corn as did the undisturbed soil. They concluded that the reduction of active inoculum in the disturbed soil was an important ecological factor in subsequent plant succession.

Daft and Hacskeylo (1976) found that most of the herbaceous plants sampled from anthracite and bituminous coal wastes in Pennsylvania were infected with endomycorrhizae; five naturally occurring plant species had both nodules and endomycorrhizae. In an inoculation test in a greenhouse, lucerne that was nodulated and infected with endomycorrhizae grew 700 percent better in limed (pH 6.4) anthracite waste than control lucerne plants. Regardless of inoculation treatment, however, lucerne plants died in limed (pH 5.8) or nonlimed (pH 2.7) bituminous wastes and unlimed (pH 4.1) anthracite wastes. In a later greenhouse study, Daft and Hacskeylo (1977) reported that red maple seedlings with endomycorrhizae were about 400 percent larger and contained more major elements in foliage after 70 days growth in limed (pH 6.2) anthracite wastes than seedlings without endomycorrhizae.

As discussed, the survival of endomycorrhizal inoculum on strip-mined lands appears important in plant succession. Various mechanical procedures carried out during strip mining can affect inoculum survival. Ponder (1979) assayed recently graded strip-mined coal spoil and found that plants grown in the spoil formed abundant endomycorrhizae in the greenhouse. He concluded that grading during reclamation could be an important means of dispersing endomycorrhizal inoculum in spoil. In unpublished reports¹, survival of endomycorrhizal fungi in piles of topsoil removed prior to strip mining of coal in Wyoming and North Dakota was found to be

significantly reduced after 2- to 3-years-storage as compared to survival in non-disturbed topsoil or topsoil immediately replaced over the mine spoil.

CONCLUSION

It should be apparent from this discussion and earlier reviews (Marx 1975, 1976, 1977a) that mycorrhizae are not only essential to growth and development of trees in natural forest ecosystems, but are especially significant to their performance on strip-mined lands and other adverse sites. This biological fact has not been applied on any practical scale because procedures to manage the fungi have not been available to the land manager. The availability of commercial inoculum of *P. tinctorius* to tailor seedlings in the nursery prior to outplanting is the first step in closing this technology gap. We still have a long way to go, however, in the selection, inoculum production, manipulation, and management of other important ectomycorrhizal fungi, as well as all endomycorrhizal fungi. Research in these and related areas must continue so that sound scientific practices can be used in the forestation of adverse sites and in reforestation of routine forest sites.

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