SURVIVAL AND GROWTH OF PINE SEEDLINGS ON STRIP-MINED SITES

by

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(ABSTRACT)

The effects of cultural treatments including ectomycorrhizal inoculation, chemical weed control, and slow-release fertilization on survival, growth, ectomycorrhizal colonization, and foliar nutrient levels of container-grown white (*Pinus strobus* L.), loblolly (*P. taeda* L.), and Virginia pine (*P. virginiana* Mill.) seedlings were studied on a recontoured and a flat bench strip-mine site in southwestern Virginia. One-half of the seedlings was inoculated with *Pisolithus tinctorius* (Pers.) Coker and Couch (Pt). A 21 g Agriform starter tablet was placed in the soil at a depth of 10 cm within 10-15 cm of each seedling in one-half of the plots at planting. Glyphosate was applied to one-half of each plot prior to planting and again later in the growing season.

First year survival was not different between sites and was not significantly affected by the cultural treatments. Abundant precipitation and high soil moisture levels throughout the initial growing season may have accounted for the excellent first year survival. The combination of chemical weed control and fertilization significantly increased the growth of all three species. Pt inoculation enhanced seedling growth to some extent but high amounts of natural ectomycorrhizal colonization masked some of the effects of Pt. Levels of foliar nitrogen closely reflected the effects of each treatment on seedling growth, indicating that it was the growth-limiting nutrient for pine seedlings on these strip-mined sites.

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INTRODUCTION

Bituminous coal is an abundant natural resource in southwestern Virginia. The people of Buchanan, Dickenson, Lee, Russell, Scott, Tazewell, and Wise counties (Figure 1) depend on coal mining for their economic base. The history of this rugged, mountainous region is characterized by resource exploitation followed by shifts to new resources. Agriculture and lumbering preceeded mining as sources of livlihood. Relative costs and availability of various energy sources indicate that it is reasonable to expect coal will continue to be mined from this region until it is thoroughly depleted or until economic conditions no longer favor its extraction. A future without coal as the economic mainstay must be considered now.

Over thirty years of surface mining have left a patchwork of drastically disturbed sites thoughout this region of Virginia. Drastically disturbed areas are those where native vegetation and animal communities have been eliminated and most of the topsoil is lost, altered, or buried (Box, 1978). Most sites are environmentally, economically, and socially unproductive even when

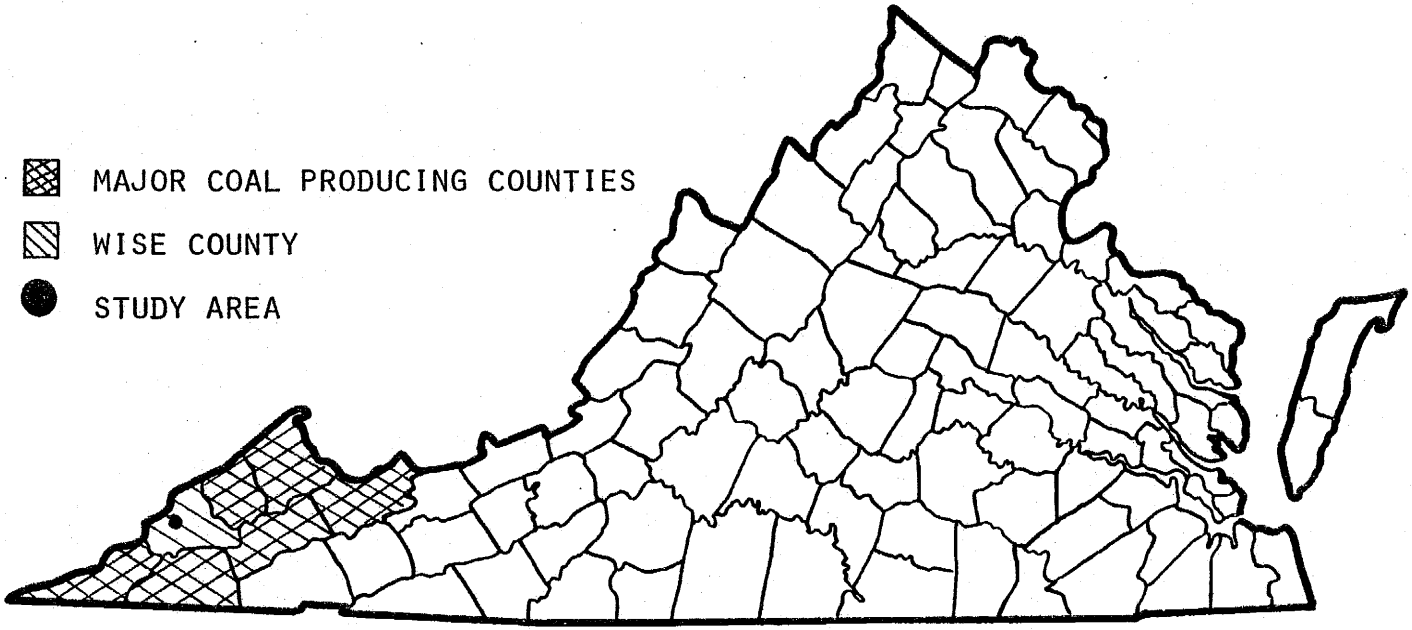

Figure 1. Coal producing region of Virginia and location of study area in Wise County.

reclamation regulations are followed. Meeting regulation requirements through the establishment of herbaceous cover usually helps to impede erosion; however, the herbaceous cover established on most mining sites is limited in terms of realizing the full productive potential of the land.

There is a challenge to establish a balance between resource utilization and conservation. Returning these disturbed coal spoils into productive land for agriculture, wildlife, water management, recreation, and perhaps industrial and residential development will help to achieve this balance. Revegetation research on these disturbed areas should be oriented toward these goals. Mays and Bengtson (1978) suggested that land of all kinds is too valuable to allow disturbed areas to remain with no further use or benefit in mind.

The Penn-Virginia Resources Corporation is addressing some of the problems and potential solutions regarding strip-mine reclamation through a cooperative interdisciplinary project with Virginia Tech on contour surface-mined land in Wise County. This particular study has been conducted to provide information for reforestation efforts in the region.

On many disturbed sites reforestation is a justifiable alternative for reclamation. Trees can be grown and managed for pulpwood, sawtimber, Christmas trees, or for wildlife habitat depending on land-use objectives. In addition, the establishment of new forests generally enhances aesthetic and environmental quality. In the past fifty years most research has focused on tree species' adaptability for revegetation and stabilization of strip-mines (Plass, 1975; Geyer, 1973; Miles et al., 1973; Czapowskyj, 1970; Czapowskyj and McQuilkin, 1966). In general, pines (Pinus spp.) have proven to be suitable because of their ability to survive and grow in harsh soil environments. However, each mine, or at least each mining region, is a unique case depending on local geology, mining practices, and edaphic factors. Research findings from one area do not necessarily hold for other areas because of the complex environmental factors involved. Bengtson et al. (1973a) emphasized that a slight change in a particular environmental parameter can change an environment favorable for a certain species into an inhospitable or toxic one.

Information concerning establishment and growth of tree seedlings on strip-mined lands in southwestern Virginia is very limited. Three pine species were chosen for this study based on their ability to survive and grow in harsh soil environments. Virginia pine (P. virginiana Mill.) and eastern white pine (P. strobus L.) are native to this region. Virginia pine grows well on poor eroded sites and is a good source of pulpwood (Plass and Burton, 1967). White pine thrives on many different sites and, once established, grows rapidly. It is a valuable source of christmas trees and sawtimber. Southwestern Virginia is West of the natural range of loblolly pine (*P. taeda* L.); however, loblolly pine has been planted on some coal spoils in this region. It is relatively easy to establish and grows rapidly on many types of disturbed sites producing pulpwood and lumber (Plass and Burton, 1967).

If the potential productivity of these sites is to be realized, intensive cultural treatments must be used to promote seedling establishment and growth. Treatments such as ectomycorrhizal inoculation, chemical weed control, and slow-release fertilization should help to achieve acceptable survival and growth to meet desired productivity levels.

Although successful pine seedling establishment on strip-mined sites in southwestern Virginia has been reported (Marx and Artman, 1979), these disturbed sites present an array of problems for reforestation. Failures in pine seedling establishment are common on drastically disturbed sites unless the seedlings are colonized by appropriate ectomycorrhizal fungi at planting time. There is substantial evidence that *Pisolithus tinctorius* (Pers.)

Coker and Couch (Pt) is ecologically adapted to mine-spoil conditions. Therefore, seedlings colonized with this ectomycorrhizal fungus should show improved survival and growth.

Surface-mine revegetation with dense herbaceous cover has generally been successful in this region. This vegetation, however, tends to compete with desirable tree seedlings for soil water, nutrients, and light. Certain herbaceous plants also have been shown to have an allelopathic effect on tree seedlings (Fisher, 1980; Larson and Schwarz, 1980; Priester and Pennington, 1978; Gilmore, 1977; Walters and Gilmore, 1976; Reitveld, 1975). When herbaceous vegetation competes significantly with tree seedlings, the competing vegetation can be controlled to release limited site resources. Little information is available on the effects of chemical weed control on surface-mine reforestation.

A third problem frequently encountered in strip-mine reforestation efforts is the lack of adequate supplies of available nutrients, particularly nitrogen (N) and phosphorus (P). Plant-available N and P in the original surface layers are often buried deeply during mining operations. Fertilizer applications are usually desirable to promote seedling establishment and growth; however, the effectiveness of these applications depends, in part, on the extent of herbaceous competition. Weed competition can be greatly stimulated by applications of soluble fertilizers and over-fertilization may result in overtopping of tree seedlings by excessive development of herbaceous cover (Mays and Bengtson, 1978).

Mays and Bengtson (1978) reported that strip-mine spoils may be highly porous, low in cation exchange capacity, and often lacking much of the fertilizer retention capacity of natural soils. Conventional fertilizer sources may leach rapidly through these disturbed soils. Slow-release fertilizer tablets offer a potential solution to this problem. Tablets placed adjacent to seedling roots provide maximum benefit for the seedling while minimizing the effect on competing weeds.

The effects of these three cultural treatments on early survival and growth of white, Virginia, and loblolly pine are not well documented for coal spoils in southwestern Virginia. Furthermore, the interactions among these treatments and the various edaphic factors influencing each treatment have not been thoroughly studied. The purpose of this research was to investigate the effects of these cultural treatments and a variety of edaphic factors on pine seedling survival and growth on two strip-mined sites in southwestern Virginia. The following objectives were established:

(1) quantify the survival and growth of *Pinus virginiana* Mill., *Pinus strobus* L., and *Pinus taeda* L. on strip-mined lands in southwestern Virginia.

(2) evaluate the effects of ectomycorrhizal inoculation, chemical weed control, and slow-release fertilization on the survival and growth of *P. virginiana*, *P. strobus*, and *P. taeda*.

(3) identify the edaphic factors affecting survival and growth on a recontoured site and a flat bench site.

LITERATURE REVIEW

The principal areas of investigation regarding reforestation of surface-mined lands have been the search for species tolerant to disturbed-land conditions and the development of appropriate cultural practices for tree establishment (Paone et al., 1978). This study was established to evaluate three cultural treatments used for establishing pine seedlings on strip-mine spoils already supporting herbaceous growth -- ectomycorrhizal inoculation, chemical weed control, and slow-release fertilization. Relevant literature concerning these three treatments is summarized below.

Ectomycorrhizal Inoculation

Mycorrhiza is the term used for a wide range of symbiotic associations between the fine feeder roots of plants and highly specialized root-inhabiting fungi (Marx, 1980). Anatomically, mycorrhizal feeder roots can be separated into three classes -- ectomycorrhizae, endomycorrhizae, and ectendomycorrhizae. Ectomycorrhizae are the predominant class occurring in forest tree species throughout the world. The association is characterized by the fungal hyphae growing internally around the primary cortical cells of the roots forming the Hartig net which may completely replace the middle lamellae between cortical cells. This Hartig net is the major distinguishing feature of ectomycorrhizae (Kormanik et al., 1977).

Plants with abundant ectomycorrhizae have an increased, physiologically active, root-fungus surface area for nutrient and water absorption. This increase in surface area is due to the multi-branching morphology of most ectomycorrhizae and from the extensive vegetative growth of fungal symbiont hyphae into the soil (Marx, 1977a). Extension of hyphae into surrounding soil assures maximum nutrient and water absorption by the host plant.

It is well-established that ectomycorrhizal roots increase phosphate uptake (Ford, 1982; Harley, 1978; Smith, 1974; Bowen and Theodorou, 1967). However, Kormanik et al. (1977) pointed out that although it is generally accepted that absorption of other nutrients is enhanced by ectomycorrhizae, results from studies on increased uptake of other macro- and micro-nutrients by ectomycorrhizal plants have been quite variable. These authors hypothesized that differential nutrient uptake may reflect the relative concentrations of elements in the soil. If an element is deficient and limiting plant growth, it will probably be found in higher concentrations in ectomycorrhizal plants than in nonectomycorrhizal plants. Marx (1977a) reported that ectomycorrhizal fungi also help decompose certain complex minerals and organic substances in the soil and transport nutrients from these materials to the plant. Studies by Walker et al. (1982), Duddridge et al. (1980), and Safir et al. (1971 and 1972) showed that improved water uptake was positively correlated with mycorrhizal colonization.

Ectomycorrhizal fungi promote the growth and development of plants in a number of other ways. Feeder roots colonized with ectomycorrhizal fungi have increased longevity (Marx and Barnett, 1974; Bowen and Theodorou, 1967). Furthermore, ectomycorrhizae may deter infection of feeder roots by root pathogens, which are common in many tree nurseries and forest soils, through the production of antibiotics (Marx, 1973). The fungal mantle is also a mechanical barrier to cortical cell infection by these root pathogens (Marx, 1973; Marx, 1969). Slankis (1973) suggested that ectomycorrhizae are involved in the synthesis and stimulation of growth hormones within the host plant. Ectomycorrhizal fungi provide host plants with auxins, cytokinins, gibberellins, and growth-regulating B vitamins.

Increased hormonal levels within the host plant could significantly influence growth and development.

Ectomycorrhizal fungi are abundant in soils of natural forests. Consequently, regenerating seedlings usually develop ectomycorrhizal associations in recently harvested forests. In contrast, pine reforestation of adverse, drastically disturbed sites usually fails unless seedlings are colonized by ectomycorrhizal fungi at planting time.

Some of the first observations and studies of ectomycorrhizae on surface-mine spoils were reported by Schramm (1966). He concluded that early ectomycorrhizal development with Pt was vital for seedling establishment of several tree species on anthracite spoils in Pennsylvania. Since Schramm's work, numerous other reports on the occurence of Pt on coal spoils and other adverse sites have been published (Medve et al., 1977; Marx and Barnett, 1974; Hile and Hennen, 1969; Lampky and Peterson, 1963). Pisolithus tinctorius was the dominant ectomycorrhizal fungus on pine roots in strip-mined coal spoils in Kentucky, Virginia, Alabama, Pennsylvania, Tennessee, Indiana, Missouri, and Ohio. Marx and Artman (1979) suggested that if Pt is frequently associated with pines growing on adverse site such as coal spoils, it can be assumed that Pt ectomycorrhizae contribute to the health of seedlings on these sites. A worldwide distribution and broad host range are additional chraracteristics of Pt (Marx, 1977b).

Marx and Artman (1979) noted that the poor performance of nursery seedlings after outplanting on many coal spoils may be partially due to the fact that the fungal symbiont on their roots at planting cannot tolerate adverse spoil conditions. There is evidence that Pt is ecologically adapted to extremes in spoil conditions frequently occurring on strip-mines. Studies by Marx et al. (1970), Marx and Bryan (1971), and Schramm (1966) demonstrated that pine seedlings with Pt have increased tolerance to extremes in soil acidity, high sulfur (S), aluminum (Al), and manganese (Mn) levels, and high soil temperatures. This ability of Pt to tolerate adverse soil conditions probably accounts for the variable response of pines to Pt on high and low quality reforestation sites. Marx et al. (1977a) reported that more growth stimulation due to the presence of Pt occurred on low quality sites than on better quality sites where nonmycorrhizal control seedlings grew well.

Marx et al. (1978) showed that lack of ectomycorrhizal fungi in former treeless areas could be circumvented by introducing ectomycorrhizal fungi into nursery soils in the form of duff or the upper layer of mineral soil from a mature forest. This method was reliable, but costly, and potentially dangerous because of the possibility of introducing harmful pathogens. Efficient experimental procedures have been developed to artificially inoculate fumigated nursery soils with pure cultures of Pt in order to form abundant Pt ectomycorrhizae on both bare-rooted and containerized pine seedlings (Ruehle, 1980; Marx and Artman, 1978; Marx et al., 1978; Ruehle and Marx, 1977; Marx et al., 1976; Marx and Barnett, 1974). Production of either a pure culture of Pt vegetative mycelia in a mixture of vermiculite, peat moss, and nutrient substrate or basidiospores mixed with a carrier such as vermiculite are used to inoculate nursery soils. Most methods for culturing vegetative inoculum of Pt follow the basic procedures of Marx and Bryan (1975).

The main factors influencing susceptibility of tree roots to ectomycorrhizal colonization are photosynthetic potential and soil fertility. High light intensity and low soil fertility enhance mycorrhizal development (Marx, 1977a). Roots growing rapidly because of high soil fertility were not highly susceptible to colonization (Marx et al., 1977b). In addition, factors directly affecting the mycorrhizal fungus, such as extremes in soil temperature, pH, and moisture, and presence of antagonistic soil microorganisms can affect survival of the symbiont (Marx, 1980).

The introduction of Pt into nursery soils under a variety of treatments has significantly improved pine seedling quality in comparison to seedlings with naturally occurring ectomycorrhizal fungi such as _Thelephora terrestris_ (Ehrh.) Fr. Marx et al. (1976) artificially introduced inocula of Pt into soils of a nursery in North Carolina. After one growing season, growth stimulation by Pt ectomycorrhizae from vegetative inoculum was 140 percent on loblolly pine seedlings and approximately 100 percent on Virginia and white pine seedlings. In a similar study on nursery plots in Georgia (Marx and Bryan, 1975), inocula of Pt were used to infest fumigated soil and synthesize ectomycorrhizae on loblolly pine seedlings. Under a number of different mulch and temperature conditions, Pt completely colonized roots of the loblolly seedlings during an eight month growth period. The biomass production of Pt ectomycorrhizae was twice that of seedlings ectomycorrhizal with naturally occurring fungi such as _T. terrestris_. More recently, Marx et al. (1978) tested the effects of fumigation on growth and ectomycorrhizal development of loblolly pine seedlings. They found that inocula of Pt, _T. terrestris_, and _Cenococcum geophilum_ Fr. (syn. _C. graniforme_ (Sow.) Ferd. and Winge) were more effective in forming ectomycorrhizae in fumigated soil than in nonfumigated soil.

Vozzo and Hacskaylo (1971) and Marx and Artman (1978) also reported the beneficial effects of seedlings inoculated with Pt in the nursery.

Unfortunately, limited information is available concerning the value of ectomycorrhizae to containerized seedlings. Containerized seedlings have only recently been viewed as a valuable alternative to conventional bare-root seedling production. Containerization has a number of advantages over bare-root production including more efficient use of quality seed and flexible seedling production due to extended planting periods (Balmer, 1974). Marx and Barnett (1974) were the first to inoculate containerized loblolly pine seedlings with Pt basidiospores. In a study published in 1977, Ruehle and Marx investigated the best combination of growing medium, fertilization, and inoculation techniques for satisfactory ectomycorrhizal development on container-grown loblolly pine seedlings. Their results indicated that Pt has potential for widespread use as a symbiont in containerized programs if the proper combination of fertilizer and growing medium is used. More recently, a number of studies have reported successful Pt inoculation of a variety of container-grown tree species (Riffle and Tinus, 1982; Navratil et al., 1981; Maronek and Hendrix, 1980; Dixon et al., 1979; Molina, 1979).

Vegetative Pt inoculum has been successfully produced with industrial fermentation equipment. Marx et al. (1982) recently reported that commercially produced inoculum of Pt was used to form abundant ectomycorrhizae on container-grown seedlings of a variety of tree species.

The value of Pt ectomycorrhizae, using both bare-root and containerized seedlings, to the improved survival and growth of pine seedlings has been demonstrated on disturbed sites in a number of studies. Results of a study conducted on severely eroded sites in the Copper Basin in Tennessee showed that bare-rooted loblolly and Virginia pine seedlings colonized with Pt were better adapted for rapid growth on these sites (Berry and Marx, 1978). Nursery-grown bare-root seedlings of loblolly and shortleaf pine (*Pinus echinata* Mill.) with Pt ectomycorrhizae survived and grew significantly better than seedlings with *T. terrestris* after three years on an acid coal spoil in Kentucky and four years on an acid coal spoil in Virginia. Seedlings with Pt grown on the Kentucky spoil had significantly more N and less iron (Fe), S, Mn, and Al in needles than seedlings with *T. terrestris* after three years (Marx and Artman, 1979). More recently, Berry (1982) reported that container-grown seedlings of pitch x loblolly hybrid pine (*P. x rigitaeda*) with Pt ectomycorrhizae grew faster than seedlings with naturally occurring ectomycorrhizae on coal spoils in Alabama and Tennessee. Seedlings with Pt also had lower foliar concentrations of potentially toxic Mn. Results of a study by Ruehle et al. (1981) indicated that mycorrhizal treatment with Pt provided no consistent advantage for survival and growth of containerized seedlings on reforestation sites in Arkansas and Oklahoma. In the same study, bare-root seedlings with ectomycorrhizae formed by Pt showed increased survival and growth over control seedlings. The conflicting results of these recent studies suggest that more research is needed on ectomycorrhizal container-grown seedlings planted in various environments.

Weed Control

Establishing a closed pine stand may be a desirable way to reduce or permanently stop erosion from bare sites. However, pines often require up to ten years after planting to drop sufficient litter to fully protect a site (Duffy, 1974). Mays and Bengtson (1978) suggested that it is generally better to establish a quick cover of herbaceous plants after grading and to plant trees into this cover, usually several months to a year later. According to Vogel and Curtis (1978), planting of both trees and herbaceous species is often desirable and is required in reclamation of surface-mine spoils in the East. The herbaceous species provide rapid erosion control, and the trees provide long-term cover and contribute to the reestablishment of a forest ecosystem.

Unfortunately, herbaceous cover usually competes with planted tree seedlings, especially when the herbaceous cover is established first. Competitive effects are usually the result of allelopathy, decreases in soil nutrients, soil moisture, and intensity and quality of light (Klingsman and Ashton, 1975). These authors suggested that increased seedling mortality and slower growth rates in the presence of herbaceous competition may result in increased reforestation costs. Under circumstances where herbaceous cover competes with desirable tree species, the herbaceous species can be considered as weeds.

The amount of available soil moisture is usually the most important consideration in evaluating the effects of vegetative competition. Grasses in particular deplete root zone moisture near young pines. Greaves et al. (1978b) reported that heavy stands of grass may deplete moisture to the point where conifer seedlings must survive for several months each year with minimum available moisture.

Grasses which deplete moisture early in the summer usually cause the most seedling mortality (Newton and

Knight, 1981). Eleven species of tree seedlings, including loblolly and Virginia pine, were interplanted in stands of sericea lespedeza (Lespedeza cuneata (Dumont) G. Don) on kaolin clay strip-mine spoils in Georgia and South Carolina (May et al., 1973). First year survival of all species was below fifty percent and those that did survive grew poorly. The authors hypothesized that competition for moisture influenced seedling survival and growth.

Results of a study on coal spoils in Alabama by Bengtson and Mays (1978) indicated that grass competition was more significant with respect to moisture than nutrients in restricting loblolly pine growth because nutrient concentrations in pine foliage were similar on bare and grass-seeded plots.

In a field study on coal mine spoils in Kentucky, Vogel (1973) measured the effect of herbaceous cover on tree survival and growth. In some plots grasses -- K-31 fescue (Festuca arundinacea Schreb.) and weeping lovegrass (Eragostis curvula Schrad.) -- alone or grass with legumes -- sericea lespedeza and Korean lespedeza (Lespedeza stipulacea Maxim.) -- were planted with four tree species -- Virginia pine, loblolly pine, cottonwood (Populus deltoides Bartr.) and sycamore (Platanus occidentalis L.) In other plots trees were planted without grasses or legumes. After three growing seasons, the herbaceous vegetation (95 percent ground cover) had not significantly decreased tree survival, but had significantly inhibited tree growth. A cover of grass alone suppressed tree growth the most. It is interesting to note that during the fourth and fifth years, growth of trees in the grass plus legume plots exceeded the growth of trees in the other plots. It was assumed that the lespedeza was providing N which was deficient in the other treatment plots.

Bengtson et al. (1973b) found that after three years of growth on a coal spoil in Alabama, loblolly pines had difficulty surviving when seeded with bermuda grass (_Cynodon dactylon_ (L.) Pers.) and K-31 fescue. Although seedling survival was still low, pines planted one year after grass seeding were better able to survive grass competition.

Ruehle (1980) reported that dense grass cover caused considerable mortality of container-grown pine seedlings in sludge-amended plots in borrow pits. Results from a more recent study (Ruehle et al., 1981) showed that dense weed cover caused considerable overtopping and competition to container-grown pines but not to bare-root pines. This result suggests that the use of container-grown seedlings may require intensive site preparation to reduce first year weed competition.

Herbaceous vegetation is not necessarily detrimental to tree seedling survival and growth. Struthers and Vimmerstedt (1965) reported that planting both trees and forage on mine spoils contributed to faster soil formation. They also reported that herbaceous cover did not compete with the trees. Results of a study on spoil banks in eastern Kentucky indicated that survival and growth of white and loblolly pines were not significantly affected by being planted into a stand of established K-31 fescue (Plass, 1968). In some cases, the amount of available nutrients may be higher beneath herbaceous vegetation through litter production and increased nutrient cycling (Greaves et al., 1978b; Vogel, 1973). These authors also suggested that legumes may increase total N under their canopies by nitrogen fixation or by accumulating large amounts of N-rich litter in newly formed organic layers. In addition, temperature extremes, which can be fatal on strip-mine spoils (Schramm, 1966), are moderated by the presence of herbaceous cover (Richardson, 1958).

When the net effect of herbaceous vegetation in a tree-seedling environment is negative, the competing vegetation must be controlled to release limited resources, particularly available soil moisture, for the desired tree species. Greaves et al. (1978b) pointed out that the competitive influence of vegetation depends on its stage of development and growth habit compared to that of the seedling. Carpenter and Alpers (1981) recommended that successful reforestation of strip-mine sites containing dense herbaceous vegetation required effective control of the ground cover. They found that water stress of European alder (Alnus glutinosa L.) seedlings was increased by herbaceous vegetation.

Nonchemical methods of weed control tend to disturb soils, and are of limited duration (Newton and Knight, 1981). Selective weed control which does not physically damage the site can be obtained using herbicides. Greaves et al. (1978a) suggested that the objectives in releasing conifer seedlings are to increase the amount of light and to decrease herbaceous competition for soil moisture and nutrients. These objectives can often be achieved without complete eradication of herbaceous cover, thereby maintaining some degree of effective erosion control.

Several reports have described significant responses in pine growth to chemical weed control during the first few years of plantation establishment. Haines (1978) evaluated loblolly pine seedling growth on plots where vegetation was controlled using a variety of herbicides including simazine, paraquat, 2,4-D, and 2,4,5-T. Herbicide treatments were much more beneficial to seedling growth than discing or mowing treatments. Atrazine applied as a preplanting treatment was evaluated for the control of perennial grasses and the establishment of jeffrey pine (P. jeffreyi Grev. and Balf.) and ponderosa pine (P. ponderosa Laws.) transplants in Nevada (Eckert, 1979). The atrazine treatment reduced grass biomass by an average of 72 percent over a three year period and increased the average survival of transplants from one percent on the control plots to 66 percent on the treatment plots. High survival and increased growth of these transplants with weed control were attributed to low soil moisture tension and increased N accumulation through the initial growing season and subsequent years. Two studies by Nelson and coworkers (1981a and 1981b) demonstrated the benefits of weed control to loblolly pine seedlings during the first three years of growth. In the 1981a study, seedlings with 100 percent weed control for two years using hexazinone were 100 percent taller, nearly 300 percent greater in ground line diameter, and 1200 percent larger in volume than trees in control plots. The authors noted that significant gains in growth were also made after one year. In the 1981b study, precipitation measurements and plant moisture measurements indicated that weeds depleted the soil moisture necessary for maximum pine height development. Weed control with hexazinone increased pine biomass by up to 12 times that on unsprayed plots.

Holt et al. (1975) reported conflicting results from weed control experiments using combinations of simazine and atrazine. One test site demonstrated a significant response of 1-0 loblolly pine seedlings in height increment and stem diameter. Remeasurements after two growing seasons indicated that the released seedlings maintained an increase in growth over the untreated seedlings. A second test site showed no reponse to weed control because of high rainfall and a lack of vegetative development in control plots.

Research on the effects of glyphosate (trade name, Roundup) on tree-seedling establishment is lacking. However, because of its nonselectivity, glyphosate offers considerable promise for control of a wide variety of herbaceous weeds. Glyphosate provides effective control of perennial grasses and broadleaf weeds. Klingsman and Ashton (1975) and Newton and Knight (1981) have presented relevant information regarding this chemical.

Few reports are available concerning the effects of herbicides on ectomycorrhizal fungi. Marx and Barnett (1974) speculated that herbicides are generally selective enough to be of minimum harm to mycorrhizal fungi. Smith and Ferry (1979) presented results which indicated that simazine did not inhibit ectomycorrhizal development in Scotch pine (P. sylvestris L.) and Austrian pine (P. nigra Arnold), and it may, under some conditions, actually enhance mycorrhizal formation. Iloba (1976) found that amitrole and dalapon, applied at recommended rates, adversely affected ectomycorrhizal colonization of Scotch pine. Kelley and South (1980) evaluated the effects of various herbicides and rates of application on growth of Pt in culture. Their results indicated that Pt was insensitive to all of the herbicides tested at recommended application rates. The effects of glyphosate on ectomycorrhizal colonization of white, loblolly, and Virginia pines are presently unknown.

Results of most studies show that increased pine seedling growth can be obtained when weed competition is controlled. However, there have been some conflicting results regarding weed control. Further research is needed to fully elucidate the effects of herbaceous competition on seedling establishment and growth on strip-mined sites. A strong response to chemical weed control may provide improved survival and growth of pine seedlings on coal spoils with dense herbaceous cover. Other potential benefits from chemical weed control, particularly the enhancement of early fertilization effects, suggest further investigation.

Slow-Release Fertilization

The effectiveness of fertilizer applications in promoting survival and growth of tree species depends, in part, on the extent of herbaceous competition on the site. Bengtson (1977) stated that there are many situations where N and P applied at planting or soon thereafter have proven to be effective. But these successful fertilization applications were almost always accompanied by successful weed control. Bengtson (1977) also noted that weed competition is greatly stimulated by applications of soluble fertilizers. Overfertilization, especially with N, may result in overtopping of tree seedlings by excessive development of herbaceous cover (Mays and Bengtson, 1978). This usually results in increased seedling mortality and decreased growth. Optimal response to fertilization can be expected when soil moisture is available for a large part of the growing season. Favorable responses may decrease if soil moisture is limited.

The lack of N in spoil materials of eastern coal fields has been reported by many workers including Wittwer et al. (1979), Bennet et al. (1978), Mays and Bengtson (1978), Vogel (1975), Bengtson et al. (1973a), Czapowskyj (1973), Plass and Vogel (1973),and Vogel and Berg (1973). During mining operations, surface layers which contain plant-available N are usually buried too deeply to be available to plants.

Phosphorus supplies are often moderately to severely deficient on strip-mined sites (McFee et al., 1981; Wittwer et al., 1979; Mays and Bengtson, 1978; Bengtson et al., 1973; Czapowskj, 1973; Plass and Vogel, 1973; Vogel and Berg, 1973; Berg and May, 1969). Plass and Vogel (1973) analyzed ten samples from each of 39 spoils in southern West Virginia. They found P concentrations very low in 52 percent, low in 35 percent, and medium in 13 percent of the samples tested.

Subsoil minerals on strip-mined sites usually contain potassium (K) in sufficient amounts to provide enough K release from natural weathering of spoil for plant growth. The literature contains little evidence that deficiencies in secondary and micro-nutrients limit plant growth on mine spoils. Spoils of high acidity may have contents of available Fe, Mn, Al, and Zn which approach phytotoxic levels (McFee et al., 1981; Barnhisel and Massey, 1969; Berg and Vogel, 1968).

Fertilizer applications to coal-mine spoils and other drastically disturbed sites have usually resulted in enhanced tree seedling growth. In greenhouse experiments conducted by Bengtson et al. (1973a), loblolly pine seeded on coal-mine spoil responded dramatically to a N-P fertilizer. This response was enhanced when mycorrhizal inoculum in the form of fresh pine duff was added to the spoil at the time of seeding. Zarger et al. (1973) evaluated the effects of varying rates of N and P alone and in combination, on early growth of 1-0 loblolly pines outplanted for one year on a reclaimed coal-mine spoil in northeastern Alabama. Growth increases of as much as 125 percent were observed when N and P were broadcast in combination.

In a subsequent study, Bengtson and Mays (1978) reported that growth of established loblolly pine seedlings on infertile sandstone-derived coal spoil was greatly increased by broadcast application of N-P fertilizer. In contrast, Funk and Kramer (1965) reported that after two years, white pine survival was decreased and growth was unaffected by fertilization pellets on strip-mined land in Ohio.

More recently, Berry and Marx (1980) found that a 560 kg/ha application of commercial 10-10-10 fertilizer did not improve loblolly seedling growth on a borrow pit in South Carolina. It is of interest to note that in this study loblolly seedlings grown on plots treated with dried sewage sludge were significantly larger than seedlings grown on plots without the sludge.

Mays and Bengtson (1978) indicated that strip-mine spoils may be highly porous, low in cation exchange capacity, devoid of plant roots, and often lacking much of the fertilizer retention capacity of natural soils. Conventional fertilizer sources may leach rapidly through these disturbed soils. Slow-release fertilizers offer a potential solution to this problem.

Slow-release fertilizer tablets were first developed in the late 1950's by Austin and Strand (1960). Sharma (1979) pointed out some of the advantages of slow-release fertilizers which include reduced nutrient loss via leaching and runoff, reduced chemical and biological immobilization reactions in the soil which result in plant-unavailable forms, reduction of rapid nitrification and N loss through ammonia volatilization and denitrification, reduced seedling damage from high local concentrations of salts, reduced leaf burn from heavy rates of surface-applied fertilizers, and availability of nutrients during the entire growing season.

Urea-aldehyde condensation products are a commonly used form of slow-release fertilizer. Agriform slow-release tablets (Sierra Chemical Company, Milpitas, CA) are in this fertilizer group. They contain urea which reacts with formaldehyde to form compounds which are sparingly soluble in water (Sharma, 1979).

Berry (1979) used two sizes of Agriform slow-release tablets and three levels of dried sewage sludge to fertilize loblolly pine seedlings on a severly eroded, infertile site in the Tennessee Copper Basin. When the seedlings were planted, fertilizer tablets or sludge were placed 8 cm deep in closing holes or slits created with a planting bar. Survival was not affected by any of the treatments. However, the relative effectiveness of the treatments on seedling volume was evident after the second and third seasons. At the end of three seasons, the 21 g and 9 g fertilizer tablets increased seedling volume 20 and 9 fold over that of the unfertilized control seedlings, whereas the 90,60, and 30 g sludge treatments increased volume by eight, four, and three fold over control seedlings. The author enumerated the benefits of slit applications of nutrients which include convenience on rugged terrain, low cost, and minimal stimulation of competing vegetation.

Wittwer et al. (1979) tested the effects of 9 g fertilizer tablets and bark mulch on the survival and growth of three species of oaks (<u>Quercus</u> spp.) and Virginia pine seeded on strip-mine spoils in southeastern Kentucky. The fertilizer tablets were placed approximately 2 cm away from each seed spot at the time of planting. First-year survival of all tree species was significantly improved by the combination of mulch and fertilizer. First-year height growth was significantly stimulated by the mulch and the fertilizer treatments; and the combination of treatments resulted in an additional growth increase over the individual treatments.

Considerable work has been done on the interaction between fertilization and ectomycorrhizal development. Schramm (1966) applied different nutrients to chlorotic Virginia pine seedlings on anthracite spoils in Pennsylvania. After nitrate fertilization, the seedlings regained normal color, but this recovery was temporary. This result indicated that seedlings growing in substrates low in N were dependent on ectomycorrhizal fungi to meet N requirements for adequate growth and development.

Kormanik et al. (1977) summarized the current knowledge of the effects of P levels on mycorrhizal development. High levels of soluble phosphates in the soil depressed mycorrhizal development, and low levels adversely affected plant growth. Plants grown in soils with low quantities of available phosphates showed heavy mycorrhizal development and grew much better than nonmycorrhizal ones. Little or no growth differences were observed between mycorrhizal and nonmycorrhizal plants when high levels of soluble phosphates were added to growing media.

The effect of combining fertilization and ectomycorrhizal inoculation on tree growth was illustrated by the following studies. Using severly eroded clay soils, Berry and Marx (1976) evaluated the effects of Pt inoculation and four levels of sewage sludge on the growth of shortleaf and loblolly pine seedlings. All four levels of sludge significantly increased growth of both pine species. However, Pt increased seedling growth at only one sludge level for either species. In this study, there was no significant interaction between the sludge and mycorrhizal treatments. Marx et al. (1977a) observed that in nonfertilized plots on a reforestation site in Florida, slash pine (P. elliottii Englem.) seedlings with Pt survived better and grew more than twice as fast as seedlings having natural ectomycorrhizae. In contrast, Pt did not improve either survival or growth of slash pine in fertilized plots. Fertilizer alone substantially increased growth of slash pine regardless of initial ectomycorrhizal treatment.

In a study by Marx and Artman (1979) bare-rooted loblolly and shortleaf pine seedlings inoculated with Pt had significantly greater plot volume indices (PVI)[1] than seedlings with naturally occurring ectomycorrhizae during three years of growth on an acid coal spoil in Kentucky. By

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[1] PVI,cm^3=(root collar diameter,cm)^2 x height,cm x number of seedlings surviving per plot.

the second year, seedlings of both species with slow-release fertilizer tablets and Pt inoculation had significantly greater PVI's than seedlings with any other treatment combination. However, the additive effect of the fertilizer-Pt combination was not present for the loblolly seedlings after the third year. The fertilizer-Pt combination did continue to significantly stimulate shortleaf pine growth during the third year.

Walker et al. (1982) used fertilization and Pt inoculation as treatments for Virginia pine and loblolly pine seedlings on a coal-mine spoil in Tennessee. After three years, Pt colonization had significantly increased the survival and growth of loblolly pine irrespective of fertilization; however, after two years, Virginia pine was not significantly affected by Pt colonization. Broadcast fertilizer increased seedling growth but reduced survival for both species irrespective of Pt inoculation. The poor survival of fertilized seedlings was attributed to increased herbaceous competition and an imbalance in shoot/root ratios. In contrast to the results of Marx and Artman (1979), Walker et al. (1982) reported no significant additive effect of the two treatments on PVI. This was probably a result of the poor survival of fertilized seedlings.

The variable results of fertilization-ectomycorrhizae experiments indicate that further research is needed to develop fertilization regimes that are compatible with other cultural treatments such as ectomycorrhizal inoculation and weed control. Information concerning the effects of these cultural treatments on survival and growth of Virginia and white pines, in particular, is lacking.

MATERIALS AND METHODS

Study Sites

The two study sites chosen for this project were located in Wise County, Virginia on strip-mined properties owned and managed by the Penn-Virginia Resources Corporation. Seedling survival and growth were evaluated on both a recontoured and a flat bench site.

The recontoured site was located at 82° 43'W longitude and 36°55'N latitude near the junction of Routes 603 and 23, approximately 8 km West of Norton. This site, which had a western aspect, was mined and recontoured in 1979. It was hydroseeded in March, 1980 with a combination of K-31 fescue, sericea lespedeza, red top (Agrostis alba L.), ladino clover (T. repens ladino L.), and annual rye (Lolium multiflorum Lam.) in mixture with 560 kg/ha of 10-20-20 fertilizer and 1681 kg/ha of wood fiber mulch. Eighteen plots were selected at this site in May, 1981. Elevation ranged from 580 to 610 m and the average slope of the plots was 38 percent.

The flat bench site was located at 82° 42'W longitude and 37° 01'N latitude near Route 620, approximately 13 km

East of the town of Norton. Elevation ranged from 790 to 825 m. The average slope of plots on this site was less than two percent. The bench site was equally divided between two adjacent benches of similar age and site conditions. The lower bench was mined in 1977 and hydroseeded in 1978 with a combination of K-31 fescue, annual rye, red top, ladino clover, and red clover (_Trifolium_ _pratense_ L.) in mixture with 409 kg/ha of 16-27-14 fertilizer and 9354 liters/ha of Conweb mulch. The upper bench was mined in 1978 and hydroseeded in 1979 in the same manner as the lower bench. Nine plots on each bench were selected in September, 1980.

The climate of Wise County is characterized by moderately cold winters and relatively cool summers with precipitation distributed evenly throughout the year. The mean annual precipitation for Wise County is 121 cm (Table A1). The growing season, defined as the period between the average date of the last frost in Spring (May 5) and the average date of the first frost in Fall (October 9) is 157 days. This is one of the shortest growing seasons in Virginia.[2]

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[2] Climatological Summary No. 20-44, Wise, VA.

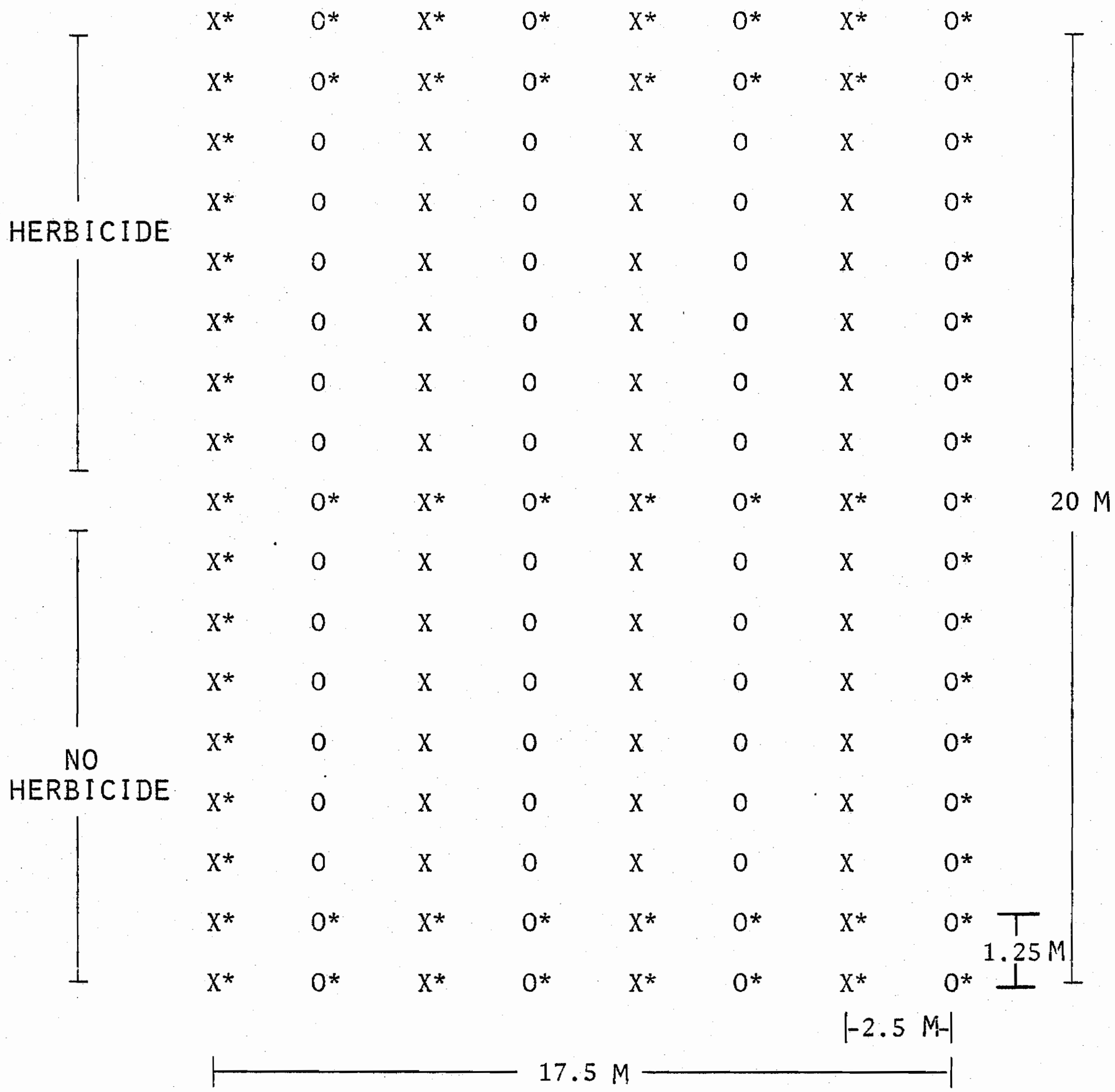
Plot Design

On each of the two study sites three tree species (*P. taeda*, *P. virginiana*, and *P. strobus*), and two fertilizer treatments were evaluated in factorial combination. In addition, two mycorrhizal treatments and two herbicide treatments were imposed on these combinations as split plots (Table A2).

Each of the thirty-six 350 m² (17.5 X 20.0 m) plots located at the two study sites was planted with 136 trees of one of the three species using a 1.25 X 2.5 m spacing. In addition, each plot was separated from adjacent plots by at least 3 m on all sides. Of the 136 trees planted, 64 were buffer trees and 72 were test trees. Each plot was divided into four split plots. This accomodated the mycorrhizal and herbicide treatments. The two mycorrhizal treatments were alternated among the eight rows of each plot, whereas the two herbicide treatments were randomly divided between two halves of each plot (Figure 2).

Seedling and Inoculum Production for Outplanting

Loblolly, Virginia, and eastern white pine seeds were obtained from the Virginia Division of Forestry. Seeds of all three species were soaked in distilled water for 36 hours, then bubbled in one percent hydrogen peroxide for 30



X*	O*	X*	O*	X*	O*	X*	O*
X*	O*	X*	O*	X*	O*	X*	O*
X*	O	X	O	X	O	X	O*
X*	O	X	O	X	O	X	O*
X*	O	X	O	X	O	X	O*
X*	O	X	O	X	O	X	O*
X*	O	X	O	X	O	X	O*
X*	O	X	O	X	O	X	O*
X*	O*	X*	O*	X*	O*	X*	O*
X*	O	X	O	X	O	X	O*
X*	O	X	O	X	O	X	O*
X*	O	X	O	X	O	X	O*
X*	O	X	O	X	O	X	O*
X*	O	X	O	X	O	X	O*
X*	O	X	O	X	O	X	O*
X*	O*	X*	O*	X*	O*	X*	O*
X*	O*	X*	O*	X*	O*	X*	O*

X = INOCULATED SEEDLING
O = UNINOCULATED SEEDLING
* = BUFFER SEEDLING

Figure 2. Plot design.

minutes, drained, and stored in plastic bags at 2 C. After stratification, germination was at least 75 percent for all three species.

Spencer-Lemaire Hillson root trainers were filled with a sterilized peat-vermiculite mixture. Each root trainer cavity was seeded with 2-3 seeds between January 6th and January 8th, 1981. After germination, seedlings were thinned leaving the most vigorous seedling in each cavity.

For the first four weeks seedlings were watered with approximately 15 ml of tap water per cavity as needed to maintain adequate moisture for vigorous growth. Weekly nutrient applications were then made as outlined in Tables A3 and A4.

Inoculum was prepared in 250 petri dishes, each containing a mixture of 90 cc vermiculite, 8 cc peat, and 60 ml Hagem nutrient solution (Table A5). After autoclaving the plates and their contents for 15 minutes at 120 C and 20 psi, three Pt (strain VT 716) mycelial plugs (3-5 mm diameter) were asceptically transferred to each plate. Pt cultures were allowed to develop in these plates at room temperature for five weeks.

In order to facilitate Pt inoculum distribution to the roots, an inoculum slurry of Pt mycelia and 30 liters of distilled water was homogenized for 15 seconds in a Waring blender. Thirty liters of water were required to provide 10 ml slurry aliquots to one half of the seedlings. Prior to homogenization, all Pt growth was removed from the petri plates, placed in an 80 mesh soil sieve, and rinsed with tap water followed by distilled water (Marx, 1969). The slurry was prepared at room temperature and stored in sterilized Nalgene containers at 2 C.

To inoculate the seedlings a small hole was opened in the growth medium of each cavity by inserting an alcohol-flamed glass rod along the seedling stem to a depth of approximately 5-6 cm. This was done to increase the probability that Pt hyphal fragments in the slurry would come in direct contact with the root system. A 10 ml aliquot of inoculum slurry was applied to the soil surface of the cavity in the vicinity of the hole. The hole was closed by gently tapping the surrounding soil surface with the glass rod. Control seedlings were treated in a similar manner with 10 ml of distilled water.

In late May seedlings were removed from the greenhouse to acclimate them to outside temperatures.

Thirty-one uninoculated seedlings and 31 inoculated seedlings of each species were destructively sampled and evaluated for the presence of Pt. This entailed removing seedlings from the cavities, rinsing the roots with tap water to remove the growth medium, and observing the roots under a dissecting microscope. In addition, the roots exposed along the inside walls of 707 cavities were inspected for Pt. This provided an estimate of the percentage of uninoculated and inoculated seedlings colonized with Pt or with other ectomycorrhizal fungi at the time of outplanting.

The seedlings were outplanted on both sites during June 8,9, and 10, 1981. Three planting crews were statistically blocked as three replications to account for any variation in planting techniques among the crews.

Treatments

Three treatments were implemented in this study. These included Pt ectomycorrhizal inoculation, slow-release fertilization using 20-10-5 Agriform fertilizer tablets, and vegetative competition control using Roundup herbicide (Table A2).

The previously-described inoculation procedure was designed to produce Pt ectomycorrhizae on one half of the seedlings of each species. The rows in each plot were planted alternately with inoculated seedlings and uninoculated seedlings of a given species (Figure 2).

The second treatment entailed placing a 21 g Agriform tablet into the soil at a depth of 10 cm within 10-15 cm of each seedling (Berry, 1979) in 18 of the 36 plots at the same time the seedlings were planted. This provided fertilization for one half of the seedlings of each tree species at both sites. The fertilizer tablets are formulated to supply sufficient N, P, and K for two years of seedling growth while minimizing the risk of 'burning' due to salt effects. The specific chemical formulation of these tablets, as described by the manufacturer (Sierra Chemical Company), is listed in Table A6. The tablets are designed to release nutrients during the growing season through the activity of soil bacteria. As a control for fertilization effects, seedlings in the remaining 18 plots were planted without fertilizer tablets.

Roundup, with 41.0% isopropylamine salt of glyphosate as the active ingredient, is a water soluble liquid which is relatively nonselective and is effective on perennial, annual, and biennial species of grasses and broad-leaved weeds (Klingsman and Ashton, 1975). These are the types of vegetation which were observed on the two sites prior to this study. Due to the dense stands of herbaceous vegetation, discings of bench site plots were conducted on February 27, 1981, and on April 31, 1981.

Sixteen days prior to planting, Roundup was sprayed at a rate of 1.5 liters/ha on one randomly selected half of each of the 18 plots at the recontoured site (Fig. 2). Roundup was applied at the same rate to one randomly selected half of each bench site plot one day prior to planting. A second application of Roundup was sprayed at the same rate and on the same plot halves as described above in August to maintain total control of vegetative competition throughout the growing season.

Site Characterization

The two study sites were characterized by analyzing physical and chemical soil parameters, soil moisture, and soil temperature in each plot. In addition, the slope of each plot and weekly precipitation were measured. An average of three clinometer readings was used to estimate the percent slope for each plot.

Preliminary soil samples collected within three 400 m^2 areas at the recontoured site were air dried at room temperature, ground, and sieved through a 2 mm screen. These samples were analyzed for available P and soil pH. Available P was determined by the ascorbic acid method after double acid (0.05 N HCl and 0.025 N H2SO4) extraction. Soil pH of each sample was determined using a glass electrode in a 1:1 soil to distilled water mixture.

The high variablility of available P within the sampled areas suggested as many as 200 subsamples per composite sample for each plot to maintain the error due to sampling at 10 percent (Tables A7 and A8). In addition, the high levels of available P which resulted from the double acid extraction suggested the use of a sodium bicarbonate extraction for estimating available P. The low variability of pH values within the sampled areas indicated only seven subsamples per composite sample for each plot to maintain the sampling error at 10 percent (Tables A9 and A10). Considering the time constraints, financial constraints, and the intended use of the soil chracterization data in this study, 15 subsamples per composite sample for each plot were used to analyze the physical and chemical characteristics of the soil.

One week prior to planting, 15 soil subsamples were taken at random locations within each plot to a depth of 20 cm. Each set of 15 subsamples was combined into one composite soil sample. Soil samples were dried at room temperature, ground, and sieved through a 2 mm screen. Standard methods of analysis currently used by the Tree Nutrition Research Laboratory at Virginia Tech were used to measure total organic matter (Broadbent, 1965), particle size distribution (Day,1965), total N (Bremner, 1965), and soil pH (Peech, 1965). Available P was determined colorimetrically using the ascorbic acid method (Watanbe and Olsen, 1965) following extraction with sodium bicarbonate (Olsen et al., 1954). Total magnesium (Mg), calcium (Ca), and K were determined by extraction with 1 N ammonium acetate adjusted to pH 7 and analyzed by atomic absorption spectrophotometry (Jackson, 1958).

Because of the high percentage of coarse fragments in the soil the routine bulk density procedure used at Virginia Tech had to be modified. For each sample, a volume of soil was removed and returned to the laboratory for rock and soil particle weight determination. Each hole resulting from soil removal was lined with a plastic bag and carefully filled with clean, dry sand. The volume of each hole was estimated by measuring the volume of sand required to fill each hole. The volume of rock in each soil sample was determined in the laboratory by measuring the volume of water displaced by the rocks. The bulk density of the soil size particles could then be calculated by dividing soil dry weight by soil volume.

Potential vegetative competition as indicated by biomass per area was measured in September, 1981. In three 0.25 m^2 plots randomly located in the unsprayed half of each plot, all of the vegetation above the soil surface was clipped and returned to the laboratory for dry weight biomass determination.

Climatic and Edaphic Factors

Soil temperature was measured on a weekly basis using standard soil moisture-temperature cells and a moisture-temperature alternating current ohmmeter. The cells contain a small thermistor for temperature determination. One cell was placed in each plot at a soil depth of approximately 15 cm. At both sites, a cell was placed in the sprayed half of nine randomly selected plots and the unsprayed half of the remaining nine plots.

Soil samples were collected for gravimetric analysis from the sprayed and unsprayed halves of each plot at intervals beginning in mid June and ending in late October. Each gravimetric sample was a composite of three subsamples collected from the same areas in each plot throughout the sampling period.

Soil moisture retention curves were derived for soils from each site in order to relate percent moisture values from the gravimetric samples to water potentials (Figures A1 and A2). The water retension curves were developed by placing samples in a pressure plate and drying to five water potentials: -0.01, -0.03, -0.10, -0.30, -0.50, and -1.50 MPa).

Weekly precipitation was recorded from mid June through mid November using one rain gauge at each site. A measured volume of mineral oil was maintained in the gauges to minimize evaporation between measurement dates.

Tree Survival, Growth, and Nutrient Status

Initial percent survival and initial height were measured three weeks after planting. In late November, 1981, the final measurements of survival, root collar diameter, and height were conducted.

In late November, 1981, needle samples from each living test tree were collected and composited by treatment combination. The foliage samples were dried at 65 C and ground in a Wiley mill to pass through a 20 mesh sieve. Ten milliters of 6 N HCl followed by ignition in a muffle furnace were used to extract P, K, Ca, and Mg from each sample. Extractable cation levels were determined with an atomic absorption spectrophotometer and extractable P was measured using the Murphy-Riley ascorbic acid technique (Watanbe and Olsen, 1965). Total foliar N was determined using standard micro-Kjeldahl procedures (Bremner, 1965). In addition, 12 seedlings in each plot (three from each split plot) were destructively sampled and returned to the laboratory for detailed analyses. Root systems of these seedlings were microscopically inspected for ectomycorrhizal colonization and root and shoot dry weights were recorded. The needles from each seedling were analyzed for P, K, Ca, and Mg using the methods mentioned above.

Statistical Analysis

Each statistical analysis was conducted using Statistical Analysis System (SAS) programs (Helwig and Council, 1979).

Means and standard errors were calculated for the site characterization data. Percent slope values were not measured on the bench site because of the lack of slope on all plots. Thus, a standard error was not calculated for this parameter on the bench site.

Analyses of variance were performed using the statistical model outlined in Table A2 to test for significant differences in survival, growth, ectomycorrhizal colonization, and foliar nutrient levels between the two sites and among the three cultural treatments for each tree species. The sites and cultural treatments are all fixed effects. Arcsin transformations of survival and colonization were used in the analyses of variance. Duncan's Multiple Range test was used to compare differences among means.

Analyses of variance were also used to test for significant soil moisture and soil temperature differences between the herbicide treatments for each site. Duncan's Multiple Range test was used to compare differences among means.

Correlations between the percentage of seedling root systems colonized and growth response variables were calculated using Pearson product-moment correlations (Helwig and Council, 1979).

RESULTS

Site Characterization

The site characterization data show four noteworthy differences between the recontoured and flat bench sites (Table 1). The recontoured site had a relatively steep slope and a clay loam soil texture whereas the bench site was flat and had a loam soil texture. Nutrient levels tended to be higher on the recontoured site. However, the organic matter level and pH were higher on the flat bench site.

Initial Survival and Growth

Three weeks after planting, the trees were measured to assess if there were initial differences in height and survival between the sites or among the cultural treatments (Table 2). Results of the initial measurement indicated that Virginia pine seedlings planted on the recontoured site were significantly taller than those planted on the flat bench site. In addition, loblolly pine seedlings inoculated with Pt were significantly taller than control seedlings. No other species-site or species-treatment combinations

Table 1. Soil and site characterization of the recontoured and flat bench sites[1].

Site Parameter	Recontoured	Std Error	Flat Bench	Std Error
Slope (%)	38	1.5	<2	-
Biomass (kg/ha)	3811	197	3878	475
Particle Size Distribution (%)				
Coarse Fragments	49	1.5	43	1.5
Sand	12	0.5	29	1.1
Silt	21	0.7	16	0.7
Clay	18	0.6	12	0.6
Bulk Density (g/cc)	1.3	0.06	1.4	0.04
Organic Matter (%)	1.2	0.06	1.8	0.16
pH	5.4	0.14	6.1	0.14
N (μ mole/g)[2]	55.9	2.41	51.1	4.37
P (μ mole/g)[3]	0.24	0.01	0.20	0.01
K (μ mole/g)[4]	2.58	0.09	2.05	0.07
Ca (μ mole/g)	15.22	0.96	14.37	1.22
Mg (μ mole/g)	17.73	0.55	10.50	0.56

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[1] Values are the means of measurements from 18 plots at each site.

[2] Micro-kjeldahl digestion.

[3] Sodium bicarbonate extract.

[4] Cation levels are for ammonium acetate extracts.

Table 2. Initial pine seedling survival and height measured three weeks after planting.

Site and Cultural Treatments	White Pine Survival	White Pine Height	Loblolly Pine Survival	Loblolly Pine Height	Virginia Pine Survival	Virginia Pine Height
	(%)	(cm)	(%)	(cm)	(%)	(cm)
Mining Site						
Recontoured	97a[1]	5.1a	99a	9.2a	99a	7.3a
Flat bench	97a	5.4a	97a	10.0a	100a	6.5b
Fertilizer						
Control	98a	5.4a	97a	9.6a	100a	6.6a
Fertilized	97a	5.1a	99a	9.6a	99a	7.1a
Weed Control						
Control	98a	5.4a	98a	9.6a	100a	6.8a
Herbicide	97a	5.1a	98a	9.6a	99a	6.9a
Mycorrhizal Inoculation						
Control	99a	4.9a	97a	9.2a	100a	6.9a
Inoculated	96b	5.6a	99b	10.0b	99a	6.9a

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[1] For each site or treatment and species, means within columns not followed by the same letter are significantly different at the 0.05 level according to Duncan's Multiple Range Test.

caused significant differences in initial growth. Initial survival of all species-site and species-treatment combinations was greater than 96 percent. Uninoculated white and inoculated loblolly pine seedlings had significantly higher survival than inoculated and uninoculated seedlings, respectively.

First Year Survival and Growth

First year survival and growth of the three pine species as affected by the mining sites and mycorrhizal treatment are presented in Table 3. After one growing season, survival and growth of all three pine species were not significantly different between the two sites. Survival and growth of white and Virginia pines were not significantly affected by mycorrhizal inoculation, whereas inoculation with Pt did significantly increase the average height and diameter of loblolly pine. Although insignificant, the favorable effect of inoculation was also shown by increased volumes for all three species. Inoculation of white, loblolly, and Virginia pine seedlings increased volume by 15, 20, and 19 percent, respectively.

There was a synergistic fertilizer x herbicide interaction which significantly affected the growth of all three pine species but did not significantly influence their

Table 3. Effects of mining site and mycorrhizal treatment on first-year survival and growth of pine seedlings.

Site and Mycorrhizal Treatments	White Pine				Loblolly Pine				Virginia Pine			
	Surv.	Ht.	Diam.	Vol.^{1}	Surv.	Ht.	Diam.	Vol.	Surv.	Ht.	Diam.	Vol.
	(%)	(cm)	(mm)	(cm^{3})	(%)	(cm)	(mm)	(cm^{3})	(%)	(cm)	(mm)	(cm^{3})
Mining Site												
Recontoured	94a^{2}	6.2a^{3}	2.2a^{4}	0.33a	98a	11.8a^{3,4}	3.3a^{3}	1.81a^{4}	97a	9.9a	2.8a	1.17a
Flat bench	92a	6.7a^{3}	2.2a^{4}	0.38a	97a	12.9a^{3,4}	3.8a^{3}	2.60a^{4}	99a	10.0a	2.9a	1.13a
Mycorrhizal Inoculation												
Control	91a	6.1a	2.2a	0.33a	98a	11.7a	3.5a	2.00a	97a	9.7a	2.8a	1.05a
Inoculated	94a	6.7a	2.2a	0.38a	97a	13.0b	3.7b	2.40a	98a	10.2a	2.9a	1.25a

[1] Vol. = ht x diam.^{2}

[2] For each site or treatment and species, means within columns not followed by the same letter are significantly different at the 0.05 level according to Duncan's Multiple Range Test.

[3] Significant site x herbicide interaction occurred.

[4] Significant site x inoculation interaction occurred.

survival (Table 4). The average height, diameter, and volume of loblolly and Virginia pine seedlings was significantly greater with the combination of fertilizer and herbicide than with either fertilizer or herbicide alone. The diameter and volume of white pine seedlings were also significantly increased over that of either fertilizer or herbicide alone. White pine volume was increased by 62 and 79 percent, loblolly pine by 109 and 317 percent, and Virginia pine by 149 and 330 percent over fertilization and herbicide alone, respectively. For all three species, fertilizer alone tended to increase seedling volume more than herbicide alone. Figure 3 illustrates the relative influence of each treatment alone, and in combination, on loblolly seedling volume. Similar relationships between treatments and seedling volume existed for white and Virginia pine.

Although only the oven-dry root weight of white pine seedlings was significantly affected by mining site, root and shoot weights of all three species were higher on the flat bench site than on the recontoured site (Table 5). Root and shoot weights on the bench site were 73 and 38, 64 and 25, and 30 and 22 percent higher than root and shoot weights on the recontoured site for white, loblolly, and Virginia pine seedlings, respectively. Mycorrhizal

Table 4. Effects of fertilizer and herbicide applications on first-year survival and growth of pine seedlings.

Fertilizer Treatment	Weed Control Treatment	White Pine Surv.	White Pine Ht.	White Pine Diam.	White Pine Vol.[1]	Loblolly Pine Surv.	Loblolly Pine Ht.	Loblolly Pine Diam.	Loblolly Pine Vol.	Virginia Pine Surv.	Virginia Pine Ht.	Virginia Pine Diam.	Virginia Pine Vol.
		(%)	(cm)	(mm)	(cm^3)	(%)	(cm)	(mm)	(cm^3)	(%)	(cm)	(mm)	(cm^3)
Control	Control	93a[2]	6.4a	2.0a	0.29a	96a	10.3a	2.6a	0.74a	97a	7.8a	2.3a	0.46a
Control	Herbicide	95a	5.9a	2.1a	0.29a	96a	10.9a	3.1b	1.13ab	99a	8.1a	2.4ab	0.59a
Fertilizer	Control	93a	6.4a	2.1a	0.32a	98a	12.6b	3.7c	2.25b	98a	10.6b	2.8b	1.02a
Fertilizer	Herbicide	91a	7.0a	2.6b	0.52b	99a	15.6c	4.9d	4.71c	97a	13.4c	3.8c	2.54b

[1] Volume = height x $diameter^2$.

[2] Means within columns not followed by the same letter are significantly different at the 0.05 level according to Duncan's Multiple Range Test.

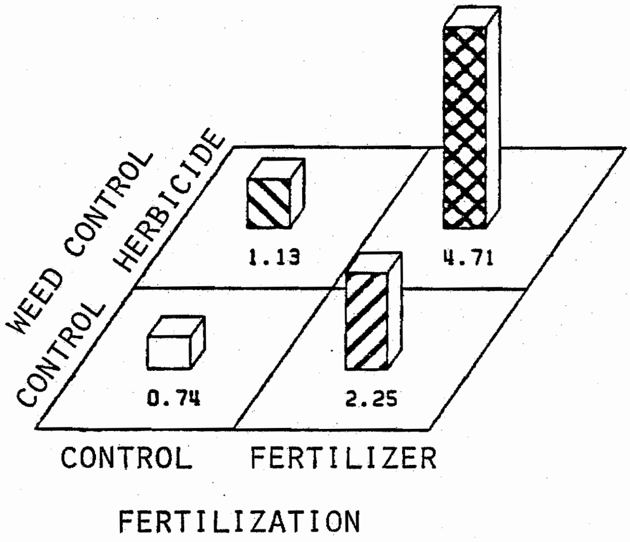

Figure 3. Interaction of fertilizer and weed control on loblolly pine seedling volume (cm^3).

Table 5. Effects of mining site and mycorrhizal treatment on first-year oven-dry root and shoot weights of pine seedlings.

Site and Mycorrhizal Treatments	White Pine		Loblolly Pine		Virginia Pine	
	Root	Shoot	Root	Shoot	Root	Shoot
	- - - - - - -	- - - - - - -	- - - - - - - g	- - - - - - -	- - - - - - -	- - - - - - -
Mining Site						
Recontoured	0.49a[1]	0.61a	0.98a	1.75a	0.98a	1.34a
Flat bench	0.85b	0.84a	1.61a	2.19a	1.27a	1.64a
Mycorrhizal Inoculation						
Control	0.67a	0.71a	1.26a	1.90a	0.97a	1.35a
Inoculated	0.67a	0.74a	1.33a	2.05a	1.28a	1.63a

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[1] For each site or treatment and species, means within columns not followed by the same letter are significantly different at the 0.05 level according to Duncan's Multple Range Test.

inoculation did not significantly effect root or shoot weights of any species; however, it did tend to increase these weights, especially in loblolly and Virginia pine seedlings. Root and shoot weights of inoculated seedlings were 0 and 4, 6 and 8, and 32 and 21 percent greater than root and shoot weights of uninoculated white, loblolly, and Virginia pine seedlings, respectively.

The same synergistic fertilizer x herbicide interaction which affected height, diameter, and volume growth, significantly influenced oven-dry root and shoot weights of all three species (Table 6). The combination of fertilizer and herbicide significantly increased root and shoot weights over either fertilizer or herbicide alone. White pine root weight was increased by 61 and 51 percent, loblolly pine by 98 and 167 percent, and Virginia pine by 177 and 188 percent over fertilization and herbicide alone, respectively. White pine shoot weight was increased by 56 and 95 percent, loblolly pine by 113 and 205 percent, and Virginia pine by 106 and 173 percent over fertilization and herbicide alone, respectively. Fertilizer alone tended to increase root and shoot weights of Virginia and loblolly pine more than herbicide alone.

Table 6. Effects of fertilizer and herbicide applications on first-year oven-dry root and shoot weights of pine seedlings.

Fertilizer Treatment	Herbicide Treatment	White Pine		Loblolly Pine		Virginia Pine	
		Root	Shoot	Root	Shoot	Root	Shoot
		- - - - - -	- - - - - -	- - - - - -	g - - - - - -	- - - - - -	- - - - - -
Control	Control	0.57a[1]	0.53a	0.70a	1.04a	0.58a	0.71a
Control	Herbicide	0.61ab	0.56a	0.89a	1.25a	0.80a	1.04ab
Fertilizer	Control	0.57a	0.70a	1.20a	1.79b	0.83a	1.38b
Fertilizer	Herbicide	0.92c	1.09b	2.38b	3.81c	2.30b	2.84c

[1] Means within columns not followed by the same letter are significantly different at the 0.05 level according to Duncan's Multiple Range Test.

Ectomycorrhizal Colonization

Of the inoculated seedlings, 4 percent of white, 26 percent of loblolly, and 23 percent of Virginia pine seedlings were colonized by Pt prior to outplanting (Table 7). No other ectomycorrhizal fungi were detected on the inoculated seedlings, and no ectomycorrhizae of any type were observed on the control seedlings.

White pine was the only species with significant differences between the two sites in Pt colonization or colonization with other ectomycorrhizal fungi after the first growing season (Table 8). There was significantly greater colonization of seedlings with both Pt and other fungal species on the bench site. The percentage of loblolly pine seedlings colonized by Pt tended to be higher on the bench site, while the reverse was true for Virginia pine. Loblolly and Virginia pine seedlings tended to be more heavily colonized by other ectomycorrhizal species on the bench site. These colonization trends resulted in a tendency for higher total colonization for each species on the bench site. Total colonization on the bench site was 314, 43, and 41 percent greater than on the recontoured site for white, loblolly, and Virginia pines, respectively.

The percentage of loblolly and Virginia pine seedlings colonized with Pt or with other ectomycorrhizal species was

Table 7. Ectomycorrhizal colonization of pine seedlings prior to outplanting.

Species and Treatment	Number of Trees Examined	Number of Trees Colonized With Pt	Number of Trees Colonized With Other Species	Percentage of Trees Colonized
White pine				
Control	152	0	0	0
Inoculated	135	6	0	4.4
Loblolly pine				
Control	158	0	0	0
Inoculated	151	40	0	26.5
Virginia pine				
Control	150	0	0	0
Inoculated	146	34	0	23.3

Table 8. Effects of mining site and cultural treatments on the percentage of pine seedlings colonized with ectomycorrhizal fungi.

Site and Cultural Treatments	Species	Trees Colonized With Pt	Trees Colonized With Other Species	Trees Colonized With Other Species or Pt
		- - - - - - - - - - -	- - - - - % - - - - -	- - - - - - - - - - -
Mining Site				
Recontoured	White pine	1a[1]	12a	14a
Flat bench		10b	49b	58b
Recontoured	Loblolly pine	18a	42a[2]	60a
Flat bench		25a	68a[2]	86a
Recontoured	Virginia pine	22a	36a	56a[3]
Flat bench		17a	69a	79a[3]
Fertilizer				
Control	White pine	4a	29a	33a
Fertilized		7a	32a	39b
Control	Loblolly pine	24a	56a	75a[4]
Fertilized		19a	54a	71a[4]
Control	Virginia pine	22a	56a	71a
Fertilized		17a	50a	64a
Weed Control				
Control	White pine	7a	21a	28a
Herbicide		4a	40b	44b
Control	Loblolly pine	21a	54a	69a
Herbicide		22a	56a	76a
Control	Virginia pine	22a	36a	57a
Herbicide		17a	69b	78b
Mycorrhizal Inoculation				
Control	White pine	1a	29a	31a
Inoculated		10a	32a	42b
Control	Loblolly pine	6a	62a	67a
Inoculated		38b	47b	79a
Control	Virginia pine	8a	58a	60a
Inoculated		31b	47b	75a

[1] For each site or treatment and species, means within columns not followed by the same letter are significantly different at the 0.05 level according to Duncan's Multiple Range Test.

[2] Significant site x herbicide interaction occurred.

[3] Significant site x inoculation interaction occurred.

[4] Significant fertilizer x inoculation interaction occurred.

slightly inhibited by fertilization (Table 8). Fertilized white pines exhibited an opposite trend.

Treatment with herbicide significantly enhanced natural colonization with ectomycorrhizal species other than Pt in white and Virginia pines resulting in significantly higher total colonization (Table 8). Although not significant, the same trends were evident in loblolly pine seedlings.

Inoculation significantly increased the percentage of loblolly and Virginia pines colonized with Pt (Table 8). The presence of Pt was also detected on some uninoculated seedlings of all three species indicating the possibility of natural Pt colonization. Uninoculated seedlings of loblolly and Virginia pine also had significantly higher colonization with other ectomycorrhizal fungi than inoculated seedlings. This resulted in no significant differences in total colonization with Pt or other fungal species between inoculated and uninoculated loblolly and Virginia pine seedlings. Although not significantly different, inoculated loblolly and Virginia pine seedlings had more total colonization with Pt and other fungal species than uninoculated seedlings. The total colonization of inoculated seedlings was 35, 18, and 25 percent higher than uninoculated seedlings of white, loblolly, and Virginia pine, respectively.

The benefits of mycorrhizal colonization were evaluated by correlating seedling growth response variables with the extent of root colonizattion by ectomycorrhizal fungi. Significant correlation coefficients are presented in Table 9. Loblolly and Virginia pine roots colonized with Pt were negatively correlated with colonization by other ectomycorrhizal fungi. Pt colonization was significantly correlated with root and shoot weight in white pine and with root collar diameter and seedling volume in loblolly pine. Colonization with other ectomycorrhizal species was significantly correlated with root and shoot weight, root collar diameter, and seedling volume in white pine, with foliar P in loblolly pine, and with root weight and foliar P in Virginia pine.

Foliar Nutrient Levels

Foliar N and P levels in loblolly, and N,P, and K levels in Virginia pine, were significantly higher on the flat bench site (Table 10). No other significant differences in foliar nutrients were detected between the two sites for any species.

Inoculated loblolly pine seedlings had significantly higher foliar P levels than uninoculated seedlings, and inoculated white pine seedlings had significantly increased

Table 9. Correlation coefficients of growth variables in relation to the extent of ectomycorrhizal colonization on pine seedlings.

Tree Species and Ectomycorrhizal Symbiont	Pt	Other Species	Root Weight	Shoot Weight	Ht.	Diam.	Vol.	Foliar P
	- - - - - -	- - - - - -	- - - - - -	- - - - r	- - - - - -	- - - - - -	- - - - - -	- - - - - -
White pine								
Pt	--	NS[1]	.199	.194	NS	NS	NS	NS
Other Species	NS	--	.528	.357	NS	.356	.309	NS
Loblolly pine								
Pt	--	-.330	NS	NS	NS	.205	.182	NS
Other Species	-.330	--	NS	NS	NS	NS	NS	.471
Virginia pine								
Pt	--	-.232	NS	NS	NS	NS	NS	NS
Other Species	-.232	--	.172	NS	NS	NS	NS	.625

[1] NS = not significant at the 0.05 level according to Pearson product-moment correlation.

Table 10. Effects of mining site and cultural treatments on pine needle nutrient levels.

Site and Cultural Treatments	Species	N	P	K	Ca	Mg
		- - - - - - - -	- - - - - - - -	%	- - - - - - - -	- - - - - - - -
Mining Site						
Recontoured	White pine	1.3a[1]	0.16a	0.36a	0.47a	0.29a
Flat bench		1.5a	0.16a	0.34a	0.53a	0.28a
Recontoured	Loblolly pine	1.2a	0.12a	0.50a[2]	0.33a[3]	0.20a[2]
Flat bench		1.8b	0.18b	0.51a[2]	0.32a[3]	0.17b[2]
Recontoured	Virginia pine	1.1a[4]	0.11a[2]	0.42a	0.37a	0.24a
Flat bench		1.5b[4]	0.16b[2]	0.52b	0.35a	0.21a
Fertilizer						
Control	White pine	1.2a[5]	0.15a	0.31a	0.51a	0.32a[5]
Fertilized		1.7a[5]	0.17a	0.38a	0.49a	0.25b[5]
Control	Loblolly pine	1.4a	0.15a	0.51a	0.34a	0.21a
Fertilized		1.6a	0.16a	0.50a	0.31b	0.16b
Control	Virginia pine	1.1a	0.12a	0.38a	0.42a	0.26a
Fertilized		1.4a	0.14a	0.56b	0.30b	0.19b

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[1] For each site or treatment and species, means within columns not followed by the same letter are significantly different at the 0.05 level according to Duncan's Multiple Range Test.

[2] Significant site x inoculation interaction occurred.

[3] Significant site x herb interaction occurred.

[4] Significant site x fertilizer interaction occurred.

[5] Significant fertilizer x herbicide interaction occurred.

Table 10. Continued.

Site and Cultural Treatments	Species	N	P	K	Ca	Mg
		- - - - - - - - - - - -	- - - - -	%	- - - - -	- - - - - - - - - - - -
Weed Control						
Control	White pine	1.3a	0.17a	0.39a	0.47a[6]	0.29a
Herbicide		1.6b	0.15b	0.31b	0.52b[6]	0.28a
Control	Loblolly pine	1.4a	0.15a	0.51a	0.31a	0.19a
Herbicide		1.6b	0.16a	0.50a	0.34b	0.18a
Control	Virginia pine	1.2a	0.13a	0.45a	0.36a	0.22a
Herbicide		1.4b	0.14a	0.48a	0.36a	0.23a
Mycorrhizal Inoculation						
Control	White pine	1.4a	0.15a	0.34a	0.49a	0.29a
Inoculated		1.4a	0.17a	0.36b	0.50a	0.28a
Control	Loblolly pine	1.4a	0.15a	0.50a	0.33a	0.19a
Inoculated		1.6a	0.16b	0.51a	0.32a	0.18b
Control	Virginia pine	1.2a	0.14a	0.46a	0.36a	0.23a
Inoculated		1.3a	0.13a	0.48a	0.35a	0.22a

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[6] Significant herbicide x inoculation interaction occurred.

levels of K (Table 10). Other foliar nutrient levels for the three species were not significantly affected by the ectomycorrhizal treatment.

Although only significant for foliar K in Virginia pine, fertilization tended to increase foliar levels of N,P, and K for all three species (Table 10). In contrast, levels of Ca and Mg were higher in unfertilized seedlings of all three species.

Herbicide applications significantly increased foliar levels of N for all three species and significantly increased P,K, and Ca levels in white pine (Table 10). Calcium levels were also enhanced by the herbicide applications in loblolly pine seedlings.

Because of its significant synergistic effect on seedling growth, the effects of the fertilizer x herbicide interaction on foliar N and P were also evaluated. Foliar N levels in white pine seedlings were significantly higher with the combination of fertilizer and herbicide than with either fertilizer or herbicide alone (Table 11). Although not significant, the same trend occurred in loblolly and Virginia pine seedlings. White pine foliar N was increased by 36 and 58 percent, loblolly by 13 and 13 percent, and Virginia pine by 15 and 25 percent over fertilization and herbicide alone, respectively. Phosphorus levels for the three species did not show conclusive trends in relation to the treatment combinations.

Climatic and Edaphic Factors

The herbicide treatment indirectly caused a significant increase in percent soil moisture on the recontoured site on four of six sampling dates, July 17, July 24, August 5, and August 21 (Figure 4). In contrast, the percent soil moisture contents were not significantly different between herbicide treatments at any of the measured times on the bench site (Figure 5).

In order to correlate soil moisture levels with seedling water stress, the moisture levels were converted to water potential values using moisture retention curves (Figures A1 and A2). The herbicide treatment again caused a significant increase in soil water potential on the same four sampling dates on the recontoured site (Figure 6). Water potential levels were not significantly different between herbicide treatments at any of the measured times on the bench site (Figure 7).

The herbicide treatment caused a significant increase in soil temperature at a 15 cm depth on the recontoured site on only one of nineteen sampling dates, September 9 (Figure 8). Soil temperature on the flat bench site was

Table 11. Effects of fertilizer and herbicide applications on pine needle nutrient levels.

Fertilizer Treatment	Weed Control Treatment	White Pine		Loblolly Pine		Virginia Pine	
		N	P	N	P	N	P
		- - -	- - -	- - -	%	- - -	- - -
Control	Control	1.1a[1]	0.16a	1.2a	0.14a	1.0a	0.12a
Control	Herbicide	1.2a	0.14a	1.5a	0.15a	1.2a	0.13a
Fertilized	Control	1.4a	0.18a	1.5a	0.15a	1.3a	0.14a
Fertilized	Herbicide	1.9b	0.16a	1.7a	0.16a	1.5a	0.14a

[1] Means within columns not followed by the same letter are significantly different at the 0.05 level according to Duncan's Multiple Range Test.

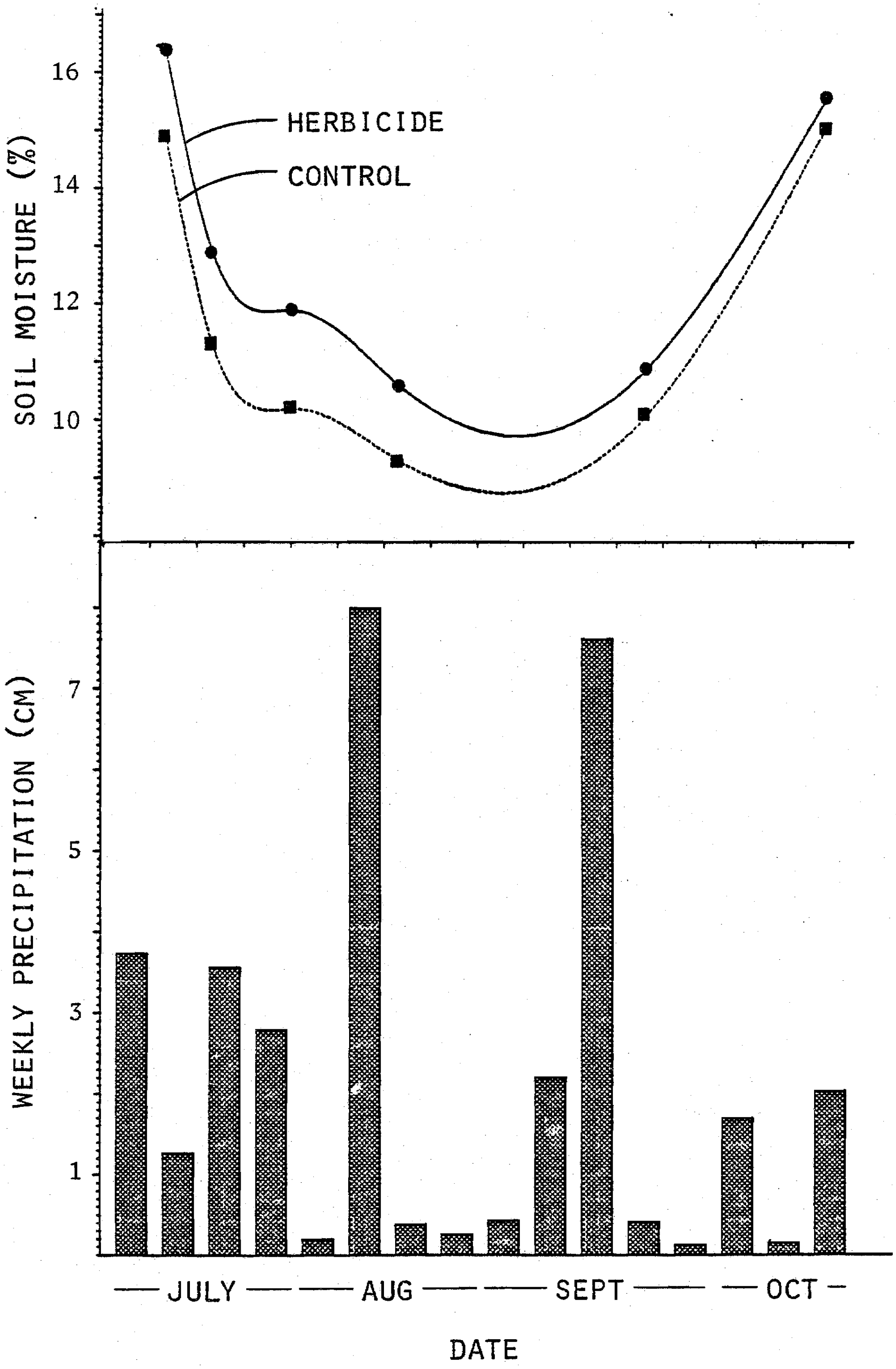

Figure 4. Soil moisture and weekly precipitation on the recontoured site.

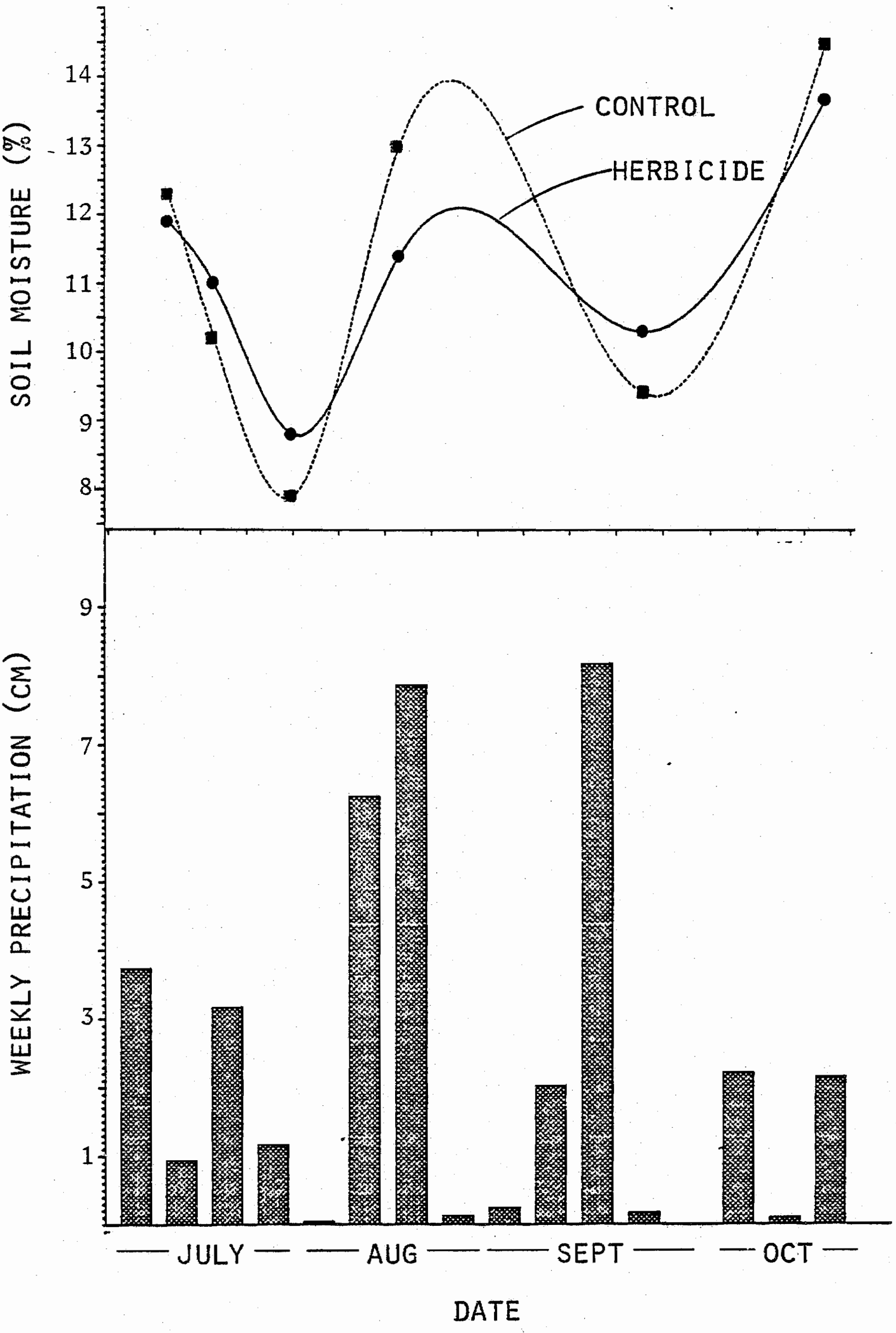

Figure 5. Soil moisture and weekly precipitation on the bench site.

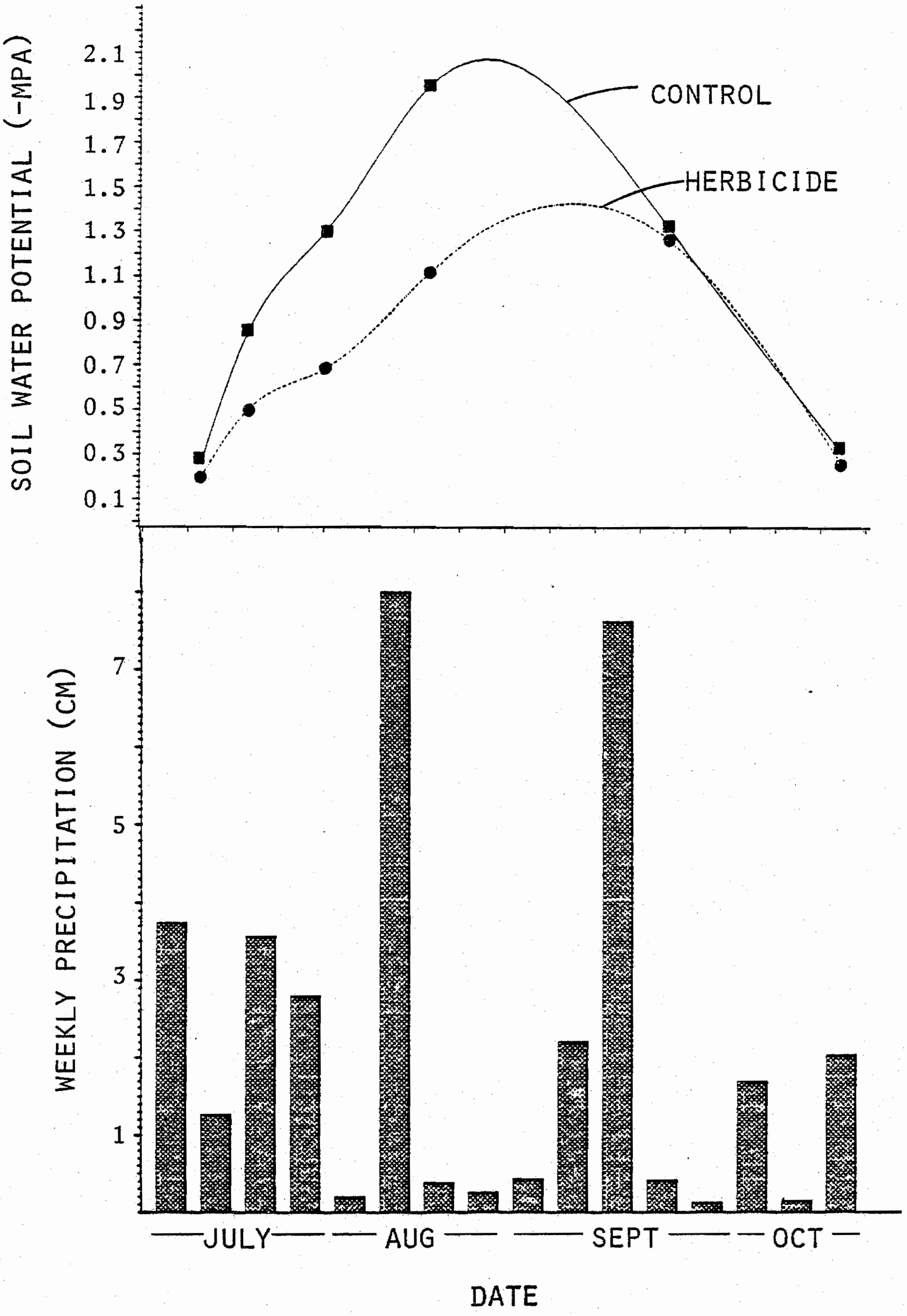

Figure 6. Soil water potential and weekly precipitation on the recontoured site.

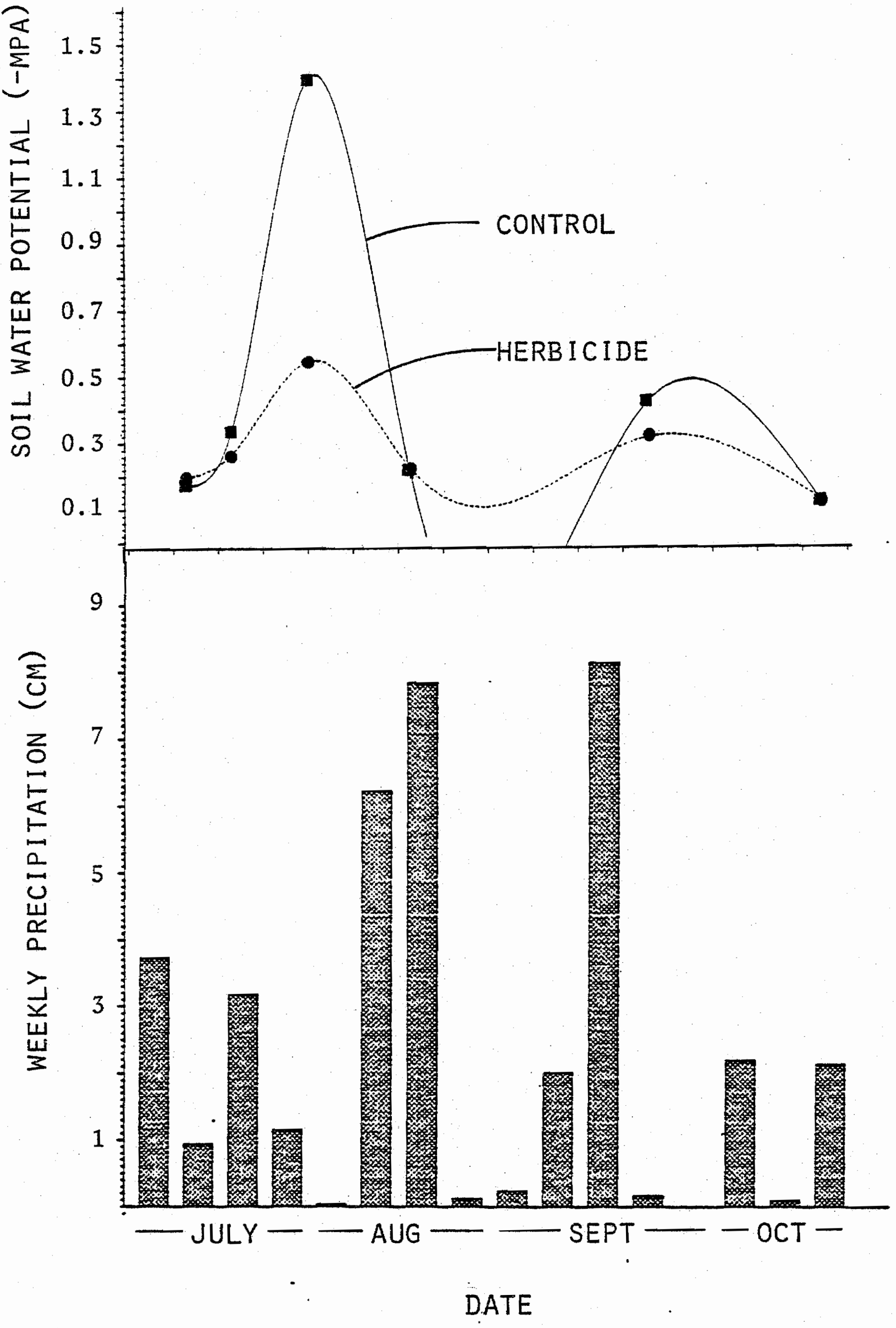

Figure 7. Soil water potential and weekly precipitation on the bench site-

significantly higher on four of nineteen sampling dates, August 19, September 2, September 23, and November 4 (Figure 9).

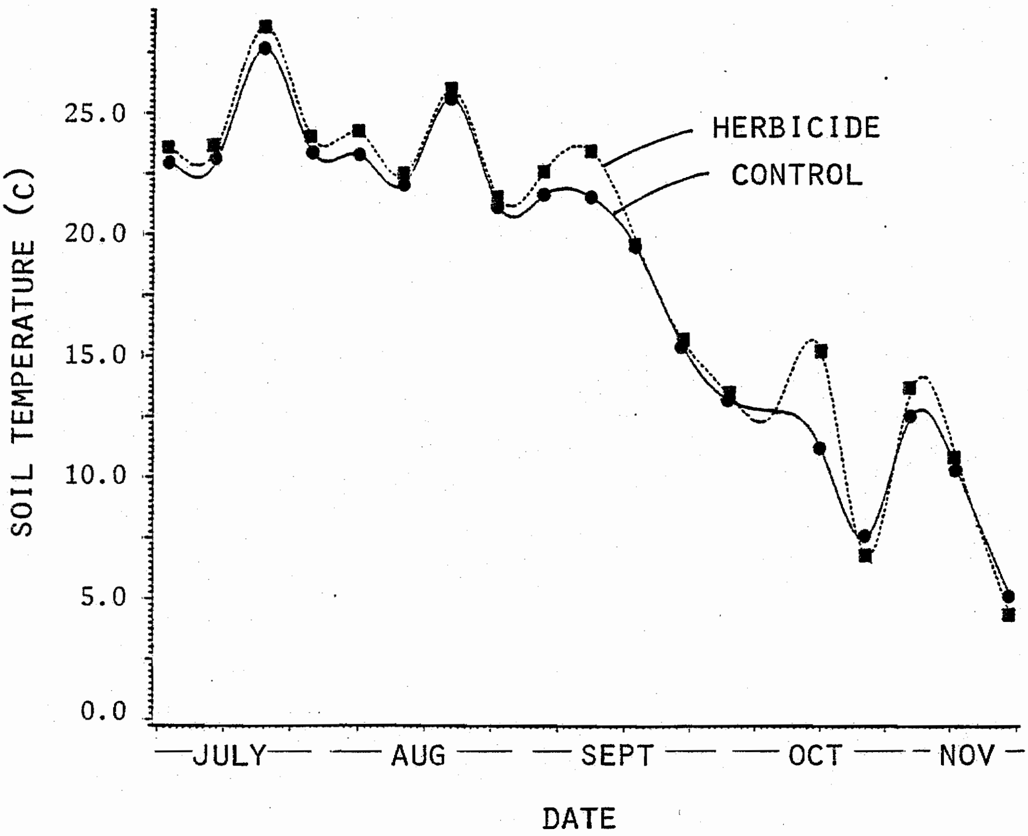

Figure 8. Effect of herbicide on soil temperature at 15 cm on the recontoured site.

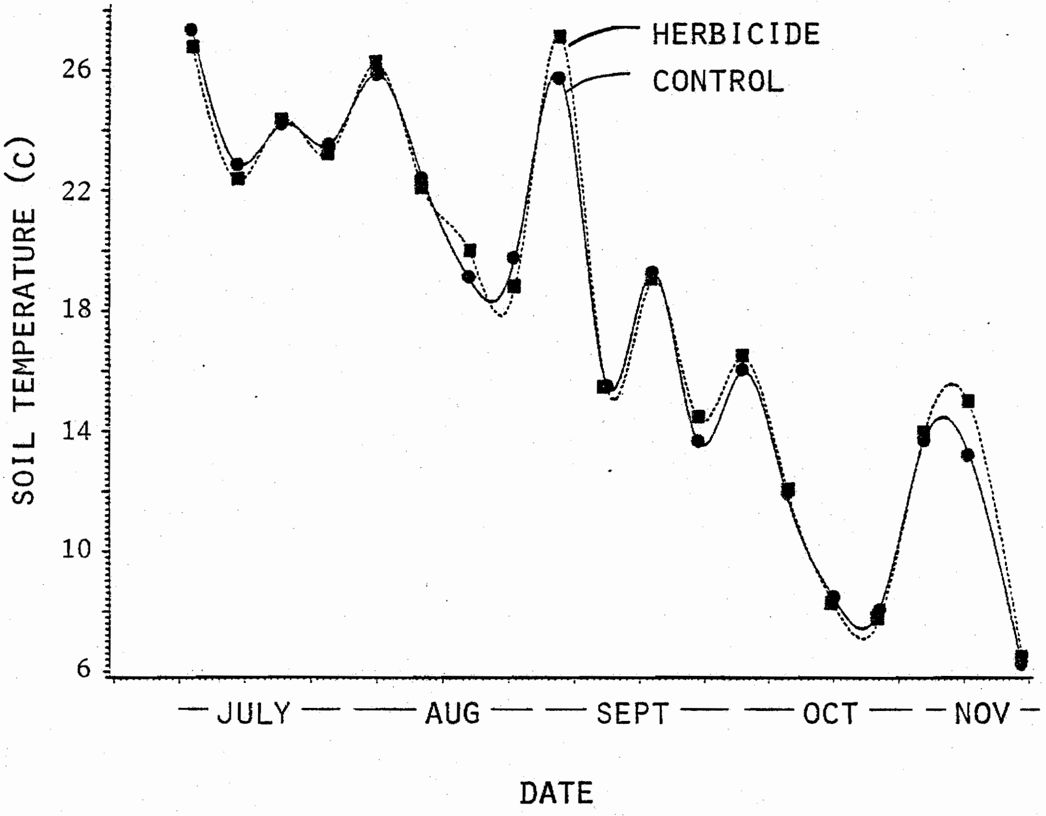

Figure 9. Effect of herbicide on soil temperature at 15 cm on the bench site.

DISCUSSION

Site Characterization

Most strip-mine soils when initially established are relatively devoid of organic matter (Mays and Bengtson, 1978; Vogel, 1975; Czapowskyj, 1973). Therefore, the higher levels of organic matter on the flat bench site as compared with the recontoured site may be attributed to earlier hydroseeding which provided an additional year or two of litter accumulation and incorporation.

The higher soil nutrient levels found on the recontoured site can be explained by two related factors, fertilization application and soil texture. Fertilizer was applied to the recontoured site at a higher rate and one year later than it was applied to the flat bench site. In addition, the finer-textured soil of the recontoured site had a better capacity to retain nutrients than the coarser-textured soil of the bench site. Note that the recontoured soil had 50 percent more clay-sized particles and 31 percent more silt-sized particles than the bench soil. Furthermore, the bench soil had almost two and a half times more sand-sized particles than the recontoured site.

Survival and Growth

None of the cultural treatments on the two sites significantly affected first year pine seedling survival. In fact, first year survival for all three treatments on both sites was excellent. This was probably due to ample and well-distributed rainfall during the growing season (Figures 4 and 5). Soil moisture levels can be limiting to seedling growth, especially where substantial herbaceous competition exists (Newton and Knight, 1981; Bengtson and Mays, 1978; Greaves et al., 1978b; May et al., 1973). However, rainfall levels were adequate to maintain soil water-potential levels above -1.5 MPa for all but one sampling date during the growing season. Therefore, the seedlings rarely, if ever, experienced moisture stress conditions during the first growing season.

Despite the slightly higher soil nutrient levels on the recontoured site, the average height, diameter, volume, root weight, and shoot weight of all three species tended to be higher on the bench site which had a higher rate of natural ectomycorrhizal colonization. Marx (1977a) showed that ectomycorrhizal colonization is stimulated by low soil nutrient levels. In addition, better drainage and aeration of the coarser soils on the bench site may have allowed greater root elongation and stimulated mycorrhizal development. It is well-documented that ectomycorrhizae increase the capacity for nutrient and water absorption (Walker et al., 1982; Duddridge et al., 1980; Harley, 1978; Smith, 1974; Bowen and Theodorau, 1967) which in turn could account for the observed growth differences.

The benefit of Pt inoculation to seedling growth was evident even though Pt colonization was not very prevalent and colonization with other native ectomycorrhizal fungi occurred. Loblolly and Virginia pine seedlings, which showed greater increases in seedling volume, root weight, and shoot weight than white pine when inoculated, had higher percentages of seedlings colonized with Pt than white pine. Berry (1982), Walker et al. (1982), Ruehle (1980), and Marx and Artman (1979) have reported similar growth improvement of Pt-inoculated seedlings on disturbed sites in the South.

Chemical weed control generally tended to increase seedling growth; however, it did not significantly increase growth unless it was used in combination with fertilization. The lack of a strong effect of herbicide on seedling growth may be attributed to the regular and abundant precipitation which occurred throughout the growing season on both sites. This also suggests that the effects of competing vegetation on light and nutrient availability were not limiting to first-year-seedling growth. The amount of available soil moisture is often the most important consideration in evaluating the effects of vegetation competition. If soil moisture had been less available, it is likely that the effects of the herbicide on seedling growth would have been dramatic considering the presence of substantial herbaceous vegetation on both sites. Carpenter and Alpers (1981), Eckert (1979), Haines (1978), and Vogel (1973) have reported striking seedling responses to weed control on densely vegetated sites.

Fertilization alone showed a tendency to increase seedling growth more than the herbicide treatment alone. This suggests that during the initial growing season, when soil moisture was not limiting, a lack of soil fertility was probably limiting seedling growth. Nevertheless, the most substantial growth stimulation occurred where fertilizer and herbicide were combined. The slow-release fertilizer appeared to be effective in providing seedlings with adequate nutrients and, at the same time, not stimulating unwanted herbaceous competition during the initial year of pine seedling establishment. The benefits of fertilization to early seedling growth in this study are similar to the findings of Berry (1979) and Marx and Artman (1979). The improved seedling growth of all three species with the combination of fertilizer tablets and weed control suggests that both treatments are important in circumventing potential nutrient and moisture deficiencies on these sites.

Ectomycorrhizal Colonization

The ectomycorrhizal colonization data from the destructive samples showed surprisingly large numbers of seedlings colonized with indigenous ectomycorrhizal fungi on the two sites (Table 8). Marx (1977a) suggested that drastically disturbed sites tend to be deficient in native ectomycorrhizal species which are ecologically adapted to harsh conditions. The results of this study indicate that on these two sites propagules of indigenous ectomycorrhizal species were successful in colonizing seedlings of all three species.

The percentage of uninoculated and inoculated seedlings colonized with Pt increased by the end of the first growing season indicating that Pt was probably one of the native fungi on these two sites. Other ectomycorrhizal fungi were not evident when seedling root systems were examined prior to outplanting. Evidence regarding the benefits of ectomycorrhizal inoculation with Pt on these sites is not conclusive considering the fact that less than 40 percent of the seedlings of any species were colonized with Pt.

Fertilization did not significantly inhibit ectomycorrhizal colonization for any of the pine species. In fact, it significantly increased the total number of white pine seedlings colonized with Pt or other ectomycorrhizal species. The literature contains reports indicating that high soil fertility levels tend to inhibit mycorrhizal formation (Dixon et al., 1979; Marx, 1977a; Marx et al., 1977b). The slow-release fertilizer tablets apparently provided sufficient quantities of nutrients without excessively increasing rhizosphere fertility.

Chemical weed control did not significantly affect the number of trees colonized with Pt. However, it stimulated sufficient colonization by other fungi to result in increases in total ectomycorrhizal colonization. The lack of dense vegetation where herbicide was applied probably increased the incidence of wind-borne spores of mycorrhizal fungi landing in proximity to the seedlings. In addition, the quantity of light was increased where vegetation was controlled. High light intensity stimulates ectomycorrhizal formation (Marx, 1977a).

Smith and Ferry (1979) reported that simazine stimulated mycorrhizal development in Scotch pine and Austrian pine. The direct effects of glyphosate on mycorrhizal fungi of conifer seedlings is unknown. Perhaps root growth was stimulated sufficiently by the herbicide treatment to result in more favorable colonization conditions. Another explanation for the stimulatory effects involves the influence of glyphosate on the soil microbiological balance. The survival of mycorrhizal symbionts is often influenced by the presence of antagonistic soil microorganisms (Marx, 1980). Mycorrhizal development could be stimulated if some of the antagonistic microorganisms were sensitive to glyphosate.

Competition within the rhizosphere was evident from the negative correlations between the percentage of a root system colonized with Pt and colonization with other ectomycorrhizal fungi (Table 9). The significantly lower number of inoculated loblolly and Virginia pine seedlings colonized by other ectomycorrhizal fungi indicated that if Pt was present, conditions for colonization by other fungi were not favorable. The colonization of uninoculated seedlings with indigenous fungi probably masked most of the well-documented growth benefits attributed to Pt colonization.

Since less than 40 percent of the seedlings of any species were colonized with Pt it was not possible to accurately evaluate the effects of Pt on seedling growth. However, the seedlings which were destructively sampled provided data which reflected the relationships between the extent of ectomycorrhizal colonization and growth responses. The lack of correlations between the percentage of individual root systems colonized by ectomycorrhizal fungi and growth response variables is notable especially where Pt was involved. The strong relationship between ectomycorrhizal colonization and P uptake is well-documented in the literature (Ford, 1982; Harley, 1978; Smith, 1974; Bowen and Theodorou, 1967). However, only the correlation between colonization with other ectomycorrhizal species and foliar P levels in loblolly and Virginia pine seedlings was significant (r=.471 and .625 for loblolly and Virginia pine, respectively).

The general lack of significant correlations between the percentage of roots colonized and growth response variables suggested that the fertilizer and herbicide treatments might have superceded the effects of mycorrhizal colonization. The extent of colonization was more strongly correlated with growth response on unfertilized plots than on fertilized plots (Table A11). However, differences between unfertilized and fertilized seedlings were probably not sufficient to fully explain the lack of significant correlations between the extent of root colonization and growth. Correlation analyses which accounted for the herbicide treatment indicated that the herbicide did not affect the relationship between the percentage of roots colonized and growth (Table A12).

As previously mentioned, inoculation appeared to improve seedling growth; however, the correlation analyses did not conclusively support this relationship. It is possible that the most important growth variable in this system was not included in these analyses. Foliar analysis of needle samples collected from each tree in the field and composited by treatment combination suggested that N may be the limiting nutrient on these sites (Tables 10 and 11). Unfortunately, there was insufficient plant tissue from the destructive samples to conduct a foliar analysis of N. There may have been a strong relationship between the percentage of roots colonized and the level of N in the seedling foliage which could account for the apparent improvement in seedling growth on these sites.

Foliar Nutrient Levels

The interpretation of foliar nutrient levels in this study is difficult because of the lack of reference standards for young conifer seedlings growing on strip-mines. Nevertheless, many of the edaphic influences and seedling growth trends were reflected in the foliar nutrient levels.

The significantly higher values for foliar N and P of loblolly and Virginia pine seedlings on the bench site can be attributed to a combination of the higher number of seedlings colonized with ectomycorrhizal fungi and to the presence of dense stands of N-fixing sericea lespedeza prior to the study. In contrast, the recontoured site was predominantly vegetated with K-31 fescue. The ectomyorrhizae may have improved N and P uptake and the incorporation of sericea into the soil prior to the study may have provided additional N. Bengtson and Mays (1978) reported that sericea was capable of improving the N status of loblolly pines planted on coal-mine spoil. The tendency for better seedling growth on the bench site correlates well with the higher N and P foliar levels (Tables 10 and 11). Foliar N and P values on the recontoured site were at loblolly pine threshold levels of 1.2% N and 0.10% P (Berry and Marx, 1980; Bengtson and Mays, 1978; Wells et al., 1973). Potassium, Ca, and Mg levels on both sites were well above critical levels for loblolly pine (Bengtson and Mays, 1978; Wells, 1970).

The foliar analysis showed that all three of the cultural treatments increased foliar N (Table 10). This correlates well with the stimulatory effect of the cultural treatments on seedling growth. Levels of P, K, Ca, and Mg were above critical levels for all species-treatment combinations. Fertilization significantly decreased the concentration of Ca in the needles of loblolly and Virginia pine and Mg in the needles of all three species. This may be the result of a dilution effect caused by greater tree growth and needle weight of fertilized seedlings. The herbicide treatment probably increased foliar N through its stimulatory effect on mycorrhizal colonization. Furthermore, the lower N levels in unsprayed seedlings may reflect the competition of herbaceous vegetation for soil nutrients. The relatively weak response of seedlings to mycorrhizal inoculation is reflected by the slightly higher N levels of inoculated seedlings. Apparently, N uptake by indigenous ectomycorrhizal fungi masked the benefits of Pt.

Although only significant for white pine seedlings, the effect of the fertilizer x herbicide interaction on foliar N and P supports the suggestion that N is the limiting nutrient on these sites. All P values were above critical levels known for loblolly pine. The benefit of the combination of fertilization and chemical weed control to seedling growth is reflected in the relatively high foliar N levels. Furthermore, seedlings of all three species receiving neither fertilization nor herbicide were at or below the critical level for foliar N (Table 11).

Climatic and Edaphic Factors

The dense cover of herbaceous vegetation on these reclaimed sites had the potential to provide significant competition for soil moisture. Greaves et al. (1978b) suggested that the amount of available soil moisture during portions of the growing season is usually the limiting factor where vegetative competition is involved. On the recontoured site, the herbicide treatment caused a significant increase in soil moisture and soil water potential levels; however, the water potential levels on the unsprayed halves of plots rarely reached generally-accepted stress levels (-1.5 MPa).This was probably due to adequate precipitation throughout the growing season.

Despite the relatively coarse texture of soils on the bench site, moisture did not appear to be a growth-limiting factor as evidenced by similar soil water-potentials on the sprayed and unsprayed plots. Three related factors account for this: First, there was ample and well-distributed precipitation throughout the growing season. Second, there was a compaction layer approximately 20 cm below the soil surface at the bench site. This is a common occurence on strip-mined sites which are often exposed to repeated passes with heavy equipment. The layer was too deep to affect first-year root penetration of seedlings; however, it did tend to restrict drainage. On numerous occasions throughout the growing season, standing water was observed on some of the plots. Third, the flatness of the bench site restricted surface drainage.

Soil temperature extremes can be fatal on strip-mine spoils (Schramm, 1966). Richardson (1958) reported that the presence of herbaceous cover moderated temperature extremes on coal spoils. Soil temperatures in this study were significantly increased as a result of the herbicide treatment on only one of the sampling dates on the recontoured site and on only four of the sampling dates on the bench site. The high soil moisture levels probably buffered soil temperature fluctuations. Since the herbicide had a beneficial effect on seedling growth, it can be surmised that soil temperatures did not reach critical levels. Although soil temperatures at 15 cm showed weekly fluctuations, it may have been more informative to measure soil temperatures closer to the surface, where greater extremes in temperature probably occurred.

SUMMARY AND CONCLUSIONS

Abundant precipitation and high soil moisture levels throughout the initial growing season on both study sites probably accounted for the excellent first year survival of all three species. High soil moisture levels may have also accounted for the insignificant effect of the three cultural treatments on first year seedling survival.

Seedling growth was not significantly affected by varying conditions between the two sites. However, growth parameters, ectomycorrhizal colonization with indigenous fungal species, and foliar nutrient levels indicated that seedlings on the bench site were more vigorous after one growing season. The fact that the recontoured site had slightly higher soil nutrient levels than the bench site prior to this study, suggests that the edaphic (soil moisture, temperature, texture, nutrients, pH, and bulk density) and biotic (herbaceous vegetation and mycorrhizal fungi) factors influencing tree seedling growth on these reclaimed strip-mined sites were very complex.

Seedling growth was enhanced to a small extent by Pt inoculation. But successful colonization with ecologically-adapted native ectomycorrhizal fungi, in combination with low inoculation success, diluted much of the widely-reported benefits of Pt to pine seedlings growing in harsh environments. Pt colonization was not essential for seedling survival and vigorous growth on these sites.

Chemical weed control alone and slow-release fertilization alone tended to increase seedling growth; however, the most significant growth stimulation of all three pine species occurred where fertilizer and herbicide were combined. This suggests that these cultural treatments improved the nutrient and moisture status of the seedlings.

Levels of foliar N closely reflected the effects of each treatment on seedling growth. This indicates that available soil N was the growth-limiting nutrient on these two sites.

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APPENDIX

Table A1. Average monthly precipitation and temperature for Wise County, Virginia.

	Month											
	J	F	M	A	M	J	J	A	S	O	N	D
Precipitation (cm)	9.1	9.1	12.7	10.7	11.7	9.9	13.7	9.6	8.4	8.1	9.4	8.9
Average Maximum Monthly Temperature (C)	5	7	12	18	23	26	27	27	24	18	12	7
Average Minimum Monthly Temperature (C)	-5	-4	0	5	9	12	15	15	11	5	1	-3

Table A2. Main effects, interactions, and error terms used for survival, growth, ectomycorrhizal colonization, and foliar nutrient level hypothesis testing.

	Source[1]	df
Main Plot	R	2
	F	1
	R x F (Error$_a$)	2
Main Plot	S	1
	S x F	1
	S x R x F (Error$_b$)	4
Sub Plot	H	1
	H x F	1
	H x S	1
	H x S x F	1
	H x S x R x F (Error$_c$)	8
Sub Plot	I	1
	I x F	1
	I x S	1
	I x H	1
	I x S x F	1
	I x H x F	1
	I x H x S	1
	I x H x S x F	1
	I x H x S x R x F (Error$_d$)	16

[1] R = Replication; F = Fertilizer; S = Site; H = Herbicide; I = Mycorrhizal Inoculation.

Table A3. Nutrient application schedule for seedlings.

Date	Application
1/8/81 - 2/13/81	Water with tap water as needed (15 ml/seedling).
2/20/81	Water with nutrient solution. Supplemental watering with tap water as needed.
2/27/81 - 5/1/81	Water with nutrient solution + Fe once/week. Supplemental watering with tap water as needed.
5/6/81	Flush cavities with water.
5/7/81 - planting	Water with tap water as needed (15 ml/seedling).

Table A4. Nutrient sources and application rates.

Nutrient	Source	Elemental Application Rate
		mg/seedling/wk
N	$(NH_4)_2SO_4$ & KNO_3	0.50
P	H_3PO_4	0.32
K	KNO_3	0.42
Fe	Greenol^R	0.18

Table A5. Modified Hagem nutrient solution.

Ingredient	Quantity
Malt Extract	5.0 g
Dextrose	5.0 g
KH_2PO_4	0.5 g
$MgSO_4 \cdot 7H_2O$	0.5 g
$FeCl_3$ (1% solution)	0.5 ml
Biotin (5 µg/ml)	1.0 ml
Thiamine HCl (1 mg/ml)	1.0 ml
Distilled Water	to 1000 ml
H_3PO_4	adjust pH to 5.0

Modess (1941).

Table A6. Agriform fertilizer tablets - guaranteed analysis[1].

Ingredient	Percentage
Total Nitrogen (N) - 7% water soluble N 13% water insoluble N	20.0
Available Phosphoric Acid (P_2O_5)	10.0
Soluble Potash (K_2O)	5.0
Calcium (Ca)	2.6
Sulfur (S)	1.6
Iron (Fe)	0.35

[1] Derived from ureaformaldehyde, calcium phosphate, potassium sulfate, calcium sulfate, and ferrous sulfate. Potential basicity: 5% or 45.4 kg calcium carbonate equivalent per ton.

Table A7. Available soil phosphorus values of preliminary samples[1].

	Area 1	Area 2	Area 3
	- - - - - - - - - - - -	μ moles/g - - - - - - - - - - - -	
	0.543	1.281	1.893
	1.212	3.693	3.146
	0.634	2.276	1.512
	1.503	2.006	0.390
	1.138	4.630	0.650
	0.646	1.549	1.818
	0.485	2.375	0.257
	0.743	2.137	0.512
	0.565	2.618	1.861
	0.692	2.189	0.345
$\overline{x}$	0.816	2.475	1.238
S^2	0.344	0.994	0.956

---

[1] Double acid extract.

[2] S = Standard deviation.

Table A8. Sampling intensity based on the variability of soil phosphorus values[1].

Area	Percent Sampling Error 10	 20	 30
	- - number of samples	- - -	
1	60	15	7
2	55	14	7
3	200	50	23

[1] Determination of sampling size based on: $n = \frac{t^2 S^2}{S_{\bar{x}}^2}$ where

n = sample size, t = t value from Student's t distribution, S^2 = variance of preliminary sample, $S_{\bar{x}}$ = standard error of the mean.

Table A9. Soil pH values of preliminary samples.

	Area 1	Area 2	Area 3
	5.82	6.39	6.70
	6.40	7.78	7.11
	5.40	7.19	6.21
	6.21	6.94	4.94
	6.08	7.81	5.16
	5.59	6.62	6.37
	5.54	7.10	5.12
	5.94	7.04	5.25
	5.70	6.99	6.22
	5.72	7.04	5.08
$\overline{x}$	5.84	7.09	5.82
S[1]	0.32	0.44	0.79

[1] S = Standard deviation.

Table A10. Sampling intensity based on the variability of soil pH values[1].

Area	Percent Sampling Error 10	 20	 30
	- - number of samples - -		
1	2	1	1
2	2	1	1
3	7	2	1

---

[1] Determination of sampling size for soil samples based on soil pH variability: $n = \frac{t^2 S^2}{S_{\bar{x}}^2}$ where n = sample size, t = t value from Student's t distribution, S^2 = variance of preliminary sample, $S_{\bar{x}}$ = standard error of the mean.

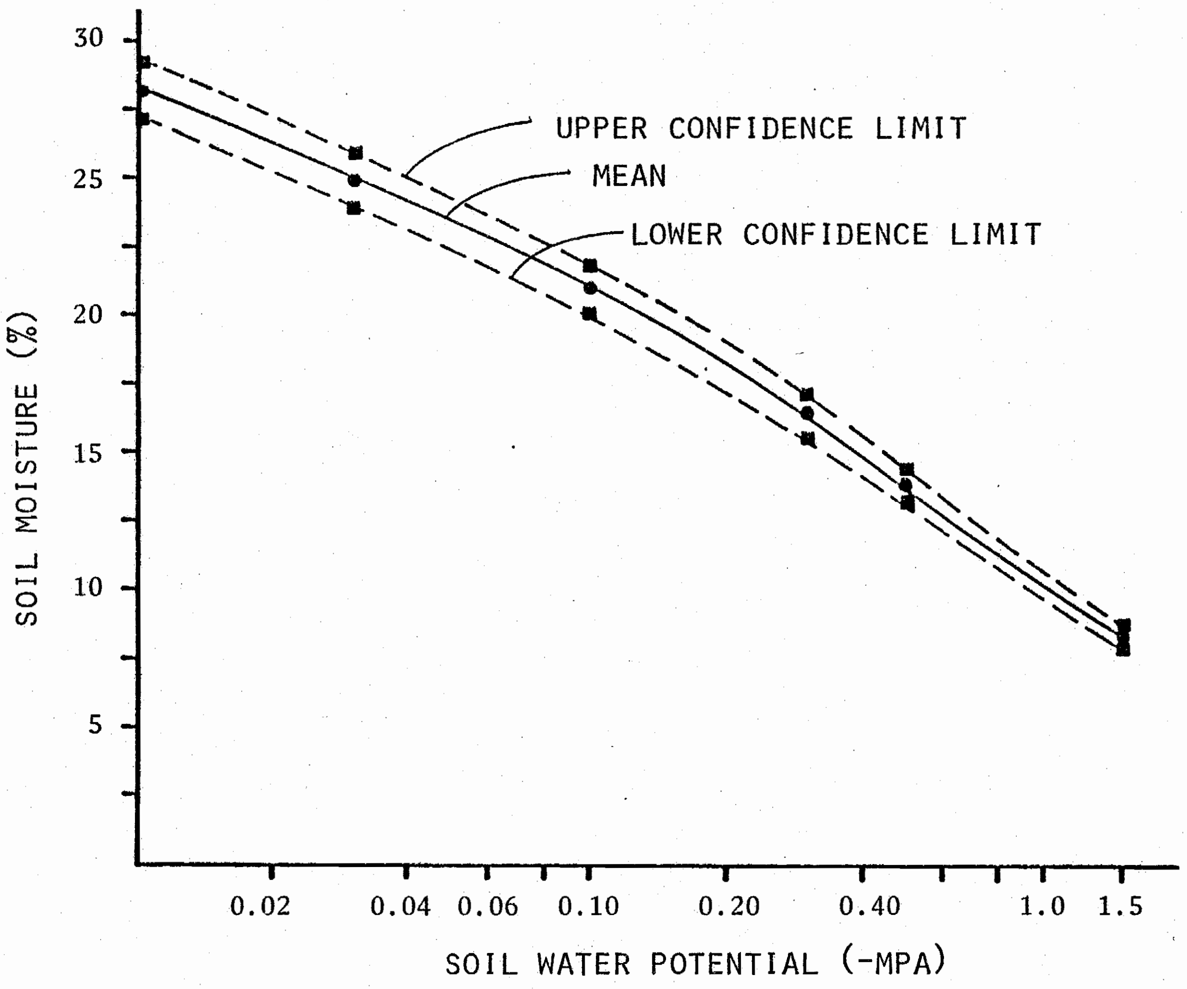

Figure A1. Soil moisture retention curve for the recontoured site with 95% confidence limits

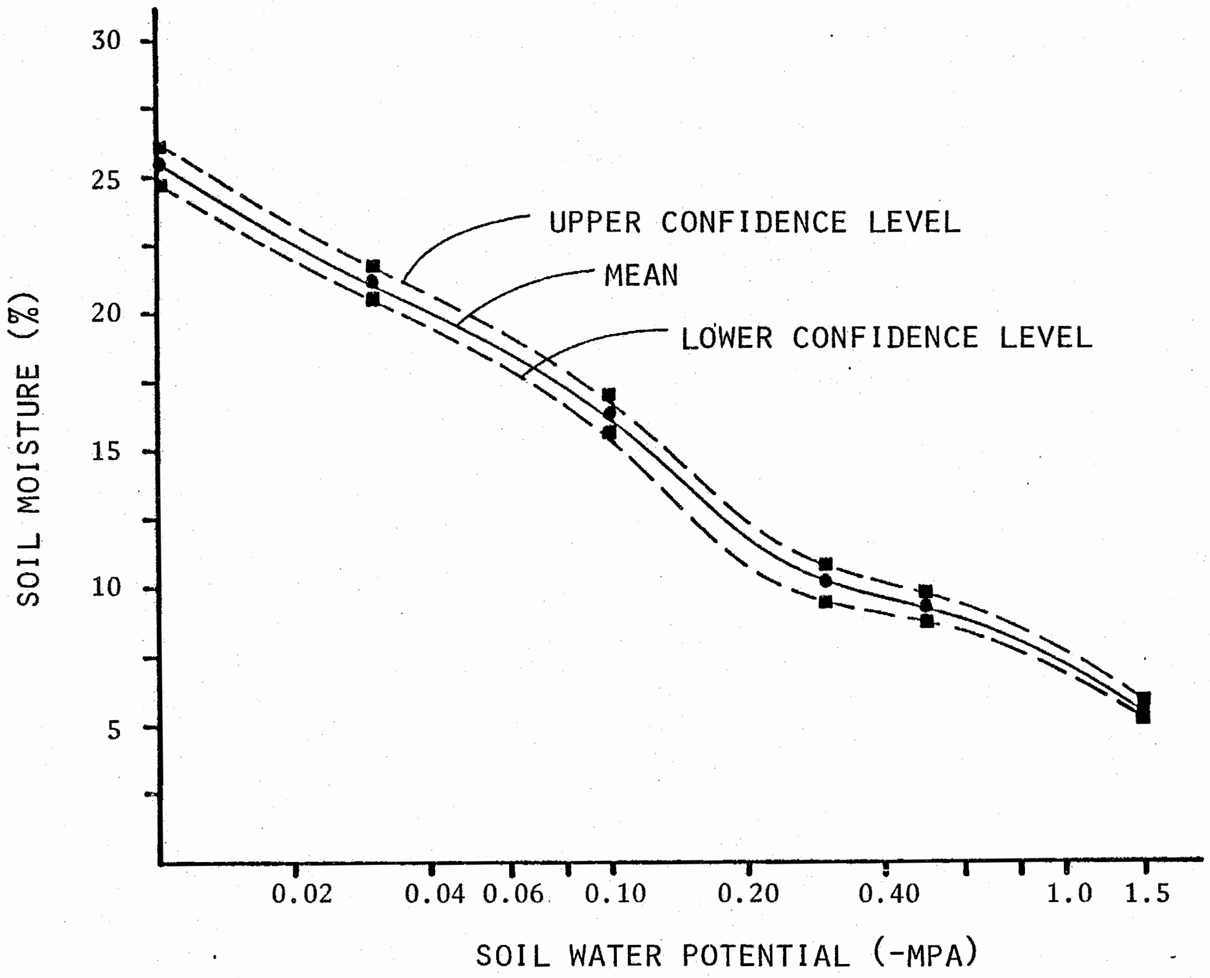

Figure A2. Soil moisture retention curve for the bench site with 95% confidence limits.

Table A11. Correlation coefficients of growth response variables in relation to fertilization and to the percentage of ectomycorrhizal colonization of pine seedling roots.

Tree Species, Fertilizer Treatment, and Ectomycorrhizal Symbiont	Pt	Other Species	Root Weight	Shoot Weight	Ht.	Diam.	Vol.	Foliar P
	- - - - - - - -	- - - - - - - -	- - - - - - - -	- - - - r	- - - - - - - -	- - - - - - - -	- - - - - - - -	- - - - - - - -
White pine - Control								
Pt	--	NS[1]	NS	NS	NS	NS	NS	NS
Other Species	NS	--	.528	.490	NS	.407	.436	NS
White pine - Fertilized								
Pt	--	NS	.263	NS	NS	NS	NS	NS
Other Species	NS	--	.544	.392	NS	.373	.322	NS
Loblolly pine - Control								
Pt	--	-.334	NS	.236	NS	.266	.274	NS
Other Species	-.334	--	.484	.364	.261	NS	NS	.662
Loblolly pine - Fertilized								
Pt	--	-.327	NS	NS	NS	.260	.244	.248
Other Species	-.327	--	NS	NS	NS	NS	NS	.249
Virginia pine - Control								
Pt	--	-.267	NS	NS	NS	NS	.274	NS
Other Species	-.267	--	.416	.400	.329	NS	NS	.761
Virginia pine - Fertilized								
Pt	--	NS	NS	NS	-.307	NS	NS	NS
Other Species	NS	--	.237	NS	NS	NS	NS	.519

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[1] NS = not significant at the 0.05 level according to Pearson product-moment correlation.

Table A12. Correlation coefficients of growth response variables in relation to chemical weed control and to the percentage of ectomycorrhizal colonization on pine seedling roots.

Tree Species, Herbicide Treatment and Ectomycorrhizal Symbiont	Pt	Other Species	Root Weight	Shoot Weight	Ht.	Diam.	Vol.	Foliar P
	- - - - - - - - - - - - - - - - - - r - - - - - - - - - - - - - - - - - -							
White pine - Control								
Pt	--	NS[1]	.369	.419	NS	NS	NS	NS
Other Species	NS	--	.578	.421	NS	.484	.480	.348
White pine - Herbicide								
Pt	--	NS	NS	NS	NS	NS	NS	NS
Other Species	NS	--	.483	.305	NS	.266	.239	NS
Loblolly pine - Control								
Pt	--	-.260	NS	NS	NS	.245	.256	NS
Other Species	-.260	--	NS	NS	NS	NS	NS	.507
Loblolly pine - Herbicide								
Pt	--	-.395	NS	NS	NS	NS	NS	NS
Other Species	-.395	--	NS	NS	NS	NS	NS	.463
Virginia pine - Control								
Pt	--	-.253	NS	NS	NS	NS	NS	NS
Other Species	-.253	--	.256	NS	NS	NS	NS	.519
Virginia pine - Herbicide								
Pt	--	NS	NS	NS	NS	NS	NS	NS
Other Species	NS	--	NS	NS	NS	NS	NS	.675

[1] NS = not significant at the 0.05 level according to Pearson product-moment correlation.

VITA

Stephen Hanley Schoenholtz, the son of Irving and Shirley Schoenholtz, was born in Kingston, Pennsylvania on March 21, 1954. Upon graduating from high school, he enrolled at Wesleyan University. After completing two and a half years of study at Weselyan, Stephen transferred to the Pennsylvania State University where he received B.S. degrees in Forest Science and Biology in 1979. Stephen was employed by the United States Department of Agriculture as a gypsy moth survey research technician for one year prior to enrolling in an M.S. program at Virginia Tech. He received his M.S. degree in Forest Biology in March, 1983, under the direction of Dr. James A. Burger. Stephen and Gloria Bernadette Hanley married in June, 1982. He is pursuing a career in forest biology research.

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