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ECTOMYCORRHIZAL ESTABLISHMENT AND SEEDLING RESPONSE ON VARIOUSLY TREATED DEEP-MINE COAL REFUSE

by

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STATEMENT OF TRANSMITTAL

Special Research Report SR-89 transmitted herewith has been prepared by the Coal Research Section of the College of Earth and Mineral Sciences Experiment Station. Each of the Special Reports listed below presents results obtained in connection with one of the research projects supported by the Department of Environmental Resources of the Commonwealth of Pennsylvania or a technical discussion of related research. The following is a list of Special Research Reports issued to date:

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SUMMATION OF RESULTS

The environment of most deep-mine refuse banks is too harsh for plants to become established naturally and grow well. Efforts to re-vegetate such banks have been sporadic, and the results have been either minimally successful or too expensive for widespread application.

It has long been known that certain fungi are capable of forming mycorrhizae on the roots of tree seedlings which enable the seedlings to grow better under conditions of nutrient deficiencies. Recent studies have revealed that specific ectomycorrhizal symbionts are able to survive under conditions of high acidity, high temperatures, or low moisture. Therefore a study was established to determine if such ectomycorrhizal symbionts could tolerate the harsh conditions found on refuse banks and if they could increase seedling growth on such banks either with or without additional treatment.

The experiments were conducted in a greenhouse in pots of spoil material. A completely randomized design with factorial arrangements of four fixed factors (spoil, fungal symbiont, lime treatment, and fertilization treatment) was used. There were three levels of bituminous spoil (Brandy Camp spoil, Cresson spoil, and Cramer spoil), three levels of fungal symbiont (Pisolithus tinctorius (Pers.) Coker & Couch, Cenococcum graniforme (Sow.) Ferd. & Winge, and a control), two levels of lime treatment (final pH of 6.0 and a control), and two levels of fertilization treatment (1.0 tons/acre and a control).

The spoils, named after the community nearest the bank, varied widely in chemical and physical characteristics. The highly acid Brandy Camp spoil and the somewhat less toxic Cresson spoil were

essentially devoid of vegetation. Conditions on the Cramer spoil were favorable enough so that over a period of time a variety of plant species had become naturally established on it.

European white birch (Betula pendula L.) and red pine (Pinus resinosa Ait.), which are among the species most tolerant of the environmental stresses found on spoil banks, were used in the study, with each species constituting a separate experiment. At the end of each experiment, the categorical mycorrhizal establishment, the height, the total dry weight, the root dry weight, the top dry weight, and the shoot/root ratio were determined for each seedling. The means for the various treatments and the interactions were evaluated statistically.

Both tree species were successfully inoculated with both mycorrhizal fungi, with Pisolithus mycorrhizae being the more abundant. This is the first report of the definite association between European white birch and Pisolithus tinctorius. Mycorrhizal development varied among banks and among amendments.

Of the mycorrhizal treatments, only the Pisolithus inoculations of the red pine seedlings resulted in a significant increase in growth. In comparison to the control plants, growth response was greater in the pots treated with lime or fertilizer than in those inoculated with mycorrhizal fungi. The greatest response on the highly acid Brandy Camp spoil was to the lime treatment; on the less acid Cramer and Cresson spoils, to the fertilizer treatment.

The results of this experiment are sufficiently promising so as to encourage additional ectomycorrhizal studies, especially field plantings

of seedlings with abundant Pisolithus mycorrhizae on their roots. Meanwhile, any refuse-bank plantings should include fertilizer application and on the highly acid banks lime application as well.

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I. INTRODUCTION

In mining and processing coal, large amounts of wastes are generated. Characteristically in large deep-mining operations, the wastes accumulate as virtual mountains of fragments of shale, coal, and rock. These piles are referred to as refuse or spoil banks.

By virtue of different compositions of spoil wastes, refuse banks vary both chemically and physically. Some banks respond favorably to planting practices and even revegetate naturally. These banks, however, are by far fewer than those which remain essentially barren even after 20 to 50 years of abandonment. The barren sites are sources of air and water pollution and remain as eyesores to the public, especially to the immediate communities and to prospective new industry.

Conditions limiting vegetation survival and growth on these barren banks include the following chemical, physical, and environmental factors: high acidity; nutrient deficiencies; toxic salt concentrations; lethal surface temperatures; moisture stresses; unfavorable texture, structure, and stoniness; erosion and slides; steep topography; frost heaving of seedlings; wind desiccation and blasting by small wind-driven particles; pooled run-off water; low organic-matter contents; absence of microorganisms and symbiotic fungi; and refuse fires. Usually the interaction of two or more factors impose conditions too harsh for natural revegetation.

Possible solutions to the dilemma of revegetation of these toxic spoil banks include the following:

1. Covering with soil material and planting.
2. Applying lime, fertilizer, and mulch.

3. Grading the steep banks and applying the above amendments.
4. Inoculating seedling stock with specific mycorrhizal fungi for increased vigor to withstand the environment.

The first three methods have been employed by some mining companies on their own refuse piles in response to public sentiment. These methods have either been minimally successful or far too expensive for extension to wide-scale revegetation of the many acres of barren, abandoned refuse piles. Therefore, the search continues for an economic means of successful revegetation to effectively mask the disturbances, curtail pollution and make these sites productive again.

If growth of transplanted seedlings could be increased by inoculation with specific mycorrhizal fungi, then seedling survival would be enhanced, especially during the first year in the harsh environments. Mycorrhizal symbionts adapted to droughty conditions and high temperatures might enable the seedlings to obtain a foothold in the refuse through increased nutrient absorption and uptake. The seedlings might then be able to overcome the other major limiting factors and become established on the spoil banks.

Some banks, however, may be too unfavorable for specific mycorrhizal symbionts alone to enhance seedling survival, growth, and vigor. In such cases, supplemental amendments, such as lime and fertilizer, might have to be added to ensure seedling establishment.

Such conclusions are largely speculative, however, since little work has been done involving the study of seedling response with specific mycorrhizal fungi in spoil wastes. Therefore a study designed to supply information on the subject was initiated. Its specific objectives were:

1. To determine if selected tree species could be successfully inoculated with the selected fungal symbionts.
2. If so, to determine the degree of mycorrhizal occurence on a variety of spoils both untreated and treated with lime, fertilizer, or both.
3. To determine the seedling response to the selected mycorrhizal fungi under the various treatments.

II. REVIEW OF THE LITERATURE

The specific environment of toxic refuse banks offers many limitations to seedling growth and survival. However, with specific fungal symbionts, vegetation may be able to survive the harsh habitats. After reviewing the results of many studies, D.H. Marx (unpublished report) stated that ectomycorrhizae:

1. "Increase the root absorbing surface by an estimated 1000X in certain cases when compared to nonmycorrhizal short roots.

2. Exert a selective ion absorption from mixtures of various nutrients and more rapid accumulation of N, P, K, Ca, and Na than do nonmycorrhizal roots.

3. Absorb and assimilate nutrients from very slightly soluble soil minerals, organic substances, and forest litter which are not readily available to nonmycorrhizal roots.

4. Function as protective barriers against root infection by certain pathogenic fungi. Mechanisms involve mechanical and chemical barriers created by the symbiont. Many symbionts produce very potent antibiotics."

Schramm (1966) noted that the only successful original colonists of bare and predominantly nitrogen-deficient anthracite wastes were either nitrogen-fixing plants or certain species with ectomycorrhizae. He further reported that if the tree seedlings did not become mycorrhizal in early stages of growth, the seedlings generally became moribund and eventually died on the less favorable spoil banks. Therefore, the purpose of this literature review is to correlate the factors required for mycorrhizae formation with the main habitat properties of harsh refuse banks limiting survival of vegetation. The manner by which ectomycorrhizae aid vegetation survival on unfavorable habitats will also be discussed.

On most natural soils, the roots of vegetation are teeming with mycorrhizal fungi. However, unless certain conditions exist, creating a favorable environment for mycorrhizal development, the fungal symbiont is lacking. The general requirements of most mycorrhizal fungi for establishing root symbiotic relationships include the following: simple carbohydrates, available nitrogen, root metabolites, substrate temperature optima of 20 to 30°C for most species, pH optima of 5.0 to 6.0 for most species, adequate oxygen supply, sufficient host light intensity, low nutrient status of substrate and host roots, and sufficient substrate moisture.

The virulence of a mycorrhizal fungus, i.e. the ability of the fungus to produce auxins in concentrations that will ensure the degree of morphogenesis necessary for the establishment of mycorrhizal symbiosis (Slankis, 1958), can be altered by environmental factors such as abnormal host physiology or unfavorable soil characteristics (Trappe, 1962). These environmental factors are common on refuse banks and include the following: extreme waste reaction, nutrient deficiencies and toxic salt concentrations, lethal surface temperatures, and drought. An extensive review of the chemical and physical properties of toxic deep-mine spoil banks in Pennsylvania can be found in Thompson (1971).

Waste Reaction (pH)

The high acidity of most refuse banks is the result of the oxidation of iron pyrites from waste coal and overlying shale producing sulfuric acid. Potter et al (1951) observed a pH range of 3.5 to 6.5 on West Virginia spoils. Horn (1968) reported extremely acid strip hyphen mine spoils of Pennsylvania ranging from 2.60 to 3.45.

In early work with strip-mining operations, Croxton (1928) found that distribution of natural vegetation was closely correlated with the hydrogen ion concentration of the surface material. He reported that the vegetation was well established under favorable conditions above pH 4.5 and generally failed below that point. Arnon and Johnson (1942) have shown that, at a pH of 4.0 or below, hydrogen ions limit plant growth. Potter et al (1951) similarly found little revegetation on spoils with a pH lower than 4.0. Wheeler (1965) attributed much of the vegetation failure in the bituminous region of the Applachian Plateau to conditions of extreme acidity. Knabe (1965) similarly believed that extreme soil reaction was the factor most frequently preventing the natural revegetation of industrial waste. He stated that plants were not adapted to survive in soils with pH values below 3.5 or above 8.5. Some mycorrhizal fungi, however, have been reported to exist under more acid conditions.

Mycorrhizae of trees develop most extensively in acid soils (Hacskaylo, 1957). Richards (1961) similarly reported that mycorrhizae of Pinus did not develop profusely on trees growing in neutral or alkaline soils. The acidophilic nature of various mycorrhizal fungi has been investigated in synthetic culture media (How, 1940; Mikola, 1948; Norkrans, 1949). How (1940) observed the lowest pH tolerance for optimum growth of an ectomycorrhizal fungus studied in culture to be 3.0. Mikola (1948) reported Cenococcum graniforme (Sow.) Ferd. & Winge to be favored by very strong acidity with a pH optimum of about 4.0 and pH limits of 2.4 to 7.0.

Tesic (1958) similarly reported that mycorrhizal fungi grew best in acid soils (pH 4.0 to 5.0) and that some would form fruiting bodies only in acid media. In a review by Hatch (1937), Melin was reported as showing prolific growth of mycorrhizal fungi in culture at a pH value of 3.0, which is similar to the pH values of the raw humus soils of Sweden. Björkman (1941) and Richards and Wilson (1963) also have shown ectomycorrhizal formation on roots to be greatest under acid conditions.

Goss (1960) and others have shown that as pH of the soil approaches neutrality, different mycorrhizae of trees develop. Marx and Zak (1965) restated the probability that pH of a soil influences the distribution of different tree root-fungal associates and the formation of different mycorrhizal associations. In their work with slash pine seedlings (Pinus elliottii) Engelm. in aseptic culture, Cenococcum graniforme, Laccaria laccata (Scopoli ex Fr.) Berk. & Boome, and Suillus luteus (L. ex Fr.) S.F. Gray grew at pH levels of 4.0, 4.6, 5.6, and 6.6. Best development was at the less acid levels. Control seedlings without ectomycorrhizae were significantly less vigorous than those with mycorrhizae, especially at the lower pH values. Cenococcum was noted as contributing strongly to seedling growth at pH 4.0 and 4.6. Schramm (1966) observed Pisolithus tinctorius (Pers.) Coker & Couch and other ectomycorrhizal sporophores on moderately acid (pH 4.6 to 5.2) anthracite wastes of Pennsylvania.

Knabe (1965) pointed out that detrimental effects on plant growth may be caused at marginal pH values by the fixation of nutrient elements such as phosphorus and by the release of greater amounts of manganese,

aluminum, or other metals into the soil solution. He believed, however, that very low pH values were directly fatal to plants. Berg (1965) concluded that the primary source of toxicity to vegetation on acid spoils seemed to be one of the elements or salts brought into solution by the acidity, rather than the acidity itself.

Spoil Nutrient Deficiencies, Salt Toxicities

The acid iron sulfates and sulfuric acid from oxidations of iron sulfides in the spoil rock material attack other rock fragments that subsequently release soluble salts. These salts in many cases augment the spoil acidity and are in concentrations toxic to plant survival. The greater acidity resulting from sulfuric acid and high soluble salts precipitates many essential nutrients from the soil solution, rendering them unavailable to plants. Reports, however, of exactly which nutrients are toxic differ because of the extreme variability in spoil material.

Stiver (1949) analyzed strip-mine materials in Indiana and found no deficiencies of potassium, calcium, magnesium, or boron. He found phosphorus and nitrogen contents to be low, but phosphorus was present in greater amounts than in the adjacent undisturbed land. Hart and Byrnes (1960), in other chemical analyses, studied 22 strip-mine spoil banks of wide ranges in Pennsylvania and found phosphorus to be very deficient and potassium abundant when compared to agricultural standards. Struthers (1964) analyzed 19 strip-mine spoils in Ohio and found limited amounts of calcium and magnesium and high levels of soluble aluminum, iron, and manganese salts. Similar large quantities of toxic salts of sulfate were found in the top four feet of spoil materials. Knabe (1964)

reported investigations of German strip-mine banks of high acidity (pH 2.0 to 3.5) and found high soluble manganese and aluminum contents, but lack of phosphoric acid, potassium, and magnesium.

In medium to extremely acid Kentucky spoils, Cummins et al (1965) found low concentrations of organic matter, total soluble salts, and exchangeable calcium. Available sulfur, aluminum, iron, and manganese were found to be present in high concentrations in several localities. Horn (1968), in a similar study of Pennsylvania strip-mine spoils, found in the acid spoils very low concentrations of potassium, phosphorus, and exchangeable calcium and magnesium. Organic matter and nitrogen were practically non-existent. Horn, however, reported variable concentrations of iron, manganese, and aluminum in the spoil banks investigated.

Coleman et al (1958) reported that aluminum was the most important toxic element in acid spoils and that element toxicities may exist in soils with pH ranges of 5.0 to 5.5. Lorie (1962) similarly reported tree survival and growth on Iowa spoils to be inversely related to total soluble salts, especially exchangeable aluminum. Berg (1965) related concentrations of hydrogen ions, sulfate, toxic salts, and iron to plant growth on extremely acid strip-mine spoils of Kentucky. He found plant yield to be inversely proportional to the element or salt concentrations. Berg believed that aluminum and manganese were the toxic elements most limiting plant growth on acid spoils.

Beyer (1969) studied the effects of pH and aluminum toxicity on several tree species recommended for harsh strip-mine spoils in Pennsylvania. He concluded that high concentrations of hydrogen and aluminum are probably an important contributing factor to revegetation

failures on unfavorable spoils. Berg and Vogel (1971) studied the effects of aluminum and manganese toxicity on vegetation and believed these soluble salts limit vegetation on many adverse spoil banks.

The benefits afforded vegetation by mycorrhizae under conditions of low fertility have been studied by many investigators. The earliest indications that ectomycorrhizae were of benefit to trees appeared in experiments wherein seedlings growing in soils devoid of mycorrhizal fungi ceased growth after one or two years. Hatch (1936) introduced mycorrhizal and nonmycorrhizal seedlings into prairie soils that were devoid of trees and lacked mycorrhizal fungi. His results showed that the mycorrhizal seedlings were capable while the nonmycorrhizal seedlings were incapable of incorporating sufficient quantities of nitrogen, phosphorus, and potassium. He explained the enhanced mycorrhizal tree growth as the increase of root-absorbing surfaces in direct contact with the soil particles.

McComb (1938) showed that in prairie soils with low content of phosphorus, mycorrhizae enabled seedlings to survive and absorb this element more rapidly than nonmycorrizal seedlings. Hacskaylo (1961) similarly reported successful establishment of several pine species in Puerto Rico when he inoculated the soil with mycorrhizal fungi. Hacskaylo and Vozzo (1967) made further studies with mycorrhizal inoculations on adverse soils in Puerto Rico. They successfully inoculated nonmycorrhizal seedlings of Pinus caribaea Morelet and observed large increases of seedling growth. Fertilizer amendments did not offset the stunting of nonmycorrhizal seedlings that were not inoculated.

Trappe and Strand (1969) inoculated severely stunted, phosphorus-deficient Douglas fir seedlings (Pseudotsuga menziesii) (Mirb.) Franco with ectomycorrhizal fungi in a newly developed western Oregon nursery. Prior to inoculation, the seedlings failed to respond to fertilization during their second growing season and proved to be nonmycorrhizal. No ectomycorrhizal fungi were present in the soil because of land leveling and extensive fumigation in development of the nursery and because of previous long use by agriculture. Inoculation with subsequent mycorrhizal formation significantly increased phosphorus uptake and growth of seedlings during the third growing season. Seedling growth was then enhanced by further fertilization.

Even on nitrogen-deficient substrates, ectomycorrhizal trees have been seen to flourish. Björkman (1949) found that soils, especially forest soils that were deficient in available nitrogen, produced seedlings with abundant mycorrhizae. He also observed extensive mycorrhizal development in seedlings growing on soil with low available phosphate but high available nitrogen. Björkman summed up the mycorrhizal establishment as follows: "A severe lack of available nitrogen or phosphorus hampers the formation of mycorrhizae as well as growth, but moderate scarcity of one or the other of these nutrients is a condition for mycorrhizal infection." This conclusion was confirmed by Doak (1955), Hacskaylo and Snow (1959), and Slankis (1964).

Schramm (1966) reported mycorrhizal vegetation on the nitrogen-deficient anthracite coal wastes of Pennsylvania. Richards and Voigt (1964) indentified Pisolithus tinctorius sporophores associated with two

ectomycorrhizal pine trees on barren sandy soils that were nitrogen deficient.

The source of nitrogen to trees and the symbionts on these areas are uncertain. Hacskaylo (1967) stated that thus far, no results positively indicate nitrogen fixation by mycorrhizal fungi. Richards and Voigt reported the possibility of nitrogen fixation by free-living organisms in nitrogen-deficient soils of Australia. Schramm observed coal wastes to be devoid of many organisms and believed sufficient atmospheric nitrogen reached the substrate via precipitation to support tree growth. The hyphae then absorbed, translocated, and accumulated nitrogen in the root tissues from the minute nitrogen in rainwater.

The importance of mycorrhizae in selective absorption and translocation of certain elements in impoverished soils has been well established. Mikola (1948) studied phosphorus uptake by mycorrhizal fungi. He investigated pH changes in phosphate-buffered nutrient solutions by Cenococcum graniforme and indicated the possibility of incorporating phosphates in mycorrhizal root systems through increased fungal utilization. Kramer and Wilbur (1949) then observed the ability of mycorrhizal pine roots and the inability of nonmycorrhizal pine roots to accumulate radioactive phosphorus.

In early papers, Melin and Nilsson (1950, 1952, 1953, 1954, and 1955) have demonstrated a movement of phosphate and other nutritive material from the mycorrhizal mycelium to host pine seedlings. Harley and his coworkers (1950, 1952a, 1952b, 1953, 1954a, 1954b, and 1955) studied the uptake of phosphate by excised roots of beech and reported transport of phosphate from fungus to host in the excised mycorrhizal

tips. Harley and McCready (1952a, 1952b) found that 90% of the phosphorus absorbed by the excised mycorrhizae was retained in the fungal sheath. They concluded that the sheath acted as a partial barrier to the absorption of phosphorus by the central root core. Melin and Nilsson (1958) confirmed previous observations that nutritive elements are transferred from the fungal symbiont to the mycorrhizal host.

More recently, Morrison (1962a) studied labelled isotopes of phosphorus and found P^{32} in mycorrhizal seedling shoots to far exceed that in nonmycorrhizal shoots. Ritter and Lyr (1965) observed the uptake of labelled orthophosphate (P^{32}) in soluble and poorly soluble forms by mycorrhizal Pinus silvestris L. seedlings. They similarly reported higher phosphorus absorption and translocation by the seedlings with mycorrhizae than by the nonmycorrhizal seedlings.

In other nutrient studies, Melin and Nilsson (1952) reported increased uptake of labelled nitrogen in pine seedlings by means of mycorrhizae. Carrodus (1966) studied nitrogen absorption by beech mycorrhizae as affected by temperature, pH, and root carbohydrate level. Ammonia and nitrates were seen to represent good and poor sources of nitrogen respectively for mycorrhizae absorption. Ammonia absorption was seen to be greatly depressed at temperatures above 20°C, at pH values below 6.0, and at low carbohydrate concentrations in the root tissue. Carrodus concluded that ammonia is incorporated into organic compounds and the rate of absorption depends upon metabolic rates and availability of carbohydrates in the roots.

Melin and Nilsson (1955) studied the uptake of labelled calcium in mycorrhizal pine seedlings. They found that considerable amounts of

calcium ions were transferred from a distant source to the mycorrhizal roots and that the calcium was distributed throughout the seedlings.

Melin et al (1958) exposed mycorrhizal seedlings of Pinus virginiana Mill. to Na^{22} and observed that large amounts of sodium were absorbed and translocated into the host by the mycorrhizae.

Harley and Wilson (1959) extended the ion uptake investigation by studying potassium absorption of mycorrhizae. Potassium was another element found to be selectively absorbed and translocated to the host.

Morrison (1962b) investigated the effect of mycorrhizae on sulfur uptake and translocation. He observed no benefit of the mycorrhizae he studied on the uptake of sulfur. He concluded that sulfur had a more direct pathway through the mycelium than the other nutrients studied in earlier reports. Many of the other nutrients investigated had been actively absorbed by mycorrhizae in which the mycorrhizae served as metabolic sinks for their accumulations.

The stimulating effect of mycorrhizal fungi on conifer seedlings was further reported to result from heightened metabolism associated with the transfer of phosphorus and growth stimulators from the fungus to the seedlings (McComb and Griffith, 1946). Following mycorrhizal formation, a marked change and enlargement of the tree seedling root system results. According to various authors, the fungus partner makes available to the tree, water (Cromer, 1935) and nutrients (Hatch, 1937; McComb, 1943; Routien and Dawson, 1943). McComb suggested growth stimulation of mycorrhizal seedlings was due to increased respiratory activity with higher levels of phosphorus and growth stimulators derived from the fungus. Routien and Dawson explained increased salt

absorption of mycorrhizal pine seedlings to be associated with heightened respiration and carbonic acid production.

After many studies over a period of years, it has been conclusively proven that mycorrhizae play a very active role in nutrient uptake of trees. Investigations of the sources of these elements and the mycorrhizae ability of absorbing elements from different soil constituents has also been studied. Rosendahl (1942) reported that some mycorrhizal fungi he studied were able to increase the solubility of potassium from orthoclase used as a sole potassium sorce. Nonmycorrhizal seedlings lacked the ability to obtain the potassium from orthoclase. Phosphorus in apatite was not avialable to any of the mycorrhizal seedlings or nonmycorrhizal seedlings tested. Routien and Dawson (1943) stated that the hydrogen ion production by respirative carbonic acid of mycorrhizal roots may enable decomposition of the highly insoluble ferric ions. They suggested that a major role of mycorrhizae was the mobilization and absorption of iron.

Stone (1949) failed to demonstrate any effect of mycorrhizae on phosphorus uptake from crushed phosphatic minerals. Meyer and König, in Ritter and Lyr (1965), explained the inability of phosphorus absorption by mycorrhizae from mineral constituents. They concluded that mycorrhizal fungi were not active in utilizing apatite and other calcium phosphates because the fungi did not produce acids necessary to obtain phosphates from mineral constituents. Ritter and Lyr (1965) further hypothesized that increased growth of mycorrhizal seedlings in clay pots was possibly a result of mobilization of mineral nutrients from the walls of the pots by fungal hyphae.

Several workers (Hatch, 1937; Björkman, 1949; Fowells and Krauss, 1959; Hacskaylo and Snow, 1959; Richards and Wilson, 1963) have shown that an inverse relationship exists between nutrient availability and the degree of mycorrhizal infection in Pinus. Hatch showed that as nitrogen, phosphorus, potassium, and calcium decreased in the soil, mycorrhizal establishment increased. He demonstrated experimentally that plants with mycorrhizae absorbed 75% more potassium, 234% more phosphorus, and 86% more nitrogen than plants without mycorrhizae growing in the same substrate.

Hatch (1937) concluded that the predisposing factor in mycorrhizae formation was plant nutritional status, especially that of nitrogen, phosphorus, and potassium. He believed that when the internal concentrations of these elements were high and balanced, the seedlings were resistant to infection by mycorrhizal fungi. Mitchell et al (1937), Björkman (1949), Doak (1955), Fowells and Krauss (1959), and Hacskaylo and Snow (1959) further concurred that low levels of available nutrients, especially nitrogen and phosphorus, increased mycorrhizae abundance. Fertilization, especially phosphate, served as a substitute, in part at least, for mycorrhizal infection (Routien and Dawson, 1943). Addition of complete inorganic fertilizers to soils favored increases in root sizes but reduced the incidence of mycorrhizae (Hacskaylo, 1961).

Hacskaylo and Snow (1959) cited Björkman's work of 1942 in which he concluded that the presence of free soluble sugars in root tissues was the predisposing factor for mycorrhizal establishment. Björkman believed that with high soil fertility and with a high rate of plant photosynthesis soluble carbohydrates would be utilized in tissue

formation. Carbohydrate reserves would be low in the roots, and no energy source would be available to initiate or support a symbiotic association. He believed light to be directly important in establishing carbohydrate reserves in the roots.

Björkman experimentally showed that a high carbohydrate concentration of roots was necessary for mycorrhizae formation. By stem girdling mycorrhizal seedlings, he was able to cut off the supply of carbohydrates to the roots. He observed mycorrhizal infection in these seedlings to be greatly reduced. Schramm (1966) found that chlorotic seedlings that had been partially heat girdled were nonmycorrhizal on anthracite coal wastes.

Roots with a high carbohydrate reserve release exudates into the rhizosphere. Rovira (1959) reported that exudates of roots influenced mycorrhizal establishment. Melin and Das (1954), Melin (1954), and Melin (1962) reported that the nutritional status of the host affected the production of the "M-factor" which stimulates the growth of many mycorrhizal fungi in pure culture. The concentration of root carbohydrates was directly proportional to the production of the "M-factor" (Melin, 1962).

Spoil Temperatures and Drought

Excessive heating and drying of the soil surface layers have long been recognized to be main causes of seedling mortality in regions with a dry growing season. Since a moist surface cannot easily be heated to dangerous levels by strong insolation (Maguire, 1955), the effects of soil surface heating and soil drought as limiting vegetation will be discussed simultaneously.

Rapid drying and heating of the surface layers of spoil material is common even under normal weather conditions during the spring and summer. Deeley (1970) observed temperatures of 70°C on very dark-colored strip-mine spoils in Pennsylvania in late spring and early summer. Schramm (1966) recorded temperatures of 75°C on fully insolated anthracite coal wastes during normal summer seasons in Pennsylvania. He attributed vegetation failure of the wastes to be caused mainly by lethal surface temperature.

Heat injury to plants is characterized by bark necrosis in the zone of the root collar causing collapse of the non-woody stem tissues. If a complete circle of tissue is affected, termed "stem girdle", the plant dies. If the stem is only partially girdled, the plant may survive but carbohydrate translocation from the seedling tops to the roots is reduced. This in turn may limit mycorrhizal establishment (Björkman, in Hacskaylo and Snow, 1959; Schramm, 1966).

Several investigators have studied the effect of soil moisture on mycorrhizae abundance. Hacskaylo (1957) reported abundance of new mycorrhizae may be correlated with higher soil moisture levels which generally occur during spring and autumn as compared with the lower moisture levels in summer, when rainfall is less and transpiration rates are high. He also reported that low temperatures in winter limited the development of mycorrhizae by suppressing root and fungal growth.

Palmer (1954) concluded that conditions of drought intensified the breakdown of most mycorrhizal short roots and conditions of plentiful moisture promoted regeneration of new roots. He postulated that periods

of drought encouraged Cenococcum graniforme mycorrhizal development, but noted that the mycorrhizae formed were rarely found to have cortices in which the cells appeared healthy. Therefore, Palmer suspected a somewhat pathogenic tendency on the part of Cenococcum.

Abundance of Cenococcum similarly has been reported to be related to soil moisture (Slankis, 1958; Worley and Hacskaylo, 1959). Worley and Hacskaylo (1959) showed that C. graniforme mycorrhizae on seedlings of Pinus virginiana increased as the available soil moisture decreased. Hacskaylo (1961) believed C. graniforme to be a more vigorous mycorrhizal former under droughty conditions because of the possible reduced vigor of the other competing mycorrhizal fungi. Cromer (1935) reported the ability of certain mycorrhizal fungi to increase the uptake of water.

Little work has been done on determining the heat tolerance of mycorrhizal fungi. Hacskaylo et al (1965) studied the effect of temperature on several mycorrhizal fungal species in pure culture. C. graniforme, with optimum growth at 24°C, was severely restricted at 32°C; thus it is not exceptionally heat tolerant.

Marx and his coworkers have recently studied the effect of fungal symbionts on seedling survival at high temperatures. Marx et al (1970) found root substrate temperature to have a significant influence, in aseptic synthesis of ectomycorrhizae, on survival and growth of loblolly pine seedlings (Pinus taeda L.). Control seedlings with no ectomycorrhizae died at root substrate temperatures above 29°C during the second month of development. Pine seedlings with ectomycorrhizae formed by Thelephora terrestris (Ehrh.) Fr. survived and displayed good growth at

29°C, while seedlings with the fungal symbiont Pisolithus tinctorius survived and grew well at both 29°C and 34°C.

Marx and Bryan (1971) studied the effects of Thelephora terrestris and Pisolithus tinctorius on survival of Pinus taeda seedlings growing at 40°C. Of the nonmycorrhizal control seedlings at 40°C, 45% survived, while 70% of the ectomycorrhizal seedlings with T. terrestris survived and 95% of the P. tinctorius seedlings survived. The nonmycorrhizal pine seedlings that lived after five weeks at 40°C were stunted with severe chlorosis at the tips of the needles. After five weeks at 40°C, the pine seedlings with ectomycorrhizae formed by T. terrestris had only minor chlorosis of needle tips while all the living seedlings (19 of 20 planted) with P. tinctorius grew as well as those seedlings used for comparisons growing at 25°C. Seedlings with no symbiont and seedlings with T. terrestris as a symbiont showed reduced growth. Marx and Bryan hypothesized that P. tinctorius may have furnished metabolites to the seedlings at high temperatures (40°C) which rendered them more heat tolerant. Another factor that they believed that may have contributed to the greater survival was the greater abundance of mycorrhizae formed by P. tinctorius than by T. terrestris initially on the roots. Schramm (1966) observed similar enhancement of vegetative growth with ectomycorrhizal seedlings of P. tinctorius and other fungi on anthracite waste banks where high temperatures are common.

The ability of specific ectomycorrhizal seedlings and the inability of nonmycorrhizal seedlings to survive in soils of high temperature (Marx and Bryan, 1971), suggests new techniques in reforestation of areas exposed to high temperatures. Thelephora terrestris, a fungal

symbiont normally found on nursery stock (Marx et al, 1970b), is incapable of performing well at high temperatures; thus nursery transplants are usually unsuccessful in soils of high temperature. Establishing Pisolithus tinctorius on nursery stock to be transplanted to fully-insolated refuse sites may, however, be a forestry practice that could enable successful revegetation of these problem sites.

III. PROCEDURE

The study was done in a greenhouse with spoil material brought in from the field. It featured a randomized design with factorial arrangements of four factors (spoil, fungal symbiont, lime treatment, and fertilization treatment). There were three levels of spoil, three levels of fungal symbionts (two fungi and one control), two levels of lime treatment (one lime rate and one control), and two levels of fertilization treatment (one fertilization rate and one control). Two tree species were used in the study, with each species constituting a separate experiment. There were two replications of each of the 36 treatments for each species.

The three spoils used in the study represented a wide range of conditions. These wastes were selected from preliminary field investigations of deep-mine bituminous refuse banks of central Pennsylvania (W.H. Davidson, unpublished data). A waste bank with a reaction of pH 4.8 resulting from mining the Lower Freeport coal seam was selected as a source of low-toxic spoil. This bank is located near Cramer, Pa. and is referred to as the Cramer refuse bank. The second spoil, associated with the Lower Kittanning seam, is noted for its high acidity. This bank, located near Brandy Camp, Pa. has a reaction of pH 2.6. The third spoil site, located near Cresson, Pa., resulted from mining both the Lower Kittanning and Lower Freeport seams. The Cresson bank has an average pH of 3.4. All three refuse banks have been abandoned for at least 20 years. From each location, spoil material was screened through one-inch mesh and bagged for later greenhouse use.

More than 95% of the spoil collected from all three banks passed through the one-inch mesh.

Vegetation and root samples were observed on the three banks selected for the study. Both the Brandy Camp and Cresson banks were completely barren, but the Cramer bank was colonized with trees of many species, including aspen, fire cherry, oak, and birch. Herbaceous species were growing in the accumulating litter layer. Pisolithus sporophores were found fruiting on the Cramer bank. Root samples were observed for mycorrhizal presence with a 10X hand lens in the field. Roots appeared mycorrhizal, but the mycorrhizal development was not ascertained by histological procedures.

The mycorrhizal fungi used in the experiment were Cenococcum graniforme and Pisolithus tinctorius. Cenococcum was chosen as a potential symbiont on mine spoils because of its tolerance to drought (Palmer, 1954; Worley and Hacskaylo, 1959; Trappe, 1962). Pisolithus was selected for its adaptability to survive in soils of high temperature (Marx et al, 1970a) and its abundant occurrence on similar adverse habitats (Schramm, 1966; Lowy, 1964; Lampky and Peterson, 1963).

The tree species studied were European white birch (Betula pendula L.) and red pine (Pinus resinosa Ait.). These species were selected because of their high tolerance to toxic strip-mine spoils and their adaptability to the climate of the region (Horn, 1968) and because they represented two contrasting types of trees--Angiosperm and Gymnosperm. The birch experiment differed from the pine experiment in that the seedlings were separated into two blocks according to their initial development. The pine experiment was set up as a random design.

Isolates of Pisolithus tinctorius (isolate 49) and Cenococcum graniforme were obtained from Dr. L.F. Grand, Department of Plant Pathology, North Carolina State University, Raleigh, N.C. The inoculum of each species was grown in pint Mason jars with screw lids for two months by a modified procedure of Marx and Zak (1965). In this procedure, 300 mls of screened vermiculite, 20 mls of peat moss, and 200 mls of Modified Melin-Norkrans (MMN) nutrient solution (Marx, 1969) were placed into each jar. The jar mixture was then autoclaved for 30 minutes at 20 psi, after which time the pH was measured to be 5.5. Inoculum discs, one-half inch in diameter, were then removed from six-week-old plate cultures of P. tinctorius and C. graniforme growing on MMN agar medium and placed into the sterile Mason jar mixtures. Control jars were treated similarly but were not inoculated. The jar lids, capped with aluminum foil, were not sealed tightly in order to permit gas exchange.

The birch seeds were stratified for 60 days in moist sand at 5°C and removed by flotation. The red pine seeds were not stratified. Surface sterilization of both seed species was accomplished by first soaking them in 1% hydrogen peroxide for 48 hours at 5°C, and then in 15% hydrogen peroxide for 15 minutes for the pine seeds and 30% hydrogen peroxide for 15 minutes for the birch. These concentrations and time limits were established as a result of preliminary tests. Hydrogen peroxide is also known to stimulate germination of many conifer seeds (Trappe, 1961).

The seeds were aseptically germinated and seedlings were grown by Dr. D.H. Marx, Principal Plant Pathologist at the Southeastern Forest

Experiment Station, USDA Forest Service, Athens, Ga. in a spore-free growth chamber which has been described by Marx and Bryan (1969). The day-length in the growth chamber was 15½ hours; the range in air temperature was 20 to 27°C; and the range in soil temperature was 18 to 25°C. The soil mixture, consisting of one part horticultural grade screened peat moss and three parts white masonry sand, had been steamed for 7 hours at 80°C on three alternate days. Each seedling received 50 mls of water twice a week.

After two months, the Mason jar fungal inoculum was incorporated into the rooting medium, and the seedlings were transplanted into 2½- x 2½- x 4-inch plastic rose pots within the growth chamber. One-third of the seedlings were not inoculated and served as controls. The seedlings were watered twice a week with 50 mls of water and received 50 mls of MN salts once during the three-month mycorrhizal synthesis period. After this period of time, the seedlings were transferred to a greenhouse on The Pennsylvania State University campus.

Equal weights of spoil material were placed in each of two-gallon polyethylene pots for the greenhouse study. The lime requirements of the spoils were determined by calcium hydroxide titrations to obtain a pH range of 6.0 to 6.5. The pH for the optimum performance of most forest tree seedlings as well as for mycorrhizal fungi has been observed in a soil pH range of 5.0 to 6.5 (Theodorou and Bowen, 1969).

Pulverized limestone was added on a weight basis, corrected for soil-size particles (less than 2mm) of each spoil, and thoroughly mixed into each pot of spoil receiving the acidity treatment. pH had dropped after two weeks and supplemental limestone was incorporated

into the top four inches of each pot as determined by lime time-lapse studies. These studies consisted of adding varying weights of limestone to constant weights of the three spoil materials. The spoils were watered periodically for two weeks and pH's were monitored daily. The pH's were measured using a 1:1 spoil:water ratio and a glass electrode pH meter according to Jackson (1958).

With the additional limestone, no correction was made for soil-size particles since the larger particles were apparently quite active in producing acid. The total rates of limestone application in the study were: 5.0 tons/acre for the Brandy Camp spoil; 1.5 tons/acre for the Cresson spoil; and 0.5 tons/acre for the Cramer spoil.

A 5-10-5 complete fertilizer was also added on a weight basis and thoroughly mixed through the pots of spoil receiving the fertilization treatment. Nitrogen consisted of both nitrate and ammonical forms. The rate used for all three spoil types was 1.0 tons/acre, which approximates the rate used in similar fertilizer studies on spoil banks by Bengtson et al (1971), Adams et al (1970), and the U.S. Forest Service (1969).

On the outset of transplanting the seedlings to spoil pots, a yellowing and chlorotic stunted condition of the seedlings was noted. In order to avoid possible death by shock of transplant, 50 mls of one-half concentration Hoagland's solution was added to each seedling. The seedlings were transplanted two weeks later. The pine seedlings were transplanted with their entire rose pot rooting medium, which served as an initial buffer zone between the young roots and the toxic spoil wastes. One-half of the rose pot rooting medium was transferred with each birch transplant since there were two birch seedlings growing in

each rose pot. Approximately 30 mls of additional inoculum were added to the root systems of both species to ensure mycorrhizal development. Similarly, 30 mls of the control Mason jar mixture were added to the nonmycorrhizal seedlings. Sample roots of seedlings were studied under an 18-power binocular microscope to determine mycorrhizal development.

Throughout the duration of the study, pH was monitored every three weeks, and above-ground heights of birch seedlings were taken weekly. pH values were determined by a 1:1 ratio of spoil:water and a 1:1 ratio of spoil:0.01M calcium chloride. Both methods yielded similar pH values. Since there was little height growth of any of the pine seedlings, no weekly height measurements were taken during the study. No noticeable color nutrient deficiencies or toxicities were observed in the foliage of either species.

The pots were watered every two to three days with approximately 100 to 200 mls of tap water. This kept drainage and nutrient loss from the bottoms of the pots to a minimum. Solar radiation was supplemented with fluorescent lights to maintain a day-length of 16 hours. During the period of the study, both the spoil temperature and air temperature ranged between 14 and 25°C.

At harvest, cold water was passed through the spoil material to separate the root systems from the spoil. With further cleaning of the roots by rinsing in water, there was no doubt that the turbulence broke some mycorrhizae from the roots. No attempt was made to recover these fragments.

The mycorrhizal development of the seedling roots was visually assessed under 18X magnification. The presence of obvious fungal mantles

on the ectomycorrhizal short roots distinquished the mycorrhizal from nonmycorrhizal short roots. Ectomycorrhizal abundance was expressed as the percentage of short roots with mycorrhizae according to the method of Richards and Wilson (1963). Cenococcum could be differentiated from other fungi because it characteristically has black, thick hyphae radiating from black, monopodial (club-shaped) mycorrhizae. Pisolithus has thick, golden brown hyphae and generally forms complex coralloid or bifurcate mycorrhizae of the same color.

Randomly selected mycorrhizae were fixed in weak formalin-acetic acid (FAA), dehydrated with a tertiary butyl alcohol (TBA) series, embedded in paraffin and serially sectioned (7μ in thickness), and stained in safranine-fast green (Marx and Davey, 1969) for histological examination. Contaminating mycorrhizae on the root systems were analyzed in the same manner.

The birch seedlings were harvested after 12 weeks, and the following measurements were taken: total height above root collar; categorical percent mycorrhizal establishment of the root system; and separate weights of the root and shoot after being dried in an oven for 48 hours at 70°C. The same measurements were made for the pine seedlings after 15 weeks.

An analysis of variance was conducted using the following dependent variables: total seedling height; total dry weight; root dry weight; top dry weight; and shoot/root ratio. The means of the significant main effects and first order and second order interactions were separated by Duncan's Least Significant Difference Test.

IV. RESULTS AND DISCUSSION

Red Pine Experiment

The growth data for the pine experiment are presented in Table 44 of the Appendix. The results of the analyses of variance based on these data are given in Table 1. The total heights, total dry weights, top dry weights, root dry weights, and shoot/root ratios were all significantly affected by amendments and mycorrhizal fungi. Also a main factor, spoil bank, had a significant effect on the first four of these dependent variables. Furthermore, a first order interaction, spoil bank x amendment, significantly influenced total dry weights, top dry weights, and root dry weights. Total dry weights of the seedlings differed from the other dependent variables by also being significantly affected by the first order interaction, spoil bank x mycorrhizal fungus.

Total Height

Total height growth was significantly greater on the Cramer and Cresson spoil material than on the Brandy Camp spoil (Table 2). The chemical composition and distribution of particle sizes of the three spoil banks, especially Brandy Camp, differed widely (Table 48 of the Appendix). The Brandy Camp spoil had the lowest pH values for all the treatments (Table 47 of the Appendix). The greater acidity, higher salt toxicity, and greater amounts of large particles were possible causes of the poor seedling response on the Brandy Camp spoil.

A thorough investigation of the chemical and physical properties of the Cresson and Brandy Camp spoils has been made by G.W. Welsh (unpublished data).

TABLE 1.

Results of analysis of variance

for red pine seedlings.

Source	Variable				
	Tot. Height	Top Dry Weight	Root Dry Weight	Tot. Dry Weight	Shoot/Root Ratio
Banks (B)	.01[a]	.01	.01	.01	-
Amendments (A)	.01	.01	.01	.01	.01
Fungi (F)	.01	.01	.05	.01	.01
B x A	-	.01	.01	.01	-
B x F	-	-	-	.01	-
A x F	-	-	-	-	-
B x A x F	-	-	-	-	-

[a]Significance level

TABLE 2.

Mean total heights of pine seedlings

for the spoil banks.

Spoil Bank	Mean Total Height[a]
	cm.
Cramer	6.24
Cresson	6.15
Brandy Camp	5.72

[a]Any two means not scored by the same line were significantly different at the .01 level according to Duncan's Least Significant Difference test.

Average heights of the pine seedlings did not differ significantly among the fertilizer, lime plus fertilizer, and lime amendments nor between the control and lime treatments (Table 3). However, the control seedlings were significantly shorter than those of the fertilizer and lime plus fertilizer treatments. Apparently, either the low nutrient status of these banks was a more serious problem to the red pine, an acid-tolerant species, than was the high acid condition, or the spoils, especially the Cresson and Cramer, were overlimed. Red pine grows better on moderately acid soils than on neutral or slightly basic soils.

Seedlings inoculated with Pisolithus tinctorius were significantly taller than those in the other two fungal treatments (Table 4). However, the difference in mean height between the control seedlings and the seedlings inoculated with Cenococcum graniforme was not significant. Pisolithus seemed to be more tolerant of harsh environments as indicated by the observation that there were more mycorrhizae from Pisolithus on root systems than from Cenococcum or naturally invading ectomycorrhizal fungi (Table 5). The Pisolithus-inoculated seedlings may have been able to absorb more nutrients than either the poorly ectomycorrhizal or nonmycorrhizal seedlings.

None of the interactions were significant in respect to seedling heights.

Top Dry Weight

The results of the statistical analysis of the dry weights of the above ground portion of the red pine seedlings were very similar to those for the total heights (Tables 6, 7, and 8). Seedling response was poorer on the Brandy Camp spoil, poorer on the control than on those

TABLE 3.

Mean total heights of pine seedlings

for the various amendments.

Amendment	Mean Total Height[a]
	cm.
Fertilizer	6.35
Lime + Fertilizer	6.14
Lime	5.96
Control	5.68

[a]Any two means not scored by the same line were significantly different at the .01 level according to Duncan's Least Significant Difference test.

TABLE 4.

Mean total heights of pine

seedlings for the mycorrhizal fungi.

Mycorrhizal Fungus	Mean Total Height[a]
	cm.
Pisolithus tinctorius	6.56
Cenococcum graniforme	5.80
Control	5.74

[a]Any two means not scored by the same line were significantly different at the .01 level according to Duncan's Least Significant Difference test.

TABLE 5.

Final mycorrhizal development

of pine seedlings.

Treatment[a]	Fungal Inoculant	Inoculated Short Roots		Naturally Invaded Short Roots	
		Rep 1	Rep 2	Rep 1	Rep 2
BC-L	Pisolithus	4[b]	4	0	0
BC-LF	Pisolithus	1	2	0	0
BC-F	Pisolithus	3	2	0	0
BC-X	Pisolithus	1	3	0	0
BC-L	Cenococcum	1	1	0	0
BC-LF	Cenococcum	1	0	0	0
BC-F	Cenococcum	1	1	0	1
BC-X	Cenococcum	1	3	0	0
BC-L	Control	-	-	0	0
BC-LF	Control	-	-	0	0
BC-F	Control	-	-	0	1
BC-X	Control	-	-	1	0
Cs-L	Pisolithus	4	4	0	0
Cs-LF	Pisolithus	4	4	0	0
Cs-F	Pisolithus	4	3	0	0
Cs-X	Pisolithus	4	4	0	0
Cs-L	Cenococcum	1	1	0	0
Cs-LF	Cenococcum	1	1	0	0
Cs-F	Cenococcum	1	1	0	0
Cs-X	Cenococcum	1	1	0	0
Cs-L	Control	-	-	0	0
Cs-LF	Control	-	-	0	0
Cs-F	Control	-	-	0	0
Cs-X	Control	-	-	0	0
C-L	Pisolithus	4	4	0	0
C-LF	Pisolithus	2	1	0	0
C-F	Pisolithus	3	3	0	0
C-X	Pisolithus	3	4	1	0

TABLE 5. (Continued)

Treatment[a]	Fungal Inoculant	Inoculated Short Roots Rep 1	Inoculated Short Roots Rep 2	Naturally Invaded Short Roots Rep 1	Naturally Invaded Short Roots Rep 2
C-L	Cenococcum	0[b]	0	0	0
C-LF	Cenococcum	0	1	0	0
C-F	Cenococcum	0	0	0	1
C-X	Cenococcum	0	0	0	0
C-L	Control	-	-	0	0
C-LF	Control	-	-	0	0
C-F	Control	-	-	1	0
C-X	Control	-	-	0	0

[a] BC = Brandy Camp
Cs = Cresson
C = Cramer

[b] 0 = 0%
1 = 1-25%
2 = 26-50%
3 = 51-75%
4 = 76-99%

L = Lime
LF = Lime + Fertilizer
F = Fertilizer
X = Control

TABLE 6.

Mean top dry weights of pine seedlings for the spoil banks.

Spoil Bank	Mean Top Dry Weight[a]
	grams
Cresson	0.48
Cramer	0.43
Brandy Camp	0.24

[a]Any two means not scored by the same line were significantly different at the .01 level according to Duncan's Least Significant Difference test.

TABLE 7.

Mean top dry weights of pine seedlings for the various amendments.

Amendment	Mean Top Dry Weight[a]
	grams
Fertilizer	0.47
Lime + Fertilizer	0.42
Lime	0.39
Control	0.26

[a]Any two means not scored by the same line were significantly different at the .01 level according to Duncan's Least Significant Difference test.

TABLE 8.

Mean top dry weights of pine seedlings for the mycorrhizal fungi.

Mycorrhizal Fungus	Mean Top Dry Weight[a]
	grams
Pisolithus tinctorius	0.52
Cenococcum graniforme	0.34
Control	0.29

[a]Any two means not scored by the same line were significantly different at the .01 level according to Duncan's Least Significant Difference test.

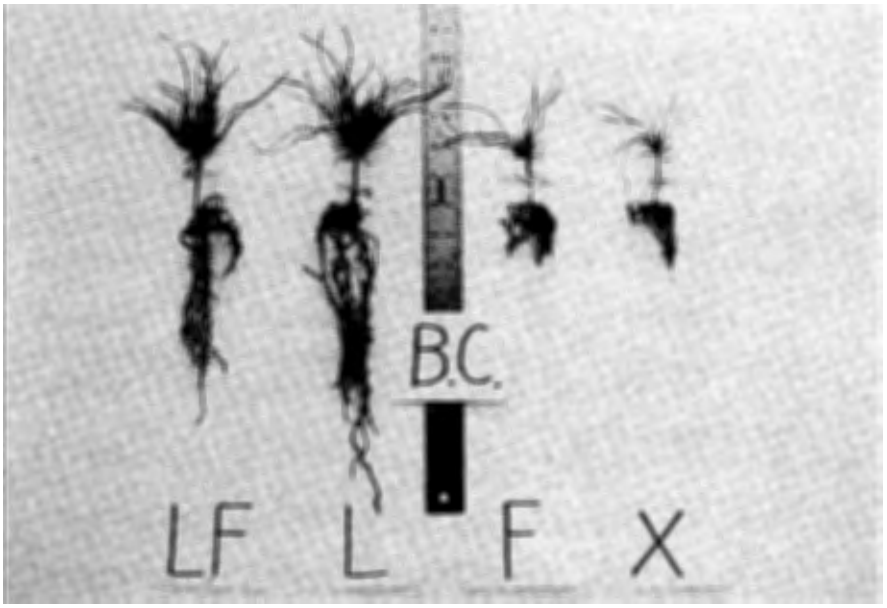
treated with amendments, and better on those inoculated with Pisolithus. In addition, there was a significantly greater response to the fertilizer treatment than to the lime treatment.

The tops of the seedlings that received the lime and fertilizer treatment, either singly or in combination, were thicker-stemmed and taller with more needles than the control seedlings (Figure 1). The elongated twisted needles, abnormal for red pine, may have resulted from altering the pine physiology. During the two-week period in January prior to transplant to the spoil materials, the seedlings may have begun to enter dormancy as a result of exposure to normal winter daylengths and temperatures somewhat cooler in the greenhouse than in the growth chamber. This incomplete period of dormancy prior to transplant may have affected the pine physiology.

The results of the analysis of the top dry weights differed from those of the total heights in that there was a significant bank x amendment interaction. On the Brandy Camp spoil, seedling response was greater with the lime treatments; on the Cresson and Cramer spoils, it was greater with the fertilizer treatments (Table 9 and Figure 2). This reinforces the point made previously that either the Cresson and Cramer spoils were overlimed or that lime was not needed to enhance red pine growth on these spoils.

Root Dry Weight

The seedling response in root growth was also similar to the height response (Tables 10 and 11). Root growth was better on the Cresson and Cramer spoils and better on those treated with amendments.



LF = lime + fertilizer, L = lime, F = fertilizer, X = control

RESPONSE OF PINE SEEDLINGS TO AMENDMENTS ON THE BRANDY CAMP SPOIL

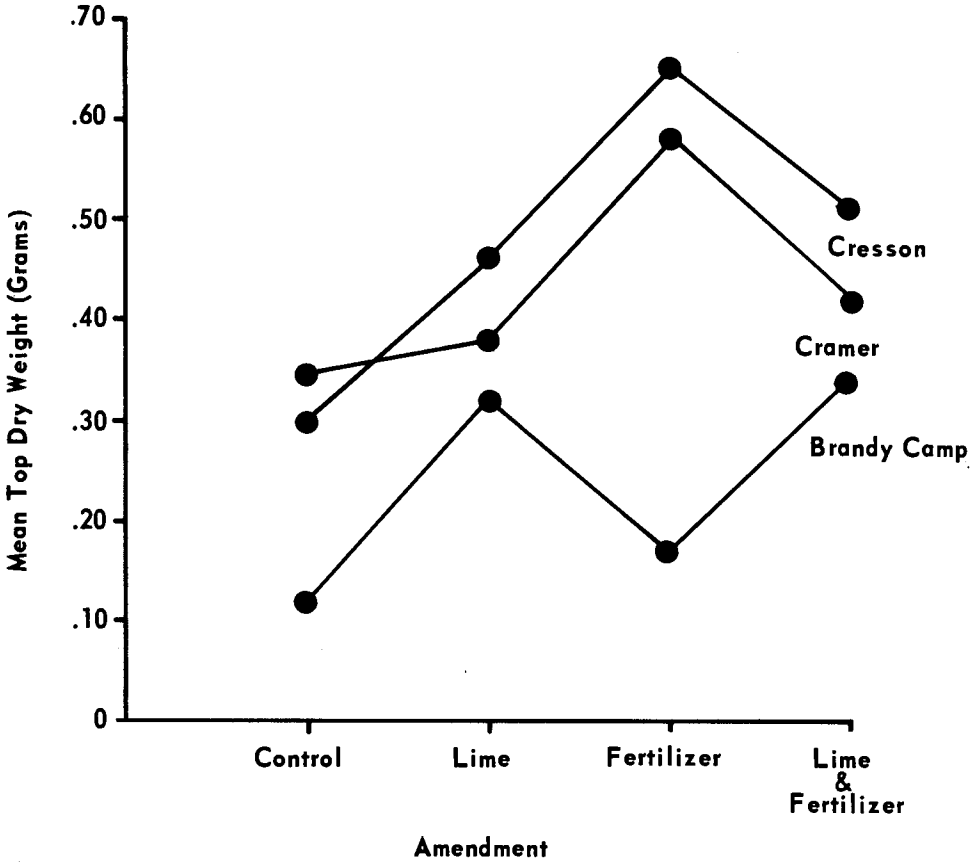
Figure 1

TABLE 9.

Mean top dry weights of pine seedlings for the various combinations of banks and amendments.

Combination		Mean Top Dry Weight[a]
Spoil Bank	Amendment	
		grams
Cresson	Fertilizer	0.65
Cramer	Fertilizer	0.58
Cresson	Lime + Fertilizer	0.51
Cresson	Lime	0.46
Cramer	Lime + Fertilizer	0.42
Cramer	Lime	0.38
Cramer	Control	0.35
Brandy Camp	Lime + Fertilizer	0.34
Brandy Camp	Lime	0.32
Cresson	Control	0.30
Brandy Camp	Fertilizer	0.19
Brandy Camp	Control	0.12

[a]Any two means not scored by the same line were significantly different at the .01 level according to Duncan's Least Significant Difference test.



TOP DRY WEIGHTS OF PINE SEEDLINGS FOR THE VARIOUS COMBINATIONS OF BANKS AND AMENDMENTS

Figure 2

TABLE 10.

Mean root dry weights of pine seedlings for the spoil banks.

Spoil Bank	Mean Root Dry Weight[a]
	grams
Cresson	0.28
Cramer	0.24
Brandy Camp	0.15

[a]Any two means not scored by the same line were significantly different at the .01 level according to Duncan's Least Significant Difference test.

TABLE 11.

Mean root dry weights of pine seedlings for the various amendments.

Amendment	Mean Root Dry Weight[a]
	grams
Lime	0.247
Lime + Fertilizer	0.236
Fertilizer	0.229
Control	0.176

[a]Any two means not scored by the same line were significantly different at the .01 level according to Duncan's Least Significant Difference test.

There was, however, no significant difference of root response between the lime and fertilizer amendments. Also, seedlings inoculated with Pisolithus did not differ from those inoculated with Cenococcum (Table 12). Both fungal treatments resulted in significantly greater root weights than the control.

Seedlings in the unamended spoils, especially Brandy Camp, had little root development outside the soil transplanted with the seedlings. The lime and fertilizer amendments, however, ameliorated the adverse chemical conditions of the spoils and enhanced root growth into the spoil materials. On the larger root systems of seedlings in the amended spoils, mycorrhizal abundance by Cenococcum and Pisolithus was much greater than on the smaller root systems of seedlings in the unamended spoils (Table 5, pages 33 and 34). Either the abundant mycorrhizae resulted from improved physiological conditions of the larger seedlings, or conversely, the mycorrhizae themselves effected the increased growth response by improving nutrient absorption.

As with top dry weights, there was a significant bank x amendment interaction. Root growth was greater with the lime treatments on the Brandy Camp spoil and with fertilizer on the Cresson and Cramer spoil materials (Table 13 and Figure 3). However, on the Cresson spoil root response was also greatly enhanced with lime. The Cramer spoil probably was overlimed in that root growth was no better than on the control.

Total Seedling Dry Weight

The total weight results were also similar to the seedling height results (Tables 14, 15 and 16). The mean total seedling weight was

TABLE 12.

Mean root dry weights of pine seedlings for the mycorrhizal fungi.

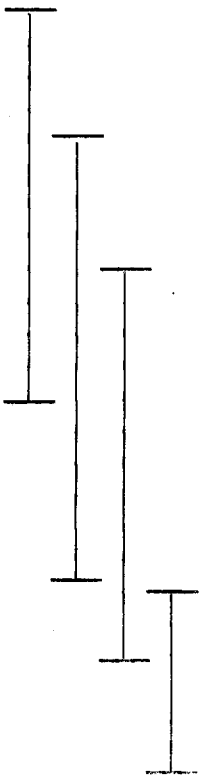
Mycorrhizal Fungus	Mean Root Dry Weight[a]
	grams
Pisolithus tinctorius	0.238
Cenococcum graniforme	0.233
Control	0.195

[a]Any two means not scored by the same line were significantly different at the .05 level according to Duncan's Least Significant Difference test.

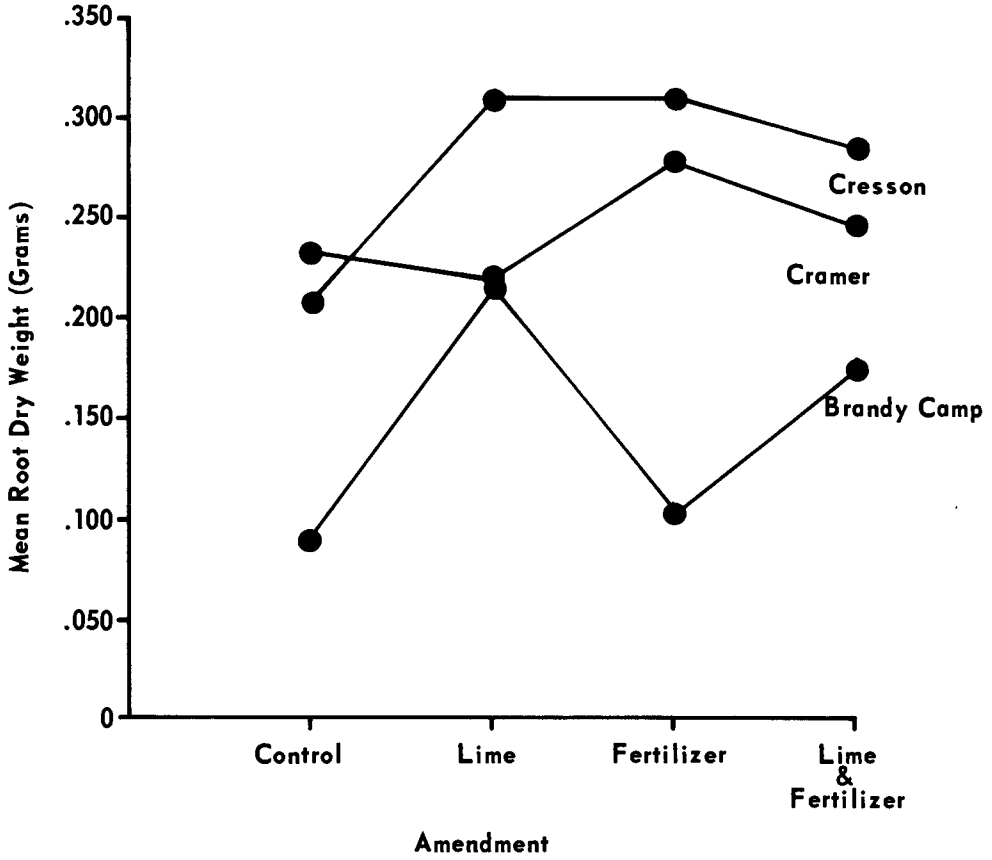
TABLE 13.

Mean root dry weights of pine seedlings for the various combinations of banks and amendments.

Combination		Mean Root Dry Weight [a]
Spoil Bank	Amendment	
		grams
Cresson	Lime	0.308
Cresson	Fertilizer	0.308
Cresson	Lime + Fertilizer	0.285
Cramer	Fertilizer	0.277
Cramer	Lime + Fertilizer	0.247
Cramer	Control	0.232
Cramer	Lime	0.218
Brandy Camp	Lime	0.215
Cresson	Control	0.207
Brandy Camp	Lime + Fertilizer	0.175
Brandy Camp	Fertilizer	0.103
Brandy Camp	Control	0.090



[a] Any two means not scored by the same line were significantly different at the .01 level according to Duncan's Least Significant Difference test.



ROOT DRY WEIGHTS OF PINE SEEDLINGS FOR THE VARIOUS COMBINATIONS OF BANKS AND AMENDMENTS

Figure 3

TABLE 14.

Mean total dry weights of pine seedlings for the spoil banks.

Spoil Bank	Mean Total Dry Weight[a]
	grams
Cresson	0.76
Cramer	0.67
Brandy Camp	0.39

[a]Any two means not scored by the same line were significantly different at the .01 level according to Duncan's Least Significant Difference test.

TABLE 15.

Mean total dry weights of pine seedlings for the various amendments.

Amendment	Mean Total Dry Weight[a]
	grams
Fertilizer	0.70
Lime + Fertilizer	0.66
Lime	0.64
Control	0.43

[a]Any two means not scored by the same line were significantly different at the .01 level according to Duncan's Least Significant Difference test.

TABLE 16.

Mean total dry weights of pine seedlings

for the mycorrhizal fungi.

Mycorrhizal Fungus	Mean Total Dry Weight[a]	
	grams	
Pisolithus tinctorius	0.76	—
Cenococcum graniforme	0.58	|
Control	0.49	—

[a]Any two means not scored by the same line were significantly different at the .01 level according to Duncan's Least Significant Difference test.

greater on the Cresson and Cramer spoils, greater on those with amendments, and greater on those inoculated with Pisolithus. As with the mean root weight, there was no significant difference between any of the amendments. In addition, mean total dry weights, like the mean top weights, were significantly affected by the bank x amendment interaction (Table 17 and Figure 4). In comparison with their respective controls, the total weights were almost two times as great on the fertilized Cresson spoil, one and a half times as great on the fertilized Cramer spoil, and two and a half times as great on the limed Brandy Camp spoil. Seedlings on the Cresson spoil treated with fertilizer weighted almost five times as much as the seedlings on the untreated Brandy Camp spoil.

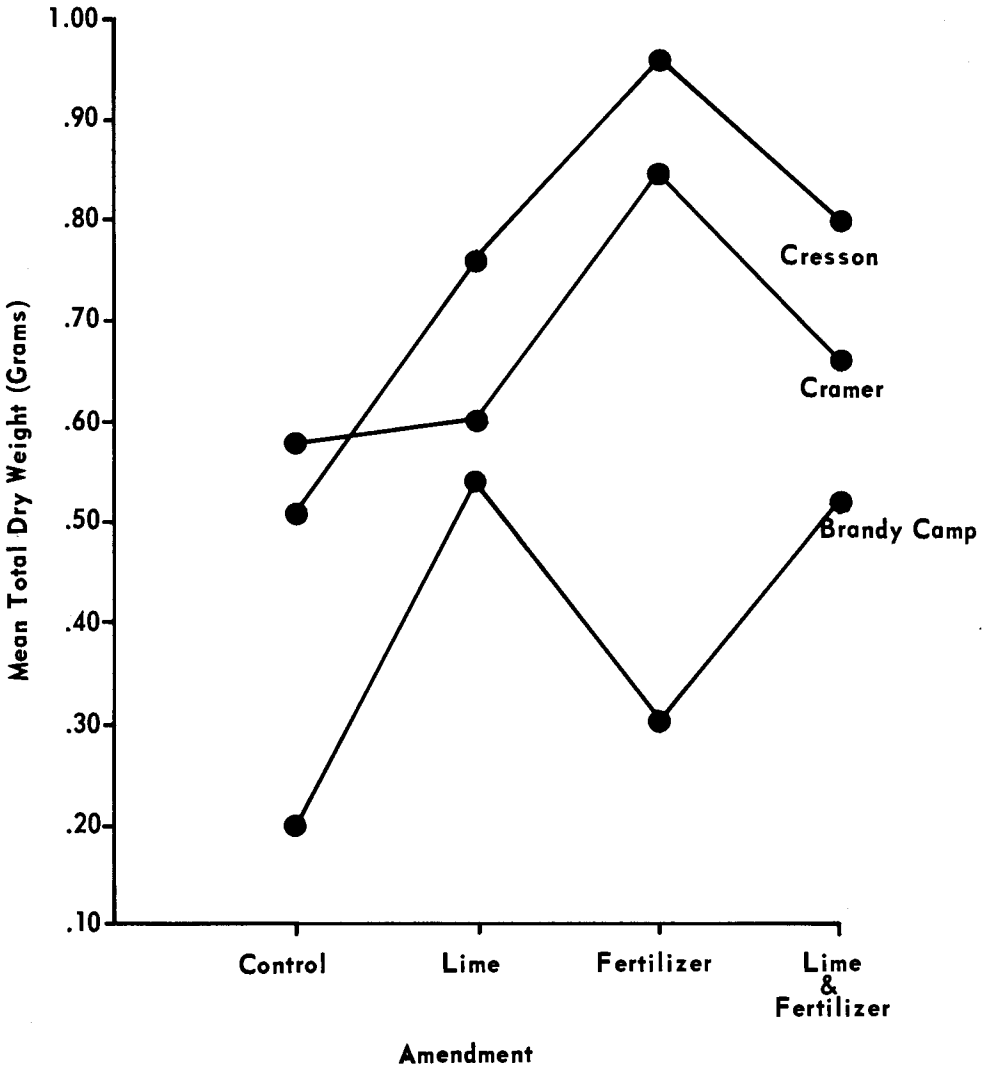
The major difference between the results of the analysis of the total dry weights of seedlings with those of the preceding analyses was that there was a significant bank x fungus interaction. The response of the seedlings on the Brandy Camp spoil to the fungal inoculations differed from that on the Cramer and Cresson spoils (Table 18 and Figure 5). For all three fungal treatments on the Brandy Camp spoil, total seedling weights were low and not significantly different from one another. On the Cresson bank, response was significantly greater by the Pisolithus-inoculated seedlings than by the Cenococcum-inoculated or control seedlings. The same pattern existed on the Cramer bank but at a reduced level of response. This was probably due to the greater mycorrhizal establishment on the seedlings growing in the Cresson spoil (Table 5, pages 33 and 34).

TABLE 17.

Mean total dry weights of pine seedlings for the various combinations of banks and amendments.

Combination		Mean Total[a]
Spoil Bank	Amendment	Dry Weight
		grams
Cresson	Fertilizer	0.96
Cramer	Fertilizer	0.85
Cresson	Lime + Fertilizer	0.80
Cresson	Lime	0.76
Cramer	Lime + Fertilizer	0.66
Cramer	Lime	0.60
Cramer	Control	0.58
Brandy Camp	Lime	0.54
Brandy Camp	Lime + Fertilizer	0.52
Cresson	Control	0.51
Brandy Camp	Fertilizer	0.30
Brandy Camp	Control	0.20

[a]Any two means not scored by the same line were significantly different at the .01 level according to Duncan's Least Significant Difference test.



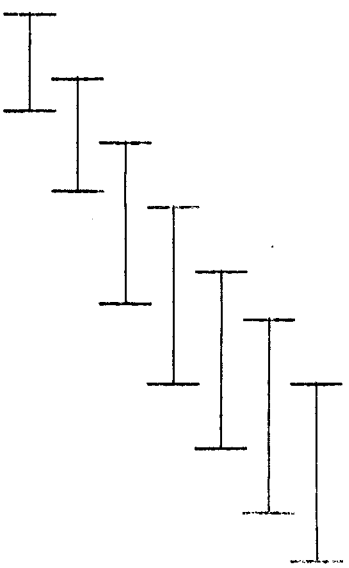
TOTAL DRY WEIGHTS OF PINE SEEDLINGS FOR THE VARIOUS COMBINATIONS OF BANKS AND AMENDMENTS

Figure 4

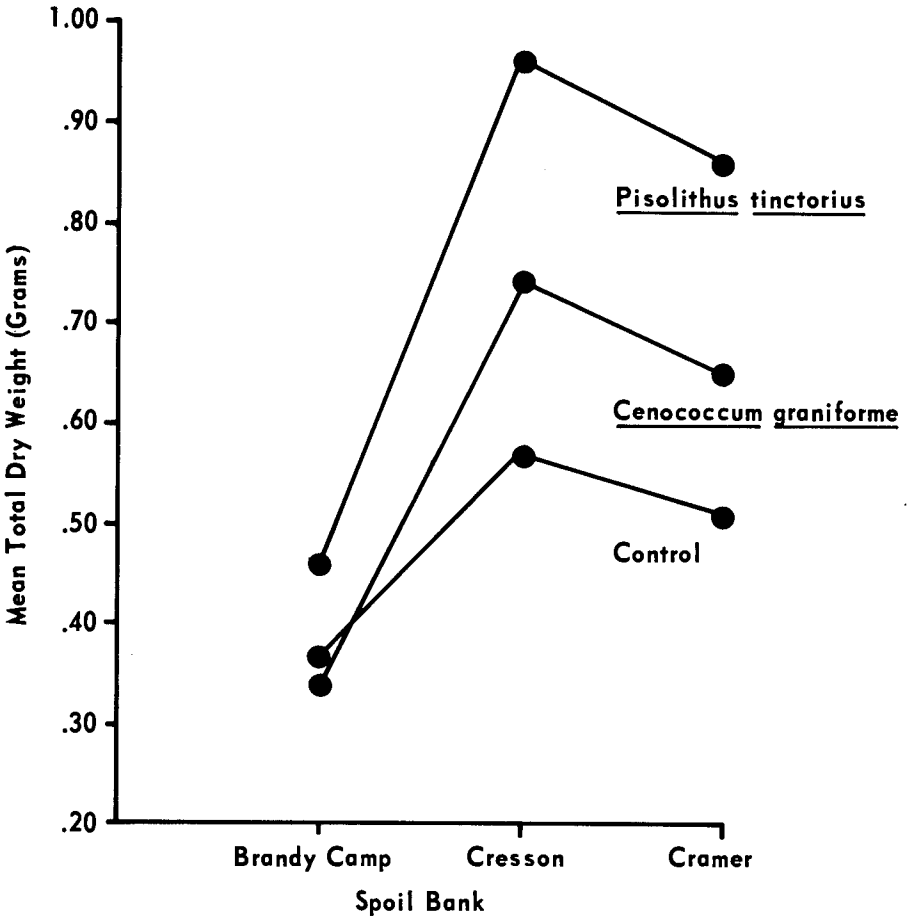
TABLE 18.

Mean total dry weights of pine seedlings for the various combinations of banks and fungi.

Combination		Mean Total Dry Weight[a]
Spoil Bank	Fungus	
		grams
Cresson	Pisolithus	0.96
Cramer	Pisolithus	0.86
Cresson	Cenococcum	0.74
Cramer	Cenococcum	0.65
Cresson	Control	0.57
Cramer	Control	0.51
Brandy Camp	Pisolithus	0.46
Brandy Camp	Control	0.37
Brandy Camp	Cenococcum	0.34



[a]Any two means not scored by the same line were significantly different at the .05 level according to Duncan's Least Significant Difference test.



TOTAL DRY WEIGHTS OF PINE SEEDLINGS FOR THE VARIOUS COMBINATIONS OF BANKS AND FUNGI

Figure 5

Shoot/Root Ratio

Since the statistical analyses for top weights and root weights of the seedlings yielded similar results, the shoot/root ratios would not be expected to differ between treatments. However, an analysis showed that both amendments and fungi resulted in significant differences in the shoot/root ratios of the seedlings (Tables 19 and 20). Both the fertilizer and the lime plus fertilizer treatments resulted in significantly higher shoot/root ratios than the control. In addition, the fertilizer treatment resulted in a significantly higher shoot/root ratio than the lime treatment. The mean shoot/root ratio of the Pisolithus-inoculated seedlings was significantly greater than those of the control and Cenococcum-inoculated seedlings.

Seedlings growing on poor sites generally have better-developed root systems relative to their above-ground portions. Such a differential development may enable the seedlings to survive on the poor sites, especially those which exhibit high moisture stress. Field studies will be needed to determine whether the higher shoot/root ratios of mycorrhizal seedlings and of fertilized seedlings will be of any added benefit or detriment to the performance of the seedlings.

Mycorrhizal Establishment

From visual inspection under magnification, it appeared that the seedlings were nonmycorrhizal when transplanted into the spoils. Black-colored mycelium of Cenococcum and golden brown mycelium of Pisolithus were observed on most of the inoculated root systems but no mycorrhizae had formed. Evidently, the initial inoculation was not successful.

TABLE 19.

Mean shoot/root ratios of pine seedlings

for the various amendments.

Amendment	Mean Shoot/Root Ratio[a]
Fertilizer	2.07
Lime + Fertilizer	1.89
Lime	1.56
Control	1.40

[a]Any two means not scored by the same line were significantly different at the .01 level according to Duncan's Least Significant Difference test.

TABLE 20.

Mean shoot/root ratios of pine seedlings

for the mycorrhizal fungi.

Mycorrhizal Fungus	Mean Shoot/Root Ratio[a]
Pisolithus tinctorius	2.25
Control	1.49
Cenococcum graniforme	1.45

[a]Any two means not scored by the same line were significantly different at the .01 level according to Duncan's Least Significant Difference test.

The failure of the initial inoculation may have been caused by any of the following factors: low virulence of the inoculum, insufficient time for mycorrhizal development, unfavorable seedling physiology, improper soil conditions or environment. However, inoculation at time of transplant resulted in subsequent mycorrhizal establishment of both symbionts by the time the study was terminated. Possibly the additional inoculum and longer mycorrhizal synthesis period enabled successful symbiosis.

The number of mycorrhizal roots from both the introduced symbionts and natural invaders varied depending upon spoil and amendment (Table 5, pages 33 and 34). There was little mycorrhizal formation by naturally invading fungi. Such invasion generally appeared on the fertilizer and control amendments.

Mycorrhizae formed by Pisolithus occurred in greatest abundance on each spoil receiving the lime treatment. On the Brandy Camp spoil, mycorrhizal establishment with lime was greater than with the other amendments; on the Cramer spoil, it was greater with lime than with the lime plus fertilizer amendment; on the Cresson spoil, it was uniformly high with all the amendments. With the lime treatment on all spoils, precipitated nutrients may have become available in concentrations low enough to favor mycorrhizal establishment. Possibly on the Cramer and Brandy Camp spoils, lime plus fertilizer resulted in a nutrient status too high and well-balanced for mycorrhizal formation. The nonlimed Brandy Camp spoil may have been too acidic for mycorrhizal formation, while the less acidic and toxic conditions of the nonlimed Cresson spoil may have favored mycorrhizal formation. This apparent interaction between

banks and amendments in regard to mycorrhizal formation by Pisolithus would need to be verified in a study for a longer period of time with more replications.

In all instances of substantial mycorrhizal establishment, Pisolithus mycorrhizae occurred on the roots growing from the transplanted soil into the spoil. Since the seedlings generally were largest on the Cresson and Cramer spoils, available photosynthates must have been amply supplied to the roots for support of the mycorrhizae. Similarly, the limed Brandy Camp spoil may have improved the seedling physiological state and chemical status of the spoil to encourage the abundance of the symbiont.

Throughout the three spoils, mycorrhizal formation with Cenococcum was either low or nonexistent, especially on the Cramer spoil. There was no pattern of mycorrhizal establishment with amendments on any of the spoils. Cenococcum evidently was not tolerant of the spoil environment since the mycorrhizae were restricted to the transplant soil around the main mass of roots. No Cenococcum mycorrhizae were observed on roots growing out into the spoil material, even on the ameliorated spoils.

European White Birch Experiment

The growth data for the birch experiment are presented in Table 45 of the Appendix. The results of the analyses of variance based on these data are given in Table 21. As in the pine experiment, the total heights, total dry weights, top dry weights, root dry weights, and shoot/root ratios were all significantly affected by amendments. The first four dependent variables were similarly affected by banks and by the first order interaction bank x amendment. In addition, total heights

TABLE 21.

Results of analysis of variance

for birch seedlings.

Source	Variable				
	Tot. Height	Top Dry Weight	Root Dry Weight	Tot. Dry Weight	Shoot/Root Ratio
Banks (B)	.01[a]	.01	.01	.01	-
Amendments (A)	.01	.01	.01	.01	.01
Fungi (F)	-	-	-	-	-
Replications (R)	-	-	.01	.01	-
B x A	.01	.01	.01	.01	-
B x F	.05	-	.05	.01	-
B x R	-	-	-	.05	-
A x F	-	-	-	-	-
A x R	-	-	.01	.01	-
F x R	-	-	.01	.01	-
B x A x F	.05	-	-	.05	-
B x A x R	-	-	-	-	-
B x F x R	-	-	-	-	-
A x F x R	-	-	.05	.05	-

[a]Significance level

were significantly affected by the bank x fungus interaction and by the bank x amendment x fungus second order interaction; root dry weights, by replication, bank x fungus, amendment x replication, fungus x replication, and by amendment x fungus x replication; total dry weight, by replication, bank x fungus, bank x replication, amendment x replication, fungus x replication, bank x amendment x fungus, and amendment x fungus x replication.

Total Height

Mean total height was greatest on the Cresson spoil, least on the Brandy Camp spoil, and intermediate on the Cramer spoil (Table 22). In contrast with the red pine experiment, mean heights of the white birch seedlings on all three banks were significantly different from one another at the .01 level. The better performance on the Cresson as compared to the Cramer spoil was unexpected since the Cramer spoil had apparently better conditions for plant growth (Tables 47 and 48 of the Appendix).

Total heights were significantly different at the .01 level among all the amendments. In decreasing order of height growth, the treatments were lime plus fertilizer, fertilizer, lime, and control (Table 23). The suspected overliming was evidently not as serious for white birch as it was for red pine.

In addition to the significant main effects of bank and amendment on height growth, there was a significant interaction between the two (Table 24 and Figure 6). Seedling response on the Cresson and Cramer spoils was mainly due to fertilization (Figures 7 and 8). Unlike the red pine, there was a large increase in seedling height with the

TABLE 22.

Mean total heights of birch seedlings

for the spoil banks.

Spoil Bank	Mean Total Height[a]
	cm.
Cresson	39.6
Cramer	33.9
Brandy Camp	21.2

[a]All means were significantly different at the .01 level according to Duncan's Least Significant Difference test.

TABLE 23.

Mean total heights of birch seedlings

for the various amendments.

Amendment	Mean Total Height[a]
	cm.
Lime + Fertilizer	55.7
Fertilizer	38.0
Lime	21.3
Control	11.3

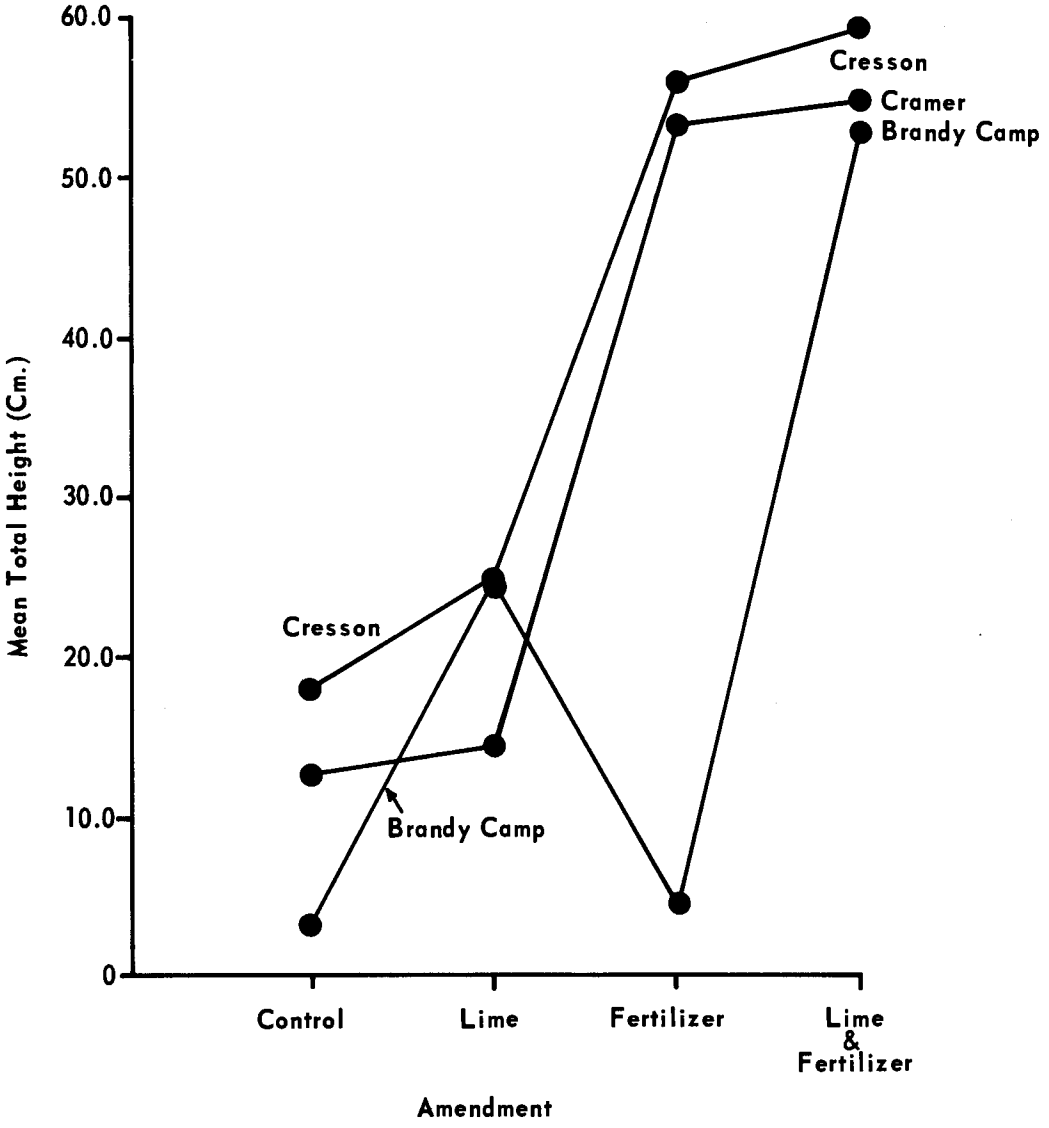
[a]All means were significantly different at the .01 level according to Duncan's Least Significant Difference test.

TABLE 24.

Mean total heights of birch seedlings for the various combinations of banks and amendments.

Combination		Mean
Spoil Bank	Amendment	Total Height[a]
		cm.
Cresson	Lime + Fertilizer	59.5
Cresson	Fertilizer	56.0
Cramer	Lime + Fertilizer	54.8
Cramer	Fertilizer	53.6
Brandy Camp	Lime + Fertilizer	52.7
Cresson	Lime	24.9
Brandy Camp	Lime	24.5
Cresson	Control	18.1
Cramer	Lime	14.6
Cramer	Control	12.6
Brandy Camp	Fertilizer	4.4
Brandy Camp	Control	3.2

[a]Any two means not scored by the same line were significantly different at the .01 level according to Duncan's Least Significant Difference test.

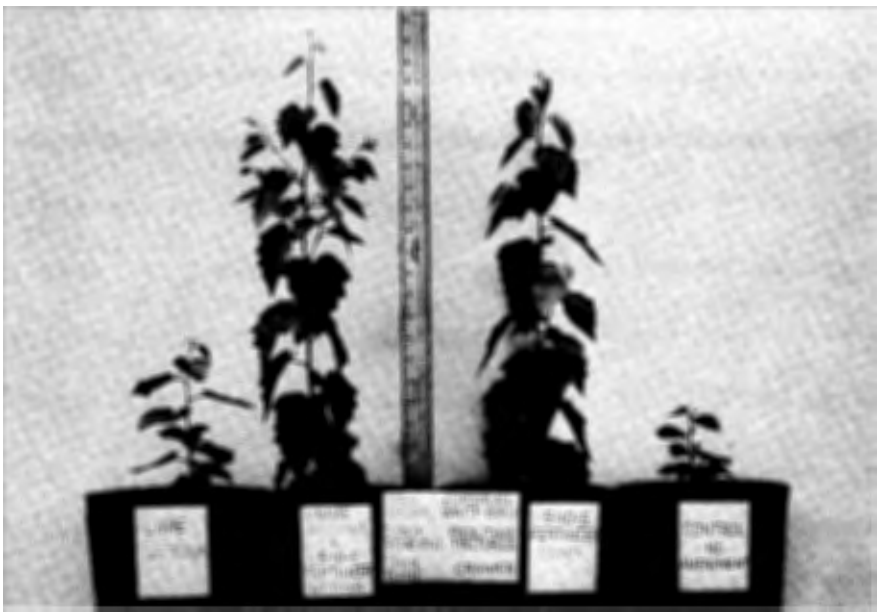


TOTAL HEIGHTS OF BIRCH SEEDLINGS FOR THE VARIOUS COMBINATIONS OF BANKS AND AMENDMENTS

Figure 6

HEIGHT RESPONSE OF BIRCH SEEDLINGS WITH THE VARIOUS AMENDMENTS ON THE CRESSON SPOIL

Figure 7



HEIGHT RESPONSE OF BIRCH SEEDLINGS WITH THE VARIOUS AMENDMENTS ON THE CRAMER SPOIL

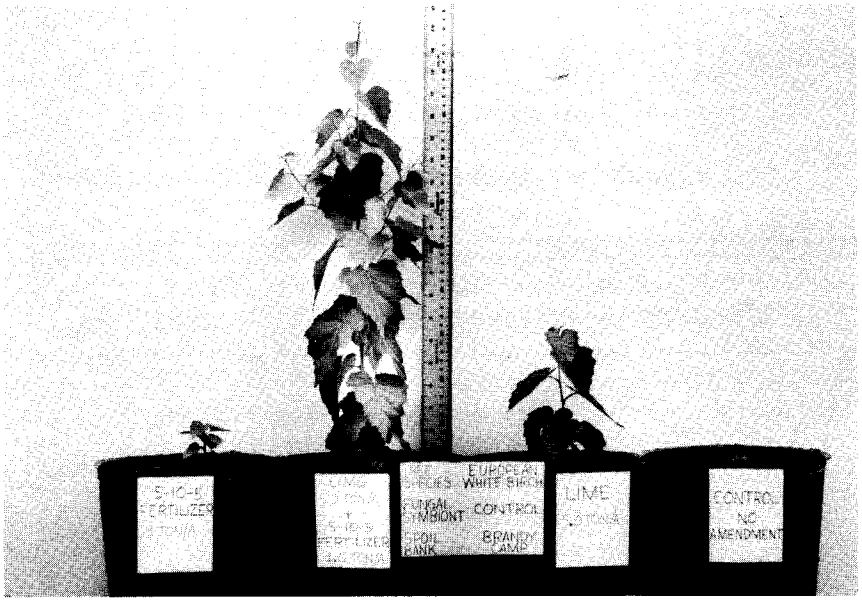
Figure 8

application of both lime and fertilizer to the Brandy Camp (Figure 9). In fact, the seedlings were almost as tall as those on the Cramer and Cresson spoils treated in the same way (Figure 10).

Even though there were no significant differences in height growth for the main effect of fungus, there were significant interactions of bank x fungus and bank x fungus x amendment. Inoculation with Pisolithus tinctorius significantly increased height growth on the Cresson spoil (Table 25). Cenococcum graniforme inoculations, however, slightly depressed growth below the control. Palmer (1954) reported a somewhat pathogenic tendency of Cenococcum as a mycorrhizal former under certain conditions. Cenococcum also resulted in less mycorrhizal establishment than did Pisolithus (Table 26).

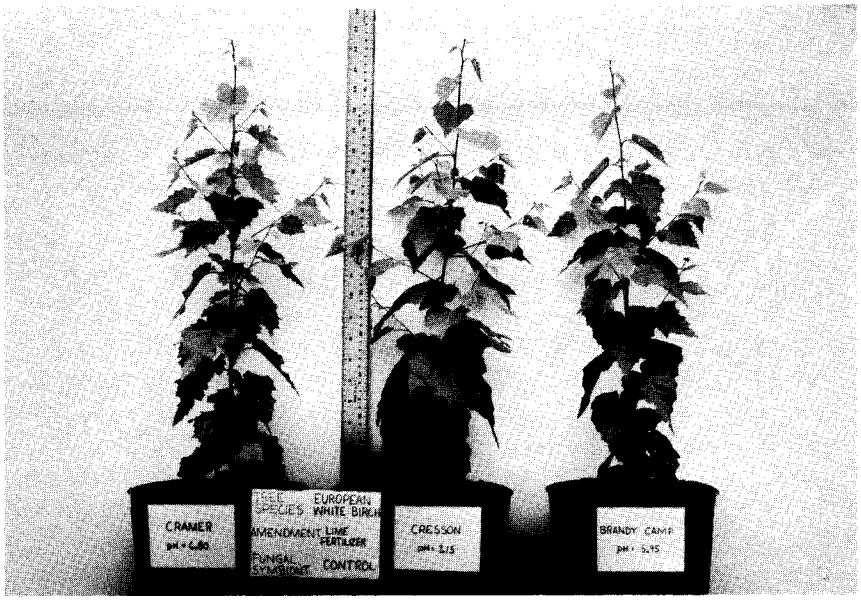
On both Brandy Camp and Cramer spoils, neither fungal symbiont significantly influenced seedling growth. Both symbionts resulted in a similar degree of mycorrhizal establishment on Brandy Camp. The generally impoverished, acidic nature of the Brandy Camp spoil may have been too harsh for the mycorrhizae to benefit the birch. Conversely, the Cramer spoil may have had generally high levels of nutrients and low acidity, thus restricting mycorrhizal influence on seedling growth. Mycorrhizae by both Cenococcum and Pisolithus were poorly established on the Cramer spoil.

The significant second order interaction of bank x amendment x fungus was largely a result of increased seedling response to Pisolithus inoculation on the lime treatment on the Brandy Camp spoil and on the control and lime plus fertilizer treatments on the Cresson spoil (Figure 11). There was a decreased response to the same fungus on the



HEIGHT RESPONSE OF BIRCH SEEDLINGS WITH THE VARIOUS AMENDMENTS ON THE BRANDY CAMP SPOIL

Figure 9



HEIGHT RESPONSE OF BIRCH SEEDLINGS WITH THE LIME PLUS FERTILIZER AMENDMENT ON THE THREE SPOILS

Figure 10

TABLE 25.

Mean total heights of birch seedlings for the various combinations of banks and fungi.

Combination		Mean
Spoil Bank	Fungus	Total Height[a]
		cm.
Cresson	Pisolithus	45.1
Cresson	Control	38.1
Cramer	Cenococcum	35.8
Cresson	Cenococcum	35.7
Cramer	Pisolithus	33.2
Cramer	Control	32.7
Brandy Camp	Control	21.9
Brandy Camp	Pisolithus	21.6
Brandy Camp	Cenococcum	20.2

[a]Any two means not scored by the same line were significantly different at the .05 level according to Duncan's Least Significant Difference test.

TABLE 26.

Final mycorrhizal development

of birch seedlings.

Treatment[a]	Fungal Inoculant	Inoculated Short Roots		Naturally Invaded Short Roots	
		Rep 1	Rep 2	Rep 1	Rep 2
BC-L	Pisolithus	4[b]	4	0	0
BC-LF	Pisolithus	2	3	0	0
BC-F	Pisolithus	1	3	0	0
BC-X	Pisolithus	1	1	0	0
BC-L	Cenococcum	1	1	2	1
BC-LF	Cenococcum	1	1	4	3
BC-F	Cenococcum	2	3	0	0
BC-X	Cenococcum	4	4	0	0
BC-L	Control	-	-	1	1
BC-LF	Control	-	-	1	2
BC-F	Control	-	-	0	0
BC-X	Control	-	-	0	0
Cs-L	Pisolithus	1	3	0	0
Cs-LF	Pisolithus	3	1	0	0
Cs-F	Pisolithus	1	3	0	0
Cs-X	Pisolithus	2	4	0	0
Cs-L	Cenococcum	1	1	0	0
Cs-LF	Cenococcum	1	1	0	1
Cs-F	Cenococcum	1	1	2	1
Cs-X	Cenococcum	3	1	0	0
Cs-L	Control	-	-	0	0
Cs-LF	Control	-	-	1	1
Cs-F	Control	-	-	1	0
Cs-X	Control	-	-	2	3
C-L	Pisolithus	1	1	0	0
C-LF	Pisolithus	1	1	0	0
C-F	Pisolithus	1	1	0	0
C-X	Pisolithus	2	2	0	0

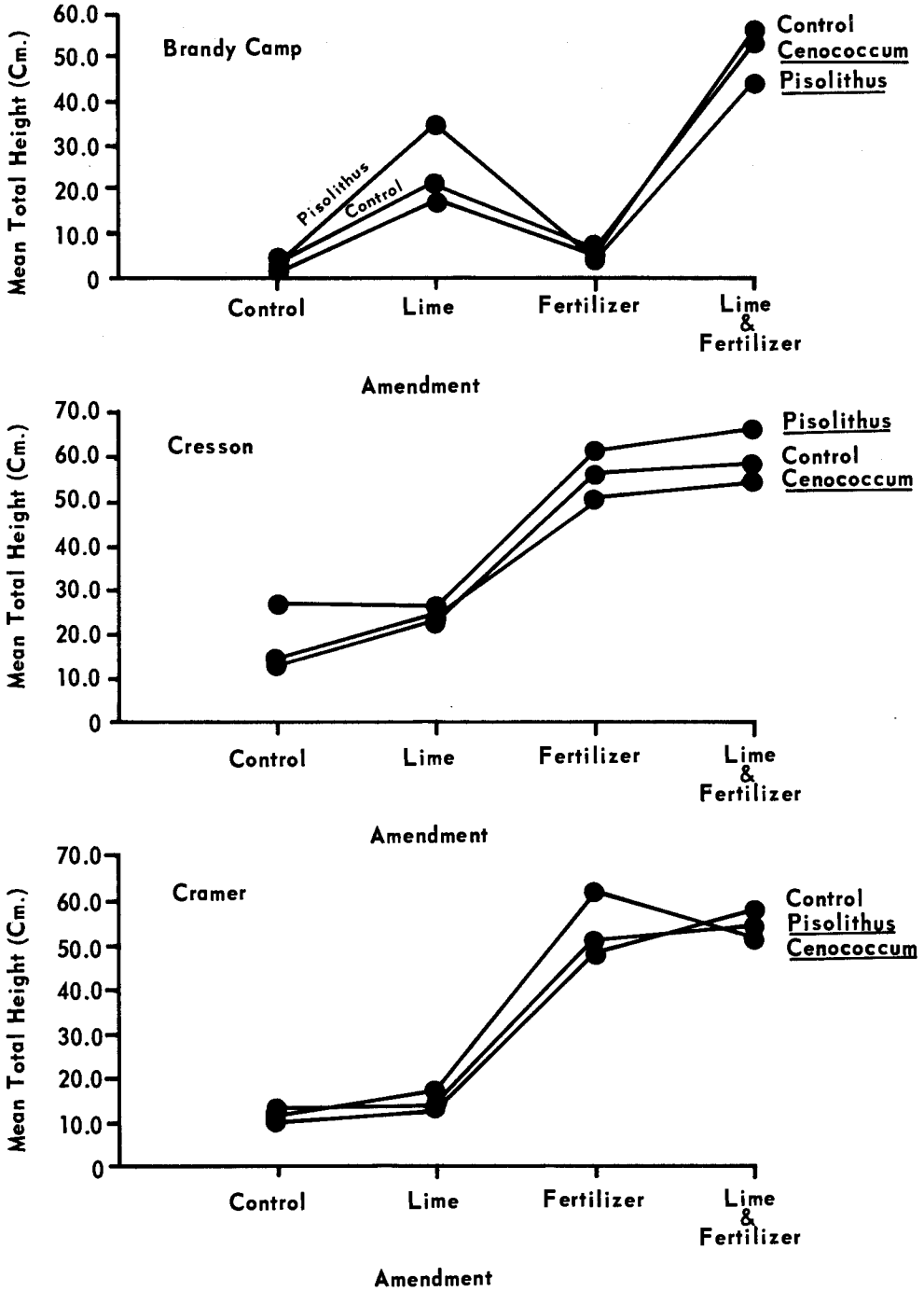
TABLE 26. (Continued)

Treatment[a]	Fungal Inoculant	Inoculated Short Roots		Naturally Invaded Short Roots	
		Rep 1	Rep 2	Rep 1	Rep 2
C-L	Cenococcum	1[b]	1	1	0
C-LF	Cenococcum	0	0	0	0
C-F	Cenococcum	1	1	0	0
C-X	Cenococcum	2	1	0	0
C-L	Control	-	-	0	0
C-LF	Control	-	-	0	0
C-F	Control	-	-	2	1
C-X	Control	-	-	0	0

[a] BC = Brandy Camp
Cs = Cresson
C = Cramer

[b] 0 = 0%
1 = 1-25%
2 = 26-50%
3 = 51-75%
4 = 76-99%

L = Lime
LF = Lime + Fertilizer
F = Fertilizer
X = Control



TOTAL HEIGHTS OF BIRCH SEEDLINGS FOR THE VARIOUS COMBINATIONS OF BANKS, AMENDMENTS, AND FUNGI

Figure 11

lime plus fertilizer treatment on the Brandy Camp spoil. In addition, there was an increased response to Cenococcum in the fertilizer treatment on the Cramer spoil. The increased response to Pisolithus seemed to be positively correlated with the degree of mycorrhizal development on the roots of the white birch seedlings (Table 26).

Top Dry Weights

The results of the analysis of the top dry weights were similar to those of the height growth analysis. Although the mean top weight of seedlings grown on the Brandy Camp spoil was significantly lower than that of seedlings grown on the Cresson and Cramer spoils, seedlings of the latter two spoils did not differ significantly from one another in respect to top dry weights (Table 27). As with the height growth response, seedling top weights significantly decreased with the following order of amendments: lime plus fertilizer, fertilizer, lime, and control (Table 28).

Again there was a significant bank x amendment interaction, with fertilization resulting in the greatest response on the Cramer and Cresson banks, but lime on the Brandy Camp spoil (Table 29 and Figure 12). However, unlike the height analysis, the seedling top weights on the Cramer bank treated with lime plus fertilizer were significantly less than those treated with fertilizer alone, while the inverse was true on the Cresson spoil. The lime plus fertilizer treatment on the Cresson spoil and the fertilizer treatment on the Cramer resulted in seedlings with thicker stems and usually profuse branching (See Figures 7 and 8 on page 62). Evidently too much lime

TABLE 27.

Mean top dry weights of birch seedlings

for the spoil banks.

Spoil Bank	Mean Top Weight[a]
	grams
Cresson	4.19
Cramer	4.02
Brandy Camp	1.77

[a]Any two means not scored by the same line were significantly different at the .01 level according to Duncan's Least Significant Difference test.

TABLE 28.

Mean top dry weights of birch seedlings

for the various amendments.

Amendment	Mean Top Weight[a]
	grams
Lime + Fertilizer	6.57
Fertilizer	4.61
Lime	1.50
Control	0.62

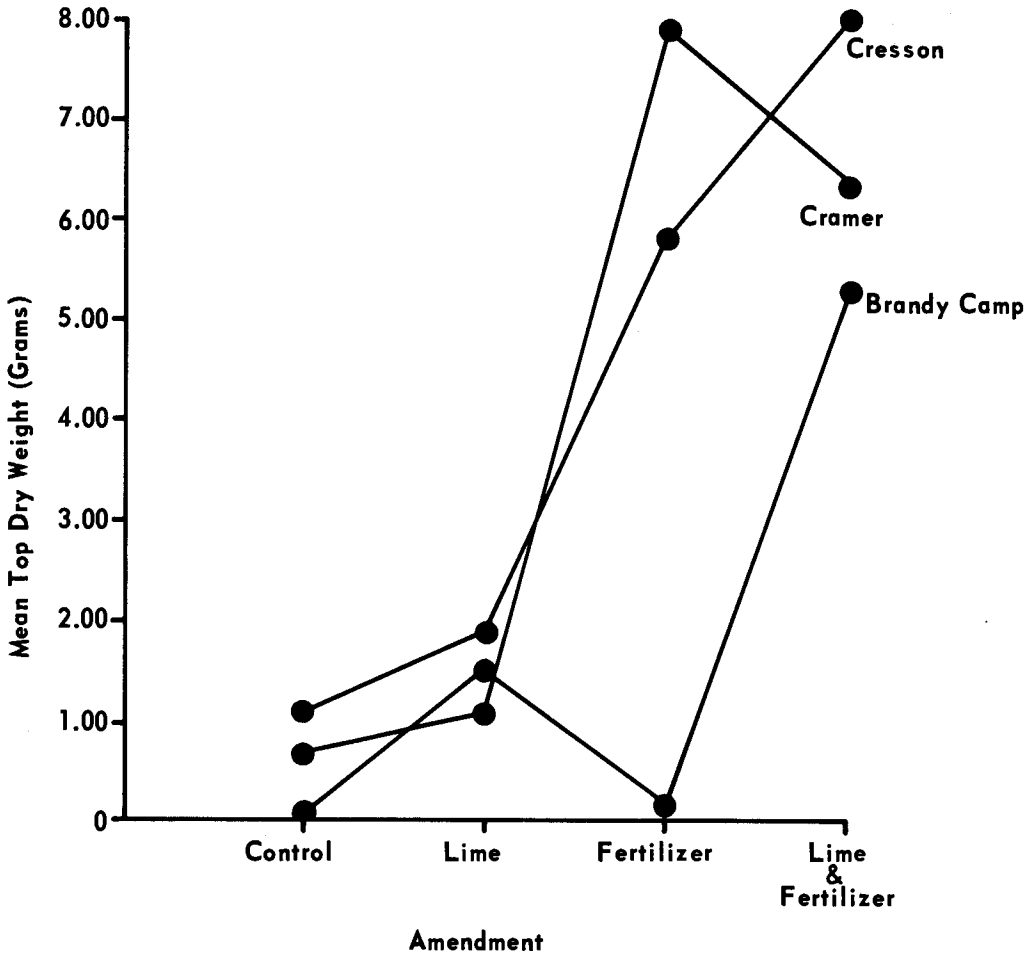
[a]All means were significantly different at the .01 level according to Duncan's Least Significant Difference test.

TABLE 29.

Mean top dry weights of birch seedling for the various combinations of bank and amendments.

Combination		Mean Top Dry Weight[a]
Spoil Bank	Amendment	
		grams
Cresson	Lime + Fertilizer	8.00
Cramer	Fertilizer	7.87
Cramer	Lime + Fertilizer	6.38
Cresson	Fertilizer	5.80
Brandy Camp	Lime + Fertilizer	5.33
Cresson	Lime	1.88
Brandy Camp	Lime	1.52
Cramer	Lime	1.10
Cresson	Control	1.09
Cramer	Control	0.72
Brandy Camp	Fertilizer	0.18
Brandy Camp	Control	0.06

[a]Any two means not scored by the same line were significantly different at the .01 level according to Duncan's Least Significant Difference test.



TOP DRY WEIGHTS OF BIRCH SEEDLINGS FOR THE VARIOUS COMBINATIONS OF BANKS AND AMENDMENTS
Figure 12

had been applied to the Cramer spoil for optimum growth of the birch seedlings. Nevertheless, they seemed to be more tolerant and responsive to higher pH values than were the pine seedlings.

There were no other significant interactions in the top weight analysis.

Root Dry Weight

The results of the analysis of the root dry weights were almost identical to those of the top dry weights in regards to the main effects (Tables 30 and 31) and the bank x amendment interaction (Table 32 and Figure 13). However, in addition there was one other main effect, replication, and four other significant interactions: bank x fungus, amendment x replication, fungus x replication, and amendment x fungus x replication.

The bank x fungus interaction was largely a result of the much greater root dry weights of the seedlings inoculated with Pisolithus and growing on the Cresson spoil relative to the pattern of root dry weights for the other combinations of fungal treatments and banks (Table 33).

Because of the wide variation in the white birch seedlings at the time the study was begun, the seedlings were separated into two groups. The better developed ones were placed in Replication 1 and the poorer, in Replication 2. Thus the main effect termed "Replication" was really one of difference in intital development.

The initial heights of the birch seedlings are presented in Table 46 of the Appendix. The mean heights for the replications were 2.16

TABLE 30.

Mean root dry weights of birch seedlings for the spoil banks.

Spoil Bank	Mean Root Dry Weight[a]
	grams
Cramer	3.95
Cresson	3.59
Brandy Camp	1.46

[a]Any two means not scored by the same line were significantly different at the .01 level according to Duncan's Least Significant Difference test.

TABLE 31.

Mean root dry weights of birch seedlings for the various amendments.

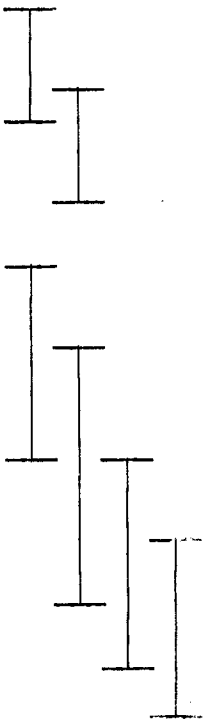
Amendment	Mean Root Dry Weight[a]
	grams
Lime + Fertilizer	5.38
Fertilizer	4.33
Lime	1.64
Control	0.64

[a]All means were significantly different at the .01 level according to Duncan's Least Significant Difference test.

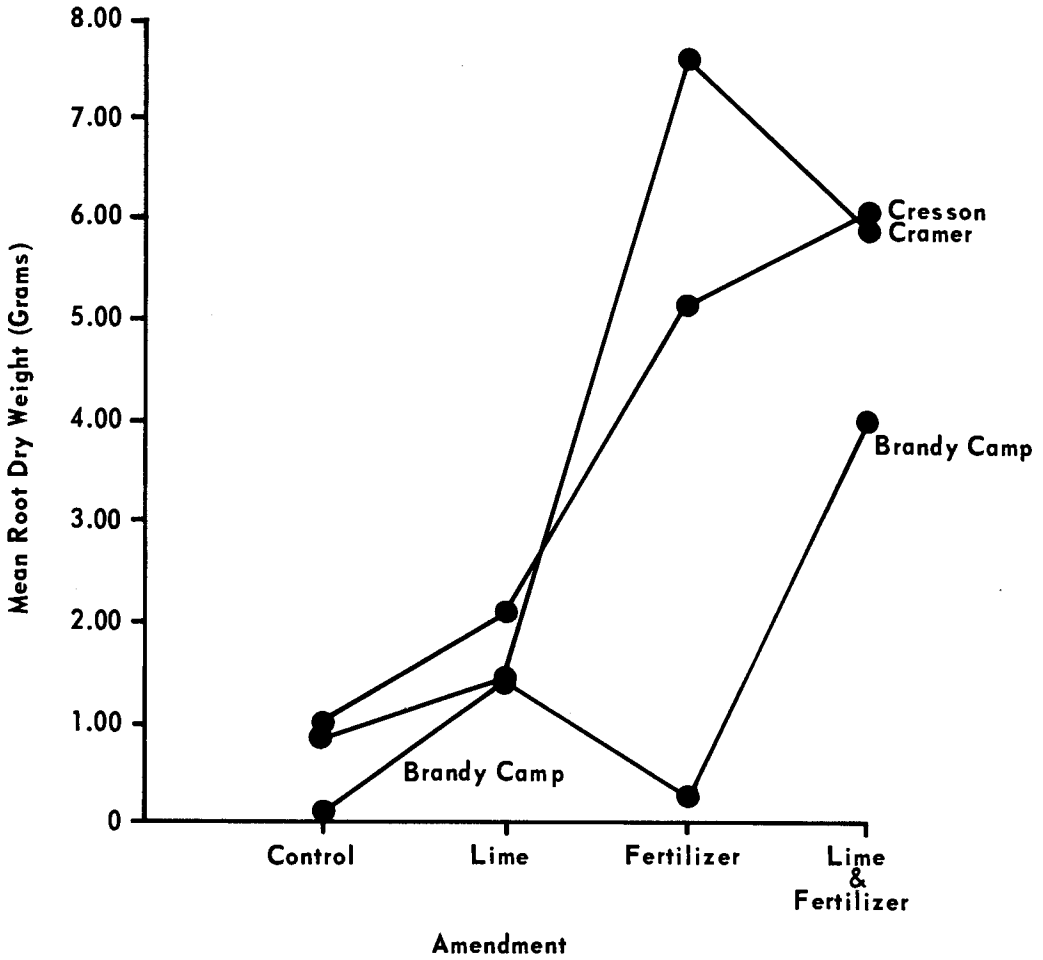
TABLE 32.

Mean root dry weights of birch seedlings for the various combinations of banks and amendments.

Combination		Mean Root Dry Weight[a]
Spoil Bank	Amendment	
		grams
Cramer	Fertilizer	7.60
Cresson	Lime + Fertilizer	6.08
Cramer	Lime + Fertilizer	5.95
Cresson	Fertilizer	5.16
Brandy Camp	Lime + Fertilizer	4.12
Cresson	Lime	2.11
Cramer	Lime	1.42
Brandy Camp	Lime	1.40
Cresson	Control	1.00
Cramer	Control	0.85
Brandy Camp	Fertilizer	0.24
Brandy Camp	Control	0.08



[a]Any two means not scored by the same line were significantly different at the .01 level according to Duncan's Least Significant Difference test.



ROOT DRY WEIGHTS OF BIRCH SEEDLINGS FOR THE VARIOUS COMBINATIONS OF BANKS AND AMENDMENTS

Figure 13

TABLE 33.

Mean root dry weights of birch seedlings

for the various combinations of banks and fungi.

Combination		Mean Root
Spoil Bank	Fungus	Dry Weight[a]
		grams
Cramer	Control	4.18
Cresson	Pisolithus	4.16
Cramer	Cenococcum	4.04
Cramer	Pisolithus	3.64
Cresson	Control	3.43
Cresson	Cenococcum	3.18
Brandy Camp	Control	1.63
Brandy Camp	Cenococcum	1.56
Brandy Camp	Pisolithus	1.20

[a]Any two means not scored by the same line were significantly different at the .01 level according to Duncan's Least Significant Difference test.

centimeters for Replication 1 and 1.75 centimeters for Replication 2. The mean root weights for Replications at the end of the study were 3.21 grams for Replication 1 and 2.79 grams for Replication 2. The latter values were significantly different from one another at the .01 level.

The initial mean heights in centimeters for the amendments were:

	Rep 1	Rep 2
Control	2.2	1.8
Lime	2.1	1.6
Fertilizer	2.1	1.8
Lime + Fertilizer	2.3	1.8

The difference in root weights between replications was significant only for the lime plus fertilizer treatment (Table 34) which was one of the two treatments which had the greatest height difference between replica replications at the beginning of the study. However, the other treatment, lime, with the greatest height difference at the beginning had a larger mean root weight for Replication 2 than for Replication 1. Thus there is no readily apparent explanation for the significant amendment x replication interaction.

The mean heights in centimeters for the fungal treatments at the beginning of the experiment were:

	Rep 1	Rep 2
Pisolithus	1.4	1.5
Cenococcum	2.6	1.8
Control	2.6	1.9

There is no clear explanation for the significant fungus x replication interaction, since only the root weights of the Cenococcum-inoculated

TABLE 34.

Mean root dry weights of birch seedlings for the various combinations of amendments and replications.

Combination Amendment	Replication	Mean Root Dry Weight[a]
		grams
Lime + Fertilizer	1	6.06
Lime + Fertilizer	2	4.71
Fertilizer	1	4.63
Fertilizer	2	4.04
Lime	2	1.78
Lime	1	1.51
Control	2	0.65
Control	1	0.64

[a]Any two means not scored by the same line were significantly different at the .01 level according to Duncan's Least Significant Difference test.

seedlings were significantly different, being considerably larger in Replication 1 than in Replication 2 (Table 35).

The significant amendment x fungus x replication interaction seems to have been due largely to a greatly reduced root weight in Replication 2 of the Cenococcum-inoculated seedlings of the lime plus fertilizer treatment as compared to Replication 1 of the same amendment-fungus combination (Figure 14). Differences between Replications 1 and 2 for other amendment-fungus combinations were not nearly as great. Again, the explanation is not apparent.

Total Dry Weight

All of the main effects and interactions that were significant in the analysis of the root dry weights were also significant in the analysis of the total dry weights, and the pattern of differences was almost the same (Tables 36, 37, 38, 39, 40, 41, and Figure 15). In addition there were three other significant interactions: bank x replication, bank x amendment x fungus, and amendment x fungus x replication.

There were some very pronounced effects on total seedling weights. The seedling weights of the Cramer and Cresson spoils were more than twice that of the Brandy Camp spoil (Table 36); on the lime plus fertilizer amendment they were about 10 times that of the control (Table 37); and on the fertilizer amendment of the Cramer spoil, they were more than 100 times that of the Brandy Camp control (Table 38). Over a longer period of time these greatly enhanced early responses would no doubt increase seedling survival.

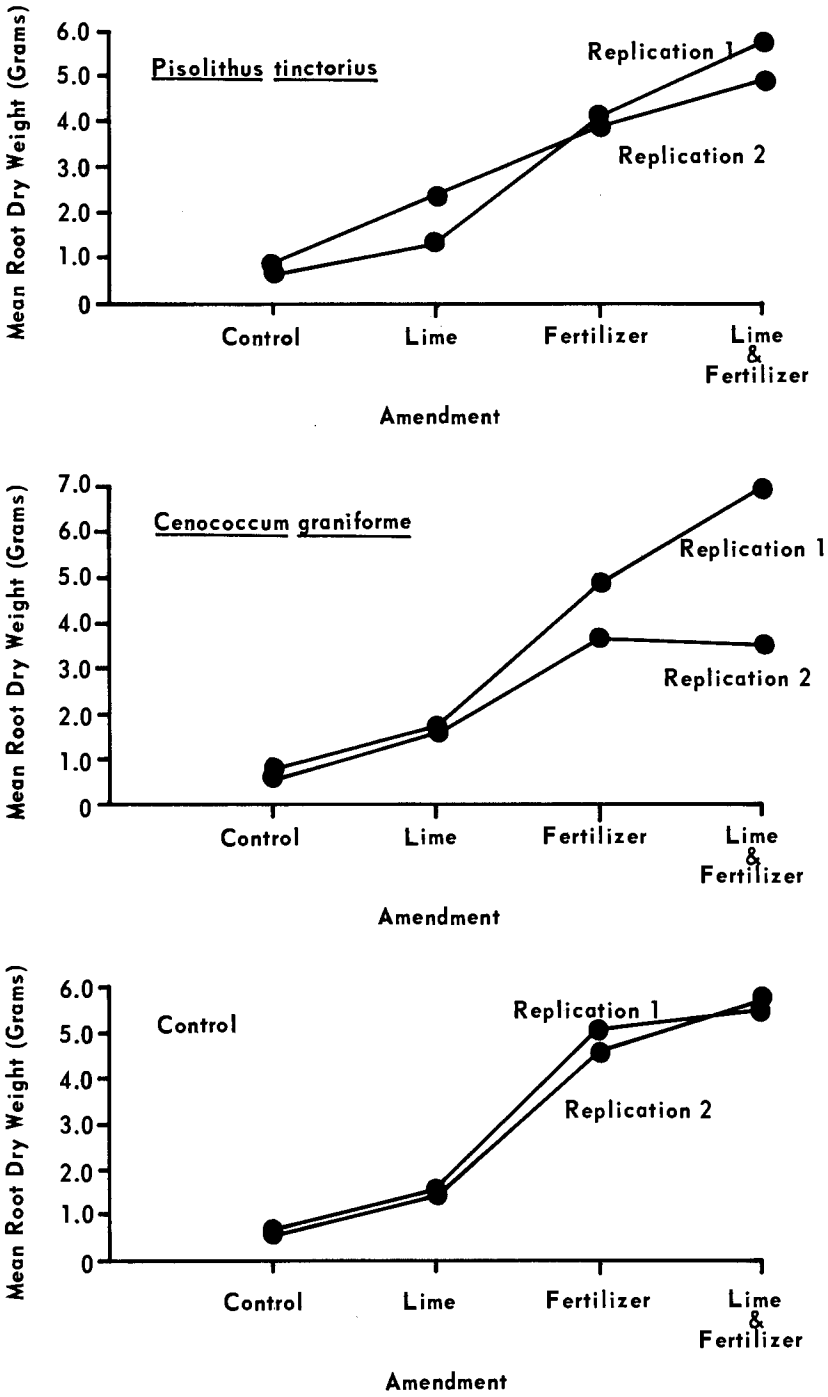
As previously noted, replication effect was actually one of difference in initial seedling development. The initial heights of the seedlings

TABLE 35.

Mean root dry weights of birch seedlings for the various combinations of fungi and replications.

Combination Symbiont	Replication	Mean Root Dry Weight[a]
		grams
Cenococcum	1	3.53
Control	1	3.14
Pisolithus	2	3.04
Control	2	3.02
Pisolithus	1	2.96
Cenococcum	2	2.32

[a]Any two means not scored by the same line were significantly different at the .01 level according to Duncan's Least Significant Difference test.



ROOT DRY WEIGHTS OF BIRCH SEEDLINGS FOR THE VARIOUS COMBINATIONS OF AMENDMENTS, FUNGI, AND REPLICATIONS

Figure 14

TABLE 36.

Mean total dry weights of birch seedlings

for the spoil banks.

Spoil Bank	Mean Total Dry Weight[a]
	grams
Cramer	7.80
Cresson	7.78
Brandy Camp	3.23

[a]Any two means not scored by the same line were significantly different at the .01 level according to Duncan's Least Significant Difference test.

TABLE 37.

Mean total dry weights of birch seedlings

for the various amendments.

Amendment	Mean Total Dry Weight[a]
	grams
Lime + Fertilizer	11.95
Fertilizer	8.95
Lime	3.14
Control	1.27

[a]All means were significantly different at the .01 level according to Duncan's Least Significant Difference test.

TABLE 38.

Mean total dry weights of birch seedlings for the various combinations of banks and amendments.

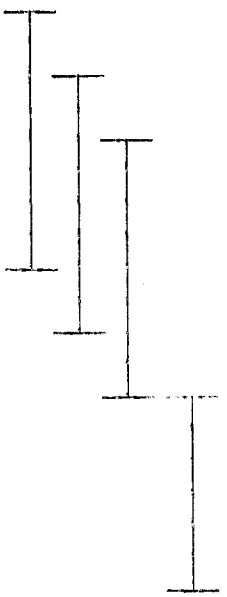
Combination		Mean Total Dry Weight[a]
Spoil Bank	Amendment	
		grams
Cramer	Fertilizer	15.46
Cresson	Lime + Fertilizer	14.09
Cramer	Lime + Fertilizer	12.32
Cresson	Fertilizer	10.96
Brandy Camp	Lime + Fertilizer	9.45
Cresson	Lime	3.99
Brandy Camp	Lime	2.92
Cramer	Lime	2.52
Cresson	Control	2.09
Cramer	Control	1.57
Brandy Camp	Fertilizer	0.42
Brandy Camp	Control	0.14

[a]Any two means not scored by the same line were significantly different at the .01 level according to Duncan's Least Significant Difference test.

TABLE 39.

Mean total dry weights of birch seedlings for the various combinations of banks and fungi.

Combination		Mean Total Dry Weight[a]
Spoil Bank	Fungus	
		grams
Cresson	Pisolithus	8.80
Cramer	Control	8.47
Cramer	Cenococcum	8.09
Cresson	Control	7.71
Cramer	Pisolithus	7.34
Cresson	Cenococcum	6.84
Brandy Camp	Control	3.63
Brandy Camp	Cenococcum	3.30
Brandy Camp	Pisolithus	2.27



[a]Any two means not scored by the same line were significantly different at the .01 level according to Duncan's Least Significant Difference test.

TABLE 40.

Mean total dry weights of birch seedlings for the various combinations of amendments and replications.

Combination		Mean Total Dry Weight[a]
Spoil Bank	Replication	
		grams
Lime + Fertilizer	1	13.15
Lime + Fertilizer	2	10.75
Fertilizer	1	9.44
Fertilizer	2	8.46
Lime	2	3.38
Lime	1	2.90
Control	2	1.28
Control	1	1.25

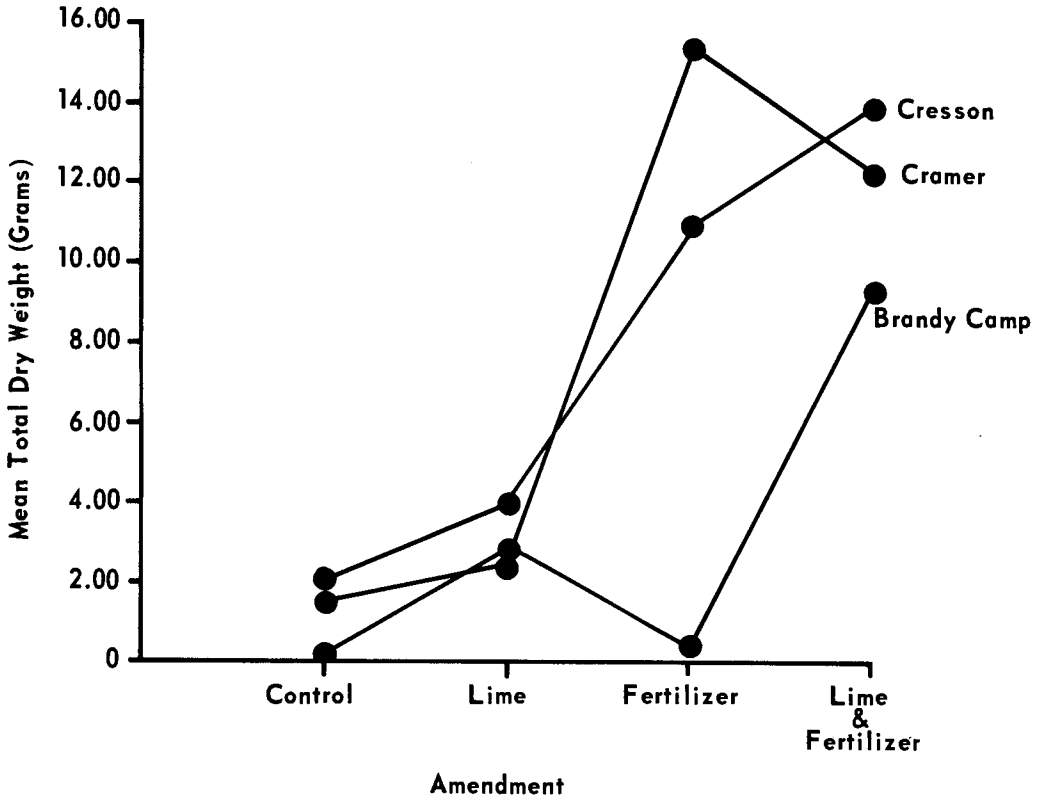
[a]Any two means not scored by the same line were significantly different at the .01 level according to Duncan's Least Significant Difference test.

TABLE 41.

Mean total dry weights of birch seedlings for the various combinations of fungi and replications.

Combination Fungus	Replication	Mean Total Dry Weight[a]
		grams
Cenococcum	1	7.01
Control	1	6.81
Control	2	6.40
Pisolithus	2	6.36
Pisolithus	1	6.24
Cenococcum	2	5.14

[a]Any two means not scored by the same line were significantly different at the .01 level according to Duncan's Least Significant Difference test.



TOTAL DRY WEIGHTS OF BIRCH SEEDLINGS FOR THE VARIOUS COMBINATIONS OF BANKS AND AMENDMENTS

Figure 15

are presented in Table 46 of the Appendix. The mean total dry weight of seedlings at the end of the study was 6.69 grams in Replication 1 and 5.97 grams in Replication 2. This difference was significant at the .01 level.

The mean heights in centimeters according to spoil bank treatment and replication were the following:

	Rep 1	Rep 2
Brandy Camp	2.1	1.7
Cresson	2.3	1.6
Cramer	2.2	1.9

The difference in total weights between replications was significant only for the Cresson spoil which had the greatest height difference between replications at the beginning of the study (Table 42).

The significant interaction of bank x amendment x fungus was largely the result of a rather erratic response to amendments by Pisolithus-inoculated seedlings on the different banks (Figure 16). In comparison to the control seedlings, the total weights of the Pisolithus-inoculated seedlings were significantly lower on the Brandy Camp spoil treated with lime plus fertilizer, higher on the Cresson spoil treated with lime plus fertilizer, and lower on the Cramer spoil treated with fertilizer. No correlation could be made however, between mycorrhizal abundance and the total dry weights of the Pisolithus-inoculated seedlings as a possible explanation for the different growth responses (Table 26, pages 66 and 67).

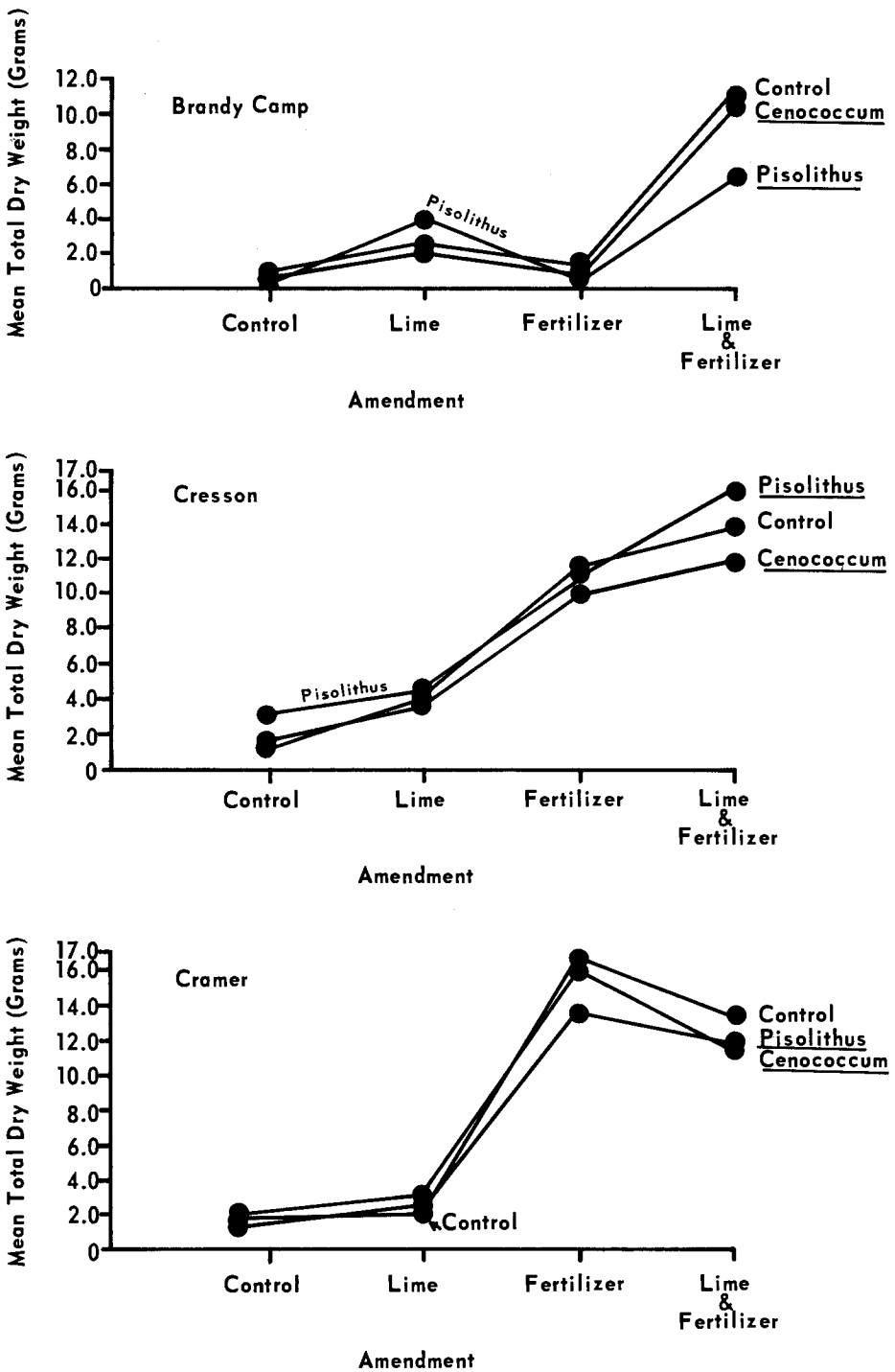
The significant amendment x fungus x replication interaction on total seedling dry weights (Figure 17) was similar to the interaction of

TABLE 42.

Mean total dry weights of birch seedlings for the various combinations of banks and replications.

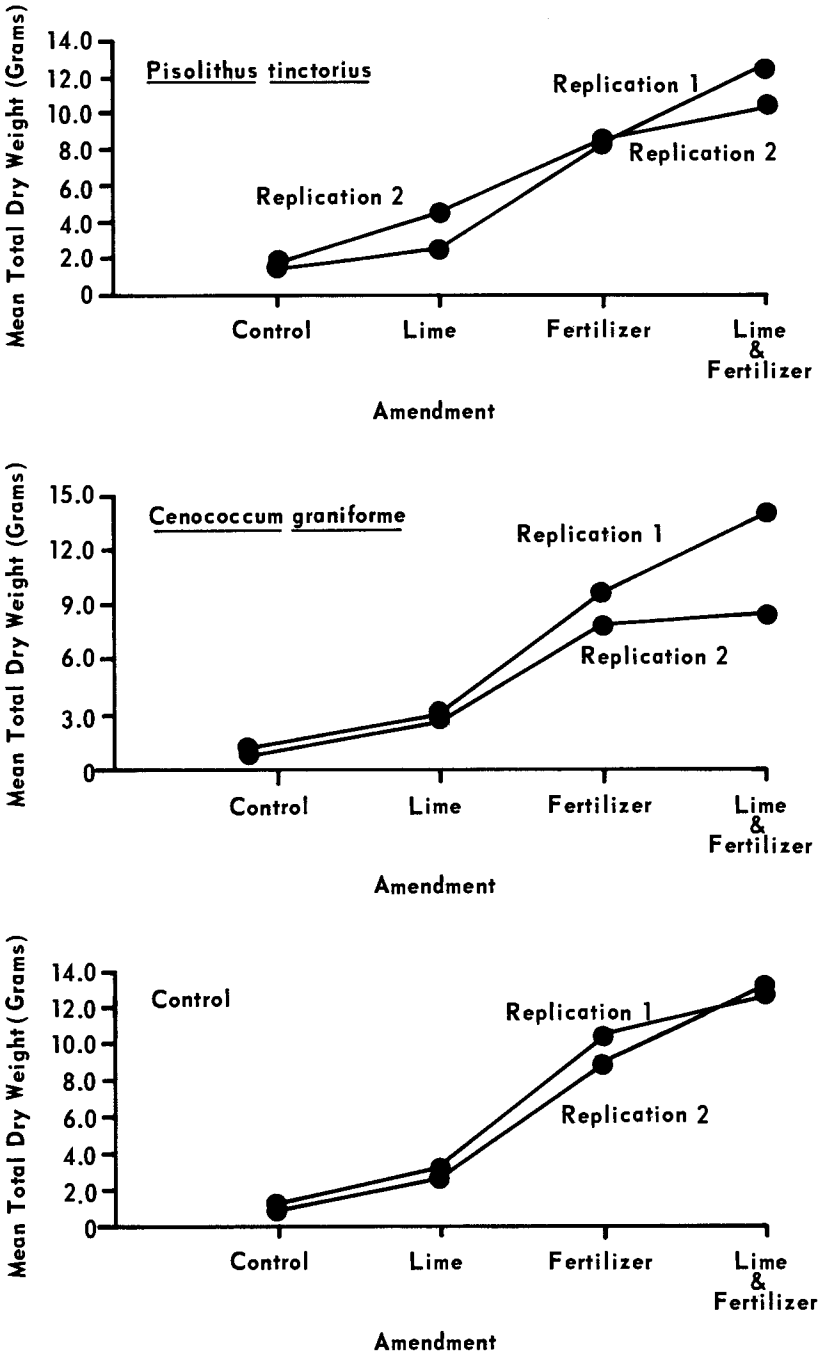
Combination		Mean Total Dry Weight[a]
Spoil Bank	Replication	
		grams
Cresson	1	8.57
Cramer	1	8.27
Cramer	2	7.66
Cresson	2	6.99
Brandy Camp	2	3.25
Brandy Camp	1	3.22

[a]Any two means not scored by the same line were significantly different at the .05 level according to Duncan's Least Significant Difference test.



TOTAL DRY WEIGHTS OF BIRCH SEEDLINGS FOR THE VARIOUS COMBINATIONS OF BANKS, AMENDMENTS, AND FUNGI

Figure 16



TOTAL DRY WEIGHTS OF BIRCH SEEDLINGS FOR THE VARIOUS COMBINATIONS OF AMENDMENTS, FUNGI, AND REPLICATIONS

Figure 17

the same factors on root dry weights. Again, the total dry weights of Cenococcum-inoculated seedlings in Replication 2 of the lime plus fertilizer treated spoil were greatly reduced as compared with Replication 1 of the same amendment-fungus combination.

Shoot/Root Ratio

The statistical analysis for top weights and root weights of the birch seedlings yielded results similar to those of the pine experiment. Moreover, like the pine, the birch shoot/root ratios differed between amendments (Table 43). The lime plus fertilizer treatment resulted in a significantly higher shoot/root ratio than did the lime, fertilizer, and control treatments. The latter three treatments did not differ significantly from one another.

Mycorrhizal Establishment

As with the pine experiment, the root systems of the birch seedlings appeared nonmycorrhizal when transplanted into the spoils. Similarly, there was an abundance of free-living mycelia of both fungi growing on the roots of most of the inoculated seedlings. Additional inoculation at time of transplant to the spoils succeeded in establishing definite mycorrhizae on the roots of seedlings with both fungal symbionts. To my knowledge, this is the first report of Pisolithus tinctorius being positively associated with European white birch (Figure 18).

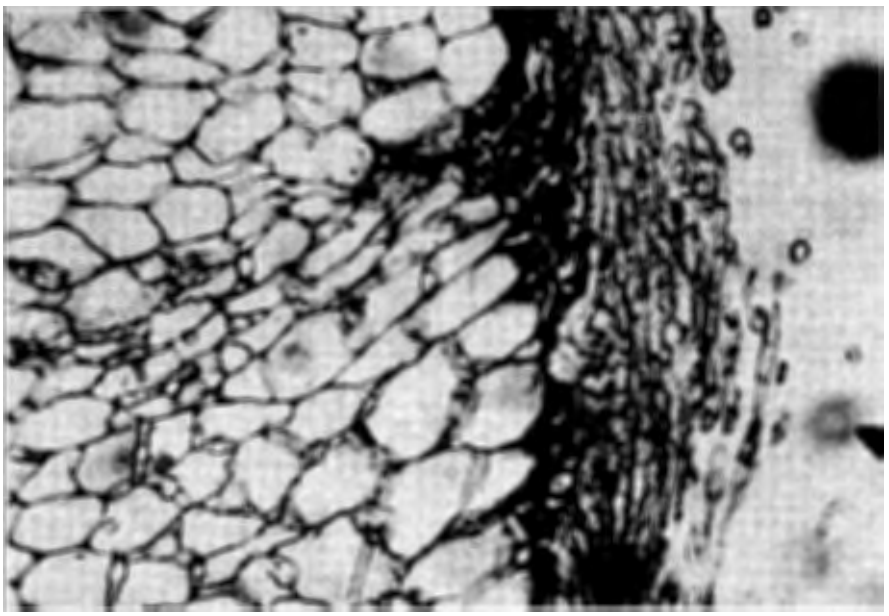
The abundance of mycorrhizal short roots differed with the various amendments and between banks (Table 26, pages 66 and 67). There was little similarity between mycorrhizal abundance in the birch and pine experiments, however. Mycorrhizae formed by both Pisolithus and

TABLE 43.

Mean shoot/root ratios of birch seedlings for the various amendments.

Amendment	Mean Shoot/Root Ratio[a]
Lime + Fertilizer	1.27
Fertilizer	0.97
Control	0.93
Lime	0.91

[a]Any two means not scored by the same line were significantly different at the .01 level according to Duncan's Least Significant Difference test.



Note fungal mantle of hyphae on right of short root.

CROSS SECTION OF ECTOMYCORRHIZAL SHORT ROOT FORMED BY Pisolithus tinctorius ON EUROPEAN WHITE BIRCH (500x)

Figure 18

Cenococcum on birch were most abundant on the Brandy Camp spoil. Cenococcum mycorrhizae were more successfully established on this spoil on the fertilizer amendment and on the control. No general trend of Cenococcum establishment was observed on the Cresson and Cramer spoils. As in the pine experiment, Pisolithus mycorrhizae were better established on the Brandy Camp spoil with the lime amendment. Unlike the pine seedlings grown in the Cresson and Cramer spoil materials, Pisolithus mycorrhizae on the birch seedlings developed somewhat better on the control than on those spoils with amendments.

Fungal inoculum was differentially distributed between the pine and birch experiments so the two experiments cannot be accurately compared for mycorrhizal abundance. The inoculum was placed in closer contact with the birch roots because smaller amounts of the planting medium were transplanted with the birch than with the pine. This may have enabled greater establishment of Cenococcum mycorrhizae with the birch. Moreover, the overall harsh characteristics of the spoil material may be unfavorable for the existence of Cenococcum mycorrhizae since this symbiont was poorly established with both seedling hosts.

Categorizing Cenococcum as being highly established on the Brandy Camp control and fertilizer spoils is misleading. As in the pine experiment, seedling roots did not grow into the spoil material but stayed within the original soil around the roots. The very small root system only became mycorrhizal within the confines of the favorable environment of the original soil. In the toxic spoil that had been ameliorated with lime, root growth extended into the spoil but was

minimally mycorrhizal with Cenococcum. Cenococcum then either was restricted in the spoil material or was a very slow mycorrhizae former as compared with Pisolithus.

Mycorrhizae formed by Pisolithus were generally most abundant on the largest birch seedlings. These seedlings, as in the pine, may have been most capable of supplying photosynthates to the roots for increased mycorrhizal establishment. On the untreated Cresson and Cramer spoils, the development of Pisolithus mycorrhizae may have been encouraged by the general deficiency of nutrients.

Invading mycorrhizal fungi formed a white mycorrhiza which Worley and Hacskaylo (1959) stated predominates in soils with relatively high moisture contents. This mycorrhiza, however, was only nominally established with the birch and generally formed in the Brandy Camp and Cresson spoils on the non-inoculated or Cenococcum-inoculated seedlings. Establishment seemed to be greatest with the addition of lime to the Brandy Camp spoil and fertilizer to the Cresson spoil. Seedlings influenced by these amendment treatments were well developed and possibly provided ample root photosynthates for the establishment and maintenance of the symbiosis. The seedlings with Pisolithus as the ectomycorrhizal symbiont were not invaded by other symbionts. Possibly, Pisolithus secreted antibiotics which warded off other mycorrhizal fungi.

More replications and a longer period of study would be needed to verify this apparent interaction between banks and amendments.

General Discussion

The birch seedlings responded greater to treatments and were more variable than the pine seedlings. Characteristically, European white

birch grows faster than red pine in the seedling stage. For example, the mean total dry weight of seedlings on the Cramer spoil receiving fertilizer was 15.46 grams for birch and 0.85 grams for pine. Differences of 1 to 4 grams were not uncommon between the two replications of the birch seedlings for the Cresson spoil treated with fertilizer, while the pine seedlings generally varied in weight by less than 0.5 grams on the same spoil and with the same treatment. These differences in growth may have been augmented by upsetting the pine physiology at the outset of the experiment.

In general, seedling response to the various types of treatments was greater with chemical improvement through the application of lime, fertilizer, or both than with the biological improvement through the inoculations with mycorrhizal fungi. This was especially apparent with the birch seedlings. For example, the mean total dry weight of birch seedlings on the Cresson spoil with fertilizer was 10.96 grams while with the control it was 2.09 grams. The pine seedlings responded similarly to the same treatments on the same bank but to a lesser degree--0.96 grams with fertilizer and 0.51 grams with the control. The weights of birch on the Cresson spoil were 8.80 grams for Pisolithus-inoculated seedlings and 7.71 grams for control seedlings. With the same spoil and treatments the mean weight of the pine seedlings were 0.96 grams and 0.57 grams, respectively.

The responses of both species might have been greater with the lime treatments if the desired pH levels had been achieved. Also, seedling response might have been greater if the seedlings had been mycorrhizal

when transplanted to the spoil materials. Since it is likely that mycorrhizal establishment on both tree species was limited by spoil characteristics, it is impossible to predict what the growth would have been had the seedlings been mycorrhizal when transplanted.

V. SUMMARY AND CONCLUSIONS

Many efforts have been made to revegetate barren deep-mine refuse banks. These efforts however, have been either minimally successful or too expensive to extend to a widescale revegetation campaign of the many acres of barren spoil material.

Literature on specific deterrents of vegetation on coal waste banks as pertaining to conditions necessary for mycorrhizal establishment was reviewed. The literature revealed that specific ectomycorrhizal symbionts may enhance seedling response on poorly-vegetated or barren spoil banks. Therefore, specific ectomycorrhizal fungi adapted to various harsh conditions common to many barren spoil banks were chosen for further study. The objectives of this study were: (1) to determine if selected tree species could be successfully inoculated with the selected fungal symbionts; (2) if so, to determine the degree of mycorrhizal occurrence on a variety of spoils both untreated and treated with lime, fertilizer, or both; (3) to determine the seedling response to the selected mycorrhizal fungi under the various treatments. The results of this study may be applicable to the economic revegetation of harsh refuse banks.

The mycorrhizae study was set up in a greenhouse in pots of spoil. A completely randomized design with factorial arrangements of four fixed factors (spoil, fungal symbiont, lime treatment, and fertilization treatment) was used. There were three levels of bituminous spoil (Brandy Camp spoil, Cresson spoil, and Cramer spoil), three levels of fungal symbiont (Pisolithus tinctorius, Cenococcum graniforme, and a control),

two levels of lime treatment (final pH of 6.0 to 6.5 and a control), and two levels of fertilization treatment (1.0 tons/acre and a control). The Brandy Camp and Cresson spoils were completely barren, but the Cramer spoil was colonized with trees of many species, such as aspen, fire cherry, oak, and birch.

European white birch and red pine were the two tree species used in the study, with each species constituting a separate experiment. The birch experiment differed from the pine experiment in that the seedlings were separated into blocks according to initial development. There were two replications of each of the 36 treatments for each tree species. Seedling heights were measured weekly and pH's were taken every three weeks. At the termination of each experiment, the total seedling heights, total dry weights, root dry weights, top dry weights, and shoot/root ratios for each treatment were statistically analyzed.

Red pine was successfully inoculated with both fungi. The abundance of mycorrhizae formed by Pisolithus varied between banks and between amendments. The symbiont was best established on the Cresson spoil at all levels of amendments. It occurred generally more with the lime, control, and fertilizer amendments on the Cramer spoil and with lime on the Brandy Camp spoil. Naturally invading symbionts were infrequently observed, occurring minimally with the control and lime amendments throughout the three spoils. The fungal symbiont Cenococcum was uniformly low throughout the spoils and almost nonexistent on the Cramer spoil.

In the pine experiment, top dry weights, total dry weights, and shoot/root ratios, as influenced by the fungal symbiont Pisolithus,

were significantly greater than the corresponding responses with either Cenococcum mycorrhizae or no mycorrhizae. However, response of the total dry weight to the fungal treatment varied depending upon the spoil. On the Cresson and Cramer spoils, total seedling dry weights were significantly greater with mycorrhizae of Pisolithus than the other fungal treatments. In the Brandy Camp spoil, no fungal treatment significantly enhanced growth. Thus Pisolithus tinctorius seems to be a valuable fungal symbiont in increasing pine growth on the less-toxic, moderately acidic spoils. It is questionable whether fungal smybionts can increase growth on highly toxic, acidic spoils.

Pine growth generally was more favorable on the Cresson and Cramer spoils than on the Brandy Camp spoil. Seedling heights, top dry weights, root dry weights, and total dry weights were significantly greater on the Cresson and Cramer spoils. Fertilization was most important in increasing seedling heights, top dry weights, and shoot/root ratios, while either lime or fertilizer was important in increasing root dry weights and total dry weights. Similarly, either lime or fertilizer was important for increasing root dry weights and total dry weights on the Cresson spoil. Fertilization was needed to enhance top weights and total dry weights on the Cramer spoil. Top dry weights, root dry weights, and total dry weights were enhanced by lime on the Brandy Camp spoil. Therefore, on less toxic, moderately acidic spoils, fertilization with or without liming will generally augment red pine seedling response. On extremely toxic, acid sites, lime with or without fertilizer is needed to successfully increase seedling growth and survival.

European white birch was also successfully inoculated with both mycorrhizal fungi. This is apparently the first report of the definite mycorrhizal association between Pisolithus tinctorius and European white birch. As with the pine, the abundance of mycorrhizal short roots differed with the various amendments and between banks. With the birch, however, mycorrhizae formed by both Pisolithus and Cenococcum were most successfully established on the Brandy Camp spoil. In this spoil Cenococcum mycorrhizae were most abundant with the fertilizer and control amendments while Pisolithus mycorrhizae were best established with the lime amendment. Pisolithus establishment on birch differed from the pine in that the establishment was somewhat better on seedlings in the controls of the Cresson and Cramer spoils than on those with amendments.

Cenococcum establishment may have differed between the two seedling hosts by the differential placement of inoculum. Inoculum was initially placed closer to the birch roots than the pine roots since twice the amount of soil was transplanted with the pine. Harsh spoil characteristics generally may limit this symbiont from becoming sufficiently established. Naturally invading mycorrhizal fungi were poorly established and occurred on nonmycorrhizal short roots of Cenococcum seedlings and control seedlings on both the limed Brandy Camp and the fertilized Cresson spoils.

Total height and root dry weight on the Cresson spoil were the only growth variables affected by Pisolithus mycorrhizae. Therefore fungal inoculations of European white birch generally should be restricted to spoils of moderate acidity and toxicity.

All of the growth criteria were better on the Cresson and Cramer spoils than on the Brandy Camp spoil. The optimum amendment generally was lime plus fertilizer. On the Cresson and Brandy Camp spoils, this treatment phenomenally enhanced growth. On the Cramer spoil, as in the pine experiment, fertilization without lime resulted in the greatest seedling growth. Possibly too much lime had been applied to this spoil for optimum growth of such acid-tolerant species as red pine and European white birch.

Based on the findings of these experiments, the nutrient deficiencies of deep-mine bituminous spoil banks in Pennsylvania may be an important contributing factor to the revegetation failures of these untreated sites. Fertilization of the less toxic and moderately acidic spoils will probably enhance seedling survival and growth. Also, inoculations with Pisolithus tinctorius of seedlings to be planted on such spoils may be an economic method of obtaining successful revegetation. However, on extremely acidic, toxic spoil wastes, lime must be added. On such banks, the advisability of making fungal inoculations of seedlings is questionable.

This study may not be directly applicable to the field, however. Field studies are needed to ascertain the benefit of Pisolithus tinctorius as an introduced symbiont on survival and growth of seedlings under high temperatures and droughty conditions that generally exist on spoil banks. Under droughty conditions Cenococcum may prove advantageous to seedling survival. Furthermore, if field studies did show the inoculations to be beneficial, methods would have to be developed for the economical mass-inoculation of seedlings in the nursery with these symbionts.

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APPENDIX

TABLE 44.

Response of pine seedlings

to the various treatments.

Treatment[a]	Brandy Camp		Cresson		Cramer	
	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
			Seedling Height (cm.)			
L-Pt	6.0	7.0	5.3	6.8	7.1	6.9
L-Cg	4.7	5.1	6.6	6.3	4.9	5.6
L-X	6.3	6.8	5.5	6.3	5.4	4.7
LF-Pt	6.0	6.2	6.7	7.0	7.3	6.2
LF-Cg	5.8	5.2	6.3	6.1	5.4	7.0
LF-X	5.8	5.9	5.3	6.2	6.0	6.2
F-Pt	6.8	5.6	7.3	8.0	7.4	7.8
F-Cg	5.5	5.3	5.9	6.8	6.4	6.5
F-X	5.5	5.5	5.0	6.8	6.3	5.9
X-Pt	5.1	5.6	5.9	6.3	7.1	6.1
X-Cg	5.5	5.9	5.3	5.1	5.7	6.3
X-X	5.1	5.0	5.3	5.5	5.6	5.9
			Top Weight (grams)			
L-Pt	0.32	0.69	0.41	0.66	0.59	0.60
L-Cg	0.13	0.23	0.41	0.53	0.19	0.40
L-X	0.26	0.32	0.31	0.42	0.33	0.20
LF-Pt	0.32	0.46	0.74	0.51	0.66	0.30
LF-Cg	0.40	0.32	0.50	0.52	0.41	0.48
LF-X	0.33	0.24	0.44	0.37	0.29	0.35
F-Pt	o.28	1.18	0.82	1.03	0.62	0.84
F-Cg	0.16	0.17	0.50	0.67	0.59	0.55
F-X	0.20	0.16	0.35	0.53	0.47	0.38
X-Pt	0.13	0.17	0.51	0.61	0.52	0.58
X-Cg	0.08	0.10	0.20	0.12	0.21	0.35
X-X	0.08	0.13	0.21	0.17	0.21	0.22

TABLE 44.

Response of pine seedlings

to the various treatments.

Treatment[a]	Brandy Camp		Cresson		Cramer	
	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
			Seedling Height (cm.)			
L-Pt	6.0	7.0	5.3	6.8	7.1	6.9
L-Cg	4.7	5.1	6.6	6.3	4.9	5.6
L-X	6.3	6.8	5.5	6.3	5.4	4.7
LF-Pt	6.0	6.2	6.7	7.0	7.3	6.2
LF-Cg	5.8	5.2	6.3	6.1	5.4	7.0
LF-X	5.8	5.9	5.3	6.2	6.0	6.2
F-Pt	6.8	5.6	7.3	8.0	7.4	7.8
F-Cg	5.5	5.3	5.9	6.8	6.4	6.5
F-X	5.5	5.5	5.0	6.8	6.3	5.9
X-Pt	5.1	5.6	5.9	6.3	7.1	6.1
X-Cg	5.5	5.9	5.3	5.1	5.7	6.3
X-X	5.1	5.0	5.3	5.5	5.6	5.9
			Top Weight (grams)			
L-Pt	0.32	0.69	0.41	0.66	0.59	0.60
L-Cg	0.13	0.23	0.41	0.53	0.19	0.40
L-X	0.26	0.32	0.31	0.42	0.33	0.20
LF-Pt	0.32	0.46	0.74	0.51	0.66	0.30
LF-Cg	0.40	0.32	0.50	0.52	0.41	0.48
LF-X	0.33	0.24	0.44	0.37	0.29	0.35
F-Pt	o.28	1.18	0.82	1.03	0.62	0.84
F-Cg	0.16	0.17	0.50	0.67	0.59	0.55
F-X	0.20	0.16	0.35	0.53	0.47	0.38
X-Pt	0.13	0.17	0.51	0.61	0.52	0.58
X-Cg	0.08	0.10	0.20	0.12	0.21	0.35
X-X	0.08	0.13	0.21	0.17	0.21	0.22

TABLE 44. (Continued)

Treatment[a]	Brandy Camp		Cresson		Cramer	
	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
			Root Weight (grams)			
L-Pt	0.18	0.32	0.20	0.33	0.30	0.24
L-Cg	0.14	0.17	0.42	0.42	0.30	0.24
L-X	0.24	0.24	0.21	0.27	0.22	0.17
LF-Pt	0.08	0.18	0.32	0.29	0.27	0.20
LF-Cg	0.24	0.16	0.25	0.33	0.33	0.30
LF-X	0.23	0.16	0.25	0.27	0.18	0.20
F-Pt	0.08	0.13	0.32	0.47	0.23	0.36
F-Cg	0.13	0.08	0.31	0.32	0.33	0.22
F-X	0.13	0.07	0.17	0.26	0.29	0.23
X-Pt	0.07	0.08	0.25	0.25	0.24	0.33
X-Cg	0.08	0.13	0.22	0.17	0.16	0.31
X-X	0.07	0.11	0.17	0.18	0.17	0.18
			Total Weight (grams)			
L-Pt	0.50	1.01	0.61	0.99	0.89	0.84
L-Cg	0.27	0.40	0.83	0.95	0.35	0.62
L-X	0.50	0.56	0.52	0.69	0.55	0.37
LF-Pt	0.40	0.64	1.06	0.80	0.93	0.50
LF-Cg	0.64	0.48	0.75	0.85	0.74	0.78
LF-X	0.56	0.40	0.69	0.64	0.47	0.55
F-Pt	0.36	0.31	1.14	1.50	0.85	1.20
F-Cg	0.29	0.25	0.81	0.99	0.92	0.77
F-X	0.33	0.23	0.52	0.79	0.76	0.61
X-Pt	0.20	0.25	0.76	0.86	0.76	0.91
X-Cg	0.16	0.23	0.42	0.29	0.37	0.66
X-X	0.15	0.24	0.38	0.35	0.38	0.40

TABLE 44. (Continued)

Treatment[a]	Brandy Camp		Cresson		Cramer	
	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
			Shoot/Root Ratio			
L-Pt	1.78	2.16	2.05	2.00	1.97	2.50
L-Cg	0.93	1.35	0.98	1.26	1.19	1.81
L-X	1.08	1.33	1.48	1.56	1.50	1.18
LF-Pt	4.00	2.56	2.31	1.76	2.44	1.50
LF-Cg	1.67	2.00	2.00	1.58	1.24	1.60
LF-X	1.43	1.50	1.76	1.37	1.61	1.75
F-Pt	3.50	1.38	2.56	2.19	2.70	2.33
F-Cg	1.23	2.12	1.61	2.09	1.79	2.50
F-X	1.54	2.29	2.06	2.04	1.62	1.65
X-Pt	1.86	2.12	2.04	2.44	2.17	1.76
X-Cg	1.00	0.77	0.91	0.71	1.31	1.13
X-X	1.14	1.18	1.24	0.94	1.24	1.22

[a]L = Lime
LF = Lime + Fertilizer
F = Fertilizer
X = Control

Pt = Pisolithus tinctorius
Cg = Cenococcum graniforme
X = Control

TABLE 45.

Response of birch seedlings

to the various treatments.

Treatment[a]	Brandy Camp		Cresson		Cramer	
	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
			Seedling Height (cm.)			
L-Pt	24.2	45.6	24.4	27.7	11.2	16.3
L-Cg	17.1	18.6	24.0	24.9	21.3	12.8
L-X	23.0	18.6	27.2	21.2	13.6	12.3
LF-Pt	45.7	42.7	67.4	64.9	47.7	61.5
LF-Cg	60.8	51.3	57.3	51.4	58.3	45.6
LF-X	59.4	56.2	57.5	58.7	58.4	57.1
F-Pt	4.3	3.5	64.5	58.7	51.1	51.1
F-Cg	4.0	4.1	50.2	49.9	53.9	68.4
F-X	4.3	6.5	59.0	53.6	50.5	46.8
X-Pt	3.8	2.8	21.6	31.4	14.4	12.2
X-Cg	3.1	2.7	12.8	15.1	12.6	13.7
X-X	4.0	3.1	15.3	12.3	13.9	9.3
			Top Weight (grams)			
L-Pt	1.14	3.57	1.82	2.31	0.89	1.15
L-Cg	0.74	1.15	1.68	1.40	1.53	0.99
L-X	1.30	1.20	2.56	1.50	0.84	1.20
LF-Pt	4.60	2.83	10.02	7.64	5.94	6.06
LF-Cg	6.04	5.57	8.04	5.69	7.43	4.31
LF-X	6.49	6.45	8.84	7.80	6.28	8.07
F-Pt	0.16	0.14	5.54	6.32	7.02	7.15
F-Cg	0.17	0.17	6.20	4.76	8.11	8.39
F-X	0.15	0.27	6.84	5.14	9.07	7.46
X-Pt	0.05	0.05	1.55	1.92	0.62	0.77
X-Cg	0.04	0.09	0.78	0.72	0.81	0.69
X-X	0.11	0.03	0.86	0.69	0.67	0.75

TABLE 45. (Continued)

Treatment[a]	Brandy Camp		Cresson		Cramer	
	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
			Root Weight (grams)			
L-Pt	0.89	2.48	2.01	2.86	1.17	1.75
L-Cg	0.91	1.46	2.31	1.83	1.82	1.51
L-X	1.25	1.43	2.29	1.38	0.96	1.28
LF-Pt	3.07	2.63	8.56	6.16	5.65	6.14
LF-Cg	6.61	2.80	6.82	3.69	7.43	4.05
LF-X	4.06	5.53	5.99	5.28	6.32	6.09
F-Pt	0.21	0.21	5.23	5.45	6.78	6.27
F-Cg	0.17	0.27	5.59	3.65	8.64	8.86
F-X	0.22	0.39	6.38	4.67	8.47	8.55
X-Pt	0.05	0.06	1.13	1.70	0.62	0.72
X-Cg	0.05	0.19	0.86	0.70	1.18	0.79
X-X	0.10	0.03	0.80	0.66	0.82	0.96
			Total Weight (grams)			
L-Pt	2.03	6.05	3.83	5.17	2.06	2.90
L-Cg	1.65	2.61	3.99	3.23	3.35	2.50
L-X	2.55	2.63	4.85	2.88	1.80	2.48
LF-Pt	7.67	5.46	18.58	13.80	11.59	12.20
LF-Cg	12.65	8.37	14.86	9.38	15.03	8.36
LF-X	10.55	11.98	14.83	13.08	12.60	14.16
F-Pt	0.37	0.35	10.77	11.77	13.80	13.42
F-Cg	0.34	0.44	11.79	8.41	16.75	15.25
F-X	0.37	0.66	13.22	9.81	17.54	16.01
X-Pt	0.10	0.11	2.86	3.62	1.24	1.49
X-Cg	0.09	0.28	1.64	1.42	1.99	1.48
X-X	0.21	0.06	1.66	1.35	1.49	1.71

TABLE 45. (Continued)

Treatment[a]	Brandy Camp		Cresson		Cramer	
	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
			Shoot/Root Ratio			
L-Pt	1.28	1.44	0.91	0.81	0.76	0.66
L-Cg	0.81	0.79	0.73	0.77	0.84	0.66
L-X	1.04	0.84	1.12	1.09	0.88	0.94
LF-Pt	1.50	1.08	1.17	1.24	1.05	0.99
LF-Cg	0.91	1.99	1.18	1.54	1.02	1.06
LF-X	1.60	1.17	1.48	1.48	1.00	1.33
F-Pt	0.76	0.67	1.06	1.16	1.04	1.14
F-Cg	1.00	0.63	1.11	1.30	0.94	1.22
F-X	0.68	0.69	1.07	1.10	1.07	0.87
X-Pt	1.00	0.83	1.18	1.13	1.00	1.07
X-Cg	0.80	0.47	0.91	1.03	0.69	0.87
X-X	1.10	1.00	1.08	1.05	0.82	0.78

[a]L = Lime
LF = Lime + Fertilizer
F = Fertilizer
X = Control

Pt = Pisolithus tinctorius
Cg = Cenococcum graniforme
X = Control

TABLE 46.

Heights[a] of birch seedlings at initiation of experiment.

Treatment[b]	Brandy Camp		Cresson		Cramer	
	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
	cm.		cm.		cm.	
L-Pt	1.0	1.5	1.5	1.5	1.5	2.0
L-Cg	2.0	1.5	3.5	1.5	3.0	1.5
L-X	1.5	1.5	3.0	2.0	2.0	1.8
LF-Pt	1.0	1.5	1.5	1.0	1.5	1.5
LF-Cg	3.5	1.8	3.0	2.0	2.5	1.5
LF-X	2.5	2.5	2.5	1.8	3.0	1.8
F-Pt	1.5	1.5	1.5	1.0	1.2	1.5
F-Cg	2.0	2.2	2.0	2.2	2.0	2.2
F-X	3.2	2.0	3.0	1.5	3.5	2.5
X-Pt	2.0	1.5	1.5	1.5	1.5	1.8
X-Cg	2.2	1.2	2.5	1.8	2.8	2.0
X-X	2.5	1.5	2.5	2.0	2.5	2.5

[a] heights taken from ground line

[b] L = Lime
LF = Lime + Fertilizer
F = Fertilizer
X = Control

Pt = Pisolithus tinctorius
Cg = Cenococcum graniforme
X = Control

TABLE 47.

pH values[a] of the selected spoils taken over the duration of the experiment.

Time	Treatment[b]	Brandy Camp	Cresson	Cramer
wks.				
0	L	6.50	7.30	7.45
	LF	6.20	7.00	7.20
	F	2.60	3.30	5.10
	X	2.50	3.40	5.00
3	L	6.70	7.30	7.20
	LF	6.50	6.90	7.15
	F	2.55	3.50	5.30
	X	2.40	3.60	5.00
6	L	5.50	6.85	6.75
	LF	5.50	6.85	6.80
	F	2.50	3.35	5.25
	X	2.40	3.45	5.15
9	L	5.75	7.05	7.00
	LF	5.95	7.15	6.80
	F	2.55	3.80	5.40
	X	2.45	3.85	5.55
12	L	6.15	7.35	7.35
	LF	5.30	7.30	7.20
	F	2.70	4.05	6.10
	X	2.50	4.00	6.10
15	L	6.15	7.30	7.35
	LF	5.60	7.25	7.20
	F	2.70	4.00	6.10
	X	2.55	4.05	6.05

[a]The Brandy Camp L and LF pH values are averaged from four samples that all varied widely. All other values are averages of two samples.

[b]L = Lime
LF = Lime + Fertilizer
F = Fertilizer
X = Control

TABLE 48.

Chemical and physical analyses of the three spoil banks (W.H. Davidson, unpublished data).

Source	Cramer	Cresson	Brandy Camp
		Chemical	
Exchangeable acidity (meq. H^+/100g)	3.0	5.6	10.0
Soluble salts (millimhos/cm)	0.18	0.27	1.78
Phosphorus (ppm)	5.6	0.7	1.0
Sulfate (ppm)	270	200	3228
		Physical	
> 2" (%)	4	-	9
¼ - 2" (%)	32	25	47
2mm - ¼" (%)	44	27	29
<2mm (%)	32	45	30