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The Growth, Nutrient Absorption, and Moisture Status of Selected Woody Species in Coal Mine Spoil in Response to an Induced Infection by the Ectomycorrhizal Fungus *Pisolithus tinctorius*

Roger F. Walker
University of Tennessee Knoxville

To the Graduate Council:

I am submitting herewith a dissertation written by Roger F. Walker entitled "The Growth, Nutrient Absorption, and Moisture Status of Selected Woody Species in Coal Mine Spoil in Response to an Induced Infection by the Ectomycorrhizal Fungus *Pisolithus tinctorius*." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Ecology and Evolutionary Biology.

Clifford C. Amundsen, Major Professor

We have read this dissertation and recommend its acceptance:

Darrell C. West, Herman H. Shugart, Ronald L. Hay

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Accepted for the Council:

Vice Chancellor
Graduate Studies and Research

THE GROWTH, NUTRIENT ABSORPTION, AND MOISTURE STATUS OF SELECTED WOODY SPECIES IN COAL MINE SPOIL IN RESPONSE TO AN INDUCED INFECTION BY THE ECTOMYCORRHIZAL FUNGUS *PISOLITHUS TINCTORIUS*

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Roger F. Walker

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ABSTRACT

The growth, nutrient absorption, and internal moisture status of selected woody species in coal mine spoil in response to an induced infection by the ectomycorrhizal fungus *Pisolithus tinctorius* was studied. Nursery grown loblolly and Virginia pine seedlings infected with *Pisolithus* and control seedlings were outplanted on a coal mine spoil in Tennessee which had been previously hydroseeded with a mixture of herbaceous ground cover species. Granular fertilizer was applied by broadcasting to one-half of the seedlings of each ectomycorrhizal treatment at the rate of 112 kg/ha NPK. After three years, the survival and growth of loblolly pine infected with *Pisolithus* was superior to that of the control seedlings, and chemical analyses of foliar samples revealed that the seedlings with *Pisolithus* ectomycorrhizae had a higher foliar concentration of NO_3 and a lower concentration of Zn than the control seedlings. The survival, growth, and nutrient absorption of Virginia pine was not significantly affected by the infection with *Pisolithus* after two years, but both loblolly and Virginia pine seedlings with *Pisolithus* ectomycorrhizae exhibited an enhanced ability to absorb water during periods of high moisture stress, as determined by the pressure chamber technique. Fertilization substantially reduced the survival of the seedlings of both species.

Sweet birch and European alder were grown under high, intermediate, and low fertility regimes in sand culture containing a mycelial inoculum of *Pisolithus tinctorius* for five months and then

transplanted to coal mine spoil containing an identical Pisolithus inoculum. Control seedlings of each species were similarly grown except that no inoculum was incorporated into the potting media. The nutrient treatments initiated in the sand culture were continued throughout the study. Examinations of the roots of the sweet birch seedlings revealed that high fertility significantly reduced the development of Pisolithus ectomycorrhizae, but Pisolithus formed abundant ectomycorrhizae on the roots of sweet birch grown under the intermediate and low fertility regimes and these seedlings were significantly larger than comparable control seedlings. Chemical analyses of foliar samples revealed that sweet birch seedlings with Pisolithus ectomycorrhizae had a significantly higher foliar concentration of total N and a lower concentration of Mg and Al than the control seedlings. No ectomycorrhizal fungi were found to have infected the roots of the European alder seedlings of any of the ectomycorrhizal-nutrient treatment combinations.

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CHAPTER I

INTRODUCTION

When energy and material resources are extracted in mining operations, the impact on the site usually requires the implementation of substantial ameliorative measures to restore productivity and reduce off-site perturbations. Such is the case in the southern Appalachian Mountains where significant coal reserves lie sufficiently close to the surface to dictate the use of surface mining as the most efficient method of extraction. The extent to which this type of coal production is developed depends upon the economics of the operation as related to such factors as depth and character of overburden, depth and quality of coal seam, development of mining methodologies, and the demand and price of coal. Present economic and energy considerations have prompted the adoption of this mining practice as the method of choice for the production of coal in the foreseeable future.

The universal product of surface mining is a denuded landscape upon which vegetation must be immediately reestablished to avoid future site productivity losses associated with erosion and its subsequent effect on water quality. In many instances these sites, consisting of spoils reflecting the character of the overburden, have both physical and chemical properties which are potentially unfavorable to the establishment of vegetation. Revegetation by natural succession is thus not considered a viable option as the resulting community, reflecting the extreme variability of spoil materials, usually fails to provide adequate site protection. Subsequently, the recovery of these sites is dependent upon managed processes. Many opportunities exist for the development of artificial revegetation techniques to be incorporated in comprehensive programs of reclamation planning such that postmining ecosystems are afforded early protection conducive to subsequent establishment of stable communities.

Mycorrhizal relationships are being given major consideration in the research directed toward improved revegetation techniques for surface mine spoils. These relationships, the most prevalent symbioses of higher plants, are of particular importance to the successful establishment of woody vegetation on adverse sites. Due to mining practices which frequently result in the burial of surface strata, the status of coal spoils in relation to their ability to promote mycorrhizal relationships is questionable. Therefore, the inoculation of selected species of forest tree seedlings with the appropriate ectomycorrhizal fungus prior to outplanting has considerable potential as an invaluable tool in developing effective revegetation methods. This technique depends upon the use of a fungal species ecologically adapted for survival on harsh sites such as coal spoils, where extremes in acidity, temperature, and moisture and nutrient deficiencies are prevalent. The fungal component must be selected with due consideration given its physiological adaptability to infecting host species of a suspected or proven reclamation value. It is conceivable that a mosaic of tree-fungus associations be developed and adapted for use on surface mine sites such that initial revegetation include a diversity of species rather than continuing the current practice of establishing monospecific stands.

This dissertation reports the results of studies concerning the effects of the ectomycorrhizal fungus *Pisolithus tinctorius* (Pers.) Coker and Couch on four host species, including loblolly pine (*Pinus taeda* L.), Virginia pine (*Pinus virginiana* Mill.), sweet birch (*Betula lenta* L.) and European alder (*Alnus glutinosa* (L.) Gaertn.) in relation to the applicability of these specific host-fungus mycorrhizal associations as components of postmining communities established on sites disturbed for coal production in the southern region of the Appalachian Mountains. Due to relative differences in the degree to which preliminary mycorrhizal research had been accomplished on each of the four host species, some of the objectives of this investigation were pursued through field studies involving experimental outplantings on a southern Appalachian surface mine site, while for other objectives mine site conditions were simulated in the greenhouse using coal mine spoil materials as the growth medium. Greenhouse facilities permitted greater environmental control in those aspects of the studies for which little or no background information was available to serve as a basis for experimental design.

Specific objectives accomplished through field studies included an evaluation of the effect of *Pisolithus tinctorius* on the survival, growth, nutrient uptake, and moisture status of loblolly and Virginia pine on a routine surface mine site. Due to the demonstrated response of pine seedlings to fertilization on such sites, a fertility variable was introduced to permit examination of the interrelationships of Pisolithus and nutrient amendments as they affected these research parameters.

Objectives accomplished through greenhouse studies included an evaluation of the physiological suitability of Pisolithus tinctorius as a mycorrhizal symbiont of sweet birch and European alder and an assessment of the effect of variations in nutrient levels on infection success. This was accomplished through sand culture techniques, which afforded greater sensitivity in the regulation of nutrient levels, as well as the mine spoil growth medium. Further studies involving sweet birch and European alder produced an evaluation of the effect of Pisolithus on the growth, nutrient uptake, and moisture status of these two species in a mine spoil growth medium and an examination of mycorrhizal-nutrient level interactions as they affected these growth parameters and physiological processes.

CHAPTER II

REVIEW OF PERTINENT LITERATURE

Selection of Fungal Symbionts

Microorganisms are present in great numbers within the rhizosphere of forest trees and play important roles in numerous physiological relationships, including saprophytism, pathogenicity, and symbiosis. The most prevalent symbiosis is the mycorrhizal association involving root-inhabiting fungi and the feeder roots of the forest tree hosts. Mycorrhizal associations are so common in natural forest soils that the nonmycorrhizal tree is the exception (Marx and Bryan 1975a). Ectomycorrhizae occur naturally on many important forest tree species, including all genera of the gymnosperms such as *Pinus*, *Picea*, *Abies*, *Larix*, *Tsuga*, and *Pseudotsuga* and those of certain angiosperms, including *Betula*, *Alnus*, *Quercus*, *Carya*, *Juglans*, *Populus*, *Fagus*, and *Salix*. This form of mycorrhizae develops from fungal spores or hyphae in the rhizosphere which are stimulated by root exudates to grow vegetatively over the root surface, forming the fungus mantle. The hyphae then develop intercellularly in the root cortex, forming the Hartig net. The fungus mantle and the Hartig net are the distinguishing features of the ectomycorrhizal association. Ectomycorrhizae benefit the host by promoting increased growth and branching of the roots, thus increasing the effective surface area and facilitating the absorption of nutrients and water. The extramatrical hyphae of the fungus mantle function as additional nutrient and water absorbing entities and assure their maximum uptake from the soil.

Many species of ectomycorrhizal fungi infect the roots of forest trees in normal forest soil environments (Marks and Kozlowski 1973; Trappe 1962). Under harsh conditions, such as those that prevail on surface mine sites, certain of these fungi appear superior in their ability to survive and provide benefits to their hosts. Many workers (Hile and Hennen 1969; Lampky and Peterson 1963; Marx 1975; Medve et al. 1977; Schramm 1966) have reported the occurrence of basidiocarps of _Pisolithus tinctorius_ associated with several forest tree species on various coal spoil sites. It is logical to assume that _Pisolithus_ contributed significantly to the survival and growth of these early colonizing host species. Marx et al. (1970) found that _Pisolithus_ was capable of forming ectomycorrhizae at elevated temperatures and later found that pine seedlings with _Pisolithus_ exhibited enhanced survival and growth at higher temperatures than did seedlings with other ectomycorrhizal associations (Marx and Bryan 1971). This temperature tolerance may partially explain the prevalence of _Pisolithus_ as the primary symbiont of young volunteer seedlings growing on coal mine spoils, where surface temperatures often exceed the limits for growth of other ectomycorrhizal fungi.

The specialized and diverse nature of ectomycorrhizal fungi has presented problems for practical application because the endemic fungal species of nursery soils are often ill suited to function effectively at the ultimate outplanting site of the host tree. Experimental techniques have been developed to artificially infect pine seedlings with pure cultures of *Pisolithus tinctorius* in the laboratory and the nursery (Bryan and Zak 1961; Marx 1969; Marx and Bryan 1975b). This technique involves the production of a pure culture, vegetative mycelial inoculum of *Pisolithus* in a vermiculite-peat moss-nutrient medium substrate; the inoculum is incorporated in fumigated potting media or nursery soils. Inoculated seedlings have been shown to be superior to uninoculated seedlings in the nursery (Marx and Bryan 1975b; Marx et al. 1976), on routine reforestation sites (Marx et al. 1977a), and on adverse sites (Berry and Marx 1978; Marx 1976; Marx and Artman 1979; Walker et al. 1981). Generally, it became apparent from these studies that as site quality decreased, the value of *Pisolithus* to the seedlings increased. This was of particular importance on surface mine spoils where such adverse growing conditions as low pH, low nutrient status, high concentrations of toxic substances, elevated surface temperatures, and droughtiness were commonly encountered. This evidence suggests that the artificial inoculation of seedlings in the nursery with *Pisolithus* promoted sufficient ectomycorrhizal development to provide these seedlings a significant advantage over those grown by conventional methods.

Selection of Forest Tree Hosts

Formal and informal studies involving performance comparisons among tree species of potential value for the revegetation of surface mines were carried out extensively in the earlier years of reclamation research (Boyce and Merz 1959; Brown 1962; Czapowskyj 1970;

Czapowskyj and McQuilkin 1966; Finn 1958; Geyer 1973; Hart and Byrnes 1960; Horn and Ward 1969; Limstrom 1960; Limstrom and Deitschman 1951; Miles et al. 1973; Plass 1975). Species were evaluated with respect to such factors as survival, growth, ability to provide site stability and protection, suitability for providing food and cover for wildlife, and potential for economic returns via timber and pulpwood production. Portions of the data generated in these studies have been used in the development of planting guides to facilitate species selection (Boyce and Neebe 1959; Limstrom 1960). Many species with apparent potential for the revegetation of these sites have not been deployed due to deficiencies in establishment techniques, and there has been a decided tendency to plant extensive areas to one species (Limstrom 1964). Schramm's (1966) conclusion that early ectomycorrhizal development was essential for the successful establishment of volunteer species on anthracite wastes has resulted in the recognition of mycorrhizal relationships as an additional factor to be considered in selecting revegetation species for surface mine sites.

Loblolly and Virginia pine have been the species of choice among conifers for regenerating marginal lands in the southeastern United States for many years. These species often invade eroded and abandoned farm land where relatively low levels of available nutrients have rendered the soil unsuitable for further agricultural use (Fowells and Krauss 1959). Loblolly pine has been observed as an early volunteer species on kaolin wastes in Georgia and coal mine spoils in Tennessee (personal observation). Virginia pine frequently occurs on the Cumberland Plateau of Tennessee on poor, sandy sites and bedrock outcrops (Burton 1960) and has been observed as a volunteer species on coal spoils in Tennessee and Kentucky (personal observation) and on anthracite wastes in Pennsylvania (Schramm 1966). Outplanting trials of loblolly (Boyce and Merz 1959; Boyce and Neebe 1959; Geyer 1971; Limstrom 1960; Thor and Kring 1964) and Virginia pine (Boyce and Merz 1959; Boyce and Neebe 1959; Brown 1962; Czapowskyj and McQuilkin 1966; Geyer 1971; Limstrom 1952; Limstrom 1960) on coal mine sites in several midwestern and central and southern Appalachian states have proven these species superior in their ability to survive and grow under varied and diverse spoil conditions. In addition to their ameliorative and stabilizing qualities, loblolly and Virginia pine offer promise of short term economic returns via the production of pulpwood on such sites (Plass and Burton 1967). Extensive studies of the mycorrhizal associations of these species on various mine spoils in Tennessee, Georgia, Alabama, Kentucky, Virginia, West Virginia, Pennsylvania, Ohio, and Indiana revealed *Pisolithus tinctorius* to be their predominant, and frequently their only, naturally occurring mycorrhizal symbiont (Marx 1975; Schramm 1966). A major portion of the mycorrhizal research involving loblolly and Virginia pine on surface mine spoils has been concerned with the potential benefits of an induced infection with this fungal species. With few exceptions, preliminary research has indicated that nursery grown seedlings infected with *Pisolithus* are superior to seedlings with other symbionts on these sites (Marx 1975; Marx 1976; Marx and Artman 1979; Walker et al. 1981).

Sweet birch occurs naturally on a wide variety of less favorable sites with rocky, coarse-textured, or shallow soils (Brooks 1920; Frothingham 1915; Frothingham 1931; Leak 1958; Tryon 1943) and has been considered a species of potential value for purposes of soil protection and stabilization (Illick 1923). Tryon and Markus (1953) found it growing on century-old iron ore spoil banks in West Virginia, and it has also been identified as a volunteer species on coal spoils in West Virginia (Brown and Tryon 1960) and Pennsylvania (Schramm 1966). Plass (1975) evaluated the reclamation potential of this commercially important species on a coal spoil in eastern Kentucky and found it to exhibit excellent survival and growth after four years. Despite the evident promise of sweet birch, minimal research has been accomplished toward its development as a reclamation species for surface mines and it has not been exploited for this purpose to any appreciable degree. Preliminary examinations of the mycorrhizal associations of this species have indicated *Pisolithus tinctorius* to be its most prevalent fungal symbiont on harsh sites. Schramm (1966) concluded that an early infection with *Pisolithus* was essential for the successful establishment of sweet birch on anthracite wastes in Pennsylvania. Marx (1975) found *Pisolithus* to be the predominant, and often the only, ectomycorrhizal fungus associated with sweet birch on coal spoils throughout much of the natural range of this host.

European alder has long been advocated as a species of considerable value for the revegetation of disturbed sites in Europe (Kohnke 1941) and in recent years has gained recognition for this potential in the United States (Limstrom 1960; Bennett et al. 1978). Among the first trees to invade disturbed areas within its naturalized distribution in eastern North America, this species offers substantial ameliorative qualities due to its ability to fix atmospheric nitrogen (Tarrant and Trappe 1971). Experimental plantings on coal spoils have indicated European alder to be adaptable to a wide variety of spoil conditions (Miles et al. 1973), including extreme acidity (Funk and Dale 1961; Lowry et al. 1962), and it has been shown to be of considerable benefit as a nurse crop to other timber species of greater commercial value (Dale 1963). Funk and Dale (1961) and Lowry et al. (1962) have advocated the use of this species as a possible alternative to black locust (*Robinia pseudoacacia* L.) on surface mine sites, believing it to be superior to locust for interplanting purposes and as a potentially greater asset for the production of wood and pulpwood materials. European alder is a known host of ectomycorrhizal fungi (Trappe 1962), but examination of this relationship under the conditions prevailing on surface mine sites has been limited to general observation. Marx (personal communication) has found basidiocarps of *Pisolithus tinctorius* associated with European alder on harsh sites in the Tennessee Copper Basin.

Ectomycorrhizae and Seedling Nutrition

Many coal mine spoils, including those that are not acid, are difficult to revegetate because they are deficient in one or more of the essential plant nutrients (Vogel 1975). Most mine spoils in the

Appalachian region are deficient in plant-available nitrogen and phosphorus (Bengtson et al. 1973a; Bengtson et al. 1973b; Czapowskyj 1973; Mays and Bengtson 1978; Plass and Vogel 1973; Vogel 1975). Though nutrient requirements for the survival and modest growth of forest tree seedlings are relatively low, several workers (Bengtson et al. 1973a; Zarger et al. 1973) have reported fertilization to have a positive effect on the growth of pine seedlings on coal spoils in the southern Appalachians. More recently, Berry (1979) provided evidence that the use of starter fertilizer tablets improved initial growth of pine on severely eroded sites in the Tennessee Copper Basin. General references to the ability of a mycorrhizal association to enhance the uptake of essential nutrients by forest tree seedlings on surface mine sites have been made (Marx 1975; Marx 1976; Marx 1980; Mays and Bengtson 1978; Schramm 1966), and it is generally believed that an infection with an appropriate, ecologically adapted mycorrhizal fungus can substantially reduce the need for supplemental fertilization on these sites. However, only preliminary assessments of the effects of mycorrhizal-nutrient amendment interactions on seedling survival, growth, and nutrient absorption are available, and similar experiments have not produced wholly complimentary results. Marx and Artman (1979) reported that fertilized and nonfertilized loblolly pine seedlings infected with *Pisolithus tinctorius* exhibited greater survival and growth than either fertilized or nonfertilized seedlings with other, naturally occurring ectomycorrhizal symbionts on a coal spoil in Kentucky. Seedlings with *Pisolithus* had significantly more foliar nitrogen and less foliar sulfur, iron, manganese, and aluminum than control seedlings. Walker et al. (1981) found that Pisolithus increased the survival and growth of loblolly pine irrespective of fertilization on a spoil in Tennessee, but that fertilization reduced survival while increasing growth irrespective of mycorrhizal symbiont. However, the infection with Pisolithus was only partially capable of compensating for the reduced survival and producing the increased growth that resulted from fertilization. Discrepancies between the results of these two studies were probably attributable to differing levels of infection with Pisolithus tinctorius and variations in site characteristics and methods of fertilization.

Ectomycorrhizae and Moisture Stress

The variation in spoil characteristics encountered by plants on surface mine sites is nearly unlimited, and though the chemical properties of these sites have generally received greater attention, physical attributes also play a vital role in the success or failure of revegetation efforts. Moisture deficiencies in the upper layers of some coal spoils often limit the survival and growth of forest tree seedlings despite efforts to select species which have exhibited considerable tolerance of the adverse conditions usually prevailing on these sites. Precipitation in the Appalachian region is generally adequate for the species commonly employed in reclamation programs, although drought or near-drought conditions during the first growing season have been implicated in reduced establishment success in several studies (Albers and Carpenter 1979; Brown and Tryon 1960; Horn and Ward 1969; Limstrom and Deitschman 1951; Schramm 1966; Tackett and Graves 1979). However, adequate precipitation does not provide assurance of a satisfactory moisture status on many sites, as some mine spoils do not have sufficient capacity to retain water deposited at their surface. Spoil texture is considered to be the most important factor influencing the water holding capacity of overburden materials, as organic matter is generally lacking in surface strata (Glover et al. 1978). Some spoils dry out rapidly after rainfall because the spoil texture permits rapid infiltration and percolation of water, while others are droughty because they develop a sealed surface that prevents infiltration of moisture (Vogel 1975). The grading of surface mine sites, required in most current reclamation laws, often contributes to the problem of inadequate infiltration of spoils where fine-textured materials predominate, and has resulted in a significant reduction in the establishment success of several species in studies on relevant sites (Limstrom 1952; Limstrom 1960; Limstrom 1964; Limstrom and Merz 1949). The capacity of forest tree seedlings to thoroughly exploit the spoil volume in their vicinity for available moisture is thus of considerable importance in revegetation efforts. General references to the ability of a mycorrhizal association to facilitate the absorption of water by the host on surface mine spoils have been made by several workers (Marx 1975; Marx 1976; Marx 1980; Mays and Bengtson 1978; Schramm 1966), but studies specifically concerned with this phenomenon are lacking. It has been demonstrated that the tolerance of selected ectomycorrhizal fungi to induced water stress differs markedly (Mexal and Reid 1973, Worley and Hacskaylo 1959), and that ectomycorrhizal rhizomorphs can absorb water and facilitate its transport over significant distances through the hyphal network (Duddridge et al. 1980). Drought tolerance is a desirable trait in symbionts chosen for the inoculation of forest tree seedlings destined for use on coal spoils in the Appalachian region, and of the many ectomycorrhizal fungi, *Pisolithus tinctorius* is among those best recognized for their ability to form vigorous mycorrhizal associations on droughty sites (Trappe 1977).

CHAPTER III

MATERIALS AND METHODS

Field Studies

Inoculum Preparation

The *Pisolithus tinctorius* inoculum used in the field study of loblolly pine, designated GA 100, was produced by the Institute of Mycorrhizal Research and Development (IMRD) of the USDA Forest Service[1] by the methods of Marx (1969) and Marx and Bryan (1975b). It consisted of fungal mycelia grown on a vermiculite-peat moss-nutrient medium substrate such that the hyphae permeated the vermiculite particles. The *Pisolithus* inoculums used in the study of Virginia pine consisted of three formulations; the GA 100 as described above, the ABB 100, and the ABB 200. The ABB 100 and ABB 200 formulations, also consisting of fungal mycelia grown on the vermiculite-peat moss-nutrient medium substrate of Marx (1969) and Marx and Bryan (1975b) were prepared by Abbott Laboratories.[2] In the field studies of loblolly and Virginia pine, GA 100 designates the application of 100 ml/.093 m^2 of the inoculum produced by IMRD to the nursery bed. In the study of Virginia pine, ABB 100 and ABB 200 designates the application of 100 ml/.093 m^2

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[1]Southeastern Forest Experiment Station, Forestry Sciences Laboratory, Carlton Street, Athens, Georgia 30602.

[2]Plant Science and Agricultural Chemical Research Division, 36 Oakwood Road, Long Grove, Illinois 60047.

and 200 ml/.093 m^2, respectively, of the inoculum produced by Abbott Laboratories to the nursery bed.

Seedling Production

Loblolly pine. The Pisolithus inoculated seedlings and the control seedlings used in this study were grown by the Weyerhaeuser Company[3] by the methods of Marx and Bryan (1975b) with modifications to accommodate the prevailing site factors and soil conditions of the nursery. The nursery bed was preconditioned with 560 kg/ha of 10-20-10 (NPK) fertilizer and 1120 kg/ha of dolomitic limestone three weeks prior to inoculation and then fumigated for four days with 392 kg/ha of Dowfume® MC-2 and aerated for two weeks. Immediately before inoculation, several soil subsamples were collected from the nursery bed, combined into one composite sample, and analyzed for texture, percent organic matter, pH, total N, available P, and exchangeable K, Ca, Mg, and Mn.[4] The Pisolithus inoculum was applied in late April 1977 to designated sections of the bed at the rate of 100 ml/.093 m^2 (GA 100) and incorporated into the soil, while the remainder of the bed was left to become infested by naturally occurring ectomycorrhizal fungi for the production of control seedlings. Loblolly pine seeds (McCurtain County, Oklahoma seed source) previously stratified for 45 days at 4°C

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[3]Southern Forestry Research Center, P. O. Box 1060, Hot Springs, Arkansas 71901.

[4]Soil analysis was done by Dr. Carol G. Wells, USDA Forest Service, Forestry Sciences Laboratory, Research Triangle Park, North Carolina 27709.

were treated with Arasan® and planted immediately after the inoculation of the bed. Three applications of $(NH_4)_2SO_4$ were applied in two-week intervals at the rate of 112 kg/ha/application beginning in June 1977. The seedlings were treated with one application of Modown® (4.2 kg/ha) in May for weed control with three additional applications in monthly intervals at one-half the initial application rate (1.88 kg/ha/application), 10 applications of Fermate® (2.24 kg/ha/application) for rust protection with biweekly applications beginning in May, and three applications of DiSyston® (75 l/ha/application) in monthly intervals beginning in July for tip moth control. In September, 119 kg/ha of KCL as 0-0-60 (NPK) was applied for hardening and the bed was then laterally undercut. The seedlings were lifted by hand in January 1978 and graded to a height of 15 cm and a root collar diameter of 3 mm. Ten seedlings were randomly selected from both the inoculated and control sections of the bed, measurements were made of height, root collar diameter, and top and root fresh weights, and the ectomycorrhizal development was evaluated by IMRD by the method of Marx et al. (1976). The seedlings were stored at 5°C for two months prior to outplanting.

Virginia pine. The Pisolithus inoculated seedlings and the control seedlings used in this study were grown by the Vallonia, Indiana State Nursery[5] by the method of Marx and Bryan (1975b) with modifications to accommodate the prevailing site factors and soil conditions of the nursery. The nursery bed was fertilized with 448

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[5]Indiana Division of Forestry, Vallonia, Indiana 47281.

kg/ha of 12-12-12 (NPK) fertilizer in early April 1978, and then fumigated one month later with 448 kg/ha of Dowfume® MC-2 for four days and aerated for two weeks. Immediately prior to inoculation, several soil subsamples were collected, combined into one composite sample, and analyzed for texture, pH, total N, available P, and exchangeable K, Ca, and Mg.[6] The GA 100 and ABB 100 inoculums were applied at the rate of 100 ml/.093 m^2 and the ABB 200 at the rate of 200 ml/.093 m^2 to designated sections of the bed in mid May and incorporated into the soil, while the remainder of the bed was left to become infested by naturally occurring ectomycorrhizal fungi for the production of control seedlings. Virginia pine seeds (southern Indiana seed source) previously stratified for two months at 4°C were treated with Arasan® and planted immediately after the inoculation of the bed. In June, 448 kg/ha of 12-12-12 (NPK) fertilizer was applied as a top dressing. The bed was laterally undercut and the seedlings lifted by hand in late March 1979 and graded to a height of 15 cm and a root collar diameter of 3 mm. Ten seedlings were lifted from each section of the bed, measurements were made of height, root collar diameter, and top and root fresh weights, and the ectomycorrhizal development was evaluated by IMRD by the method of Marx et al. (1976). The seedlings were stored at 5°C for three weeks prior to outplanting.

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[6]Soil analysis was done by Dr. Carol G. Wells, USDA Forest Service, Forestry Sciences Laboratory, Research Triangle Park, North Carolina 27709.

Study Installation

Site preparation. The outplanting site for the loblolly and Virginia pine field studies was a surface mine spoil located on a west facing slope on Brushy Mountain in Campbell County, Tennessee (36°19'30"N, 84°17'30"W). The site consisted of one upper and one lower bench separated by a slope. All mining ceased in the fall of 1977 and the spoil was returned to approximate original contour. The site was fertilized with 224 kg/ha of NH_4NO_3, 224 kg/ha of triple superphosphate, and 112 kg/ha of K_2O; hydroseeded with a mixture of Kentucky 31 tall fescue (*Festuca arundinacea* Schreb.), perennial ryegrass (*Lolium perenne* L.), and Korean lespedeza (*Lespedeza stipulacea* Maxim.); and a straw-cellulose fiber-asphalt mulch was applied.

Loblolly pine. Five replicate blocks, each with four 5 x 5 m plots, were established on the spoil with a 6 m wide border separating each plot. Five spoil subsamples were collected from each plot at a depth of 0 to 20 cm, combined into one composite sample per plot, and analyzed for texture, percent organic matter, pH, total N, NO_3, NH_3, weak and strong bray P, and K, Ca, Mg, Fe, Al, Zn, Mn, Cu, S, B, and Mo. Texture was determined by the hydrometer method; percent organic matter by the Walkley-Black method; pH by use of a glass electrode on a 1:1 mixture of spoil and distilled water; total N by macro-Kjeldahl digestion; NO_3 by an Orion® specific ion electrode after extraction with $CaSO_4$; NH_3 by steam distillation; weak and strong bray P colorimetrically after extraction with HCl and NH_4F; K, Ca, and Mg by atomic absorption after extraction with $NH_4C_2H_3O_2$; Fe, Al, Zn, Mn, and Cu by atomic absorption after extraction with HCl; S turbidimetrically after extraction with

$NH_4C_2H_3O_2$; B colorimetrically after extraction with water; and Mo by the acid ammonium oxalate method (American Society of Agronomy 1965). Analyses of variance were made on all spoil data and the differences among means were evaluated with Duncan's Multiple Range Test (P=0.05). One of four treatments was randomly assigned to each of the four plots within a replicate block. The treatments in this randomized block design were GA 100 seedlings fertilized at the rate of 112 kg/ha NPK, control seedlings fertilized at an identical rate, GA 100 seedlings without fertilization, and control seedlings without fertilization. Twenty-five seedlings were planted by hand in April 1978 in each plot in five rows of five seedlings each. The spacing of seedlings within and between rows was approximately 1.25 m. One hundred fourteen grams of 15-15-15 (NPK) granular fertilizer was evenly distributed over 0.37 m^2 around each of the appropriate seedlings in early June to achieve the fertilization equivalent of 112 kg/ha of NPK (as N, P_2O_5, and K_2O).

Virginia pine. Three replicate blocks, each with four 5 x 10 m plots, were established on the spoil with a 6 m wide border separating each plot. Each plot was divided into two subplots. Five spoil subsamples were collected from each subplot at a depth of 0 to 20 cm, combined into one composite sample per subplot, and analyzed for texture, percent organic matter, pH, total N, NO_3, NH_3, weak and strong bray P, and K, Ca, Mg, Fe, Al, Zn, Mn, Cu, S, B, and Mo by the methods described above for the loblolly pine study spoil samples. Analyses of variance were made on all spoil data and the differences among means were evaluated with Duncan's Multiple Range Test (P=0.05). One of four ectomycorrhizal treatments was randomly assigned to each of the four plots within a replicate block, and each of the two subplots within a plot was assigned a fertilization treatment (either fertilized or nonfertilized). The ectomycorrhizal treatments of the seedlings in this split plot design were the Ga 100, the ABB 100, the ABB 200, and the controls infected with fungal symbionts endemic to the nursery. Twenty-five seedlings were planted by hand in April 1979 in each subplot in five rows of five seedlings each with a spacing within and between rows of approximately 1.25 m. One hundred fourteen grams of 15-15-15 (NPK) granular fertilizer was evenly distributed over 0.37 m^2 around each of the appropriate seedlings in late May to achieve the fertilization equivalent of 112 kg/ha of NPK (as N, P_2O_5, and K_2O).

Data Collection and Statistical Analysis

Survival and growth. At planting, measurements of height and root collar diameter were taken of each seedling. Survival, height, and root collar diameter measurements were taken again in March 1979, March 1980, and May 1981 for loblolly pine and March 1980 and May 1981 for Virginia pine. Height and root collar diameter measurements for each succeeding year of the studies were also expressed as relative growth indices by subtracting the initial measurements from the yearly measurements and dividing by the initial measurements. Growth parameters expressed in this manner compensate for initial differences in seedling size and variation in planting depth. The plot volume index (PVI) of Marx et al. (1977a), which incorporates both survival and growth, was employed as an indicator of overall seedling performance. The PVI was also expressed as a relative index (RPVI) by subtracting the initial PVI from the yearly PVI and dividing by the initial PVI. Analyses of variance were made on all data and the differences among means were evaluated with Duncan's Multiple Range Test (P=0.05).

Nutrient absorption. In late July 1979 and late July 1980, current-year needle subsamples were collected from each of the nine interior seedlings of the loblolly pine plots and combined into one composite sample per plot and year. The needles were 70 to 90 percent elongated at the time of sampling. Similarly, in late July 1980, current-year needle subsamples were collected from each of the nine interior seedlings of the Virginia pine subplots and combined into one composite sample per subplot. If less than five of the nine interior seedlings of a plot (for loblolly pine) or subplot (for Virginia pine) were surviving at the time of sampling, no sample was collected. Immediately after sampling, all samples were dried at 100°C for 24 hours in a forced-draft oven, ground in a Wiley mill to pass a 20-mesh screen, and sealed in air-tight glass jars. The samples were chemically analyzed for total N, NO_3, P, K, Ca, Mg, Fe, Al, Zn, Mn, Cu, S, B, and Mo. Total N was determined by macro-Kjeldahl digestion; NO_3 by an Orion® specific ion electrode after extraction with AG_2SO_4; P and B colorimetrically after dry ashing and being taken up in HCl; S turbidimetrically after fixing with $Mg(NO_3)_2$; and K, Ca, Mg, Fe, Al, Zn, Mn, Cu, and Mo by atomic absorption after dry ashing and being taken up in HCl (Association of Official Analytical Chemists 1980).

Analyses of variance were made on all data and the differences among means were evaluated with Duncan's Multiple Range Test (P=0.05).

<u>Moisture stress</u>. In early July 1980, five Tru-Chek® rain gauges were installed on the field study site such that precipitation falling on any part of the site could be detected and measured within a 24-hour period. Also, the percent ground cover of each plot of loblolly pine and each subplot of Virginia pine was determined by the guided estimate method of Phillips (1959) in order to evaluate the effect of competing vegetation on seedling moisture status. During the third week of July, a PMS® Model 600 portable pressure bomb was employed to evaluate the internal water status of the loblolly and Virginia pine seedlings of each ectomycorrhizal-fertilization treatment combination. Five seedlings from each plot (for loblolly pine) or subplot (for Virginia pine) within each replicate block were randomly selected for measurement. If there were less than five surviving seedlings in a plot or subplot, then all surviving seedlings were measured. A maximum of two replicate blocks of loblolly pine or one replicate block of Virginia pine were tested during a single day. One hour prior to measurement, an Irrometer® Model M eight-inch soil tensiometer was installed at the center of each plot or subplot to be tested to provide an indication of spoil moisture status. Measurements of xylem potential, an indicator of the internal moisture status of the seedlings, were made at dawn, when moisture stress was least, and in the early afternoon, when it was greatest, by the method of Waring and Cleary (1967). The dawn measurements were begun at 6:00 a.m. and

completed by 7:00 a.m. At the measurement of each individual seedling, the soil tensiometer reading was recorded. Small branches of similar size were selected from the first live whorl for measurement. The xylem potential of the seedlings was recorded in lb/in^2. This procedure was repeated in the early afternoon between 1:00 p.m. and 2:00 p.m. on the seedlings previously measured at dawn of the same day. All measurements of the xylem potential in lb/in^2 were converted to negative megapascals (MPa). The relative change in xylem potential was determined by subtracting the dawn measurement from the afternoon measurement and dividing by the dawn measurement. The height and root collar diameter of the tested seedlings were recorded and an estimate of seedling volume was determined by multiplying the height of each seedling by the square of its root collar diameter (Marx et al. 1977a). Analyses of variance were made on all data and the differences among means were evaluated with Duncan's Multiple Range Test (P=0.05).

Greenhouse Studies

Inoculum Preparation

The *Pisolithus tinctorius* inoculum used in the greenhouse studies of sweet birch and European alder, designated ESD 200, was produced at Oak Ridge National Laboratory by the methods of Marx (1969) and Marx and Bryan (1975b). It consisted of fungal mycelia grown on a vermiculite-peat moss-nutrient medium substrate such that the hyphae permeated the vermiculite particles. This inoculum was applied at the rate of 200 ml/.093 m^2 of both the sand and mine spoil potting media. The inoculum was stored at 5°C for six weeks prior to inoculation of the sand and for two weeks prior to inoculation of the mine spoil. A sterile mixture of vermiculite and peat moss was used for the production of control seedlings.

Potting Media Preparation

Sand. White quartz sand, screened through a U.S.A. No. 10 sieve, was acid washed for five days using 6.5 percent HCl. Prior to acid washing, the pH of the sand was determined by adding one part sand to one part distilled water (by weight), mechanically stirring the mixture for one hour, and determining the pH with a standardized glass electrode. The sand was rinsed with distilled water by placing it on 32-mesh stainless steel screen so that any silt or clay particles were removed. Rinsing continued until the pH returned to the value existing prior to the acid wash, as measured by the method described above. The sand was fumigated with 3.4 g/kg of Dowfume® MC-2 for three days and aerated for four days prior to inoculation.

Mine spoil. Fresh spoil from a coal surface mine in Campbell County, Tennessee was crushed and homogenized with a mechanical soil shredder to pass a 2.54 cm mesh screen. The spoil was fumigated with 1.3 g/kg of Dowfume® MC-2 for three days and aerated for five days prior to inoculation. Ten spoil samples were randomly collected and analyzed for texture, percent organic matter, pH, total N, NO_3, NH_3, weak and strong bray P, and K, Ca, Mg, Fe, Al, Zn, Mn, Cu, S, B, and Mo by the methods previously described for the loblolly pine field study spoil samples.

Study Installation

Four hundred thirty-two milliliters of the ESD 200 *Pisolithus tinctorius* inoculum was applied to each of six 50 x 40 x 6.4 cm seed flats filled with the acid-washed quartz sand, and each of the flats was sown with 300 sweet birch or European alder seeds (east Tennessee seed source; germination tests revealed the germination success of the seed lots of both species to be approximately 25 percent). The seeds were previously stratified under moist conditions at 3°C for eight weeks and sterilized by the water rinse sterilization method of Karrfalt[7] (personal communication). Six additional seed flats with a sterile mixture of vermiculite and peat moss replacing the ESD 200 inoculum were identically prepared and sown for the production of control seedlings. The flats were misted with distilled water for 10 minutes twice daily until germination. Each of the three flats of each species-ectomycorrhizal treatment combination was designated to receive one of three nutrient treatments; one-half concentration of Hoagland's #2 nutrient solution with micronutrients and iron, one-quarter concentration of Hoagland's #2 nutrient solution with micronutrients and iron, or one-eighth concentration of Hoagland's #2 nutrient solution with micronutrients and iron (Hoagland and Arnon 1950). Each flat received one liter of the appropriate nutrient concentration each week beginning at germination. Iron was applied with every fourth application of the nutrient solutions. The flats were watered with distilled water twice weekly, and the photoperiod was maintained at

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[7] USDA Forest Service, Southeastern Forest Experiment Station, P. O. Box 819, Macon, Georgia 31298.

16 hours with supplemental lighting. The temperature in the greenhouse was 32°C during the day and 26°C at night. After five months, four seedlings of each species and ectomycorrhizal-nutrient treatment combination, comprising one replication, were transplanted to each of 11 polyethylene containers (11.4 l capacity). Seedlings inoculated with Pisolithus in the seed flats were reinoculated by incorporating 132 ml of the ESD 200 inoculum into the mine spoil of the appropriate containers, while 132 ml of the sterile mixture of vermiculite and peat moss was applied to containers receiving control seedlings. Care was taken to insure that all seedlings within a species and ectomycorrhizal-nutrient treatment were similar in size. Nutrient treatments with concentrations identical to those described above were continued except that each container (replication) received 250 ml of the appropriate nutrient concentration every three weeks and the iron supplement was applied every 12 weeks. The seedlings were watered twice weekly with distilled water and the greenhouse conditions maintained during growth in the seed flats were continued throughout the study. Three applications of Resmethrin® at 0.39 ml/m^2/ application were applied in six-month intervals to control whiteflies. The position of the containers within the greenhouse was changed every three months to insure that all seedlings were subjected to similar overall growth conditions.

Data Collection and Statistical Analysis

Growth and ectomycorrhizal development. After five months' growth in the seed flats, eight seedlings were randomly selected from each species and ectomycorrhizal-nutrient treatment combination, measurements were made of height and root collar diameter, and an estimate of seedling volume was determined by multiplying the height of each seedling by the square of its root collar diameter (Marx et al. 1977a). The roots of these seedlings were washed free of potting medium and the percent ectomycorrhizal formation by symbiont was determined by measuring the length of all lateral roots in centimeters, counting the number of centimeters infected by each specific symbiont, and expressing the level of infection of each symbiont as a percent. This analysis was performed under magnification by mounting a transparent 1.0 cm^2 grid on the microscope stage. Each symbiont was identified by the characteristic appearance of its ectomycorrhizae. These seedlings were then dried at 100°C for 24 hours in a forced-draft oven, the top and root dry weights were measured, and the top/root ratio was determined. At transplanting, and again 18 months after transplanting at the completion of the study, measurements of height and root collar diameter were made on all seedlings growing in the mine spoil and an estimate was made of seedling volume by the method described above. Three replications of each species and ectomycorrhizal-nutrient treatment combination were randomly selected at the end of the study to assess ectomycorrhizal development. The roots were washed free of potting medium, and the percent ectomycorrhizal formation by symbiont was determined by counting all lateral roots, determining the number of lateral roots infected by each specific symbiont, and expressing the level of infection of each symbiont as a percent.

Also, the number of root nodules was determined for each European alder seedling. These seedlings were then dried at 100°C for 24 hours in a forced-draft oven, the top and root dry weights were measured, and the top/root ratio was determined. Analyses of variance were made on all data and the differences among means were evaluated with Duncan's Multiple Range Test (P=0.05). The statistical analysis of the percent ectomycorrhizal development was done separately for each ectomycorrhizal treatment.

Nutrient absorption. Eighteen months after transplanting from sand to mine spoil, seven replications of seedlings of each species and ectomycorrhizal-nutrient treatment combination were randomly selected to assess the effect of each treatment on nutrient absorption. Current-year leaves were collected from each of the four seedlings of each replication and combined into one composite sample per replication. The leaves were 70 to 90 percent expanded at the time of collection. The foliar samples were collected 10 days after the application of the final nutrient treatments. Immediately after collection, all samples were dried at 100°C for 24 hours in a forced-draft oven, ground in a Wiley mill to pass a 20-mesh screen, and sealed in air-tight glass jars. The samples were chemically analyzed for total N, NO_3, P, K, Ca, Mg, Fe, Al, Zn, Mn, Cu, S, B, and Mo by the methods previously described for the loblolly and Virginia pine field study foliar samples. Analyses of variance were made on all data and the differences among means were evaluated with Duncan's Multiple Range Test (P=0.05).

Moisture stress. Eighteen months after transplanting from sand to mine spoil, five replications of seedlings of each species and ectomycorrhizal-nutrient treatment combination were randomly selected to assess the effect of each treatment on seedling moisture status. The xylem potential of each of three randomly selected seedlings from one replication of each species and treatment combination was measured each day at dawn, when moisture stress was least, and in the early afternoon, when it was greatest, by the method of Waring and Cleary (1967) using a PMS® Model 600 portable pressure bomb. The replications to be tested were not watered for eight days prior to measurement. One hour prior to measurement, an Irrometer® Model M eight-inch soil tensiometer was installed at the center of each container (replication) to be tested to provide an indication of spoil moisture status. The dawn measurements were begun at 6:00 a.m. and completed by 7:00 a.m. At the measurement of each individual seedling, the soil tensiometer reading was recorded. Small branches of similar size were selected from the first live whorl for measurement. The xylem potential of the seedlings was recorded in lb/in^2. This procedure was repeated in the early afternoon between 1:00 p.m. and 2:00 p.m. on the seedlings previously measured at dawn of the same day. All measurements of xylem potential in lb/in^2 were converted to negative megapascals (MPa). The relative change in xylem potential was determined by subtracting the dawn measurement from the afternoon measurement and dividing by the dawn measurement. The height and root collar diameter of the tested seedlings was recorded and an estimate of seedling volume was determined by multiplying the height of each seedling by the square of its root collar diameter (Marx et al. 1977a). Analyses of variance were made on all data and the differences among means were evaluated with Duncan's Multiple Range Test (P=0.05).

CHAPTER IV

RESULTS

Field Studies: Loblolly Pine

Nursery Performance

The mechanical analysis of the nursery soil samples collected prior to the inoculation of the bed revealed the soil type to be sand with 90 percent sand, 6 percent silt, and 4 percent clay. The organic matter content was 0.5 percent. The soil chemical analysis revealed a pH of 5.5 with 142, 43, 31, 106, 12, and 24 ppm of total N, available P, and exchangeable K, Ca, Mg, and Mn, respectively. The seedlings inoculated in the nursery with *Pisolithus tinctorius* (GA 100) had 15 percent of their feeder roots infected with this symbiont and 17 percent infected with other naturally occurring ectomycorrhizal fungi, primarily *Thelephora terrestris* Ehrh. ex Fr. The total ectomycorrhizal infection of the GA 100 seedlings was 32 percent, and 90 percent of the seedlings examined were infected with *Pisolithus*. The control seedlings had 35 percent of their feeder roots infected with naturally occurring fungi, primarily *Thelephora terrestris*. None of the control seedlings examined were infected with *Pisolithus*. The GA 100 seedlings had a mean height of 25.1 cm, a mean root collar diameter of 4.6 mm, a mean top fresh weight of 12.5 g, and a mean root fresh weight of 2.7 g. The mean height of the control seedlings was 23.7 cm, the mean root collar diameter was 4.0 mm, and the mean top and root fresh weights were 10.4 g and 2.2 g, respectively.

Spoil Analysis

The mechanical and chemical analysis of the spoil samples collected at outplanting revealed that the plots designated to receive the loblolly pine seedlings were fairly uniform with respect to all of the parameters tested. No significant difference existed for any parameter between the groups of plots designated for each ectomycorrhizal-fertilization treatment combination. The means for all plots were 38.7, 33.5, and 27.8 percent sand, silt, and clay, respectively; 3.1 percent organic matter; pH 6.0; and 1049, 15, 19, 12, 61, 120, 1308, 308, 340, 12, 9.3, 136, 6.0, 187, 0.5, and 0.43 ppm, respectively, of total N, NO_3, NH_3, weak bray P, strong bray P, K, Ca, Mg, Fe, Al, Zn, Mn, Cu, S, B, and Mo. This spoil was typical of those found in the southern Appalachians with high levels of Fe, Mn, and S, although these elevated concentrations are more commonly associated with a lower pH than existed on this site. It also had high levels of Zn, Cu, and Mo. Given the narrow margin between deficient and toxic levels of several of these elements, the potential exists for one or more of them to produce toxicity symptoms in vegetation. This spoil was atypical of those in the southern Appalachians in that nitrogen and phosphorus were sufficient for moderate productivity of most of the commonly used reclamation species and there were high levels of Ca and Mg.

Survival and Growth

The survival of the loblolly pine seedlings was significantly affected after three years by an infection with *Pisolithus* and by fertilization (Table 1). Generally, *Pisolithus* promoted enhanced survival of the GA 100 seedlings, but fertilization reduced survival irrespective of the ectomycorrhizal treatment. The interaction of the two treatments resulted in some moderation of their opposite effects, but an infection with *Pisolithus* was only partially capable of compensating for the reduced survival associated with fertilization. After one year, both the nonfertilized GA 100 and the nonfertilized control seedlings had significantly greater survival than the fertilized control seedlings. After three years, the nonfertilized GA 100 seedlings exhibited survival superior to that of all of the other treatment combinations, and the fertilized GA 100 and non-fertilized control seedlings had greater survival than the fertilized control seedlings. There were also significant differences among the ectomycorrhizal-fertilization treatment combinations in the relative growth in height after three years, and substantial, although not statistically significant, differences in the relative growth in root collar diameter (Table 2). Initially, there were no significant differences among the treatment combinations in height, and only marginal differences in root collar diameter (Table 1). However, after three years, the relative growth of the GA 100 seedlings was generally greater than that of the control seedlings, and the relative growth of the fertilized seedlings was generally greater than that of the nonfertilized seedlings. The differences in survival and growth among the treatment combinations became more apparent after these parameters were calculated to plot volume indices. After three

Table 1. Survival and growth of loblolly pine with and without <u>Pisolithus tinctorius</u> and with and without fertilization on a coal spoil in Tennessee.

Year	Treatment	Percent Survival	Height (cm)	Root Collar Dia (mm)	PVI (cm^3)
Initial	GA 100 Fertilized		17.0a	4.2a	81.1a
	GA 100 Nonfertilized		16.1a	3.8b	65.9a
	Control Fertilized		15.8a	4.1ab	71.4a
	Control Nonfertilized		16.4a	3.9ab	68.8a
1	GA 100 Fertilized	63ab	23.7a	5.1a	166.8a
	GA 100 Nonfertilized	93a	20.1ab	4.4ab	115.4ab
	Control Fertilized	40b	18.6b	4.6ab	64.9b
	Control Nonfertilized	86a	19.3b	4.3b	96.4ab
2	GA 100 Fertilized	59ab	37.3a	7.2a	684.5a
	GA 100 Nonfertilized	91a	30.1a	5.8a	359.7a
	Control Fertilized	34b	35.9a	6.6a	237.1a
	Control Nonfertilized	79ab	29.8a	5.7a	329.7a
3	GA 100 Fertilized	57b	63.1a	11.1a	3291.4a
	GA 100 Nonfertilized	86a	49.5a	9.3a	2403.3a
	Control Fertilized	33c	56.8a	9.4a	559.3a
	Control Nonfertilized	71b	51.2a	8.8a	2069.5a

Means within a given year with a common letter do not differ significantly between treatments at P=0.05.

Table 2. Survival and relative growth of loblolly pine with and without Pisolithus tinctorius and with and without fertilization on a coal spoil in Tennessee.

Year	Treatment	Percent Survival	Height	Root Collar Dia	RPVI
1	GA 100 Fertilized	63ab	0.40a	0.24a	0.64a
	GA 100 Nonfertilized	93a	0.24ab	0.16b	0.63a
	Control Fertilized	40b	0.20b	0.18ab	-0.28a
	Control Nonfertilized	86a	0.16b	0.11b	0.27a
2	GA 100 Fertilized	59ab	1.27ab	0.77a	5.69a
	GA 100 Nonfertilized	91a	0.93ab	0.52ab	4.31a
	Control Fertilized	34b	1.37a	0.70a	1.48a
	Control Nonfertilized	79ab	0.79b	0.43b	2.71a
3	GA 100 Fertilized	57b	2.88a	1.80a	44.44a
	GA 100 Nonfertilized	86a	2.22ab	1.48a	43.96a
	Control Fertilized	33c	2.74ab	1.33a	5.09b
	Control Nonfertilized	71b	2.02b	1.16a	19.76b

Means within a given year with a common letter do not differ significantly between treatments at P=0.05.

years, the relative growth in plot volume index (RPVI) of the fertilized GA 100 seedlings was 773 percent greater than that of the fertilized control seedlings and 125 percent greater than that of the nonfertilized control seedlings, while the RPVI of the nonfertilized GA 100 seedlings was 764 and 122 percent greater than that of the fertilized and nonfertilized control seedlings, respectively.

Nutrient Absorption

The concentrations of NO_3 and Zn in the foliage of the loblolly pine seedlings were significantly affected during the third growing season by an infection with Pisolithus but not by fertilization (Table 3). Generally, the GA 100 seedlings had a higher foliar concentration of NO_3 and a lower concentration of Zn than the control seedlings. Specifically, the nonfertilized GA 100 seedlings had a significantly higher concentration of foliar NO_3 than the nonfertilized control seedlings, but both the fertilized and nonfertilized GA 100 seedlings had a significantly lower concentration of Zn than the fertilized control seedlings. The concentrations of total N, P, K, Ca, Mg, Fe, Al, Mn, Cu, S, B, and Mo did not differ significantly among any of the ectomycorrhizal-fertilization treatment combinations during the third growing season. During the second growing season, there were significant differences among the treatment combinations in the foliar concentrations of NO_3, Ca, Fe, S, and Mo, but these differences did not appear to reflect a response to either the ectomycorrhizal or fertilization treatments. There were no significant differences among the treatment combinations with respect to the foliar

Table 3. Foliar concentrations of elements in loblolly pine with and without Pisolithus tinctorius and with and without fertilization on a coal spoil in Tennessee.

Sample Year	Treatment	ppm													
		N	NO_3	P	K	Ca	Mg	Fe	Al	Zn	Mn	Cu	S	B	Mo
2	GA 100 Fertilized	9150a	325c	1295a	7750a	2250b	1195a	42b	70a	34a	115a	5a	1505ab	6a	0.9a
	GA 100 Nonfertilized	6650a	355ab	960a	7750a	3000a	1460a	56a	100a	33a	60a	5a	1990a	6a	0.5bc
	Control Fertilized	8200a	360a	1110a	7500a	2400ab	1150a	44b	65a	28a	94a	4a	1430b	4a	0.4c
	Control Nonfertilized	7100a	340bc	1215a	8800a	3000a	1325a	51ab	140a	30a	130a	5a	1580ab	9a	0.6b
3	GA 100 Fertilized	8550a	445ab	1740a	13300a	3000a	1900a	44a	65a	27b	83a	6a	2160a	5a	1.1a
	GA 100 Nonfertilized	9250a	460a	1690a	12950a	3400a	1880a	43a	80a	26b	102a	6a	2845a	6a	0.7a
	Control Fertilized	11050a	390ab	1780a	10850a	3850a	2000a	40a	45a	32a	67a	6a	2705a	3a	1.0a
	Control Nonfertilized	11450a	370b	1580a	10600a	3550a	1900a	36a	150a	28ab	168a	6a	2570a	7a	1.0a

Means within a given year with a common letter do not differ significantly between treatments at P=0.05.

concentrations of total N, P, K, Mg, Al, Zn, Mn, Cu, and B during the second growing season. All nutrient analyses were done using only two replications of each ectomycorrhizal-fertilization treatment combination due to the poor survival in some plots and insufficient samples.

Moisture Stress

The moisture status of the loblolly pine seedlings was significantly affected by an infection with *Pisolithus* during the third growing season, but not by fertilization (Table 4). The fertilized and nonfertilized control seedlings exhibited significantly greater internal moisture stress (greater negative xylem potential) than either the fertilized or nonfertilized GA 100 seedlings during the afternoon (p.m.) testing period. During the dawn (a.m.) testing period, the control seedlings generally exhibited greater stress than the GA 100 seedlings, although only the fertilized GA 100 and the nonfertilized control seedlings differed statistically. The relative change in xylem potential did not differ significantly among any of the ectomycorrhizal-fertilization treatment combinations. The differences among the treatment combinations in the height and root collar diameter of the seedlings tested for moisture stress were generally consistent with those of the loblolly pine study as a whole, and although the volume of these seedlings did not differ statistically, the fertilized GA 100 seedlings were substantially larger and the nonfertilized control seedlings were substantially smaller than the average. The percent ground cover of the plots

Table 4. Internal moisture status of loblolly pine with and without <u>Pisolithus tinctorius</u> and with and without fertilization on a coal spoil in Tennessee.

Treatment	Height (cm)	Root Collar Dia (mm)	Seedling Volume (cm^3)	Percent Ground Cover	Soil Moisture a.m.	Soil Moisture p.m.	Xylem Potential a.m. (-MPa)	Xylem Potential p.m. (-MPa)	Relative Change-Xylem Potential
GA 100 Fertilized	52.4a	9.2a	64.5a	71b	60ab	60a	-0.87a	-1.59a	0.88a
GA 100 Nonfertilized	46.8a	8.3a	43.4a	72b	61a	61a	-0.89ab	-1.59a	0.86a
Control Fertilized	48.0a	8.5a	47.0a	94a	58b*	60a	-1.03ab	-1.87b	0.92a
Control Nonfertilized	43.0a	6.7a	22.7a	63b	58ab*	59a	-1.05b	-1.86b	0.83a

Means with a common letter do not differ significantly between treatments at P=0.05.

*Difference in grouping due to rounding.

containing the fertilized control seedlings was significantly greater than that of the plots of the other treatment combinations (although not at P=0.01), but the moisture status of the spoil was generally consistent across all treatment combinations during both the dawn and afternoon testing periods. No measurable precipitation event occurred on this site for 15 days prior to the initiation of the loblolly pine moisture stress study.

Field Studies: Virginia Pine

Nursery Performance

The mechanical analysis of the nursery soil samples collected prior to the inoculation of the bed revealed the soil type to be loamy sand with 82 percent sand, 10 percent silt, and 8 percent clay. The soil chemical analysis revealed a pH of 4.3 with 409, 113, 63, 105, and 8 ppm of total N, available P, and exchangeable K, Ca, and Mg, respectively. The GA 100 seedlings had 20 percent of their feeder roots infected with *Pisolithus tinctorius* and 5 percent infected with other naturally occurring ectomycorrhizal symbionts, primarily *Thelephora terrestris*. The total ectomycorrhizal infection of the GA 100 seedlings was 25 percent, and 100 percent of the seedlings examined were infected with *Pisolithus*. The ABB 100 seedlings had 5 percent of their feeder roots infected with *Pisolithus* and 12 percent with *Thelephora*. The total ectomycorrhizal infection of the ABB 100 seedlings was 17 percent, and 50 percent of the seedlings examined were infected with *Pisolithus*. The ABB 200 seedlings had 22 percent of their feeder roots infected with *Pisolithus* and 5 percent infected with *Thelephora*. The total ectomycorrhizal infection of the ABB 200 seedlings was 27 percent, and 100 percent of the seedlings examined were infected with *Pisolithus*. The control seedlings had 20 percent of their feeder roots infected with naturally occurring ectomycorrhizal symbionts, primarily *Thelephora*. None of the control seedlings examined were infected with *Pisolithus*. The GA 100 seedlings had a mean height of 20.0 cm, a mean root collar diameter of 4.0 mm, a mean top fresh weight of 11.0 g, and a mean root fresh weight of 7.6 g. The ABB 100 seedlings had a mean height of 21.8 cm, a mean root collar diameter of 4.5 mm, a mean top fresh weight of 13.1 g, and a mean root fresh weight of 7.9 g. The ABB 200 seedlings had a mean height of 22.8 cm, a mean root collar diameter of 4.5 mm, a mean top fresh weight of 13.2 g, and a mean root fresh weight of 7.0 g. The mean height of the control seedlings was 18.5 cm, the mean root collar diameter was 4.3 mm, and the mean top and root fresh weights were 11.6 g and 10.3 g, respectively.

Spoil Analysis

The mechanical and chemical analysis of the spoil samples collected at outplanting revealed that the plots designated to receive the Virginia pine seedlings were fairly uniform with respect to all of the parameters tested. No significant difference existed at P=0.05 for percent clay, pH, total N, NO_3, NH_3, weak bray P, strong bray P, K, Ca, Al, Zn, Mn, S, and Mo between the groups of plots designated for each ectomycorrhizal-fertilization treatment combination, and none existed for percent sand, silt, and organic matter and Mg, Fe, Cu, and

B at P=0.01. The means for all plots were 39.7, 34.0, and 26.3 percent sand, silt, and clay, respectively; 3.8 percent organic matter; pH 5.3; and 1265, 19, 16, 10, 44, 119, 900, 268, 230, 92, 14.7, 99, 7.5, 145, 0.4, and 0.28 ppm, respectively, of total N, NO_3, NH_3, weak bray P, strong bray P, K, Ca, Mg, Fe, Al, Zn, Mn, Cu, S, B, and Mo. The high levels of Fe, Al, Mn, and S found on these plots are typical of those found on coal spoils throughout the southern Appalachians. The levels of Zn and Cu were also very high, and given the narrow margin between deficient and toxic levels of several of these elements, the potential exists for one or more of them to produce toxicity symptoms in vegetation. The variability in the surface strata of this spoil was demonstrated by the disparity in the mean pH and Al values between the plots containing the loblolly pine field study seedlings, as noted in the previous section, and those of the Virginia pine seedlings. The levels of nitrogen and phosphorus in these plots were atypically adequate, in comparison with most southern Appalachian coal spoils, for moderate productivity of most of the commonly used reclamation species.

Survival and Growth

The survival of the Virginia pine seedlings was not significantly affected by an infection with *Pisolithus* during this study, but fertilization generally reduced survival irrespective of ectomycorrhizal treatment after two years (Table 5). However, the effect of *Pisolithus* and fertilization on seedling growth was marginal throughout the study. After one year, all of the treatment combinations with *Pisolithus*

Table 5. Survival and growth of fertilized and nonfertilized Virginia pine inoculated with three formulations of Pisolithus tinctorius and control seedlings on a coal spoil in Tennessee.

Year	Treatment	Percent Survival	Height (cm)	Root Collar Dia (mm)	PVI (cm^3)
Initial	GA 100 Fertilized		23.2a	4.8ab	149.5ab
	GA 100 Nonfertilized		21.2bcd	4.3b	112.0c
	ABB 100 Fertilized		22.7ab	5.0a	156.7a
	ABB 100 Nonfertilized		21.5bc	4.9a*	145.0abc
	ABB 200 Fertilized		23.3a	4.8ab	151.4ab
	ABB 200 Nonfertilized		23.5a	4.9ab*	152.8a
	Control Fertilized		19.7d	4.6ab	116.3bc
	Control Nonfertilized		21.0cd	4.7ab	120.5abc
1	GA 100 Fertilized	100a	34.7a	6.6ab	422.0a
	GA 100 Nonfertilized	100a	30.2cd	6.0c	306.9b
	ABB 100 Fertilized	97a	33.4ab	6.9a	426.1a
	ABB 100 Nonfertilized	100a	32.6abc	6.8a	396.0a
	ABB 200 Fertilized	100a	32.9ab	6.8a	426.5a
	ABB 200 Nonfertilized	100a	33.2ab	6.7a	417.4a
	Control Fertilized	100a	29.0d	6.2c	304.6b
	Control Nonfertilized	97a	31.0bcd	6.4bc	326.6b
2	GA 100 Fertilized	77bc	72.5a	11.5ab	3117.0a
	GA 100 Nonfertilized	91ab	56.8b	9.5b	1639.8a
	ABB 100 Fertilized	73c	75.0a	12.7a	3833.4a
	ABB 100 Nonfertilized	93ab	69.5ab	11.9ab**	3271.6a
	ABB 200 Fertilized	80c	70.3ab	11.9a**	3795.5a
	ABB 200 Nonfertilized	99a	61.6ab	10.6ab	2502.0a
	Control Fertilized	88bc	66.3ab	10.9ab	2687.9a
	Control Nonfertilized	95ab	65.7ab	10.5ab	3038.6a

Means within a given year with a common letter do not differ significantly between treatments at P=0.05.

* and ** Difference in grouping due to rounding.

ectomycorrhizae except the nonfertilized GA 100 seedlings were generally larger in height and root collar diameter than either the fertilized or nonfertilized control seedlings, and all of the treatment combinations with Pisolithus except the nonfertilized GA 100 seedlings had a significantly higher plot volume index (PVI) than the control seedlings of either fertilization treatment (Table 5). After two years, however, the effect of Pisolithus was negligible, and although the relative growth in height of the fertilized seedlings was slightly better than that of the nonfertilized seedlings (Table 6), this was not reflected in a significant improvement in the relative growth in plot volume index (RPVI) due to the reduced survival associated with fertilization.

Nutrient Absorption

The concentration of total N in the foliage of the Virginia pine seedlings was not significantly affected by an infection with Pisolithus during the second growing season but was significantly affected by fertilization (Table 7). Fertilized seedlings, irrespective of ectomycorrhizal treatment, had a higher foliar concentration of total N than nonfertilized seedlings. There were also statistically significant differences among the treatment combinations in the foliar concentrations of P, Mg, Zn, Mn, S, B, and Mo, but these differences did not appear to reflect a response to either the ectomycorrhizal or fertilization treatments. The foliar concentrations of NO_3, K, Ca, Fe, Al, and Cu did not vary significantly. The analysis of foliar S was done using only two of the replications of each ectomycorrhizal-fertilization treatment combination due to insufficient samples.

Table 6. Survival and relative growth of fertilized and nonfertilized Virginia pine inoculated with three formulations of <u>Pisolithus tinctorius</u> and control seedlings on a coal spoil in Tennessee.

Year	Treatment	Percent Survival	Height	Root Collar Dia	RPVI
1	GA 100 Fertilized	100a	0.51ab	0.37a	1.79a
	GA 100 Nonfertilized	100a	0.44ab	0.40a	1.77a
	ABB 100 Fertilized	97a	0.49ab	0.40a	1.82a
	ABB 100 Nonfertilized	100a	0.54a	0.40a	2.02a
	ABB 200 Fertilized	100a	0.42b	0.43a	1.95a
	ABB 200 Nonfertilized	100a	0.42b	0.39a	1.81a
	Control Fertilized	100a	0.48ab	0.34a	1.67a
	Control Nonfertilized	97a	0.49ab	0.38a	1.69a
2	GA 100 Fertilized	77bc	2.31ab	1.50a	27.45a
	GA 100 Nonfertilized	91ab	1.81bc	1.39a	20.37a
	ABB 100 Fertilized	73c	2.50a	1.67a	32.85a
	ABB 100 Nonfertilized	93ab	2.40a	1.63a	32.75a
	ABB 200 Fertilized	80c	2.16abc	1.63a	34.44a
	ABB 200 Nonfertilized	99a	1.71c	1.27a	18.02a
	Control Fertilized	88bc	2.33ab	1.36a	23.88a
	Control Nonfertilized	95ab	2.17abc	1.29a	23.19a

Means within a given year with a common letter do not differ significantly between treatments at P=0.05.

Table 7. Foliar concentrations of elements in fertilized and nonfertilized Virginia pine inoculated with three formulations of <u>Pisolithus tinctorius</u> and control seedlings on a coal spoil in Tennessee.

Treatment	ppm													
	N	NO_3	P	K	Ca	Mg	Fe	Al	Zn	Mn	Cu	S*	B	Mo
GA 100 Fertilized	19800a	367a	1413ab	8067a	3667a	1670c	48a	207a	31ab**	261ab	5a	2930a	10ab	0.4b
GA 100 Nonfertilized	13600b	360a	1410ab	8367a	3967a	1867a	40a	277a	31a**	340a	4a	2665ab	12a	0.8a
ABB 100 Fertilized	19433a	363a	1570a	8067a	3767a	1713bc	50a	187a	28ab	298ab	6a	2545ab	9ab	0.6ab
ABB 100 Nonfertilized	14333b	347a	1433ab	7933a	3433a	1787abc	45a	250a	30ab	243ab	5a	2755ab	10ab	0.7ab
ABB 200 Fertilized	19433a	353a	1407ab	8300a	3567a	1847ab	44a	250a	25b	293ab	6a	2645ab	9ab	0.6ab
ABB 200 Nonfertilized	12633b	363a	1420ab	8400a	3863a	1803abc	53a	290a	32a	354a	5a	2425b	8ab	0.9a
Control Fertilized	17867a	380a	1337b	8867a	3667a	1797abc	51a	193a	28ab	249ab	10a	2645ab	7b	0.6ab
Control Nonfertilized	13767b	377a	1577a	8700a	3700a	1870a	54a	207a	30ab	179b	5a	2825ab	9ab	0.7ab

Means with a common letter do not differ significantly between treatments at P=0.05.

*Means of two replications of each ectomycorrhizal-fertilization treatment combination.

**Difference in grouping due to rounding.

Moisture Stress

The moisture status of the Virginia pine seedlings was significantly affected by an infection with *Pisolithus* during the second growing season, but not by fertilization (Table 8). The fertilized and nonfertilized control seedlings exhibited significantly greater internal moisture stress (greater negative xylem potential) than all of the treatment combinations with *Pisolithus* ectomycorrhizae except the fertilized ABB 100 seedlings during the afternoon (p.m.) testing period. During the dawn (a.m.) testing period, the fertilized and nonfertilized control seedlings exhibited significantly greater stress than all of the treatment combinations with *Pisolithus* except the fertilized ABB 100 and ABB 200 seedlings. The relative change in xylem potential of the fertilized and nonfertilized ABB 200 seedlings was appreciably smaller than that of the other treatment combinations. Generally, the fertilized seedlings tested for moisture stress were somewhat larger than the nonfertilized seedlings, but the effect of *Pisolithus* on the size of the tested seedlings was negligible. There was some variation among the treatment combinations in the percent ground cover of the plots, but the moisture status of the spoil was generally consistent across all treatment combinations during both the dawn and afternoon testing periods. No measurable precipitation event occurred on this site for 18 days prior to the initiation of the Virginia pine moisture stress study.

Table 8. Internal moisture status of fertilized and nonfertilized Virginia pine inoculated with three formulations of *Pisolithus tinctorius* and control seedlings on a coal spoil in Tennessee.

Treatment	Height (cm)	Root Collar Dia (mm)	Seedling Volume (cm^3)	Percent Ground Cover	Soil Moisture a.m.	Soil Moisture p.m.	Xylem Potential a.m. (-MPa)	Xylem Potential p.m. (-MPa)	Relative Change-Xylem Potential
GA 100 Fertilized	50.0a	8.8abc	41.3ab	67a	63ab*	63ab**	-1.55ab	-2.07ab	0.37ab
GA 100 Nonfertilized	45.4a	8.2bc	31.5bc	47ab	60b	61c***	-1.58ab	-2.11ab	0.38ab
ABB 100 Fertilized	46.9a	9.0ab	38.9abc	62a	63a*	65a	-1.66bc	-2.21bc	0.37ab
ABB 100 Nonfertilized	48.2a	8.9abc	38.8abc	67a	64a	65a	-1.57ab	-2.14ab	0.45a
ABB 200 Fertilized	50.3a	9.3a	44.4a	52ab	60b	61bc***	-1.66bc	-2.10ab	0.31b
ABB 200 Nonfertilized	46.5a	8.3bc	32.4bc	34b	60b	61bc***	-1.51a	-1.95a	0.31b
Control Fertilized	46.3a	8.8abc	37.0abc	48ab	61ab	61bc***	-1.78c	-2.41c	0.41ab
Control Nonfertilized	46.5a	8.1c	30.8c	65a	61ab	63abc**	-1.78c	-2.39c	0.40ab

Means with a common letter do not differ significantly between treatments at P=0.05.

*, **, and *** Differences in grouping due to rounding.

CHAPTER V

RESULTS

Greenhouse Studies: Sweet Birch

Quartz Sand and Mine Spoil Analyses

The pH of the quartz sand used in the sand culture experiments of sweet birch and European alder was 5.5 prior to the acid wash treatment, and it returned to this value after rinsing with distilled water. The mechanical and chemical analysis of the mine spoil samples collected prior to transplanting revealed that the coal spoil material used as a potting medium in the sweet birch and European alder greenhouse studies was fairly uniform with respect to most of the parameters tested. The mean, minimum, and maximum values for all samples were 43.3 (35.2 to 52.4) percent sand, 30.4 (21.6 to 38.4) percent silt, and 26.3 (20.0 to 28.4) percent clay; 2.4 (1.9 to 2.6) percent organic matter; pH 5.6 (5.0 to 5.9); and 848 (790 to 910) ppm of total N, 35 (26 to 42) ppm of NO_3, 17 (14 to 28) ppm of NH_3, 8 (7 to 8) ppm of weak bray P, 75 (45 to 83) ppm of strong bray P, 103 (99 to 107) ppm of K, 1790 (1600 to 1900) ppm of Ca, 300 (290 to 320) ppm of Mg, 520 (473 to 683) ppm of Fe, 13 (2 to 21) ppm of Al, 8.8 (8.0 to 11.2) ppm of Zn, 158 (150 to 173) ppm of Mn, 5.7 (5.5 to 6.3) ppm of Cu, 478 (464 to 485) ppm of S, 0.5 (0.5 to 0.6) ppm of B, and 0.53 (0.38 to 0.71) ppm of Mo. This coal spoil material was typical of that found in the southern Appalachians with low levels of readily available P and high levels of Fe, Mn, and S, although the latter are more commonly associated with a spoil material having a lower pH value than that of these samples. The levels of Zn, Cu, and Mo were also high, and the potential exists for one or more of these metallic elements to produce toxicity symptoms in vegetation. This spoil material was atypical of that found in the southern Appalachians in that nitrogen was sufficient for moderate productivity of most of the commonly used reclamation species and there were high levels of Ca and Mg.

Initial Ectomycorrhizal Evaluation

The concentration of nutrients applied to the ESD 200 sweet birch seedlings significantly affected the level of infection of the feeder roots by *Pisolithus tinctorius* after five months' growth in the quartz sand potting medium (Table 9). Generally, the seedlings grown under lower fertility regimes had a higher level of infection with this symbiont, although the percent infection by *Pisolithus* of the seedlings grown in the lowest (1/8 Hoagland) concentration of Hoagland's solution was somewhat lower than that of the seedlings grown in the intermediate (1/4 Hoagland) concentration. However, both of these treatment combinations had a significantly higher level of infection with *Pisolithus* than the ESD 200 seedlings grown in the high (1/2 Hoagland) nutrient concentration. The concentration of Hoagland's solution applied to these seedlings also significantly affected the level of infection by other naturally occurring ectomycorrhizal symbionts. Other than *Pisolithus*, the only ectomycorrhizal fungus found infecting the roots of the ESD 200 sweet birch seedlings after five months in the sand culture was *Thelephora terrestris*, and only

Table 9. Growth and ectomycorrhizal development of sweet birch inoculated with Pisolithus tinctorius and control seedlings after five months in sand culture with three concentrations of Hoagland's solution #2 with micronutrients and iron.

Treatment	Height (cm)	Root Collar Dia (mm)	Seedling Volume (cm^3)	Dry Weight (g) Top	Dry Weight (g) Roots	Dry Weight (g) Total	Top/Root Ratio	Percent Infection* P. tinctorius	Percent Infection* Other	Percent Infection* Total
ESO 200 1/2 Hoagland	37.0a	5.0a	9.3a	3.0a	1.6a	4.6a	2.0a	3b	20a	23b
ESO 200 1/4 Hoagland	26.0b	3.4c	3.1b	0.8b	0.4b	1.3b	1.9a**	33a	1b	34a
ESO 200 1/8 Hoagland	17.9c	2.7d	1.7b	0.7b	0.4b	1.0b	2.0a	24a	0b	24ab
Control 1/2 Hoagland	38.4a	4.3b	7.5a	2.3a	1.6a	3.9a	1.5bc	0	11a	11a
Control 1/4 Hoagland	22.1bc	3.2cd	2.5b	0.8b	0.6b	1.4b	1.4c	0	7b	7b
Control 1/8 Hoagland	17.2c	2.7d	1.3b	0.6b	0.3b	0.8b	1.9ab**	0	3c	3c

Means with a common letter do not differ significantly between treatments at P=0.05; all means except percent infection were rounded to one decimal place.

*Grouping applies only to means within ectomycorrhizal treatments.

**Difference in grouping due to rounding.

the seedlings grown in the high nutrient concentration had a significant infection with this symbiont. Thelephora was also the only ectomycorrhizal symbiont found infecting the feeder roots of the control seedlings. The percent infection by Thelephora of the control seedlings grown in the high nutrient concentration was higher than that of the seedlings grown in the intermediate nutrient concentration, which was higher than that of the seedlings grown in the low nutrient concentration. The size and weight of the seedlings evaluated for ectomycorrhizal development generally reflected the fertility of the sand culture, as determined by the nutrient treatments, and although the ESD 200 seedlings were often larger than the control seedlings within each nutrient treatment, the differences were usually subtle and statistically nonsignificant. However, the differences in top and root weights between the ESD 200 and control seedlings became more apparent when the top/root ratios were calculated, and the top/root ratio of the control seedlings was generally superior to that of the ESD 200 seedlings. This disparity can probably be explained by an inadvertent loss of portions of some root systems during lifting.

Seedling Growth

The height, root collar diameter, and volume of the sweet birch seedlings were significantly affected after five months by an infection with Pisolithus and by the fertility of the quartz sand potting medium (Table 10). Initially, seedling size reflected the fertility of the sand culture, as determined by the nutrient treatments, but often the

Table 10. Growth of sweet birch with and without Pisolithus tinctorius grown in mine spoil with three concentrations of Hoagland's solution #2 with micronutrients and iron.

Time of Measurement	Treatment	Height (cm)	Root Collar Dia (mm)	Seedling Volume (cm^3)
Initial	ESD 200 1/2 Hoagland	34.2a	3.4a	4.2a
	ESD 200 1/4 Hoagland	25.4b	3.1b	2.5b
	ESD 200 1/8 Hoagland	13.5d	2.2d	0.7c
	Control 1/2 Hoagland	31.9a	2.8c	2.5b
	Control 1/4 Hoagland	17.8c	1.8e	0.6c
	Control 1/8 Hoagland	11.8d	1.5f	0.3c
18 Months	ESD 200 1/2 Hoagland	72.7a	5.6ab	24.1a
	ESD 200 1/4 Hoagland	65.4b	5.7a	23.0a
	ESD 200 1/8 Hoagland	39.5d	4.6c	9.7b
	Control 1/2 Hoagland	73.4a	5.3b	21.6a
	Control 1/4 Hoagland	47.8c	4.2d	8.8b
	Control 1/8 Hoagland	29.9e	3.6e	4.2c

Means within a given time of measurement with a common letter do not differ significantly between treatments at P=0.05.

seedlings with Pisolithus were as large, and sometimes larger, than the control seedlings grown at a higher fertility level. At transplanting, the heights of the ESD 200 and the control seedlings grown in both the high and low nutrient concentrations were similar, but the ESD 200 seedlings grown in the intermediate nutrient concentration were significantly larger than the control seedlings grown at this fertility level. The initial disparity in seedling size within nutrient treatments was more pronounced with respect to root collar diameter. The ESD 200 seedlings grown in the high and intermediate nutrient concentrations had a significantly larger root collar diameter than all of the other treatment combinations, and the ESD 200 seedlings grown in the low nutrient concentration had a larger root collar diameter than the control seedlings grown in either the intermediate or low nutrient concentrations. Similar results were found with respect to the initial determination of seedling volume. The ESD 200 seedlings grown under the high fertility regime were significantly larger than the seedlings of all of the other treatment combinations, the ESD 200 seedlings grown under the intermediate fertility regime were similar in volume to the control seedlings grown under the high fertility regime, and the volume of the ESD 200 seedlings grown under the low fertility regime was similar to that of the control seedlings grown at the intermediate and low fertility levels.

The height, root collar diameter, and volume of these seedlings were also significantly affected by an infection with Pisolithus and by fertility after 18 months in the mine spoil potting medium (Table 10). At the end of the study, seedling size still reflected the fertility of the potting medium, as determined here by the nutrient treatments applied to the mine spoil, but the disparity in size within each nutrient treatment between the seedlings with Pisolithus and the control seedlings was even more pronounced. The lone exception was the seedlings grown under the high fertility regime, where the ESD 200 and control seedlings were similar in size with respect to all three of the growth parameters. However, the heights of the ESD 200 seedlings grown in the intermediate and low nutrient concentrations were significantly greater than that of the control seedlings grown at the intermediate and low fertility levels, respectively. Also, the root collar diameter of the ESD 200 seedlings grown in the intermediate nutrient concentration was similar to that of the ESD 200 seedlings grown in the high nutrient concentration and significantly larger than that of all of the other treatment combinations, and the root collar diameter of the ESD 200 seedlings grown in the low nutrient concentration was significantly larger than that of the control seedlings grown at the intermediate and low fertility levels. When the measurements of height and root collar diameter were calculated to seedling volume, the effect of Pisolithus on overall seedling growth under the intermediate and low fertility regimes was made very apparent. The volume of the ESD 200 seedlings grown under the intermediate fertility regime was 161 percent greater than that of the control seedlings grown at this fertility level, and the volume of the ESD 200 seedlings grown under the low fertility regime was 131 percent greater than that of the control seedlings grown at the low fertility level.

Nutrient Absorption

There were significant differences among the ectomycorrhizal-nutrient treatment combinations in the concentrations of total N, NO_3, P, K, Ca, Mg, Fe, Al, Zn, Mn, Cu, B, and Mo in the foliage of the sweet birch seedlings (Table 11). The differences in the foliar concentrations of total N, Mg, and Al reflected overall differences within nutrient treatments and between ectomycorrhizal treatments in the absorption of these elements from the mine spoil potting medium. The seedlings with Pisolithus generally had a higher foliar concentration of total N and lower concentrations of Mg and Al than the control seedlings. The concentration of total N in the foliage of the ESD 200 seedlings grown under all three of the fertility regimes was similar, but within each nutrient treatment, the ESD 200 seedlings had a significantly higher foliar concentration of total N than the control seedlings, and the ESD 200 seedlings grown under the low fertility regime had a higher concentration of total N than the control seedlings grown at the intermediate fertility level. In contrast, the foliar concentration of Mg was similar for the control seedlings of all three of the nutrient treatments and the ESD 200 seedlings grown under the high fertility regime, but the control seedlings grown under the intermediate fertility regime had a significantly higher concentration of Mg than the ESD 200 seedlings grown at this fertility level, and the control seedlings grown under the low fertility regime

Table 11. Foliar concentrations of elements in sweet birch with and without <u>Pisolithus tinctorius</u> grown in mine spoil with three concentrations of Hoagland's solution #2 with micronutrients and iron.

	ppm													
Treatment	N*	NO_3**	P	K	Ca	Mg	Fe	Al	Zn	Mn	Cu	S***	B	Mo
ESD 200 1/2 Hoagland	14633a	582a	2900a	12471a	21000ab	6300ab	130abc	75c	210a	3206ab	5ab†	5633a	61b	1.9b
ESD 200 1/4 Hoagland	12967ab	226c	2171cd	11043ab	19000b	5129c	113bc	77c	179b	2724b	4bc††	4100a	63b	1.7b
ESD 200 1/8 Hoagland	12783ab	198c	2343bc	11643ab	21000ab	5571bc	168ab	111bc	193ab	4964a	5a†	4167a	61b	1.8b
Control 1/2 Hoagland	11100bc	208c	2500abc	10157ab	24143a	6586a	77c	73c	213a	2833b	4abc††	8233a	74ab	1.7b
Control 1/4 Hoagland	8800c	308bc	2786ab	9971b	22857ab	6471a	140abc	160ab	206ab	3063ab	4abc††	5467a	76ab	2.5ab
Control 1/8 Hoagland	8300c	362b	1686d	9571b	21700ab	6614a	205a	194a	209a	2749b	4c††	7700a	83a	3.0a

Means with a common letter do not differ significantly between treatments at P=0.05.

*, **, and *** Means of six, five, and three replications, respectively, of each ectomycorrhizal-nutrient treatment combination.

† and †† Differences in grouping due to rounding.

had a higher concentration of Mg than the ESD 200 seedlings grown at either the intermediate or low fertility levels. Also, the foliar concentration of Al did not differ significantly within the high nutrient treatment, but the control seedlings grown under the intermediate fertility regime had a higher concentration of Al than the ESD 200 seedlings grown at either the high or intermediate fertility levels, and the control seedlings grown under the low fertility regime had a higher concentration of Al than the ESD 200 seedlings of any nutrient treatment. The differences among the treatment combinations in the foliar concentrations of NO_3, P, K, Ca, Fe, Zn, Mn, Cu, B, and Mo were random and did not appear to reflect a response to either the ectomycorrhizal or nutrient treatments. The foliar concentration of S did not differ significantly among any of the treatment combinations. The analyses of total N, NO_3, and S were done using only six, five, and three replications of each ectomycorrhizal-nutrient treatment combination, respectively, due to insufficient samples.

Moisture Stress

The internal moisture status of the sweet birch seedlings was not significantly affected by an infection with Pisolithus but was significantly affected by the fertility of the mine spoil potting medium (Table 12). Generally, larger seedlings exhibited greater moisture stress (greater negative xylem potential) and seedling size reflected the fertility of the mine spoil, as determined by the nutrient treatments. Also, with the exception of the seedlings grown

Table 12. Internal moisture status of sweet birch with and without <u>Pisolithus tinctorius</u> grown in mine spoil with three concentrations of Hoagland's solution #2 with micronutrients and iron.

Treatment	Height (cm)	Root Collar Dia (mm)	Seedling Volume (cm^3)	Soil Moisture a.m.	Soil Moisture p.m.	Xylem Potential a.m. (-MPa)	Xylem Potential p.m. (-MPa)	Relative Change-Xylem Potential
ESD 200 1/2 Hoagland	72.0a	5.9a	26.4a	62a	62a	-1.22c	-1.45c	0.21a
ESD 200 1/4 Hoagland	61.4b	5.6a	20.2b	59a	59a	-0.60ab	-0.81ab	0.35a
ESD 200 1/8 Hoagland	36.8d	4.9b	10.2c	65a	65a	-0.57a	-0.76a	0.34a
Control 1/2 Hoagland	74.1a	5.6a	23.4ab	55a	55a	-0.85b	-1.08b	0.32a
Control 1/4 Hoagland	52.7c	4.9b	12.9c	37a	37a	-0.77ab	-1.06ab	0.44a
Control 1/8 Hoagland	28.7d	4.0c	4.6d	36a	36a	-0.68ab	-0.83ab	0.26a

Means with a common letter do not differ significantly between treatments at P=0.05.

under the high fertility regime, the control seedlings consistently exhibited greater moisture stress than the seedlings with Pisolithus during both the dawn (a.m.) and afternoon (p.m.) testing periods, but the differences were subtle and statistically nonsignificant. Within the high nutrient treatment, however, the ESD 200 seedlings exhibited significantly greater stress than the control seedlings during both testing periods. The relative change in xylem potential did not differ significantly among any of the ectomycorrhizal-nutrient treatment combinations. Generally, the ESD 200 seedlings tested for moisture stress were significantly larger than the control seedlings within all except the high nutrient treatment, as was the case previously noted for the sweet birch study as a whole. Also, there were substantial, though statistically nonsignificant, differences between the treatment combinations in the moisture status of the mine spoil potting medium, and the moisture deficit of the spoil containing the ESD 200 seedlings was consistently greater (higher reading) than that of the spoil containing the control seedlings. This disparity was largely attributable to the greater size of the ESD 200 seedlings and the subsequent elevated transpirational losses from the limited spoil moisture reserve.

Final Ectomycorrhizal Evaluation

The concentration of nutrients applied to the ESD 200 sweet birch seedlings also significantly affected the level of infection of the feeder roots by *Pisolithus tinctorius* after 18 months' growth in the mine spoil potting medium (Table 13). Again, the percent

Table 13. Growth and ectomycorrhizal development of sweet birch inoculated with Pisolithus tinctorius and control seedlings after 18 months in mine spoil with three concentrations of Hoagland's solution #2 with micronutrients and iron.

Treatment	Height (cm)	Root Collar Dia (mm)	Seedling Volume (cm^3)	Dry Weight (g) Top	Roots	Total	Top/Root Ratio	Percent Infection* P. tinctorius	Other	Total
ESD 200 1/2 Hoagland	74.9ab	6.0a	28.6a	5.3a	3.5a	8.8a	1.5a	32b	0	32b
ESD 200 1/4 Hoagland	65.8b	5.6a	22.0a	4.0ab	3.4a	7.4a	1.2a	74a	0	74a
ESD 200 1/8 Hoagland	36.7d	4.6b	8.1b	2.0c	1.8b	3.8b	1.1a	67a	0	67a
Control 1/2 Hoagland	76.3a	5.3ab	22.2a	5.1a	3.6a	8.7a	1.5a	0	29a	29a
Control 1/4 Hoagland	48.0c	4.6b	11.3b	2.7bc	2.1b	4.8b	1.6a	0	28a	28a
Control 1/8 Hoagland	27.5d	3.8c	4.3b	1.2c	1.4b	2.7b	1.0a	0	13b	13b

Means with a common letter do not differ significantly between treatments at P=0.05; all means except percent infection were rounded to one decimal place.

*Grouping applies only to means within ectomycorrhizal treatments.

infection with Pisolithus of the ESD 200 seedlings grown under the intermediate fertility regime was somewhat higher than that of the seedlings grown at the low fertility level, although the difference was not statistically significant, but both of these treatment combinations had a significantly higher percent infection with this symbiont than the seedlings grown under the high fertility regime. No ectomycorrhizal symbiont other than Pisolithus was found infecting the roots of the ESD 200 sweet birch seedlings during this evaluation. The level of infection by naturally occurring ectomycorrhizal fungi of the feeder roots of the control seedlings was also significantly affected by the fertility of the mine spoil, as determined by the nutrient treatments. The only symbiont found infecting the roots of the control seedlings was Thelephora terrestris. The percent infection by Thelephora of the control seedlings grown under the high and intermediate fertility regimes was similar, and the seedlings of both of these treatment combinations had a significantly higher percent infection than the control seedlings grown at the low fertility level. The ESD 200 seedlings evaluated for ectomycorrhizal development were usually larger than the control seedlings within all except the high nutrient treatment, as was noted previously for the sweet birch study as a whole, and the size and weight of the seedlings of both ectomycorrhizal treatments generally reflected the fertility regime under which they were grown. The top/root ratio of these seedlings did not differ significantly among any of the ectomycorrhizal-nutrient treatment combinations.

Greenhouse Studies: European Alder

No ectomycorrhizal fungi were found infecting the feeder roots of the European alder seedlings of any of the ectomycorrhizal-nutrient treatment combinations after five months' growth in the sand culture (Table 14) or after 18 months' growth in the mine spoil potting medium (Table 15). However, the results of the growth, nutrient absorption, and moisture status evaluations of these seedlings are included here for future reference in Table 16, Table 17, and Table 18, respectively. Any differences among the treatment combinations in the parameters included in these results were attributable to the nutrient treatments, variation in root nodulation, or random variability.

Table 14. Growth and ectomycorrhizal development of European alder inoculated with Pisolithus tinctorius and control seedlings after five months in sand culture with three concentrations of Hoagland's solution #2 with micronutrients and iron.

Treatment	Height (cm)	Root Collar Dia (mm)	Seedling Volume (cm^3)	Dry Weight (g)			Top/Root Ratio	Percent Infection		
				Top	Roots	Total		P. tinctorius	Other	Total
ESD 200 1/2 Hoagland	15.3a	3.0a	1.4a	2.1ab	1.9ab	4.0ab	1.1a	0	0	0
ESD 200 1/4 Hoagland	13.4ab	2.6ab	1.0ab	1.9ab	1.7abc	3.6abc	1.2a	0	0	0
ESD 200 1/8 Hoagland	11.6bc	2.1bc	0.5b	1.8ab*	1.6bc**	3.4bc***	1.2a	0	0	0
Control 1/2 Hoagland	15.2a	2.8a	1.3a	2.2a	2.0a	4.1a	1.1a	0	0	0
Control 1/4 Hoagland	13.0bc	2.2bc	0.6b	1.9ab	1.6bc**	3.6abc	1.2a	0	0	0
Control 1/8 Hoagland	11.2c	1.9c	0.4b	1.8b*	1.6c**	3.4c***	1.2a	0	0	0

Means with a common letter do not differ significantly between treatments at P=0.05; all means except percent infection were rounded to one decimal place.

*, **, and *** Differences in grouping due to rounding.

Table 15. Growth, ectomycorrhizal development, and nodulation of European alder inoculated with *Pisolithus tinctorius* and control seedlings after 18 months in mine spoil with three concentrations of Hoagland's solution #2 with micronutrients and iron.

Treatment	Height (cm)	Root Collar Dia (mm)	Seedling Volume (cm^3)	Dry Weight (g)			Top/Root Ratio	Percent Infection			Root Nodules
				Top	Roots	Total		*P. tinctorius*	Other	Total	
ESD 200 1/2 Hoagland	45.0a	9.1a	40.4a	6.4a	8.5ab	14.9ab	0.8a	0	0	0	14a
ESD 200 1/4 Hoagland	50.3a	9.1a	43.3a	6.3a	11.5a	17.8a	0.7a	0	0	0	10ab
ESD 200 1/8 Hoagland	37.5a	9.0a	36.5a	5.1ab	11.3a	16.4a	0.6a	0	0	0	7bc
Control 1/2 Hoagland	55.1a	7.6a	37.2a	5.5ab	7.0ab	12.5ab	0.8a	0	0	0	2c
Control 1/4 Hoagland	56.3a	8.4a	54.3a	4.9ab	7.4ab	12.3ab	0.7a	0	0	0	4c
Control 1/8 Hoagland	34.6a	8.4a	31.2a	2.8b	5.1b	7.9b	0.6a	0	0	0	3c

Means with a common letter do not differ significantly between treatments at P=0.05; all means except percent infection were rounded to one decimal place.

Table 16. Growth of European alder with and without *Pisolithus tinctorius* grown in mine spoil with three concentrations of Hoagland's solution #2 with micronutrients and iron.

Time of Measurement	Treatment	Height (cm)	Root Collar Dia (mm)	Seedling Volume (cm^3)
Initial	ESD 200 1/2 Hoagland	15.0a	3.5a	1.9a
	ESD 200 1/4 Hoagland	8.2c	2.5b	0.6c
	ESD 200 1/8 Hoagland	5.3d	2.0c	0.2d
	Control 1/2 Hoagland	11.4b	2.6b	0.8b
	Control 1/4 Hoagland	3.1e	1.7d	0.1d
	Control 1/8 Hoagland	2.6e	1.4e	0.1d
18 Months	ESD 200 1/2 Hoagland	40.5ab	7.9ab	28.6a
	ESD 200 1/4 Hoagland	39.1ab	8.0ab	32.6a
	ESD 200 1/8 Hoagland	35.0b	8.1a	29.8a
	Control 1/2 Hoagland	47.3a	7.0b	34.1a
	Control 1/4 Hoagland	47.6a	7.1b	37.9a
	Control 1/8 Hoagland	34.6b	7.3ab	30.9a

Means within a given time of measurement with a common letter do not differ significantly between treatments at P=0.05.

Table 17. Foliar concentrations of elements in European alder with and without *Pisolithus tinctorius* grown in mine spoil with three concentrations of Hoagland's solution #2 with micronutrients and iron.

Treatment	ppm													
	N	NO_3	P	K	Ca	Mg	Fe	Al	Zn	Mn	Cu	S*	B	Mo
ESD 200 1/2 Hoagland	25914a	971a	1214a	14200a	15857b	4457c	132c	85ab	209ab	6443c	14ab	4900c	59a	1.7ab
ESD 200 1/4 Hoagland	26200a	860a	1057a	14257a	15714b	4157c	172c	70b	159d	6917c	15a	3250c	56a	1.3b
ESD 200 1/8 Hoagland	26371a	687b	1300a	14129a	15929b	4700c	185c	71b	170cd	6849c	14ab	4625c	50a	1.2b
Control 1/2 Hoagland	25571a	857a	1329a	10400b	22286a	7871a	767a	132ab	221a	12000a	14ab	5175bc	58a	1.7ab
Control 1/4 Hoagland	23029b	697b	1071a	10286b	21000a	6129b	373bc	133ab	200abc	10250ab	13ab	7775ab	56a	1.6ab
Control 1/8 Hoagland	23314b	579b	1171a	10443b	20071a	6343b	570ab	146a	183bcd	8461bc	12b	9375a	50a	1.9a

Means with a common letter do not differ significantly between treatments at P=0.05.

*Means of four replications of each ectomycorrhizal-nutrient treatment combination.

Table 18. Internal moisture status of European alder with and without Pisolithus tinctorius grown in mine spoil with three concentrations of Hoagland's solution #2 with micronutrients and iron.

Treatment	Height (cm)	Root Collar Dia (mm)	Seedling Volume (cm^3)	Soil Moisture a.m.	Soil Moisture p.m.	Xylem Potential a.m. (-MPa)	Xylem Potential p.m. (-MPa)	Relative Change-Xylem Potential
ESD 200 1/2 Hoagland	48.1abc	9.3a	45.8a	76a	76a	-2.03ab	-2.36ab	0.17a
ESD 200 1/4 Hoagland	49.3abc	9.5a	48.8a	80a	80a	-2.39b	-2.84ab	0.18a
ESD 200 1/8 Hoagland	39.3bc	8.8a	34.6a	80a	80a	-2.27b	-2.89b	0.29a
Control 1/2 Hoagland	59.2a	8.4a	51.0a	74a	74a	-1.81a	-2.29a	0.28a
Control 1/4 Hoagland	56.8ab	8.9a	48.9a	71a	71a	-2.32b	-2.84ab	0.23a
Control 1/8 Hoagland	36.1c	9.0a	34.1a	72a	72a	-2.28b	-2.87ab	0.27a

Means with a common letter do not differ significantly between treatments at P=0.05.

CHAPTER VI

DISCUSSION

Field Studies

Loblolly Pine

It can be concluded that *Pisolithus tinctorius* ectomycorrhizae significantly improved the performance of loblolly pine on this mine spoil. This ecologically adapted fungal symbiont provided the host a greater physiological tolerance of the adverse conditions that prevailed on this site. It is believed that the increased capability for the absorption of moisture and nutrients afforded by *Pisolithus* was of particular importance in this study due to competition with the ground cover species planted on the spoil. The establishment of an herbaceous ground cover, required by most state reclamation laws and regulations in the southern Appalachian region, provided a degree of realism in that it presented a competition variable usually ignored in reclamation studies, and herbaceous species often prove to be formidable competitors for light, moisture, and nutrients with the woody species planted on these sites. Nevertheless, it has become increasingly desirable to revegetate coal spoils simultaneously with both ground cover species, which provide early protection from erosion, and with woody species, which provide long-term site protection and a potential economic return in the form of timber and pulpwood. The results of this study indicate that the loblolly pine seedlings infected with *Pisolithus* were superior in their ability to survive and grow under these conditions, even though they had only a moderate level of infection by this symbiont. It is probable that the infection with Pisolithus was of particular importance during the first growing season, a critical period in seedling establishment, due to the occurrence of extended dry periods. Also, it is possible that the poor conditions of the first year served to encourage the development of new Pisolithus ectomycorrhizae on the roots of these seedlings due to the competitive advantage this symbiont has on harsh sites. However, higher initial levels of infection with Pisolithus of loblolly pine seedlings destined for outplanting on adverse sites would undoubtedly prove to be even more beneficial to overall seedling performance.

Fertilization was found to be both a positive and a negative factor in the establishment of loblolly pine on this site. The application of fertilizer during the first growing season moderately enhanced the relative growth in height and root collar diameter of these seedlings, but fertilization proved to be largely detrimental to survival. Czapowskyj (1973) also noted that the fertilization of forest tree seedlings on surface-mined sites was not entirely free from the risk of reducing survival, although surviving seedlings generally exhibited a favorable growth response. Several possible explanations exist for the relatively high mortality of the fertilized seedlings in this study. The application of fertilizer to the spoil surface, which simulated the hydroseeding method of application commonly employed in the steep terrain of the southern Appalachian coal fields, stimulated the growth of the herbaceous ground cover species, resulting in increased competition for light, moisture, and nutrients. In some instances, the herbaceous species overtopped the pine seedlings. It is also probable that fertilization during the first growing season stimulated excessive top growth, which the root system was physiologically incapable of supporting during the dry periods of the summer and fall seasons. The problem of an undesirably high top/root ratio would be accentuated by the competition with the herbaceous species. It is apparent that further research is warranted with respect to the rate, timing, and method of fertilization deployed on coal spoils when the establishment of both ground cover and woody species is desired.

A consideration of the interaction of the two treatments indicates that either of the treatment combinations with Pisolithus ectomycorrhizae were superior to those with control seedlings infected by Thelephora terrestris, as indicated by the disparity in the relative growth in plot volume index (RPVI) between the GA 100 and control seedlings after three years. Nonfertilized seedlings with Pisolithus was the combination of treatments most closely approaching commonly accepted reclamation objectives, i.e., maximization of site protection and productivity. Although the fertilized seedlings with Pisolithus exhibited a similar RPVI to that of the nonfertilized GA 100 seedlings, the fertilizer-induced lower survival of this treatment combination rendered it less desirable, as the distribution of seedlings over the spoil surface is an important consideration in the control of erosion. The fertilized control seedlings, representing the most common method of establishment of woody species on adverse sites, was the poorest of all the treatment combinations in this regard. It was apparent in this study that the infection by Pisolithus was only partially capable of compensating for the reduction in survival resulting from fertilization, although a higher level of infection by this symbiont would in all probability have reduced the mortality of the fertilized seedlings. It is also probable that an adjustment in the rate, timing, and method of fertilizer applications would enable loblolly pine seedlings to derive maximum benefit from both Pisolithus ectomycorrhizae and nutrient amendments. In a similar study, Marx and Artman (1979) found that fertilizer tablets installed in the rhizosphere were not detrimental to the survival of loblolly pine with abundant Pisolithus ectomycorrhizae on an acid coal spoil in eastern Kentucky, and that the growth of nonfertilized seedlings with Pisolithus was equal to that of fertilized seedlings with Thelephora ectomycorrhizae. However, competition with ground cover species was not a factor in their study. Nevertheless, these slow-release fertilizer tablets may prove to be more compatible with the use of pine seedlings with Pisolithus than the broadcast application of fertilizer. Research is needed to further delimit the fertilizer variable such that the improved performance afforded loblolly pine on coal spoils by an infection with Pisolithus is not compromised to the detriment of overall reclamation success.

The poor survival in several plots of the loblolly pine seedlings made it difficult to assess differences in the absorption of nutrients between the treatment combinations. The chemical analysis of a limited number of foliar samples collected during the third growing season revealed that the seedlings with *Pisolithus* generally had a higher foliar concentration of NO_3 and a lower concentration of Zn than the control seedlings. The enhanced absorption of NO_3 undoubtedly contributed to the improved overall performance of the GA 100 seedlings, although this spoil was not sufficiently devoid of the essential nutrients to inhibit the establishment of loblolly pine. The elevated concentrations of many of the potentially toxic metallic elements in this spoil, and the lower foliar concentration of Zn in the GA 100 seedlings, would tend to lend credence to the theory that some ectomycorrhizal fungi may have the ability to inactivate high concentrations of toxic metals, although this is largely a matter of conjecture and the data presented here are grossly inadequate to support such a conclusion. However, Marx and Artman (1979) found that loblolly pine with *Pisolithus* ectomycorrhizae had lower foliar concentrations of sulfur, iron, manganese, and aluminum as well as a higher concentration of nitrogen, than seedlings with *Thelephora* ectomycorrhizae in their study on the acid spoil in Kentucky. It is probable that the variation in the concentrations of NO_3, Ca, Fe, S, and Mo in the foliar samples collected during the second growing season reflected the chemical heterogeniety of the spoil materials, as the nutrients applied during the first growing season were undoubtedly depleted by the second year and the differences in foliar concentrations of these ions did not appear to reflect a response to either the ectomycorrhizal or fertilization treatments.

It can be concluded that *Pisolithus* ectomycorrhizae significantly reduced the internal moisture stress of loblolly pine on this site during periods of high moisture stress, and this was a major factor in the superior overall performance of the GA 100 seedlings. The lack of significant differences among the treatment combinations in the change in xylem potential between the dawn and afternoon measurements relative to the dawn measurement indicated that midday transpirational losses relative to the water reserved in the woody tissues and that being concurrently absorbed were fairly equal between the ectomycorrhizal treatments. However, the distinct differences in xylem potential between the GA 100 and control seedlings during the early afternoon testing period, when high transpirational losses caused midday moisture deficits, and the less pronounced differences during the dawn testing period, when the recharge of seedling water was at the maximum permitted by the moisture status of the spoil and the absorption capacity of the roots, indicated that the seedlings with Pisolithus had an enhanced capacity to absorb water under the stress conditions of midday and also to recharge the water reserve in the woody tissues of these seedlings at night. It is probable that the infection by Pisolithus resulted in an expanded root surface area and extramatrical hyphae which functioned to more thoroughly exploit the surrounding spoil volume for available moisture. The droughtiness of this site was illustrated by the high readings of the tensiometers installed in each plot; by agronomic standards, a reading of approximately 40 is the point at which drought-resistant crops require irrigation. Although the percent ground cover in the plots of the fertilized control seedlings was significantly higher than that of the other treatment combinations, this was not reflected in the moisture status of the spoil volume in these plots.

Virginia Pine

Pisolithus tinctorius ectomycorrhizae did not improve the overall performance of Virginia pine on this site after two years. There were preliminary indications that the infection with Pisolithus would prove beneficial to these seedlings; after one year, the plot volume index (PVI) of the control seedlings of both fertilizer treatments was significantly lower than that of all the treatment combinations with Pisolithus except the nonfertilized GA 100 seedlings, which had been small at outplanting. However, there was no evidence of improvement due to Pisolithus at the end of this study. Several possible explanations exist for the negligible effect of the Pisolithus ectomycorrhizae. With the exception of a trend toward droughtiness and high levels of several potentially toxic metals, this spoil was more than adequate for the growth of Virginia pine, a species noted for its hardiness on poor sites. Furthermore, ample precipitation during the first growing season greatly facilitated the early establishment of the seedling root systems and reduced the potential benefit afforded by an infection with Pisolithus. Nevertheless, it is probable that a higher initial level of infection by this symbiont would have resulted in a more substantial improvement in overall seedling performance. The insufficiency of the initial infection by Pisolithus was possibly compounded by the favorable conditions of the first growing season, which compromised the competitive advantage this symbiont has on harsh sites and slowed the development of new Pisolithus ectomycorrhizae that might have proven more beneficial during the drier second year.

The fertilization of Virginia pine during the first growing season proved to be both a positive and a negative factor in overall seedling performance. As was the case with loblolly pine, fertilization resulted in a marginal improvement in the relative growth in height of these seedlings, but it also resulted in a substantial reduction in survival. Here again, the growth of the ground cover species was stimulated by the application of the fertilizer to the spoil surface, with the resulting increase in competition between the herbaceous species and the pine seedlings. Also, it is probable that fertilization stimulated top growth of these seedlings, which posed no problem to the root system during the relatively wet first growing season, but which resulted in significant mortality during the drier second season.

The chemical analysis of the foliar samples collected during the second growing season revealed a response to the application of nitrogen during the previous year. Fertilized Virginia pine seedlings had a significantly higher concentration of foliar N than non-fertilized seedlings. Given the relatively poor survival of the fertilized seedlings, however, it is apparent that nitrogen is not a limiting factor to Virginia pine on this site, and it is probable that the application of this nutrient during fertilization only served to stimulate the excessive top growth which eventually contributed to the increase in seedling mortality. The variability in the foliar concentrations of P, Mg, Zn, Mn, S, B, and Mo can only be interpreted as a reflection of the chemical heterogeniety of this spoil, as there is no indication of a response to either the ectomycorrhizal or fertilization treatments.

It can be concluded that Pisolithus ectomycorrhizae significantly reduced the internal moisture stress of Virginia pine on this site during extended dry periods, although this was not sufficient to produce an improvement in the overall performance of these seedlings after two years. All of the treatment combinations with Pisolithus ectomycorrhizae except the fertilized ABB 100 and ABB 200 seedlings exhibited lower moisture stress than the fertilized and nonfertilized control seedlings with Thelephora terrestris ectomycorrhizae during the dawn testing period, and all except the fertilized ABB 100 seedlings exhibited less stress than the control seedlings during the afternoon testing period. This indicated that the ectomycorrhizae and extramatrical hyphae resulting from the infection by Pisolithus functioned to enhance the absorption of water during the midday periods of high transpirational losses, but they also functioned to recharge the moisture reserve in the woody tissues of these seedlings during the evening hours. It is probable that the failure of the fertilized ABB 100 seedlings to exhibit less stress than the control seedlings can be partially attributed to the low level of infection with Pisolithus of these seedlings. There was also some indication that the seedlings with higher levels of infection with this symbiont had lower midday transpirational losses relative to the water reserved in the woody tissues and that being concurrently absorbed by the root systems, as indicated by the comparatively small relative change in xylem potential of the ABB 200 seedlings. Although the data presented here is not conclusive in this regard, this would appear to be additional evidence of the superior ability of the root systems of the seedlings with Pisolithus to absorb water during midday when transpiration rates were high, but it would also seem to indicate that these seedlings began the day with a more adequate recharge of their water reserves. As was reported previously for loblolly pine, the droughtiness of this spoil was indicated by the high readings of the tensiometers installed in each plot of Virginia pine during this experiment. The differences among the treatment combinations in seedling size, percent ground cover, and spoil moisture status did not appear to be sufficient to produce corresponding differences in the moisture stress of the tested seedlings.

Greenhouse Studies

Sweet Birch

It is apparent that an induced infection of sweet birch by Pisolithus tinctorius can be accomplished through the use of a vegetative mycelial inoculum, and that the level of this infection is greatly influenced by the fertility of the potting medium. High fertility reduced the infection of sweet birch by Pisolithus in both the quartz sand and mine spoil potting media. This lends credence to the theory that this symbiont is physiologically adapted for survival and growth under conditions that would prove prohibitory to the ectomycorrhizal fungi normally occurring in routine forest soils. In a similar study, Marx et al. (1977b) found that high levels of nitrogen and phosphorus in the potting medium reduced the susceptibility of the feeder roots of loblolly pine to ectomycorrhizal development by Pisolithus, and attributed this to a lower sucrose content in these roots. There was some indication, although the data presented here are not conclusive, that extremely low fertility also reduces the infection of sweet birch by Pisolithus, or at least that there is a point beyond which progressively lower fertility levels do not result in progressively higher levels of infection. As there is an interdependence for certain resources between the fungus and host in any mycorrhizal association, it is possible that the ability of the seedlings grown under the low fertility regime to produce a sufficient excess of carbohydrates, or to produce a sufficient excess of a specific carbohydrate conducive to the growth of Pisolithus, ultimately proved to be the factor that limited the level of infection achieved by this symbiont at the low fertility level. This observation suggests a need for further investigation. It was also apparent that the fertility of the potting media influenced the level of infection by Thelephora terrestris, a naturally occurring symbiont introduced via wind-disseminated propagules. Thelephora generally exhibited a positive response to increasing fertility levels in both potting media, although competition with Pisolithus appeared to have excluded this symbiont from the roots of the ESD 200 seedlings in the mine spoil. It is obvious that Thelephora is physiologically adapted to survive and form ectomycorrhizae on the roots of this host when essential nutrients are supplied at levels exceeding those commonly existing in mine spoil materials. The evidence presented here indicates that the fertility of the medium in which sweet birch is grown predisposes the level of infection attained by the ectomycorrhizal fungi present in the rhizosphere, whether naturally occurring or artificially introduced. Any effort to manipulate this infection for practical applications will involve the selection of a fungal symbiont which is ecologically compatible with the ultimate outplanting site and the implementation of cultural practices which assure its proliferation on the roots of this host, as the candidates for the symbiotic infection of sweet birch vary greatly in their response to the application of nutrient amendments.

It can be concluded that Pisolithus tinctorius ectomycorrhizae significantly improved the growth of sweet birch in both the quartz sand and mine spoil potting media. The abundant ectomycorrhizae and extramatrical hyphae resulting from the infection by this symbiont effectively enhanced the absorption of nutrients by the host, as reflected by the disparity in size within nutrient treatments between the ESD 200 and control seedlings. With the exception of the seedlings grown under the high fertility regime, the seedlings with Pisolithus were consistently larger within nutrient treatments than the seedlings with Thelephora, and often the ESD 200 seedlings were similar in size, and occasionally larger, than the control seedlings grown under a higher fertility regime. This indicated the ability of Pisolithus ectomycorrhizae to compensate for inadequate supplies of essential nutrients with no appreciable reduction in growth.

It is probable that the superior growth of the ESD 200 seedlings can be largely attributed to an enhanced absorption of nitrogen, as seedlings with Pisolithus had a higher foliar concentration of total N than the control seedlings. Also, only within the high nutrient treatment did the significant difference in the foliar concentration of total N between the ESD 200 and control seedlings fail to be reflected by a consistently superior overall growth of the seedlings with Pisolithus, indicating that this nutrient treatment probably supplied nitrogen far in excess of that required by the host. Evidently, not only does high fertility reduce the infection of sweet birch by this symbiont, but the advantage afforded this host by a reduced infection with Pisolithus is largely compromised when essential nutrients are supplied in abundance. The significance of the higher accumulation of Mg in the leaves of the control seedlings is largely a matter of speculation, and the temptation to attribute the lower concentration of Al in the foliage of the ESD 200 seedlings to a theoretical ability of Pisolithus to inactivate high concentrations of potentially toxic metals is negated by the low concentration of this element in the mine spoil used as the potting medium. Nevertheless, the adaptability of sweet birch to the poor substrates used in this investigation, and the ability of Pisolithus to form abundant ectomycorrhizae on this host which substantially increase its growth at low fertility levels, renders obvious the potential of this combination of host and symbiont for use in the revegetation of surface mine spoils.

It is apparent that Pisolithus tinctorius ectomycorrhizae did not reduce the internal moisture stress of sweet birch in this study. This failure can be largely attributed to an unsuccessful effort to invoke moisture stress in these seedlings, as indicated by the modest values for negative xylem potential obtained during both testing periods, by withholding water from them for eight days prior to testing. Obviously, a longer period without irrigation would be more effective in bringing about the moisture deficit required in a study of this nature. Also, it is probable that the transpiration rate of these seedlings was suppressed by the subdued light in the greenhouse, despite the use of supplemental lighting. Given the efficiency of a moderate level of infection by Pisolithus in reducing the moisture stress of loblolly and Virginia pine as demonstrated in the field studies, it is likely that under conditions of sufficient stress the abundant Pisolithus ectomycorrhizae on the roots of the ESD 200 sweet birch seedlings would impart a similar, if not greater, advantage to this host.

European Alder

It can be concluded that the isolate of Pisolithus tinctorius used in the greenhouse studies was not physiologically suitable for the infection of European alder. Despite the incorporation of a mycelial inoculum of this symbiont in the sand culture prior to seeding, and reinoculation upon transplanting to the mine spoil, neither Pisolithus nor any other fungal symbiont was found infecting the roots of this host. It is probable that this ectomycorrhizal species is physiologically incompatible with European alder, and any attempt to induce a symbiotic infection involving this fungus and host using routine methods will prove unsuccessful. Any differences between the treatment combinations in the growth, nutrient absorption, or moisture status of the European alder seedlings in this study can not be attributed to an ectomycorrhizal infection. It is possible that the relatively large number of nodules on the roots of the ESD 200 seedlings was a response to residual carbohydrates in the vermiculite and peat moss used as a physical carrier in the *Pisolithus* inoculum, and these nodules may partially explain the generally high foliar concentration of total N in the ESD 200 seedlings relative to the control seedlings. All other differences within nutrient treatments between the ESD 200 and control seedlings in the absorption of essential nutrients can only be attributed to random variation. Although European alder has been espoused as a species of considerable potential for the revegetation of disturbed sites, its inability to withstand even moderate droughtiness, as indicated by the extreme values obtained for xylem potential during both testing periods, leaves some question as to its ability to survive on coal spoils. The extremely high readings of the tensiometers installed in the container of each replication tested for moisture stress offered some indication of the water requirements of this species; after eight days without irrigation, these seedlings had largely exhausted all available moisture in the mine spoil potting medium, and the onset of wilting was evident. The questionable survivability of European alder on sites that sustain moisture deficits during the growing season may render it unreliable as a revegetation species on coal spoils in the southern Appalachians unless establishment techniques are developed that counteract this deficiency. These may include, but would not be limited to, prior infection with a physiologically compatible ectomycorrhizal symbiont that has a demonstrated ability to facilitate the absorption of water.

CHAPTER VII

SUMMARY

Induced mycorrhizal relationships are being given major consideration in research efforts directed toward improved revegetation techniques for surface mine spoils. The inoculation of selected species of forest tree seedlings with the appropriate ectomycorrhizal fungus prior to outplanting has considerable potential as an invaluable tool in developing effective revegetation methods and may reduce or eliminate the need for fertilization on many of these sites. After three years, the survival and growth of loblolly pine seedlings infected with *Pisolithus tinctorius* was superior to that of seedlings infected with naturally occurring ectomycorrhizal symbionts, primarily *Thelephora terrestris*, on a coal mine spoil in Tennessee. Seedlings with *Pisolithus* ectomycorrhizae had a higher foliar concentration of NO_3 and a lower concentration of Zn than control seedlings and exhibited an enhanced ability to absorb water during periods of high moisture stress. Fertilization proved to be both a positive and a negative factor in the overall performance of these seedlings, resulting in some improvement in seedling growth but substantially reducing survival. The relatively poor survival of the fertilized seedlings was attributed to an imbalance in the top/root ratio and enhanced competition with herbaceous ground cover species. The infection with *Pisolithus* was only partially capable of compensating for the reduced survival resulting from fertilization, although more abundant Pisolithus ectomycorrhizae on the roots would have probably reduced the mortality of the fertilized seedlings.

An infection with Pisolithus did not significantly improve the overall performance of Virginia pine seedlings on this site after two years, although the seedlings infected with this symbiont exhibited an enhanced ability to absorb water during extended dry periods. The hardiness of Virginia pine on poor sites and abundant precipitation during the first growing season may have compromised the potential benefits of an infection by Pisolithus. Fertilization again substantially reduced survival, but fertilized seedlings were somewhat larger than nonfertilized seedlings and had a significantly higher foliar concentration of total N.

Pisolithus tinctorius formed abundant ectomycorrhizae on the roots of sweet birch when introduced via a vegetative mycelial inoculum, but the formation of Pisolithus ectomycorrhizae was significantly reduced by high fertility in both the sand culture and mine spoil potting media. Thelephora terrestris, introduced by way of wind-borne propagules, formed more abundant ectomycorrhizae on the roots of this host at high fertility levels. Sweet birch seedlings infected with Pisolithus and grown under intermediate and low fertility regimes were significantly larger than comparable control seedlings infected with Thelephora, and the seedlings with Pisolithus generally had a higher foliar concentration of total N and lower concentrations of Mg and Al than the control seedlings. The adaptability of sweet birch to poor substrates, and the ability of Pisolithus to form abundant ectomycorrhizae on this host which substantially increase its growth at low fertility levels, suggest that this combination of host and symbiont has considerable potential for use in the revegetation of surface mine spoils. The failure to induce a sufficient moisture deficit in the mine spoil potting medium precluded a determination of the ability of the *Pisolithus* ectomycorrhizae and extramatrical hyphae to facilitate the absorption of water by sweet birch during periods of high moisture stress.

The isolate of *Pisolithus tinctorius* used in this study did not form ectomycorrhizae on the roots of European alder, and it is unlikely that a symbiotic association incorporating this fungus and host can be achieved using routine methods of inoculation. Other ectomycorrhizal species which are physiologically compatible with European alder may prove sufficiently beneficial to this host to warrant the development and production of practical inoculums, especially if these symbionts have a demonstrated ability to facilitate the absorption of water.

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