PINUS TAEDA GROWTH AND PHOSPHORUS UPTAKE AS AFFECTED BY INTERACTIONS OF MYCORRHIZAE AND SUPPLEMENTAL PHOSPHORUS

by

Victor Lavann Ford

Dissertation submitted to the Faculty of the

Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Forestry and Forest Products

APPROVED:

D.Wm. Smith

R.J. Stipes

T.L. Sharik

J.A. Burger, Co-chairman

O.K. Miller, Jr., Co-chairman

December, 1982
Blacksburg, Virginia

PINUS TAEDA GROWTH AND PHOSPHORUS UPTAKE AS AFFECTED BY INTERACTIONS OF MYCORRHIZAE AND SUPPLEMENTAL PHOSPHORUS

by

Victor L. Ford

(ABSTRACT)

A greenhouse study was intitiated to assess P uptake, growth, colonization, total mycorrhizal P levels, and mycorrhizal polyphosphate levels in loblolly pine seedlings colonized with different ectomycorrhizal fungi and grown in a Piedmont soil. The pine seedlings were inoculated with one of four species of fungi (*Scleroderma aurantium*, *Pisolithus tinctorius*, *Thelephora terrestris*, and *Rhizopogon roseolus*). Uninoculated trees served as a control. The seedlings were grown in pots containing a Cecil sandy clay loam amended with one of the following: 75 % sand, 25% sand, unamended, 56 kg P ha^{-1}, 112 kg P ha^{-1}. They were harvested ten months after planting. Shoot lengths, root lengths, biomass, and total P of all plant parts including mycorrhizae were determined. Mycorrhizae of *T. terrestris* and *S. aurantium* were analyzed for polyphosphates, and amended soils were analyzed before planting and after harvest for double-acid extractable Al, Fe, and P.

Each fungus changed postharvest extractable P, Fe, and Al differently in the soil amendments. Seedlings colonized

with S. aurantium were larger, contained more P, and had a higher degree of mycorrhizal colonization. There were no significant differences in growth among seedlings colonized with the other three fungi, but all colonized seedlings were significantly larger and contained more P than uncolonized seedlings. Soil amendments had no effect on the total levels of mycorrhizal P. Mycorrhizae of S. aurantium increased polyphosphate levels with increasing available P in the soil amendments. The pattern of polyphosphate accumulation in T. terrestris among the soil treatments was less definitive. Accumulation of foliar P was affected by the interaction of soil and mycorrhizal treatments. Control seedlings were P deficient in all soil treatments although foliar P increased as soil P increased. The accumulation of foliar P seemed to reflect the ability of each symbiont to survive, uptake P, and transfer it to the seedling. Seedlings colonized with S. aurantium were P deficient in sand-amended soils, while seedlings colonized with R. roseolus were P deficient in fertilized soils. Seedlings colonized with either P. tinctorius or T. terrestris increased foliar P with the addition of sand and the addition of P. This study indicates that S. aurantium is adapted to Piedmont soils such as the Cecil, is able to extract more of the vast amount of unavailable P present in these soils, and hence stimulate growth and P levels in loblolly pine.

ACKNOWLEDGEMENTS

I would like to gratefully acknowledge Dr. James A. Burger, Department of Forestry, and Dr. Orson K. Miller, Jr., Department of Biology, who as faculty advisors provided advice, encouragement, and friendship during my tenure at Virginia Tech.

Appreciation is expressed to Dr. David Wm. Smith, Department of Forestry, Dr. Terry L. Sharik, Department of Biology, and Dr. R. Jay Stipes, Department of Plant Physiology and Pathology, who have served on my committee and whose advice was invaluable.

I would like to thank Mr. John L. Torbert for aid in laboratory analyses and phosphorus-fixation literature. I would also like to thank Mr. Ed Childers and Mr. Steve Keeley for help in the greenhouse portion of this research. Special appreciation is expressed to Dr. Thomas J. Tworkowski, Dr. George M. Hopper, and Mr. Stephen H. Schoenholtz who aided in harvesting the seedlings, listened to me during my planning, and gave advice when needed. I thank the Virginia Division of Forestry for supplying seed. Thanks are also due to the laboratory and graduate students

of Dr. Miller for aiding in culturing the fungi. I would like to acknowledge Dr. Robert E. Adams who served as advisor during my first year of study before accepting duty in Nepal.

Very special gratitude is expressed to my family, especially my parents, for valuable support throughout my academic career. This dissertation, along with my love, is dedicated to my wife, Rhonda, and my daughter, Christine, for being there in both the good and bad times.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Loblolly pine (Pinus taeda L.) is the principal commercial pine species in the southeastern United States. It has a wide range and can, under a variety of conditions, produce more wood volume than other commercial species. It accounts for the largest percentage of trees planted and harvested in the Southeast each year. Genetically superior loblolly pine seed and seedlings are available to industry, government, and private individuals for reforestation (Dorman, 1976). It is planted primarily on Coastal Plain and Piedmont sites for lumber, pulpwood, and veneer production (Fowells, 1965).

Coastal Plain soils which are generally low in clay and organic matter are also low in phosphorus. Clays and organic matter are the primary P retention sites, and the element is leached if these are not present. Coastal Plain soils rich in organic matter tend to hold nutrients, but regeneration practices often remove large amounts of organic matter by removal, accelerating erosion, and increasing decomposition. Phosphorus fertilization on these deficient Coastal Plain soils compensates for P deficiency and increases loblolly

pine growth (Wells et al., 1973). In contrast, Piedmont soils are high in clay content and hold phosphorus as iron and aluminum compounds. These compounds are generally insoluble in soil solution, and not available to the plant. When these soils are fertilized with P, the majority of the element combines with iron and aluminum and is not available to the trees. Wells et al. (1973) conducted loblolly pine fertilization experiments on Coastal Plain and Piedmont soils. The trees responded to phosphorus fertilization on Coastal Plain soils, but did not respond to phosphorus fertilization on Piedmont soils although soil tests showed low available P.

Piedmont soils are generally high in total P, but the P is in forms not readily available for plant uptake. Movement of P to the roots in these soils is a diffusion process based on root surface area (Lewis and Quirk, 1967). Young pine seedlings find it difficult to take up the nutrient because of an underdeveloped root system that is less able to obtain P through diffusion. The result can be a phosphorus deficient seedling which cannot capture and transfer energy from photosynthetic or respiratory processes. Meristematic activity decreases in phosphorus-stressed seedlings; consequently, needle area and height growth are restricted and the seedling cannot survive competition from other vegetation.

Colonization by a mycorrhizal fungus will enhance the seedling's ability to absorb nutrients and to grow normally. The relationship of ectomycorrhizae and loblolly pine has been investigated by several workers. The U. S. Forest Service Institute of Mycorrhizae (Marx, 1980) had good success with Pisolithus tinctorius (Pers.) Coker and Couch colonizing loblolly pine growing on a variety of sites. Uninoculated seedlings or seedlings colonized with Thelephora terrestris (Ehrh.) Fr., a very common mycorrhizal fungus on seedlings in nursery soils (Marx, 1980) were generally less successful in growth and survival than P. tinctorius - colonized seedlings. Studies have not been carried out to examine the effect of other mycorrhizal fungi on loblolly pine growth in Piedmont soils.

Ectomycorrhizae may exploit water-insoluble forms of phosphorus in the soil (Bowen, 1973). Bowen and Theodoru (1967) have shown that Pinus radiata D. Don seedlings inoculated with different ectomycorrhizal fungi can selectively mediate the uptake of different forms of P. Therefore, the potential for selection of fungi to exploit different forms of soil P is possible. Bowen (1973), however, stated that the limiting factor in P uptake from the soil is the slow diffusion rate to the root; the differential absorption of phosphorus may play only a minor

role in stimulation of tree growth in phosphorus-deficient soils. The morphology of the root affects diffusion of phosphorus (Bowen, 1973), and different species of mycobionts result in different short root morphology. The exact effect of morphological differences in colonized short roots on P uptake is not known. The role of different mycorrhizal fungi in stimulating tree growth may arise from the ability of one symbiont to capture differentially and store more nutrients than another during periods of increased availability which often result from fertilization or litter fall. In addition, the mycelium of one mycorrhizal symbiont may exploit more soil volume than that of another fungus.

Studies have shown that certain fungi predominate at specific pH ranges, temperatures, and moisture regimes (Meyer, 1975). Therefore, certain mycorrhizal fungi may be more efficient than others in a given environment in terms of nutrients absorbed from the soil per unit of carbohydrate supplied to the mycobiont. Bowen (1973) stated that the task of the ecologist-physiologist is to interpret the physiological research in an ecological framework. These interpretations should aid in assessing and selecting certain superior strains or species of fungi so that specific environment-plant-fungus combinations can be selected for maximum production.

Objectives

Since there appear to be differences among mycorrhizal symbionts in their ability to stimulate loblolly pine grown under specific conditions, a greenhouse experiment was used to evaluate the effect of different symbionts on degree of colonization, seedling biomass, and the partitioning of phosphorus to the needles, stems, and roots. The specific objectives of this study were to:

1. Compare the ability of four species of fungi to produce mycorrhizae and to enhance height, diameter, root, and biomass growth of loblolly pine seedlings growing in a Piedmont soil adjusted to five levels of available phosphorus.

2. Correlate mycorrhizal P pools with pine seedling phosphorus levels and seedling growth.

3. Determine how phosphorus is partitioned among the roots, stems, and needles of loblolly pine seedlings in each of the mycorrhizal and soil treatments.

4. Compare changes in available phosphorus among soils due to the presence of different mycorrhizal fungi.

LITERATURE REVIEW

Definition of Ectomycorrhizae

A mycorrhiza is a compound organ composed of two tissue types:

1. The cortical cells of a root.

2. The hyphae of a fungus.

Ectomycorrhizae are characterized by a mantle or sheath composed of hyphae which cover a short feeder root. The feeder root under the mantle is also referred to as the core. In the genus Pinus, short roots arise from lateral roots, and the formation of short roots is stimulated by indoleacetic acid produced by the fungus (Slankis, 1973). The short roots which differentiate as a result of the symbiosis may be monopodial, bifurcate, coralloid, or nodulate in form. The occurrence of these forms apparently depends on the fungal species, the host plant species, and the soil characteristics (Slankis, 1973). The symbiont covers the short roots with several layers of hyphae and forms the mantle. The hyphae penetrate between the cortical cells and completely replace the middle lamella without

disturbing the plasmodesmata (Nylund, 1980). The intercellular hyphae which result are called the Hartig net. All nutrients and water must pass through both the mantle and Hartig net before reaching the cortical cells or the stele.

Initial colonization results when the root makes contact with some fungal structure such as the sexual or asexual spores or hyphae (Harley, 1969). The mycelium surrounds the root and forms the mantle. As the process of mantle formation occurs, the Hartig net is also formed. As a response mechanism to invasion, the plant produces tannins and other phenolics that completely infiltrate the cortical cells (Marks and Foster, 1973).

Anatomy and Function of Ectomycorrhizal Structures

External Hyphae

External hyphae are the nutrient-gathering parts of the mycorrhizae (Bowen *et al.*, 1972). The hyphae are able to exploit more soil volume than a comparable network of fibrous roots due to the increased absorptive surface area of the hyphae. Mycelial growth uses less photosynthate per unit area of soil exploited than a nonmycorrhizal root system alone (Bjorkman, 1949) and therefore is more energy efficient. According to Bowen (1973), mycelial strands

penetrate long distances through the soil, but individual hyphae penetrate only a short distance. Formation of these strands and their growth are not well understood. However, Went and Stark (1968) postulated that the mycelium of mycorrhizal fungi could serve as a means of absorbing potentially leachable nutrients from the litter layers which could aid in preventing nutrient losses. Presently, no one has explored the differences in mycelial development among fungal species and how this leads to differential absorption from the soil.

The Mantle

The fungal mantle is a key part in the mycorrhizal relationship. The fungal sheath is nearly 40 percent of the total biomass of the mycorrhizal short root (Harley and McCready, 1952). The large mantle obtains carbonydrates from the host, and studies have shown the loss is direct and rapid (Lewis and Harley, 1965; Harley, 1978). Storage compounds, such as trehalose, mannitol, and glycogen, are accumulated in the sheath and allow the short root to survive for several days after being severed from the plant. Harley (1978) estimated that 10 percent of total carbohydrate going for wood production is diverted into maintainence of the mantle. In exchange for this vast amount

of carbohydrates, it is hypothesized that the mantle accumulates, stores, and transfers nutrients to the woody plant.

The Hartig Net

The Hartig net is a distinguishing feature of true ectomycorrhizae from ectendomycorrhizae or pseudomycorrhizae. The depth of hyphal penetration of the Hartig net into the root cortex has been used to characterize different ectomycorrhizal symbionts (Zak, 1973). This development splits the middle lamella by mechanical and enzymatic action. The presence of pectinases produced by the ectomycorrizal fungi is disputed. Marks and Foster (1973) reported that the penetration between cortical cells was a mechanical phenomenon. When root growth is slow, the penetration is increased and the hyphae are able to split the cells apart. This supports data presented by Chivers and Gust (1982), who found that actively growing roots were not colonized by mycorrhizae although strands were present on the epidermis.

The Mycorrhizosphere

The complex interaction at the interface between soil and hyphae termed mycorrhizosphere has recieved little

attention. Marks and Foster (1973) explored the close relationship of the rhizosphere to the ectomycorrhizal short roots and related this to mantle morphology and physiology. Different types of bacteria apparently predominate in the rhizosphere of different ectomycorrhizal fungi. Oswald and Ferchau (1966) reported ovoid bacteria associated with white mycorrhizae and rod bacteria associated with red mycorrhizae. Their observations suggest that each symbiont controls the rhizosphere composition so that the bacteria population is unique. Marx (1972) postulated that bacterial secretions in the rhizosphere increased disease resistance. Bowen and Roviria (1966) showed that rhizosphere bacteria could fix nitrogen in the presence of mannitol. Lewis and Harley (1969) detected mannitol secretion by mycorrhizae which suggested that bacterial nitrogen fixation could take place.

Alexander (1979) stated that rhizosphere bacteria can solubilize mineral phosphates. Foster (1981) found that the hyphae of some fungi are covered with a polysaccharide that helps prevent desiccation. This polysaccharide covering is probably very similar to the musigel sheath covering a metabolically active root. Bacteria are known to inhabit this covering and it has been postulated that this is the site of associated phosphorus liberation and nitrogen fixation (Foster, 1981).

Ocurrence of Ectomycorrhizae in the United States

There are more than two thousand species of ectomycorrhizal fungi that are presumed to be symbiotic with forest trees in North America (Kormanik et al., 1977). The majority of these fungi are Homobasidiomycetes, primarily Amanitaceae, Boletaceae, Gomphidiaceae, Cortinariaceae, Russulaceae, Tricholomataceae, Rhizopogonaceae, Clavariaceae, and Sclerodermataceae (Miller, 1980). A few orders of Ascomycetes, ie. Eurotiales, Tuberales, Pezizales, and Helotiales, are known to be ectomycorrhizal symbionts. In addition, the genus Endogone in the Zygomycetes forms ectomycorrhizae with pine and eucalyptus. Most Northern Hemisphere forest tree genera and many shrubs, fruit and nut trees form ectomycorrhizae. The most common families are the Pinaceae, Betulaceae, Fagaceae, Juglandaceae, Tiliaceae, and Salicaceae (Meyer, 1973).

Use of Ectomycorrhizae in Forestry

Need for Mycorrhizae

Recent reviews by Mikola (1973), Molina (1977), and Marx (1980) have examined the use of ectomycorrhizae in routine forest operations. Their consensus was that the greatest use of mycorrhizae in forestry was in reforestation and afforestation of lands with exotic species. Attempts to

establish exotic pines routinely failed until the essential symbionts were introduced (Kessell, 1927; Hatch, 1936; Briscoe, 1959; Clements, 1941; van Suchtelen, 1962; Madu, 1967; Vozzo and Hacskaylo, 1971). Pine and oak seedlings demonstrated a need for mycorrhizae in the afforestation of the grasslands of Russia and the Great Plains of the United States (Hatch, 1937; McComb, 1938; White, 1941; Rosendahl and Wilde, 1942; Shemahanova, 1962). The primary purpose for inoculating with symbiotic fungi is to provide seedlings with adequate mycorrhizae for the tree to survive transplanting and to establish an efficient root system. The use of ectomycorrhizae can be of major significance to artifical regeneration. The majority of past research on inoculation with ectomycorrhizal symbionts has been with nursery produced or containerized seedlings, and this trend will probably continue. However, the inoculation of seed for direct seeding operations could be an alternative to planting seedlings on rough terrain or remote sites (Marx, 1980).

Today, widescale inoculation of nursery soils and containerized seedling media with selected mycorrhizal fungi appears to be imminent. The success of these inoculation programs hinges on the selection of effective and beneficial symbionts (Molina, 1977). Few data exist on which of the

many ectomycorrhizal fungi may be the best candidates for inoculating a particular host species. Nursery management practices and outplanting sites complicate the selection process. These complications can be resolved with proper and precise research. With a better understanding, the culture and selection of the symbiotic system would be an added tool available to the forester to maximize seedling survival and growth.

No research has addressed the possibility of manipulation of symbionts in older stands. It is presumed that after a period of years those fungi adapted to a particular site will be present. However, cultural practices such as fertilization change the soil environment and may compromise the effect of native fungi. Fungi adapted to higher nutritional levels may be introduced into the stand at the time of fertilization and aid in the efficient use of the nutrients. For example, older stands are fertilized at 5 to 15 years before harvesting with nitrogen to increase biomass yields (Pritchett, 1979). The ectomycorrhizal fungus, Paxillus involutus, is a fungus that is adapted to and requires high levels of nitrogen (Trappe, 1977). The introduction of this fungus may aid in the efficient uptake and use of the nitrogen added upon fertilization.

Benefits of Ectomycorrhizae in the Field

Ectomycorrhizae benefit the growth and development of woody plants by increasing:

1. Nutrient and water absorption through increased absorptive surface area.

2. Nutrient mobilization through biological weathering.

3. Root longevity by

   a) providing mechanical and chemical barriers against root pathogens

   b) producing hormones which increase the absorptive life of the short root.

Field performance, however, is proof of the benefits supplied by the ectomycorrhizal symbionts. This can only be evaluated by properly designed, installed, and maintained field experiments over a number of years. Limited data of this type are present in the literature. Greenhouse and short-term field plantings only lend limited evidence about the selection of fungi, but provide evidence for the biological processes involved in the symbiosis. Moser (1963) followed the progress of inoculated Pinus cembra L. for

three years after planting. The mycorrhizal seedlings were inoculated with a mixture of fungi and were compared with uninoculated controls. He found that the mixture of symbionts greatly stimulated tree growth at the end of the experiment. Vozzo and Hacskaylo (1971) found that mycorrhizae in soil inoculum stimulated Pinus caribaea Morelet growth regardless of the level of fertilization. Theodorou and Bowen (1970) observed that pure cultures of Suillus granulatus (Fr.) Kuntze, Suillus luteus (Fr.) S.F. Gray, and Rhizopogon luteolus (Corda) T.M.Fr. stimulated field growth of P. radiata after two years. Their results suggested that pure cultures of S. granulatus and R. luteolus were best adapted for this pine species in Australia.

Marx (1980) summarized his work with southern pines adverse sites and routine reforestation. Pisolithus tinctorius - colonized seedlings outperformed Thelephora terrestris - colonized and uncolonized seedlings on coal spoils in Kentucky and Virginia. Thelephora terrestris - colonized seedlings were chlorotic and had few functional mycorrhizae. Similar results were found in kaolinitic spoils, borrow pits, and the Copper Basin region in southeastern Tennessee. Based on his results, Marx (1980) stated that mycorrhizal seedlings are necessary for survival

and growth on adverse sites. He also concluded that Pisolithus mycorrhizae are better adapted to adverse sites even after the soil amended with sludge or fertilizer.

For routine reforestation, Marx (1980) estimated pine growth and survival differences by plot volume index (PVI). The formula is as follows:

PVI, cm^3 = (root collar diameter, cm)^2 * (height, cm) * (No. of surviving seedlings per plot).

Pisolithus - colonized pines planted in Florida and North Carolina outperformed Thelephora - colonized pines after two years in the field. The increase of PVI due to Pisolithus colonization over Thelephora was 25 percent on good sites and 50 percent on poor sites. However, in Florida, unfertilized plots with Pisolithus had a 175 percent increase of PVI over the same plots with Thelephora, but in fertilized plots, the PVI for both fungi were the same.

Site preparation, release, fertilization, and replanting operations are labor intensive and expensive. Site adapted symbionts would be more efficient in stimulating growth so that seedlings could outgrow competing vegetation. The use of proper mycorrhizae may increase seedling growth greatly. Also, nutrients would be more efficiently used so that the site would be producing at its full potential.

Research in the proper use of the mycorrhizal symbiosis for forestation is in its infancy. Millions of hectares in

the world, particularly in Third World nations, are in need of proper afforestation and reforestation. Much more practical and basic knowledge is needed before mycorrhizal fungi can be utilized effectively in routine nursery and forest management.

Inorganic Phosphorus Availability in Acid Soils

Phosphorus Fixation

Phosphorus is found as phosphate in the soil (Bonn et al., 1979). Hydrogenation of the free oxygen groups are pH dependent. In very acid soils with a pH range of 3.5 to 5.5, the predominate form of phosphate is $H_2PO_4^{-1}$ (Bonn et al. 1979).

Phosphorus is generally immobile, except in very sandy soils, because of the numerous fixation reactions. Iron and aluminum associated with clayey soils are primarily responsible for these phosphorus fixation reactions. Highly weathered Piedmont soils that are high in kaolinitic clays and iron and aluminum oxides are generally considered problem soils in terms of low phosphorus availability for agronomic crops. These soils may contain 4.8 umoles P g^{-1} (150 ppm) dry weight soil; however, extractable P levels are less than 0.05 umoles ml^{-1} (1.5 ppm) (Torbert, 1982). Researchers have found that 90 percent of the phosphorus

added as fertilizer to acid soils forms an iron or aluminum compound which is not water soluble or readily available for plant growth (Yuan et al., 1960). The availability of specific soil phosphorus compounds depends on the nature of the compound and the soil environment.

Phosphorus fixation can be viewed as occurring by two general mechanisms:

1. Precipitation as colloids with exchangeable iron and aluminum.

2. Adsorption to the surfaces of aluminum and iron hydroxides which coat soil particles and occupy interlayer positions of 2:1 clay minerals (Hsu, 1965).

Precipitation reactions are most prevalent at low soil pH levels. At a pH less than 4.5, most aluminum exists in a trivalent form and reacts readily with soil solution phosphorus to form insoluble, amorphous particles (Bonn et al., 1979). The availability of these phosphorus compounds are subject to the solubility product principal described by Lindsay and Moreno (1960). Fresh precipitates of Fe phosphate are more soluble than fresh precipitates of Al phosphate and are more readily available to plants due to the greater surface area of the amorphous mass (Juo and

Ellis, 1968). With time, these collodial amorphous materials crystallize and become even less available. Iron phosphates crystallize faster than aluminum phosphates to become varscite as opposed to strengite. Aluminum phosphate crystals have a greater surface area than iron phosphate crystals and are thought to be more soluble and more available for plant uptake (Juo and Ellis, 1968).

Phosphorus is absorbed to the surface of these Fe and Al sesquioxides by (1) high energy chemisorption reactions via ligand exchange with hydroxyl groups at the edge or (2) low energy electrostatic attraction. This electrostatic form is more readily released into solution. The amount of phosphate attraction is dependent upon the degree of surface saturation (Sample et al., 1976).

After a period of time, the phosphate that has been precipitated or adsorbed becomes covered by subsequent deposition of new oxides. This process is called occlusion. Occlusion of chemisorbed reactants also takes place as P groups work their way in from the surface of hydrous oxides. Occluded P is generally considered not available for the plant due to the amount of material covering the phosphorus (Juo and Ellis, 1968).

Hsu (1965) concluded that, in principal, there were no differences in precipitation and adsorption of phosphorus,

but the formation of these compounds is dependent on the form of iron and aluminum available. The concentration of phosphate in solution is the summation of the activities of iron and aluminum. However, due to the surface area of clay micelles and their pH dependent charges, adsorption is the predominate mechanism of phosphorus fixation.

After studying aluminum and iron in the soil, researchers have estimated the solubility of phosphorus (Thomas, 1974). Cole and Jackson (1951) calculated the solubility of varscite, Al(OH) H PO , which dissociates as:

$$Al(OH)_2 H_2PO_4 \longrightarrow Al^{+3} + 2\ OH^- + H_2PO_4^-.$$

Their calculations on the solubility of the compound were purely theoretical and are not verifiable due to other aluminum reactions (Thomas, 1974). However, general trends could still be determined in terms of aluminum concentration as related to phosphorus concentration. If aluminum is rendered inactive in soil solution due to chelation or precipitation, phosphorus concentration in soil solution increases. If phosphorus is removed from soil solution, variscite is dissolved until an equilibrium is reached. Chang and Jackson (1957a) made similar calculations for strengite, $Fe(OH)_2\ H_2PO_4$. They found that the solubility of strengite and the equilibrium of phosphorus in solution makes it unavailable for plant growth. Thomas (1974)

concluded that based on their relative solubilities, aluminum phosphates were the prevalent form of available phosphorus at a pH range from 4 to 5.

Availability

With the fixation and formation of insoluble phosphate compounds, there are problems with plant availability. Presently, there are two mechanisms postulated for the movement and release of phosphorus from the soil. It is hypothesized that both mechanisms work cooperatively.

The first mechanism is diffusion of phosphorus to the root. Lewis and Quirk (1967) developed a model of phosphate diffusion which assumed that a zone of depletion existed around the roots. This zone of depletion resulted in a diffusion gradient in which phosphate outside of this depletion zone diffused from areas of higher concentration to areas of lower concentration. This depletion of phosphorus also created a thermodynamic instability around the sites of phosphorus fixation and affected a release of phosphate from these insoluble compounds. Lewis and Quirk's model was derived from aseptically grown wheat roots. They found that root hairs were more efficient in establishing the diffusion gradient than feeder roots due to the increased surface area. This was only true if the root hair

density was not so great that the diffusion zones would overlap. If overlapping did occur, efficiency would drop to the same level as a feeder root. Sanders and Tinker (1973) postulated that hyphae behaved like extremely efficient root hairs. The growth and maintainence of hyphae required less carbohydrate per unit surface area than root hairs. They postulated that the increased surface area created by the hyphae allowed for a steeper diffusion gradient and faster phosphate diffusion.

Ectomycorrhizal anatomy also is well adapted for phosphate uptake via diffusion. The characteristic swelling of the short root increases root surface area. Hyphae and rhizomorphs penetrate the soil from the short roots and increase the effective absorptive area. Mycorrhizal short roots are longer lived than nonmycorrhizal short roots (Meyer, 1974). The extended life allows for a greater depletion zone and diffusion gradient. By living longer, the short roots exploit each volume of soil longer without expenditure of photosynthate which then may be used in increasing the biomass of the host.

The second mechanism for the release of insoluble P is biological weathering. Phosphate may be liberated from fixed iron and aluminum forms by the action of organic acids produced by the roots, the mycorrhizae, or associated

organisms in the rhizosphere (Alexander, 1971). Henderson and Duff (1963) showed that fungal activity released metallic and silicate ions from the soil. Tan (1980) observed release of aluminum in the decomposition of soil minerals by humic acid. Tan et al. (1978) found humic acid compounds produced by the mycorrhizal fungus Pisolithus tinctorius which could be active in the dissolution of insoluble aluminum and iron phosphates. Iron minerals have been demonstrated to be dissolved by soil microorganisms (Arrieta and Grez, 1971). The chelation of iron by organic acids is the primary means by which plants uptake iron from the soil (Lindsay, 1974). Foy (1974) also stated that the efficiency of these organic acids increased with the number of carboxyl and functional hydroxyl groups and decreased with the length of the carbon chain. Mycorrhizal fungi produce these simple organic acids in culture, and it is presumed that they are produced in situ (Bowen, 1973).

Diffusion of these organic acids produced by the mycobiont or mycorrhizosphere-associated microbes may aid in the exploitation of phosphorus and the formation of the phosphate diffusion zone. The combination of diffusion and solubilization of insoluble phosphate compounds by plant produced or mycorrhizal organic acids may be the survival mechanism for phosphorus uptake in soils where iron and

aluminum predominate. The production of organic acids may account for the differences in phosphorus uptake with different mycorrhizal fungi. Some fungi are suitable for a high organic matter environment and may produce phosphatases which cleave phosphate from organic molecules. Other fungi adapted to mineral soils may produce organic acids which dissolve unavailable inorganic phosphorus.

Phosphorus Fractionation

Chang and Jackson delineated a soil P fractionation scheme based on the predominate forms of inorganic P in the soil. This procedure was later modified by Petersen and Corey (1966). The process of extracting the various forms of P involves the systematic dissolving of soil P minerals by various reagents. In theory, the P fractionation scheme removes varscite (Al-P), strengite (Fe-P), and apatite (Ca-P) from the soil and assumes that these minerals are the predomoinate forms. Water-soluble P is removed by a neutral extracting solution, 1 N NH_4Cl. This extractant removes the loosely bound P and free Ca and Al which prevents further fixation. Aluminum phosphates are removed from the soil by 0.5 N NH_4F adjusted to pH 8.2; Fe-phosphates are removed by 0.1 N NaOH; and 0.5 N H_2SO_4 is used to extract the Ca-phosphates. Between each of the last three steps, the soil

is rinsed a saturated NaCl solution to remove traces of the previous reagent. Occluded and reductant P can also be determined by the fractionation procedure. These forms comprise the bulk of soil P in Piedmont soils (Torbert, 1982), but are thought not to be plant available under most conditions. Sample and others (1976) stated that some phosphate compounds are not totally extracted in any reagent. An example is crandalite which is only 40 percent extractable in 0.5 N H_2SO_4. Overall, the fractionation procedure is useful only in obtaining relative amounts or characterizing the nature of soil P (Torbert, 1982).

Polyphosphates

Inorganic polyphosphates are phosphate polymers linked together with an anhydride bond. These bonds are equivalent to the terminal phosphate bond of ATP in terms of chemical free energy. Linear condensed polyphosphates have a general formula of $M_{(2n+2)}\ P_n O_{(3n+2)}$, where M is a cation such as NH_4^+, Mg^{+2}, Ca^{+2}, Na^+, K^+, or an amino acid. The linear condensed polyphosphates are the most prevalent in biotic systems. Cyclic condensed and branched polyphosphates do not occur in living systems (Harold, 1966). Significant amounts of polyphosphates have been found in bacteria, fungi, and algae, and the compound plays an important role in the energetics and the metabolism of nucleotides.

Histochemical detection of polyphosphate granules depends on the polymer reacting with certain dyes, such as toluidine blue. In the presence of these granules, the dye reflects a pink-purple instead of the usual blue color reaction. This color reaction is actually a metachromatic shift (Kulaev, 1979). Only high molecular weight polyphosphates are capable of undergoing this shift; pyrophosphates and triphosphates do not stain pink (Harold, 1966). The pink staining structures are called volutin granules, but care should be taken in interpreting all structures stained pink in toluidine blue to be polyphosphates because other polymeric structures are also capable of producing this metachromatic shift. Ten percent trichloroacetic acid (TCA) has been used to separate polyphosphates from other volutin granules by dissolving the polyphosphates after sectioning (Kulaev, 1979).

There are many chemical methods used in determining polyphosphate concentration. The most prevalent of these techniques are modifications of the Schmidt-Thannhauser procedure which divides polyphosphates into those soluble and those insoluble in cold acid. Polyphosphates were then hydrolyzed into the orthophosphate monomer units in 1 N acid at 100 C. Orthophosphate is then determined before and after hydrolysis to obtain the amount of phosphorus in the polyphosphate fraction (Baker and Schmidt, 1963).

Ling-Lee and others (1975) found polyphosphate granules in ectomycorrhizae, endomycorrhizae, and ectendomycorrhizae. They postulated that the uptake and storage of phosphorus may be a common denominator of all mutualistic mycorrhizal phenomena. Other workers have found and isolated polyphosphates from mycorrhizal systems, but none has characterized the dynamics or the effect on the associated phycobiont. Harley (1978) suggested polyphosphates were accumulated, stored in the mantle, and slowly released to the green plant. This benefit can clearly be seen during periods of nutrient surpluses which occur in the autumn during litter fall. These surpluses do not correspond to peak nutrient demand of the woody plant in the spring. The storage mechanism insures that the nutrients are available during times of need. Cox et al. (1975) observed polyphosphate granules in vacuoles of mycorrhizal fungus mycelia. They stated that these packets of phosphate were transported by cytoplasmic streaming along with associated cationic nutrients, degraded, and transferred to the phycobiont.

Phosphorus accumulation in mycorrhizal roots is an aerobic process. Under flooded conditions, mycorrhizae are not present due to an intolerence to low partial pressures of oxygen. Harley and others (1953) showed that uptake by

mycorrhizal beech roots was linearly related to oxygen concentration between partial pressures of 0 and 10 percent. The results of Harley's work present strong evidence that phosphorus uptake depends on respiration. The reason is that the uptake of phosphorus from soil solution depends on an ATP carrier enzyme, and polyphosphate synthesis uses ATP as a precursor. Ling-Lee and others (1975) revealed that polyphosphate granules accumulated during active phosphate uptake. To take up phosphorus properly from the soil, healthy, metabolically active mycorrhizae are needed. Conditions under which the fungus is stressed or compromised limits the efficiency of phosphorus uptake.

METHODS AND MATERIALS

Greenhouse 10 C on the Virginia Tech campus was the setting for the experiment, and the seedlings were grown from August, 1980 to June, 1981. Chemical analyses of the soil and tissue samples were conducted in the Forestry Department's tree nutrition and tree physiology laboratories. Mycorrhizal fungi were obtained in cooperation with Dr. O.K. Miller, Jr. of the Department of Biology, Virginia Tech.

Installation of the Experiment

Soil

The Cecil series is a common series found throughout the Piedmont physiographic province. The upper 60 cm of a slightly eroded Cecil sandy clay loam on a 2 to 5 percent slope (clayey, kaolinitic, thermic, Typic Hapludult) was taken from a forest site at the Reynolds Homestead Research Center, Critz, Virginia (lat. 36° 38', long. 80° 08'). After sieving (1 cm^2), the soil was placed on and covered with a black polyurethane, fumigated with methyl bromide for 72 hr, and then allowed to air for a week. The soil was

then divided into five portions and amended to provide the following five soil treatments:

1. 75% fine sand + 25% soil.

2. 25% fine sand + 75% soil.

3. Soil only.

4. Soil + 56 kg P ha^{-1} (50 lb P ac^{-1}).

5. Soil + 112 kg P ha^{-1} (112 lb P ac^{-1}).

Nutrient rates were based on pot surface area. The rates of fertilization were based on recommendations by Wahlenberg (1965). The fine, sterile silica sand used to dilute the available phosphorus in two treatments was acid washed in dilute HCl before mixing. Five water slurries were formed my mixing an eighth of the medium per treatment with water and phosphorus in the form of potassium and nitrogen salts in a cement mixer. Nitrogen and potassium were added to all five treatments at a rate of 56 kg ha^{-1}. Each slurry was poured on plastic sheets and allowed to dry. The dried slurry was pulverized and thoroughly mixed with the remaining soil in a cement mixer. The slurry method insured chemical equilibrium of the P added to the two treatments and a uniform distribution of all nutrients. The mixed soil was added to

15 cm pots prefilled to a 2 cm depth with horticultural perlite. The perlite was used to promote adequate drainage and root pruning. The pots were randomly arranged in the greenhouse, thoroughly watered, and allowed to set for four weeks.

Fungi

The fungi (Table 1) were grown in an 8:1 (v/v) mixture of vermiculite and finely ground peat. A modified Melin-Norkrans solution (Table 2) was applied at the rate of two volumes of solution to one volume of peat-vermiculite medium. The fungi were aseptically introduced from stock cultures into 1 l Erlenmeyer flasks containing 900 ml of growth medium. The flasks were closed with a sterile cotton plug after inoculation and placed in the dark. Several flasks were uninoculated to serve as a control. The growth medium was removed after four months, rinsed thoroughly with tap water, and stored for 24 hr. The procedures used are modifications from Marx and Bryan (1975).

The fungi were chosen because they are known to form mycorrhizae with loblolly pine and did so in preliminary studies. All are Homobasidiomycetes, but they represent a wide range of taxa and ecological niches. Pisolithus tinctorius (Pt) (Sclerodermatales, Pisolithaceae) has been

TABLE 1

Ectomycorrhizal fungi used in this study.

Species	Abbreviation	Culture No.
Rhizopogon roseolus (Corda) Th. Fr.	(Rr)	VT 1011[1]
Thelephora terrestris (Ehrh.) Fr.	(Tt)	VT 1010
Pisolithus tinctorius (Pers.) Coker and Couch	(Pt)	VT 929
Scleroderma aurantium Pers.	(Sa)	VT 988

[1] VT represents isolates from the culture collection at Virginia Tech Department of Biology.

TABLE 2

A modified Melin-Norkrans solution (MMN)[1] used as a nutrient medium for culturing the fungi.

Solution component	Quantity / liter
KH_2PO_4	0.5 g
$MgSO_4 \cdot 7\ H_2O$	1.5 g
$(NH_4)_2HPO_4$	0.25 g
NaCl	0.025 g
CaCl	0.05 g
D - glucose	2.5 g
Thiamine - HCl	0.05 ug
Malt extract	3.0 g
1% FeCl	1.2 ml

[1]Solution adjusted to pH 5.5 with 1 N H_3PO_4

After Marx (1969)

studied throughout the southeast under a variety of conditions (Marx, 1977). This fungus is found naturally in hot, dry rocky conditions. Thelephora terrestris (Tt) (Aphyllophorales, Thelephoraceae) also has been studied with loblolly pine. Tt predominates on moist fertile soils such as those found in nurseries (Marx et al., 1978). Rhizopogon roseolus (Rr) (Hymenogastrales, Rhizopogonaceae) was synthesized with loblolly pine by Hacskaylo and Palmer (1955). The author has collected this species and associated mycorrhizae in pine stands with a developed litter layer. No research has been accomplished using the fungus Scleroderma aurantium (Sa) (Sclerodermatales, Sclerodermataceae) on loblolly pine. Nutrient uptake by this fungus has not been explored. The author has collected this fungus on fine-textured soils under Pinus and Quercus spp.

Seed and Seedlings

Loblolly pine seeds were collected from the Virginia Division of Forestry seed orchard from clones native to the Virginia Piedmont and stored air-dried at 1 C. The seeds were separated in 90 percent ethanol and those that floated were discarded. The remaining seeds were rinsed twice with sterile water, placed in a covered beaker, and stored at 1 C for two weeks. The seeds were then bubbled in 10 percent

hydrogen peroxide for ten minutes, rinsed with sterile water, and placed in a petri dish on wetted filter paper. The dishes were placed in a growth chamber under 16 hr of light with temperatures of 22 C day and 18 C night. After two weeks, the germinated seeds were placed in seed flats with a 1:4 (v/v) mixture of rinsed inoculum to peat-vermiculite. The seed flats were covered with horticultural perlite and watered with modified Melin-Norkrans solution. The flats were transferred to the greenhouse and watered with tap water as needed. In August, one month later, the loblolly pine seedlings were planted in pots containing the moist soil amendments. Approximately 1 cm^3 of seed flat medium was transferred with each seedling to insure mycorrhizal colonization. All treatments were randomly assigned on six benches in the greenhouse. A total of 750 seedlings were planted using 30 seedlings per treatment combination. Seedlings were watered at least once per week but more often when greenhouse temperatures were high.

Harvesting the Trees

Two harvests were conducted using the first as a preliminary survey. Ten individuals from each treatment combination were sampled during January, approximately five months after planting. The preliminary harvest assessed

mycorrhizal infection and refined techniques for the final harvest. Results from the preliminary harvest are not presented. The final harvest occurred in June, ten months after planting. Shoot length, live crown length, and root collar diameter were measured at time of harvest. A soil sample was collected from each pot. Stems, roots, and needles were separated from each seedling, and stems and needles were placed into individual bags for biomass and P analysis.

Roots required additional treatment to obtain needed information before biomass and P analysis. They were rinsed thoroughly in tap water and then sonic cleaned in tap water to remove the clay. Root length and colonized root length were estimated by the line intersect method developed by Tennant (1975). The roots were photographed on a 1 x 1 cm grid with black and white film (Panatomic-X ASA 125). The negative was projected and the root intersections counted. After the roots were photographed, mycorrhizae were cut from the lateral roots, placed in borosilicate tubes, and frozen with dry ice.

Post-Harvest Tissue Analysis

Needles, Roots, and Stems

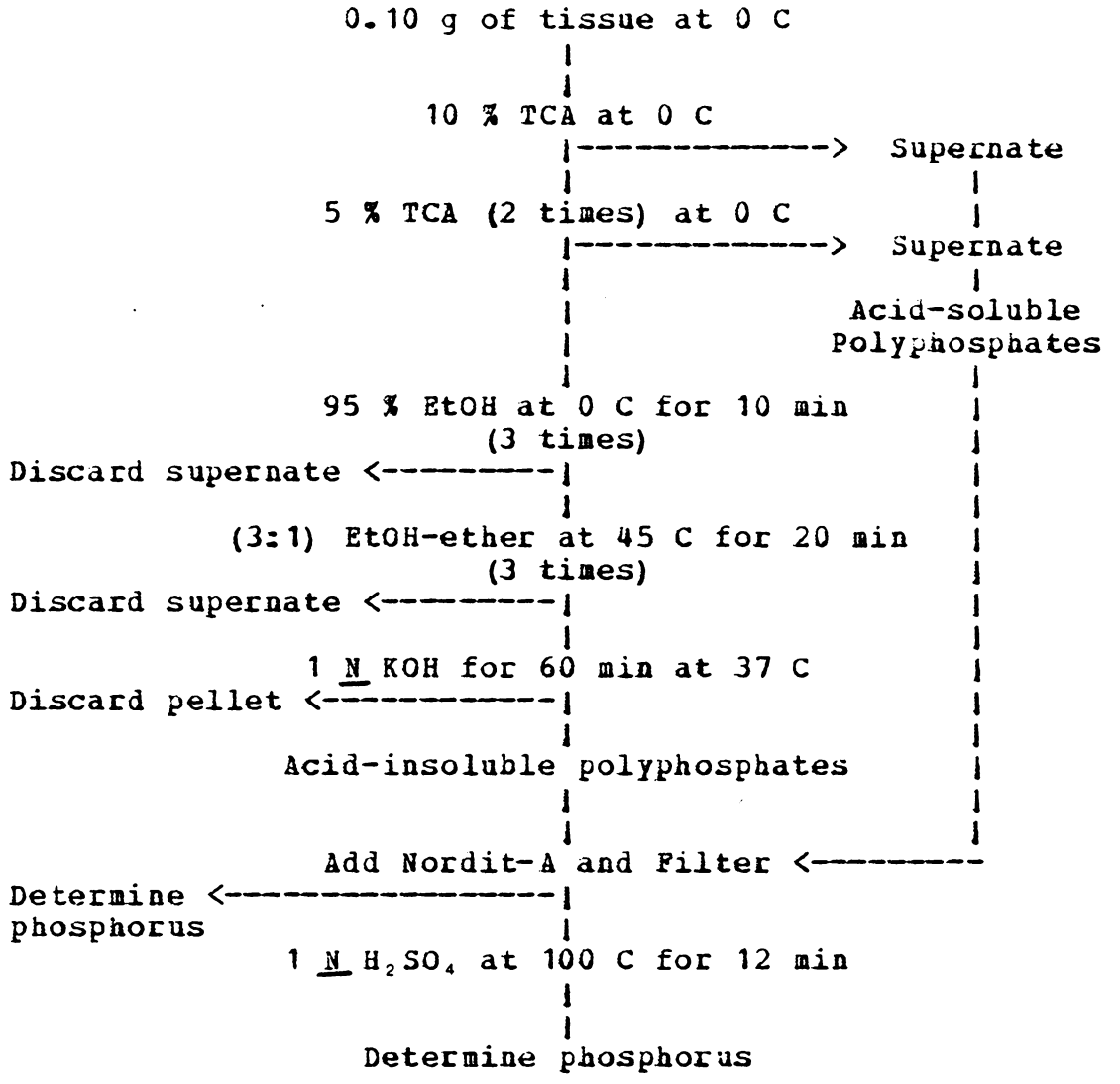
Tissue was dried at 65 C for a 48 hr period. After biomass was obtained, the tissue was ground to pass through a 10 mesh screen, and stored at 65 C. Phosphorus extraction was by dry-ashing at 425 C for 12 hr, dissolving the ash in 6 N HCl for 1 hr, bringing the solution to a 50 ml volume, and filtering with Whatman No. 4 paper. Phosphorus concentration of the extracts was determined after Murphy and Riley (1962) using a Bausch and Lomb Spec 100 spectrophotometer.

Mycorrhizae

The frozen mycorrhizae were lyphilized, weighed, and stored frozen until analysis. Total phosphorus was extracted as described for other tissue. Polyphosphates were extracted by a modified Schmidt-Thannhauser extraction as described by Baker and Schmidt (1963) (Figure 1). Inorganic phosphorus was determined by the Murphy-Riley method (Murphy and Riley, 1962).

Soil Analyses

Soils were sampled at the time of planting and harvest. Samples were pooled by treatment for the preplanting soil and by treatment combination for the post-harvest samples.



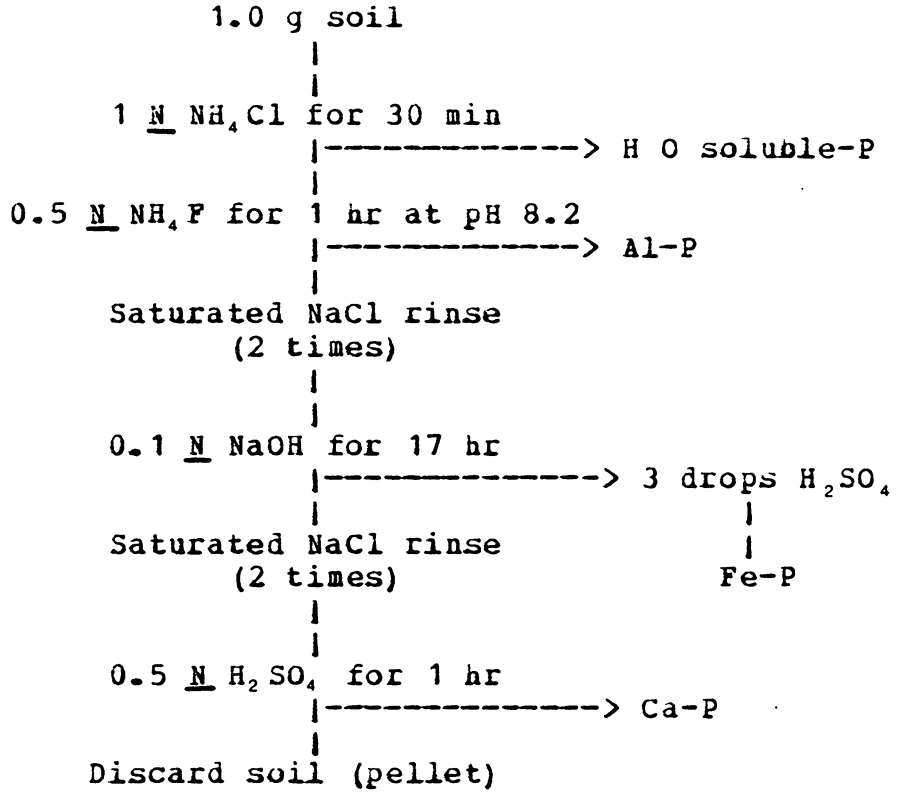
Between each step the sample was centrifuged at 10,000 rpm for 10 min.

Polyphosphates = Phosphorus after hot acid - phosphorus before hot acid

From Baker and Schmidt (1963)

Figure 1: Polyphosphate extraction and analysis sequence.

From each pooled sample four subsamples were extracted with dilute double acid (0.05 N HCl + 0.025 N H_2SO_4) and analyzed for iron, aluminum, and phosphorus. Preplanting soils were also analyzed for potassium, calcium, and magnesium. Cations were determined by atomic absorption spectrophotometry and phosphorus was determined by the Murphy-Riley method. Double acid extractions indicate a relative level of plant available nutrients (Thomas and Peaslee, 1973). Phosphorus fractionation as delineated by Chang and Jackson (1957b) and modified by Petersen and Corey (1966) (Figure 2), and total soil phosphorus by HClO digestion (Somers and Nelson, 1972) were determined for the preplanting soils. Water-soluble, Fe phosphate, Al phosphate, and Ca phosphate fractions were determined, but reductant and occluded phosphorus in the Petersen and Corey fractionation were not determined. Soil pH was obtained on replicate samples using a 2:1 (w/w) water:soil paste with distilled water. Readings were obtained using a combination electrode and an Orion 901 ionanalyzer.



Each sample is shaken for the time stated and centrifuged for 10 min at 2000 rpm. 50 ml volumes of each solution and 25 ml of the saturated NaCl rinse were added.

Figure 2: Soil inorganic P fractionation after Petersen and Corey (1966)

Statistical Analyses

The experiment was analyzed as a completely randomized design with a (5^2) factorial layout. A mixed model was used since soil phosphorus treatments were considered a random effect. This decision was made in the interest of interpolating the results among available P levels used in this experiment. The interaction mean square (fungus x soil) was used to test the F-value for the soil term. All other effects were tested using the pooled error term. Soils or seedlings which either failed to be colonized or were contaminated controls were not used in the analyses. Due to the unbalanced design created by varying mortality and lack of colonization of some individuals, the Type IV sum of squares of the GLM procedure in the Statistical Analysis System (SAS 79.5) was used (Sas Institute, 1979). The unbalanced design also created a need for least squares adjustment for means (Harvey, 1960) which was also accomplished using the GLM procedure. Least square differences were used for mean separation if significant F-values (Probability < 0.05) were obtained. If the interaction term was significant, a block chart was created to examine the interactions. The Corr Procedure of SAS 79.5 (Sas Institute, 1979) using Spearman Rank correlations was used to test relationships among variables. The correlation

was significant if the probability associated with the Spearman R was less than 0.10.

RESULTS

Soil

Characterization

Texture.

Physical characteristics of the soil used in this study were altered by the addition of sand. Table 3 contains the particle size analysis for the soil and soil-sand mixtures. The differences in soil texture resulted in a difference in water retention (Figure 3). At matrix potentials less than 0.1 MPa, considerably less soil moisture was retained by the mix containing 75 percent sand. However, the difference in soil moisture content between the 75 percent sand and the other two textures was only two percent between 0.1 and 1.0 MPa. It was estimated that the soil moisture was between 0.1 and 1.0 MPa 90 percent of the time.

Cations.

The addition of sand changed the levels of extractable cations (Table 4). Extractable potassium, calcium, and magnesium concentrations all decreased with the addition of sand but remained unchanged with the addition of P.

TABLE 3

Particle size analysis of a sand-amended Cecil sandy clay loam.

Soil-sand mix	Sand	Silt	Clay	Textural class
	--------%--------			
75% Sand	85	11	4	Loamy sand
25% Sand	66	19	15	Sandy loam
Soil	56	15	29	Sandy clay loam

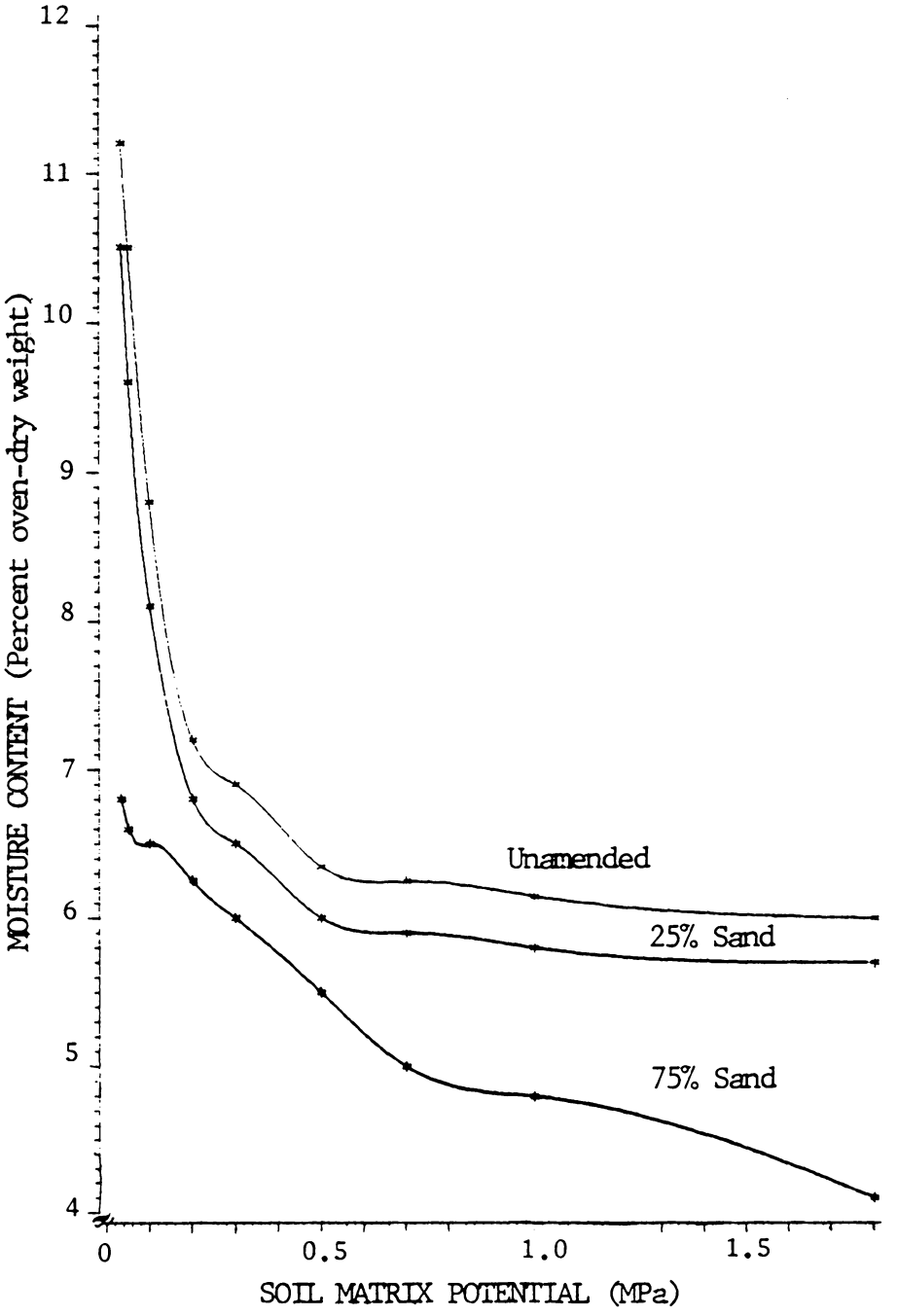


Figure 3: Moisture retention curves of a sand-amended Cecil sandy clay loam.

TABLE 4

Initial double acid-extractable potasssium, calcium, and magnesium of the amended Cecil sandy clay loam.

Soil amendments	K	Ca	Mg
	---------- umoles g^{-1} ----------		
75% Sand	3.3±0.1[1]	4.1±0.2	0.1±0.1
25% Sand	4.2±0.1	5.5±0.2	0.3±0.2
Unamended	4.3±0.1	7.1±0.5	0.6±0.1
56 kg P ha^{-1}	4.7±0.1	7.1±0.6	0.5±0.1
112 kg P ha^{-1}	4.6±0.1	7.4±0.7	0.7±0.1

[1] Standard error of the mean based on four samples.

Phosphorus.

The addition of sand and fertilizer provided the necessary soil P gradient needed to evaluate availability and uptake at different P levels. Table 5 contains the level of each soil P fraction for each soil amendment. Only a small amount of total soil P was accounted for by the four fractions obtained by the Petersen and Corey procedure or by the double acid extraction. In the unamended and sand-amended treatments, the predominate form found in the analysis was iron phosphate, but in the fertilized treatments aluminum phosphate predominated.

Changes in Soil Chemistry

Phosphorus

Differences in extractable P before and after the experiment are shown in Figure 4. An approximate ten fold difference was found between the lowest and highest preplanting levels of extractable P. All postharvest concentrations significantly decreased in extractable P from preplanting levels, but an interaction between soil amendment and mycorrhizal treatment occurred.

Colonized seedlings in 75 percent sand-amended soil depleted extractable P more than control seedlings. The 25 percent sand treatment with control and Tt-colonized

TABLE 5

Initial concentrations of different soil phosphorus fractions of the amended Cecil sandy clay loam.

Soil Amendments	Total P	Double-acid P	Water-soluble P	Al-P	Fe-P	Ca-P
	umoles P g^{-1}					
75% Sand	9.09±0.06[1]	0.19±0.01	0.08±0.01	0.32±0.01	0.34±0.12	0.06±0.01
25% Sand	11.10±1.31	0.41±0.02	0.03±0.01	0.76±0.02	1.06±0.06	0.18±0.01
Unamended	15.37±0.77	0.49±0.02	0.03±0.01	0.90±0.03	1.53±0.05	0.22±0.03
56 kg P ha^{-1}	16.41±1.11	1.06±0.03	0.10±0.01	2.42±0.14	1.97±0.15	0.31±0.02
112 kg P ha^{-1}	21.12±2.03	1.69±0.06	0.20±0.02	4.23±0.24	2.58±0.15	0.32±0.01

[1]Standard error of the mean based of four samples.

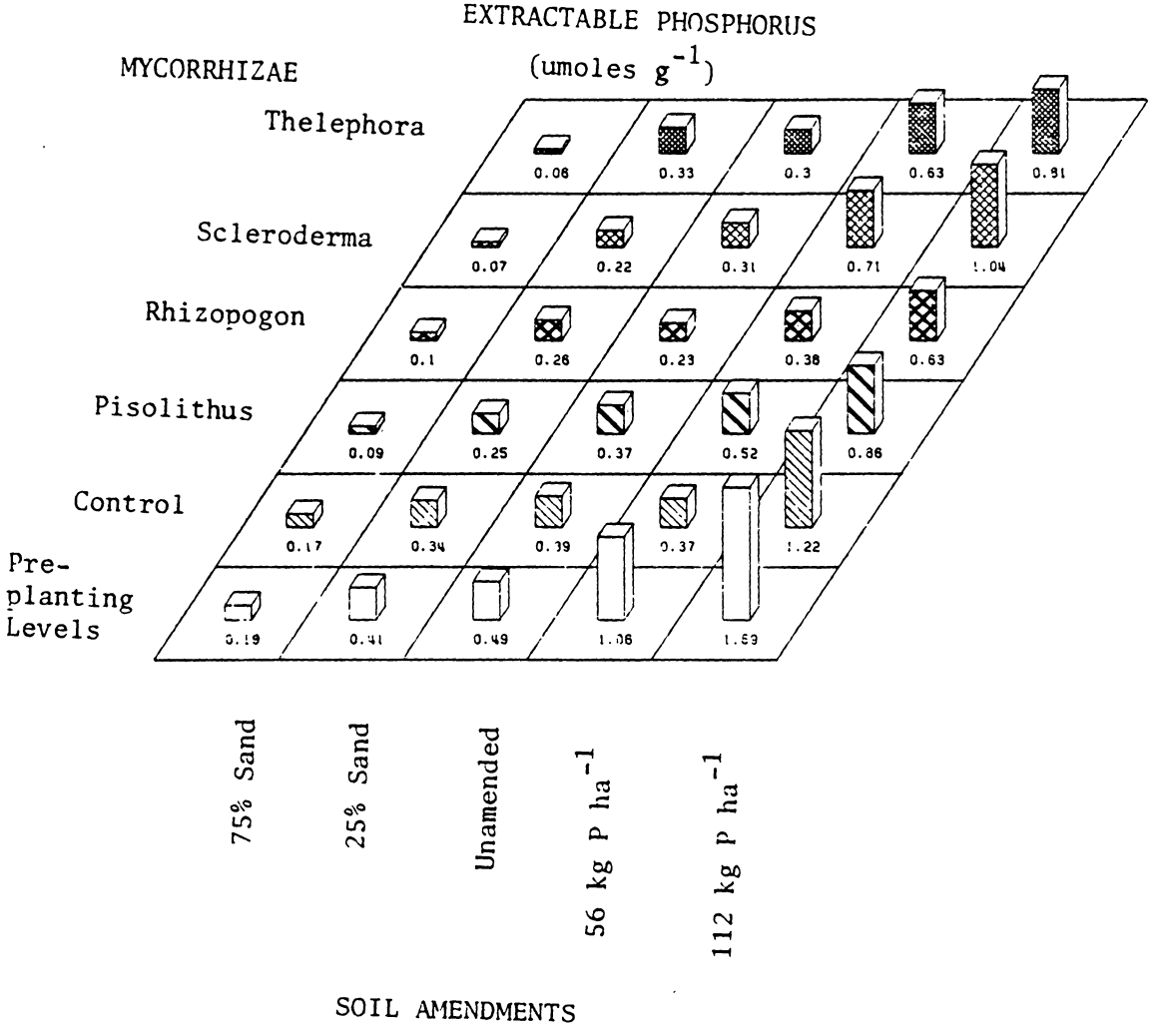


Figure 4: Preplanting and postharvest concentrations of extractable phosphorus by soil and mycorrhizal treatments.

seedlings contained approximately equal concentrations of extractable P. All other mycorrhizal treatments in 25 percent sand contained significantly less extractable P than the control. Extractable P was higher in unamended control soils than soils containing mycobionts. The difference in unamended soils with Sa, Tt, and Pt compared to unamended control soil was less than the same comparison in the 25 percent sand-amended soil. Control and Rr infested soil fertilized with 56 kg P ha^{-1} had approximately equal levels of extractable P, but all other mycorrhizal treatments had higher levels of extractable P than the control. With the addition of 112 kg P ha^{-1}, the control soil had the highest postharvest level of extractable P. This was followed by Sa, Pt, Tt, and Rr, respectively. In general, treatments with the highest preplanting levels of extractable P also had the highest postharvest levels. However, each fungus differentially mediated changes in extractable P among soil amendments.

Soil pH

The preplanting soil pH was relatively unaffected by either the addition of sand or fertilizer (Table 6), but soil pH increased during the course of the experiment. The sand amendments had less buffering capacity and increased in

pH significantly more than treatments without the sand amendments.

Extractable Iron

Extractable Fe levels decreased with the sand amendments, but did not change with the addition of P (Figure 5). A significant interaction occurred between the amended soil and mycorrhizae treatments. Amended soil without mycorrhizal fungi increased in extractable Fe over preplanting levels, but with the symbionts, it decreased or remained constant. The 75 percent sand-amended soil with Rr, Sa, and Tt-colonized seedlings decreased in extractable Fe. There were no differences in postharvest extractable Fe between any mycorrhizae treatment within any other soil treatment except for three cases: the unamended soil with Sa was significantly lower than all other mycorrhizal treatments in the unamended soil; extractable iron was higher in 25 percent sand-amended soil infested with Rr; and soils with Tt-colonized seedlings had reduced extractable Fe in soil amended with 112 kg P ha^{-1}.

TABLE 6

Preplanting and postharvest values of soil pH by soil mycorrhizal treatments.

Mycorrhizae treatment	Soil Amendments				
	75% Sand	25% Sand	Unamended	56 kg $P\ ha^{-1}$	112 kg $P\ ha^{-1}$
	----------pH----------				
Preplanting	4.43±0.03[1]	4.26±0.04	4.32±0.01	4.38±0.03	4.46±0.01
Postharvest					
Control	5.68±0.03	5.16±0.01	5.00±0.01	5.02±0.01	5.05±0.01
Pisolithus	5.57±0.05	5.08±0.04	4.94±0.02	5.04±0.03	5.08±0.05
Rhizopogon	5.76±0.01	5.07±0.01	5.10±0.01	5.00±0.01	5.14±0.05
Scleroderma	5.84±0.04	5.10±0.01	4.96±0.01	5.02±0.01	5.08±0.01
Thelephora	5.86±0.01	5.16±0.01	4.99±0.04	5.12±0.02	5.06±0.01

[1]Standard error of the mean based on two samples.

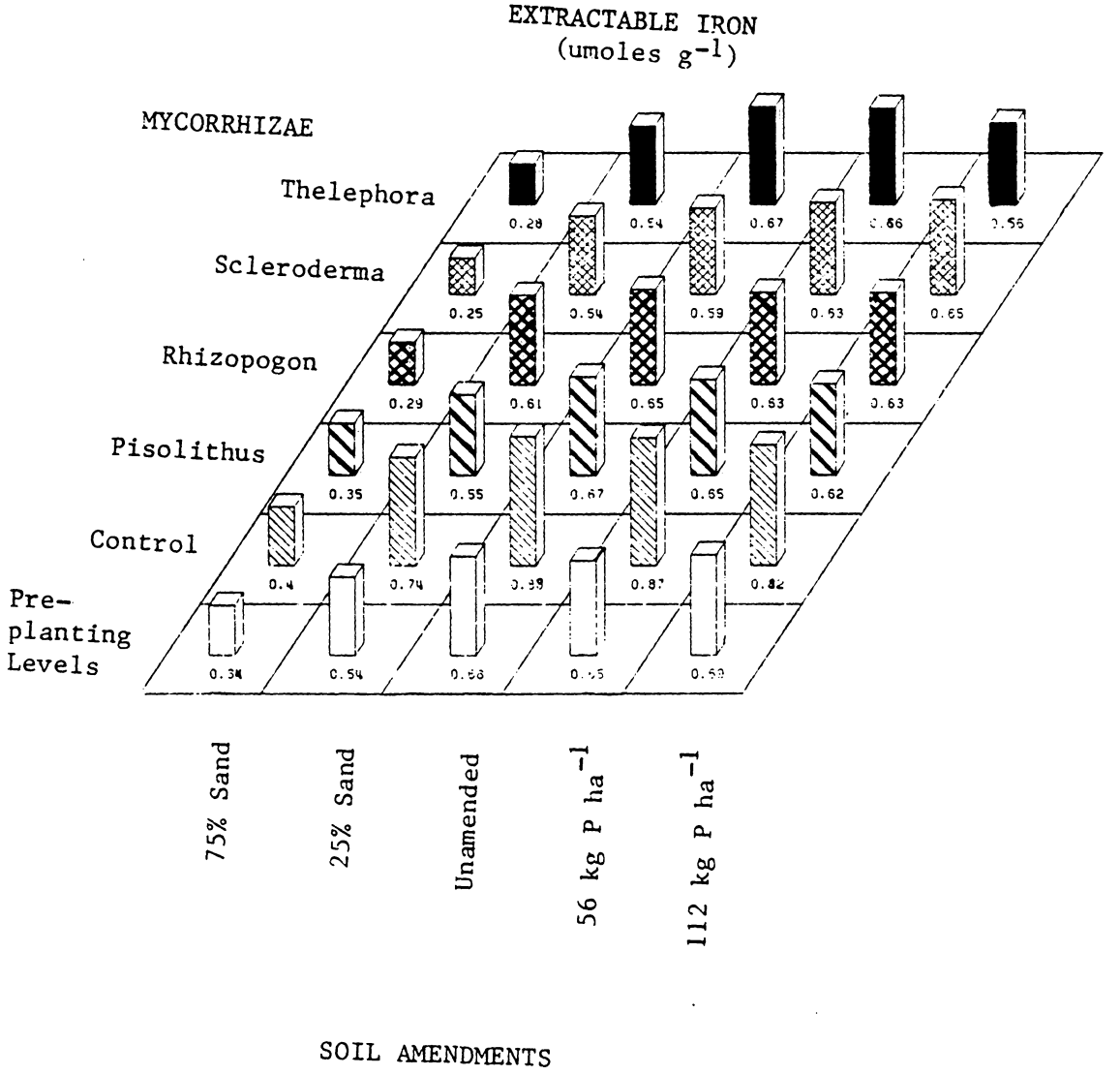


Figure 5: Preplanting and postharvest levels of extractable iron by soil amendments and mycorrhizal treatments.

Extractable Aluminum

Preplanting levels of extractable Al, like extractable Fe, decreased with sand amendments (Figure 6). Postharvest extractable Al concentrations did not change from preplanting levels for soil amendments containing control seedlings. In general, extractable Al in soil amendments with mycorrhizal fungi either decreased or did not change during the ten months. Sand-amended soils with mycorrhizal fungi significantly decreased in extractable Al from preplanting levels. In the unamended soil, only Rr, Sa, and Tt mycorrhizal treatments had decreased extractable Al. Mycorrhizal treatments showed slight increases in extractable Al in soil fertilized with 56 kg P ha^{-1}, but extractable Al decreased in soil fertilized with 112 kg P ha^{-1} when compared to soil fertilized with 56 kg P ha^{-1}. The most dramatic decrease from preplanting levels in soil fertilized with 112 kg P ha^{-1} was with Tt-colonized soil. Similar decreases were observed in extractable Fe and P levels.

Seedlings

Survival and Colonization

The soil treatments did not affect seedling survival except for soils colonized with Tt (Table 7). Tt-colonized

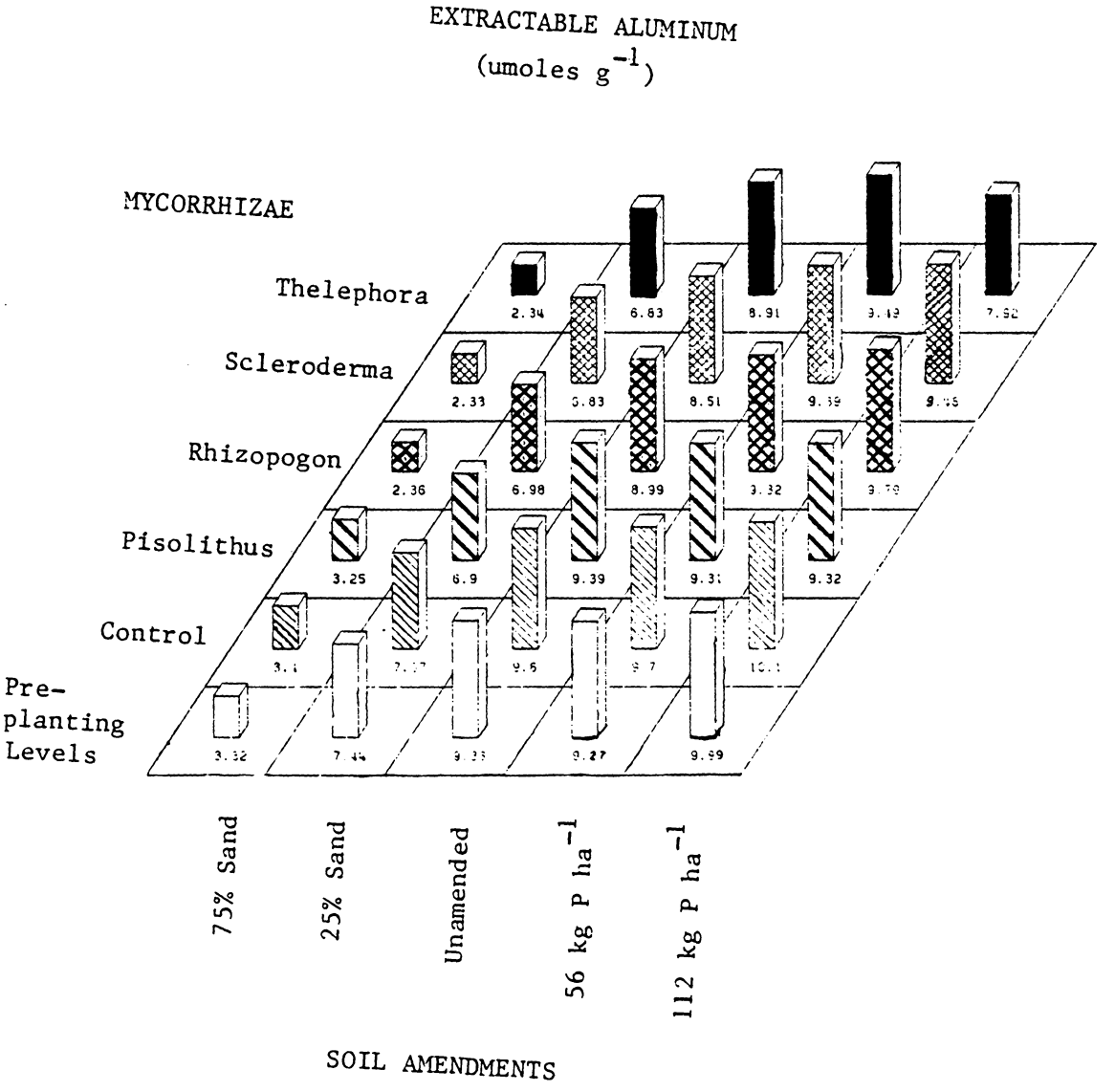


Figure 6: Preplanting and postharvest concentrations of extractable aluminum by soil amendments and mycorrhizal treatments.

seedlings had no mortality in the 75 and 25 percent sand-amended soils. Seedling survival rates for mycorrhizal treatments across all soil amendments were not substaintially different. Overall, seedlings growing in 75 percent sand-amended soil had better survival than seedlings in other soil amendments.

The percent of surviving seedlings colonized with Tt, Pt, and Sa was higher at the 75 percent sand-amended soil and soil fertilized with 112 kg P ha $^{-1}$ (Table 8). Control seedlings were less contaminated with natural Tt in the 75 percent sand-amended soil and soil fertilized with 112 kg p ha $^{-1}$. Contaminated control seedlings had only a small portion of their roots colonized. The contamination occurred after fruiting of Tt was observed in pots containing seedlings from other experiments. However, the overall number of contaminated controls was low (Table 8), and contamination of colonized seedlings was not found.

Shoot Components

Stem.

The main effects of soil and mycorrhizal treatments had a significant effect on stem components. As shown in Table 9, control seedlings were significantly shorter (25 percent)

TABLE 7

Survival of pot-grown loblolly pine by soil amendments and mycorrhizal treatments.

Mycorrhizae	Soil amendment					
	75% Sand	25% Sand	Unamended	56 kg P ha^{-1}	112 kg P ha^{-1}	Mean
	%					
Control	80	50	80	70	65	69
Rhizopogon	80	65	90	75	75	77
Thelephora	100	100	60	75	75	82
Pisolithus	95	55	80	65	50	69
Scleroderma	75	75	75	85	60	74
Mean	86	69	77	77	65	74

TABLE 8

Surviving loblolly pine seedlings with visible mycorrhizal colonization.

Mycorrhizae	Soil amendment					
	75% Sand	25% Sand	Unamended	56 kg P ha^{-1}	112 kg P ha^{-1}	Mean
	%					
Control	6	28	19	14	0	13
Rhizopogon	81	46	56	20	47	50
Thelephora	90	35	33	67	100	65
Pisolithus	100	64	56	15	90	65
Scleroderma	100	53	53	59	83	70
Mean	75	45	43	35	64	53

than colonized seedlings. Sa-colonized seedlings were significantly taller (20 percent), had a larger diameter (20 percent), and had a larger live crown (20 percent) than Rr, Tt, and Pt-colonized seedlings. Control seedlings had a significantly smaller ratio of live crown to stem length than colonized seedlings. Live crown length was significantly different for Pt and Sa-colonized seedlings, but both treatments had the same live crown ratio. However, Sa-colonized seedlings had a higher live crown ratio than Tt or Rr-colonized seedlings.

Fertilization generally increased height, live crown length and ratio, and stem diameter. Fertilized seedlings were significantly taller and had larger live crown lengths than seedlings growing in the 75 percent sand. Fertilization with 112 kg P ha^{-1} significantly increased stem diameter over unamended soil, and the 75 percent sand significantly decreased stem diameter.

The mean stem biomass of Sa-colonized seedlings (1.09 g) was twice that of all other mycorrhizal seedlings and four times larger than control seedlings (Table 10). Rr, Tt, and Pt-colonized seedlings were not statistically different from one another, but Tt and Pt-colonized seedlings were significantly different from control seedlings. Fertilization produced significantly heavier stems than

TABLE 9

Seedling stem components by soil amendment and mycorrhizae treatment.

Treatment	Height	Live crown length	Live crown ratio	Root collar diameter
	cm			mm
Mycorrhizae				
Control	13.3 a*	9.3 a	0.66 a	3.0 a
Rhizopogon	17.7 b	13.4 b	0.73 b	3.4 b
Thelephora	19.4 b	15.6 b	0.78 b	3.5 b
Pisolithus	19.3 b	15.8 b	0.79 bc	3.7 b
Scleroderma	24.4 c	20.8 c	0.84 c	5.1 c
Soil Amendment				
75% Sand	16.6 a	12.5 a	0.71 a	3.2 a
25% Sand	18.3 ab	14.0 ab	0.75 ab	3.5 ab
Unamended	18.5 ab	15.1 ab	0.77 bc	3.7 bc
56 kg P ha^{-1}	20.7 b	16.4 b	0.77 bc	4.0 cd
112 kg P ha^{-1}	20.2 b	16.8 b	0.81 c	4.3 d

* Means within the same column and treatment set with the same letter do not differ significanlty at the 0.05 level.

unamended or sand-amended soils. However, there were no significant differences between the two fertilized treatments or between the two sand-amended treatments. Soil amendments did not significantly affect stem phosphorus concentration; however, the mycorrhizal fungi increased stem phosphorus concentration over uncolonized control seedlings (Table 10).

Foliage and Shoot.

The mycorrhizae-soil amendment interaction had a significant effect on foliar biomass (Figure 7). All seedlings generally responded to increasing soil P except those colonized with Sa which decreased slightly with the addition of 112 kg P ha^{-1}. The foliar biomass of Sa-colonized seedlings was larger than all other treatments regardless of soil amendment. Tt, Rr, and Pt-colonized seedlings had a larger foliar biomass than control seedlings within the same soil treatment. The mycorrhizae-soil interaction also significantly affected shoot biomass (Figure 8).

The interaction of soil amendments and mycorrhizal treatments significantly affected foliar phosphorus concentration (Figure 9). The addition of sand tended to decrease foliar P in control seedlings, and P fertilization

TABLE 10

Stem biomass and phosphorus concentration by soil amendment and mycorrhizae treatment.

Treatment	Stem biomass	Stem phosphorus
	-----g------	-umoles P g^{-1}--
Mycorrhizae		
Control	0.29 a*	11 a
Rhizopogon	0.34 ab	20 b
Thelephora	0.47 b	21 b
Pisolithus	0.51 b	19 b
Scleroderma	1.09 c	21 b
Soil Amendment		
75% Sand	0.37 a	19 a
25% Sand	0.47 a	18 a
Unamended	0.50 a	20 a
56 kg P ha^{-1}	0.68 b	20 a
112 kg P ha^{-1}	0.68 b	15 a

* Means within a column and treatment set with the same letter do not differ significantly at the 0.05 level.

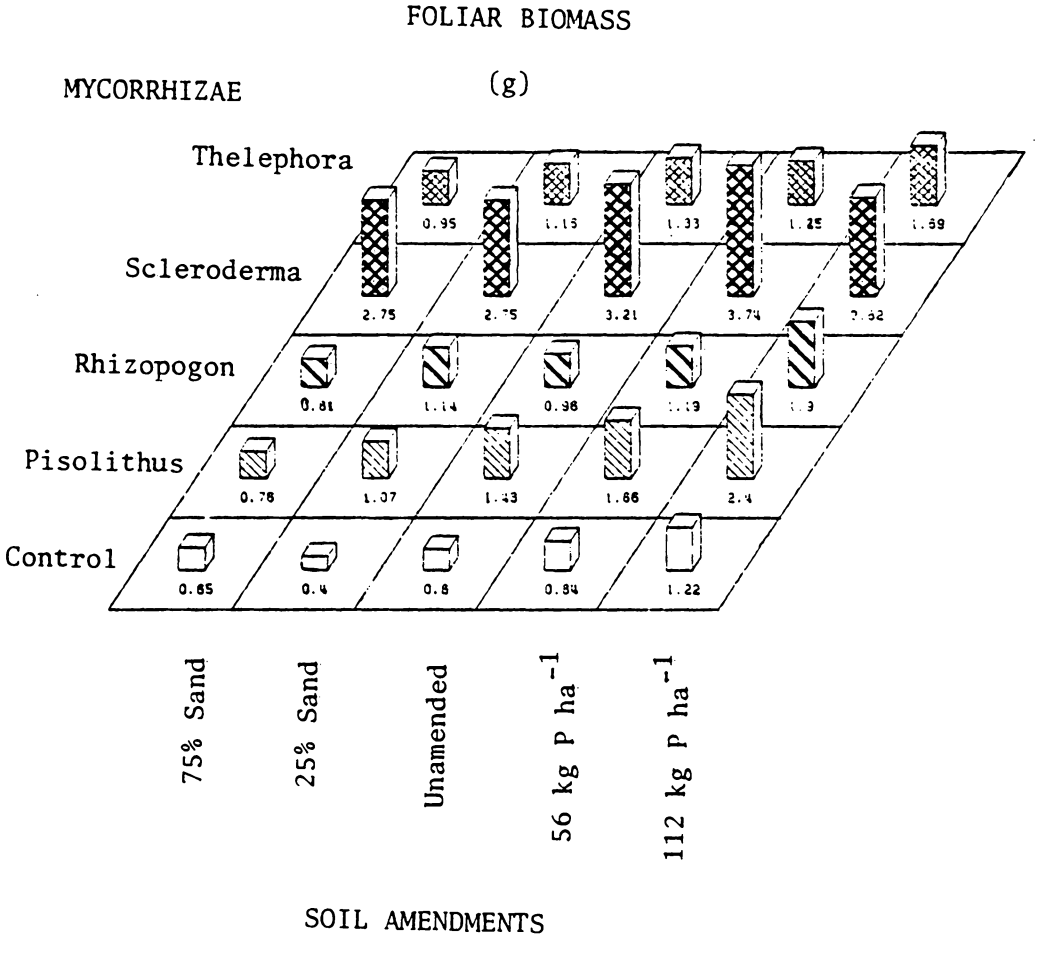


Figure 7: Foliar biomass as affected by soil and mycorrhizal treatments.

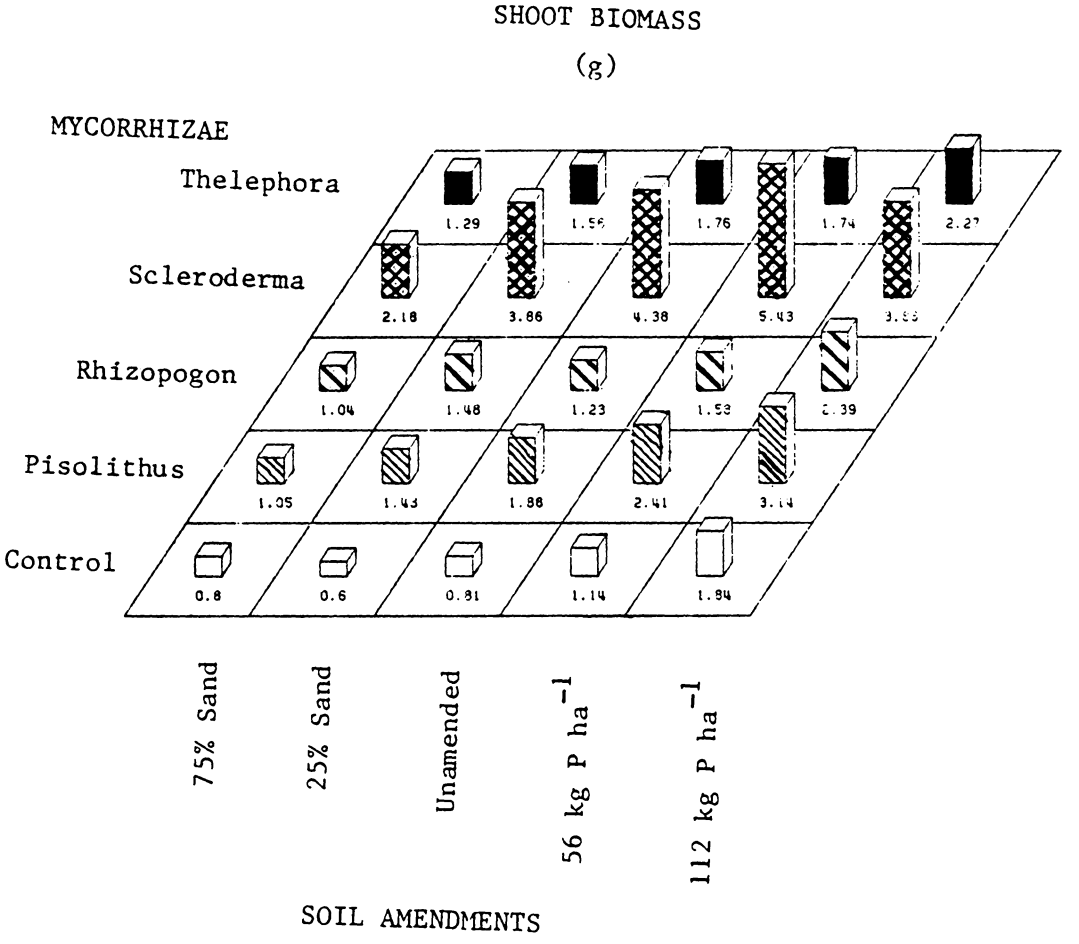


Figure 8: Shoot biomass as affected by soil and mycorrhizal treatments.

increased the foliar P levels. Added P and sand suppressed foliar P in Sa-colonized seedlings as compared to the unamended soil. Foliar P levels in Rr, Pt, and Tt-colonized seedlings were elevated by the addition of sand. These levels were also elevated by P fertilization for Pt and Tt-colonized seedlings, but the addition of P to the soil suppressed foliar P levels for Rr-colonized seedlings.

The critical level of P in loblolly pine foliage is 32 umoles P g^{-1} (0.1%) (Pritchett, 1980). Control seedlings remained below the foliar critical level of P in all soil treatments. Sa-colonized seedlings were only below the critical level in the sand-amended treatments. Tt, Rr, and Pt-colonized seedlings were at or above the critical level in 75 percent sand-amended soil, but only Tt and Rr were above the critical level in the 25 percent sand-amended soil. Pt-colonized seedlings were above the critical level for foliar P with the addition of 56 kg P ha^{-1} to the soil, and both Tt and Pt were above the critical level with the addition of 112 kg P ha^{-1}.

Belowground Components

Root length and mycorrhizal colonization.

Root length was significantly affected by the soil and mycorrhizal treatments, but not the interaction (Table 11).

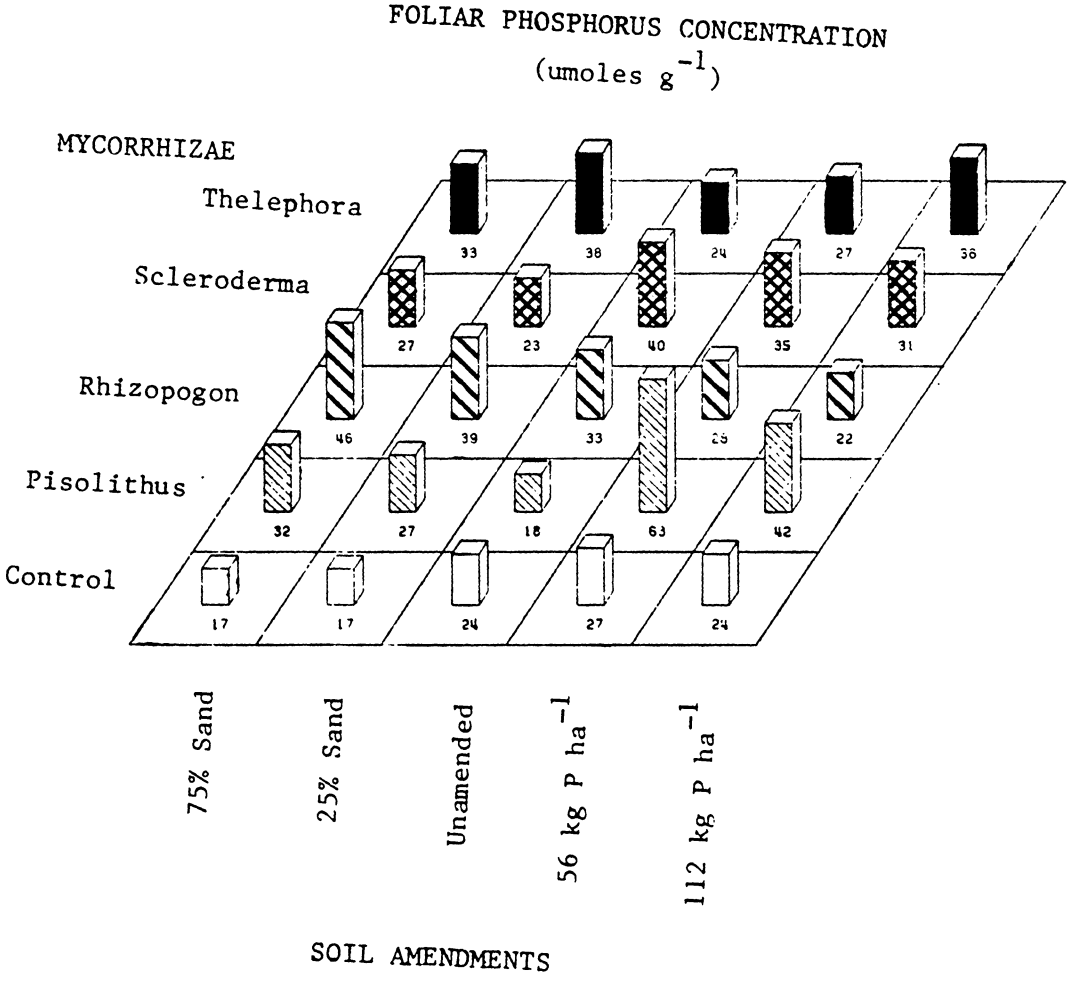


Figure 9: Foliar phosphorus concentration by soil and mycorrhizal treatments.

Sa-colonized roots were significantly longer than all other mycorrhizal treatments. Phosphorus fertilization significantly increased root growth over unamended or sand-amended soil. Soil amendments did not affect length of root colonized by mycorrhizae, but there were differences between species of symbionts. Sa colonized three times more root length (182 cm) than any other symbiont. There were no statistical differences among root lengths colonized by other fungi.

The portion of root colonized by the symbionts closely followed the same trends as length of root colonized (Table 11). Sa colonized more than twice the amount of root (35 percent) in comparison with other symbionts, and there were no differences in percentage colonization among the other fungi. Soil amendments did not affect colonization percent.

The length of uncolonized roots of colonized seedlings were about the same as total root lengths of control seedlings. There were no statistical differences among any of the mycorrhizal treatments. Fertilization increased the root length not colonized by mycorrhizal fungi. Fertilized seedlings and seedlings growing in 25 percent sand-amended soil were not significantly different, and seedlings in unamended and sand-amended soil were not significantly different.

TABLE 11

Root length and mycorrhizal colonization of loblolly pine seedlings for soil and mycorrhizal treatments.

Treatments	Root length	Colonized root length	Noncolonized root length	Root colonization
	cm			%
Mycorrhizae				
Control	343 a*	0 a	343 a	0 a
Rhizopogon	392 a	56 b	336 a	15 b
Thelephora	393 a	66 b	326 a	17 b
Pisolithus	402 a	59 b	344 a	12 b
Scleroderma	502 b	182 c	319 a	35 c
Soil Amendment				
75% Sand	347 a	67 a	281 a	16 a
25% Sand	381 a	48 a	334 ab	12 a
Unamended	354 a	75 a	280 a	18 a
56 kg P ha^{-1}	478 b	91 a	387 b	15 a
112 kg P ha^{-1}	469 b	82 a	387 b	18 a

* Means in the same column and treatment set with the same letter are not significanly different as the 0.05 level.

Biomass and total P.

Table 12 contains means for the biomass and total P for the roots and mycorrhizae. Both mycorrhizae and soil amendments affected root biomass, but not the interaction. The Sa-colonized root systems had the largest biomass of any mycorrhizal treatment (1.55 g). The biomass of Tt (0.93 g) and Pt-colonized (0.83 g) root systems were the next largest, and they were not significantly different. The biomass of Rr and Pt-colonized roots and control root systems were not significantly different. Phosphorus fertilization increased root biomass significantly over the unamended or sand-amended soils. The soil amendments did not significantly affect mycorrhizae biomass. Sa mycorrhizae were significantly larger (two to five times) in total biomass (0.26 g) per seedling than all other species of mycorrhizal fungi. There were no significant biomass differences among the other fungi.

Root phosphorus concentration was not statistically affected by soil amendment or mycorrhizae. However, Sa-colonized seedlings tended to be higher in P (30 umoles g^{-1}) than all other treatments, and colonized roots tended to have higher P content than control roots (18 umoles g^{-1}) Mycorrhizae P concentrations were different for the species of mycobiont but not the soil amendment. Pt mycorrhizae

TABLE 12

Biomass and total P concentration of roots and mycorrhizae by soil amendments and mycorrhizal treatments.

Treatment	Root biomass	Mycorrhizae biomass	Root P	Mycorrhizae P
	g		umoles P g^{-1}	
Mycorrhizae				
Control	0.59 a*	0.00 a	18 a	--
Rhizopogon	0.72 ab	0.05 b	27 a	117 a
Thelephora	0.93 c	0.07 b	23 a	103 ab
Pisolithus	0.83 bc	0.13 b	23 a	69 b
Scleroderma	1.55 d	0.26 c	30 a	108 a
Soil Amendment				
75% Sand	0.68 a	0.10 a	24 a	87 a
25% Sand	0.74 a	0.09 a	23 a	116 a
Unamended	0.85 a	0.20 a	21 a	91 a
56 kg P ha^{-1}	1.11 b	0.12 a	32 a	97 a
112 kg P ha^{-1}	1.24 b	0.13 a	22 a	108 a

* Means in the same column and treatment set with the same letter do not differ significantly at the 0.05 level.

contained a lower concentration of P (69 umoles g^{-1}) than Rr (117 umoles g^{-1}) and Sa (108 umoles g^{-1}) mycorrhizae. Total P in the mycorrhizae was very high (approximately 100 umoles P g^{-1}), and on the average five times greater than the root concentration.

Polyphosphate concentration.

Tt and Sa were the only two mycorrhizae that provided sufficient tissue for polyphosphate analyses. Soluble polyphosphates contributed 10 to 30 percent of the total mycorrhizal phosphorus. The interaction of mycorrhizae and soil treatments significantly affected acid soluble polyphosphates (Figure 10). Tt mycorrhizae contained significantly more acid soluble polyphosphates than Sa mycorrhizae, except in soil amended with 112 kg P ha^{-1} (Tt 15 umoles; Sa 19 umoles). Sa mycorrhizae increased acid soluble polyphosphates as available phosphorus in the soil amendments increased. Acid soluble polyphosphates in Tt mycorrhizae remained relatively constant across 25% sand, unamended soil, and soil fertilized with 56 kg P ha^{-1}. Polyphosphate levels were about 30 percent lower in the 75 percent sand and contained only half as much per gram in the treatment fertilized with 112 kg Pha^{-1}.

Neither soil amendments nor mycorrhizal symbionts affected acid insoluble polyphosphates (Table 13). Analysis

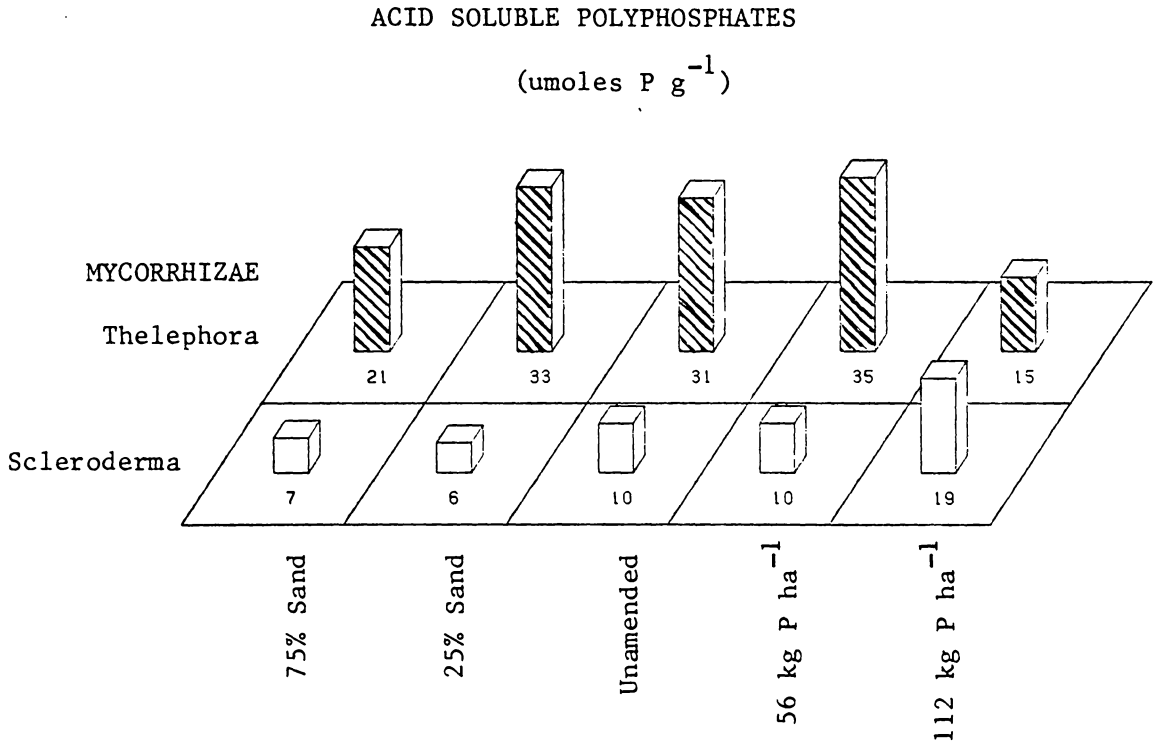


Figure 10: Acid soluble polyphosphates of loblolly pine mycorrhizae by soil and mycorrhizal treatments.

of variance showed that the interaction of symbiont species and soil amendments significantly affected total polyphosphates (Figure 11). The pattern of response is identical to that already presented in acid soluble polyphosphates (Figure 10). Acid soluble polyphosphates comprise 90 percent of the total polyphosphate levels.

Total Plant Components

Total plant components are composed of both belowground and aboveground plant parts, and include root to shoot ratio and total biomass. A root uptake efficiency and a mycorrhizae uptake efficiency values were computed. Uptake efficiency represents the amount of phosphorus absorbed per unit length of root, expressed as:

$$(B \times P) / L$$

where:

B = the total plant biomass.

P = the average phosphorus concentration of all plant parts.

L = the length of absorbing structure.

Biomass.

Control seedlings had a significantly larger root to shoot ratio (0.61) than Pt, Rr, and Sa-colonized seedlings (Table 14). Sa-colonized seedlings also had a significantly

TABLE 13

Acid insoluble polyphosphates of loblolly pine mycorrhizae for mycorrhizal and soil treatments.

Treatments	Acid insoluble polyphosphates
	--umoles P g^{-1}-
Mycorrhizae	
Thelephora	2.1 a*
Scleroderma	2.0 a
Soil Amendment	
75% Sand	1.7 a
25% Sand	2.7 a
Unamended	3.4 a
56 kg P ha^{-1}	1.4 a
112 kg P ha^{-1}	1.0 a

* Means in the same column and treatment set with the same letter do not differ significantly at the 0.05 level.

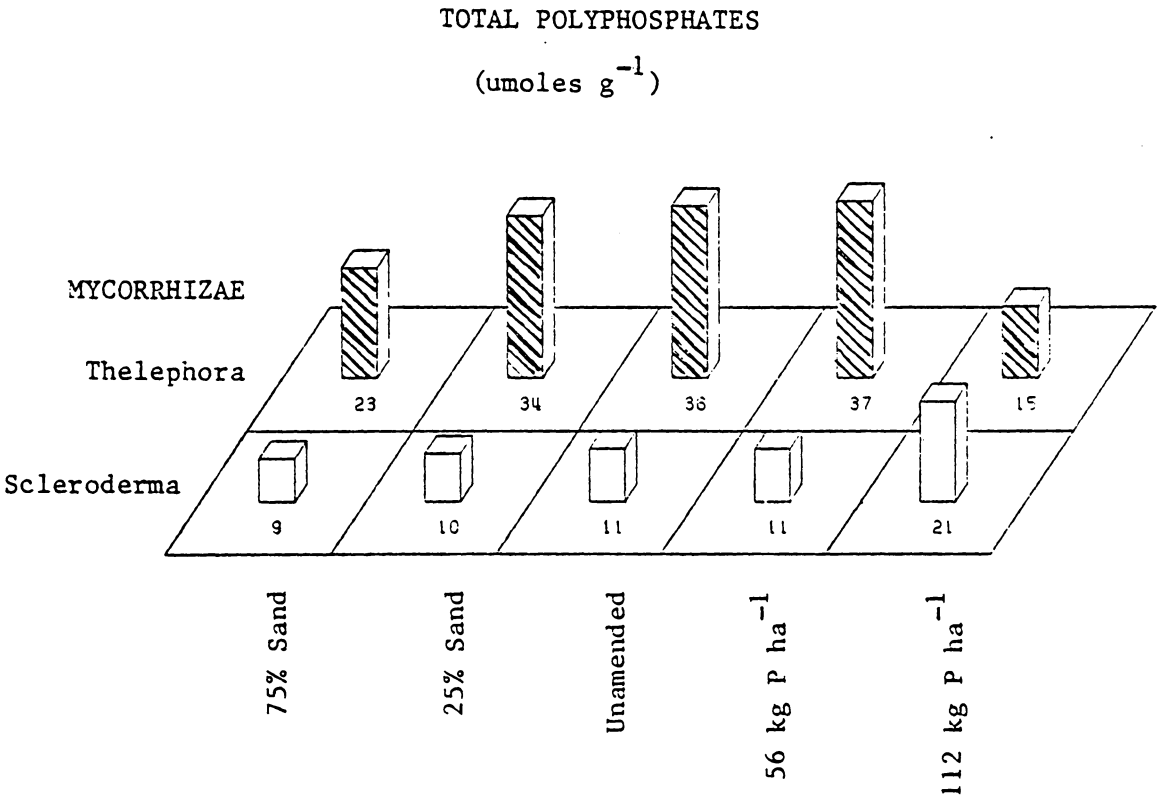


Figure 11: Total polyphosphates of loblolly pine mycorrhizae by soil and mycorrhizal treatments.

lower root to shoot ratio (0.43) than Tt-colonized seedlings (0.55). Soil amendments did not significantly affect root to shoot ratios.

Total biomass was significantly affected by the soil and mycorrhizal treatments, but not the interaction (Table 14). Sa-colonized seedlings (5.5 g) were twice as large as other mycorrhizal seedlings and three times as large as control seedlings (1.6 g). There were no significant differences among Pt, Rr, and Tt-colonized seedlings, but all colonized seedlings were significantly larger than the controls. Applied P at 112 and 56 kg P ha^{-1} produced larger seedlings (4.0 g and 3.6 g respectively) than the unfertilized soil (2.8 g) or sand-amended soil. Seedlings growing in soil fertilized with 56 kg P ha^{-1} had a larger biomass than those growing in the sand-amended treatments. Seedlings growing in unamended soil were larger than those growing in the 75 percent sand (1.9 g).

Uptake efficiency.

Mycorrhizal treatment, but not soil amendment, significantly affected root uptake efficiency (Table 15). Sa-colonized root systems had significantly higher efficiencies (0.37 umoles P cm^{-1}) than Rr-colonized (0.16 umoles P cm^{-1}) or control root systems (0.11 umoles P cm^{-1}). Pt and Tt-colonized root systems were more efficient than control root systems.

TABLE 14

Total biomass and root to shoot ratio of loblolly pine seedlings for soil amendments and mycorrhizal treatments.

Treatments	Root to shoot ratio	Total Biomass
		---g---
Mycorrhizae		
Control	0.61 a*	1.6 a
Rhizopogon	0.50 bc	2.3 b
Thelephora	0.55 ab	2.6 b
Pisolithus	0.48 bc	2.8 b
Scleroderma	0.43 c	5.5 c
Soil Amendment		
75% Sand	0.59 a	1.9 a
25% Sand	0.47 a	2.5 ab
Unamended	0.48 a	2.8 b
56 kg P ha^{-1}	0.50 a	3.6 c
112 kg P ha^{-1}	0.51 a	4.0 c

* Means in the same column and treatment set with the same letter do not differ significantly at the 0.05 level.

TABLE 15

Loblolly pine root and mycorrhizae uptake efficiencies for mycorrhizal and soil treatments.

Treatment	Root uptake efficiency	Mycorrhizal uptake efficiency
	-------umoles P cm^{-1} root length------	
Mycorrhizae		
Control	0.11 a*	--
Rhizopogon	0.16 ab	1.5 a
Thelephora	0.28 b	3.5 a
Pisolithus	0.25 b	1.7 a
Scleroderma	0.37 c	1.5 a
Soil Amendment		
75% Sand	0.29 a	2.6 a
25% Sand	0.18 a	1.7 a
Unamended	0.23 a	1.3 a
56 kg P ha^{-1}	0.23 a	2.2 a
112 kg P ha^{-1}	0.24 a	2.2 a

* Means in the same column and treatment set with the same letter do not differ significantly at the 0.05 level.

There were no statistical differences in mycorrhizal uptake efficiencies. The amount of P absorbed per length of colonized root was not affected by soil amendments.

Correlations

Polyphosphates.

Mycorrhizae P concentrations were not significantly correlated with any other variables (Table 16). Acid-soluble polyphosphate concentration had rather low, negative, but significant correlations with foliar P levels, shoot length, and live crown length. Acid-insoluble polyphosphates were similarly correlated with foliar phosphorus concentration and live crown length.

Seedling.

Root length, mycorrhizae length, root colonization, and foliar P were all significantly correlated with total biomass, shoot biomass, foliar P, stem height, and live crown length (Table 17). All correlations were highly significant with coefficients ranging from 0.26 to 0.63. Root length, mycorrhizae length, and mycorrhizae biomass had the largest R-values, while foliar P had the lowest.

TABLE 16

Correlation matrix of mycorrhizal phosphorus levels with seedling growth and foliar phosphorus levels.

	Total mycorrhizal P	Acid soluble polyphosphate	Acid insoluble polyphosphate
Total biomass	NS[1]	NS	NS
Shoot biomass	NS	NS	NS
Foliar P	NS	-0.34[2] 0.03[3]	-0.33 0.02
Shoot Length	NS	-0.26 0.04	NS
Live Crown Length	NS	-0.24 0.05	-0.22 0.07

[1]Nonsignificant correlation.
[2]Spearman R correlation coefficient.
[3]Probability associated with the Spearman R.

TABLE 17

Correlation matrix of seedling growth, mycorrhizal colonization, and phosphorus concentration variables.

	Total biomass	Shoot biomass	Foliar P	Stem height	Live crown
Root length	0.63[1] 0.01[2]	0.60 0.01	0.26 0.01	0.53 0.01	0.54 0.01
Mycorrhizae length	0.56 0.01	0.58 0.01	0.36 0.01	0.62 0.01	0.60 0.01
Root colonization	0.51 0.01	0.52 0.01	0.35 0.01	0.57 0.01	0.56 0.01
Mycorrhizae biomass	0.63 0.01	0.62 0.01	0.51 0.01	0.58 0.01	0.56 0.01
Foliar P	0.39 0.01	0.39 0.01	----	0.47 0.01	0.44 0.01

[1]Spearman R correlation coefficient.
[2]Probability associated with the Spearman R.

DISCUSSION

Experimental Environment

Time of Year

Greenhouse-moderated temperatures extended the growing season well into the fall. Although shoot growth ceased in November, inspection of the seedling roots revealed that root growth continued throughout the winter. The duration of root growth is a function of moisture and temperature, and southern pines seldom completely stop root growth in the field (Pritchett, 1979). Bilan (1967) found that one-year old loblolly pine roots continued growing until air temperatures dropped below -2 C. Zimmerman and Brown (1977) concluded that peak activities of tree root growth in temperate climates occurred in spring and fall due to favorable moisture and temperature regimes. Shoot growth resumed in February as the photoperiod was extended.

Inspection of seedling roots revealed that root colonization occurred between 8 and 10 weeks after planting. It was presumed that colonization occurred when both roots and fungi were metabolically active, but if root growth rates were too high, colonization was probably retarded.

Chivers and Gust (1982) demonstrated this principle on pot-grown Eucalyptus seedlings. Fast-growing low order roots do not become colonized, but the high order small diameter roots are readily colonized (Chivers and Gust, 1982).

Fungi have predictable seasonable cycles and mycologists predict the fruiting season from year to year. Other phases of the annual life cycle are not as accurately predicted. The time of colonization by each fungus may be different (Harley, 1969), but this is not well documented. Spores may colonize roots in the summer or fall after discharge, or they may overwinter and germinate in the spring. Harvey et al. (1978) found that maximum colonization of roots in western forests occurred in late spring. The relationship of when colonization occurs in the field, and when vegetative inoculum is most effective is not known.

Temperature

Ambient temperatures of the greenhouse reached 32 C on the average of 3 days per week during July and August. The median duration of these temperatures was 1.5 hr. When the temperatures reached 30 C, the greenhouse floors were watered which reduced the temperatures 5 to 15 C. This also raised the relative humidity from 30 percent to 100 percent. Marx and Bryan (1971) found that Pt could colonize seedlings

at 35 C, but Tt could not. Theodoru and Bowen (1971) determined that temperatures above 23 C restricted growth of Rhizopogon luteolus. Schramm (1966) observed that Pt and a few other fungi could tolerate high temperatures on anthracite spoils. Little is known about the heat tolerance of Sa and Rr. High temperatures may have reduced early colonization rates. However, mycorrhizae and rhizomorphs were observed in the soil and on the roots indicating that not all of the fungi were inactive.

Soil Texture and Moisture

Despite the difference in texture (Table 3), water retention curves showed that very little difference in percent soil moisture between 0.1 and 1.0 MPa (Figure 3). Soils were watered twice per week during August to counterbalance high transpiration and evaporation rates. Watering may have affected aeration, while in turn aeration may have affected colonization during the early stages of root growth due to the aerobic nature of mycorrhizal fungi in general (Harley, 1978). However, mycorrhizae were distibuted evenly throughout the pot and none of the seedlings seemed to be stressed by either too much or too little water. Seedling responses correlated with the continuum in soil P instead of differences in texture (Figures 7 - 11).

Nitrogen

An estimate of foliar N was determined from tissue samples composited by treatment combination. A single subsample was analyzed using standard microKjeldahl procedures (Jackson, 1958) to insure that N was not limiting normal tree growth. An overall mean of 1.28 ± 0.18 percent with a range of 1.08 to 1.41 percent was found. There were no evident trends in N levels among treatments samples. No visual symptoms of N deficiency were observed and the majority of the samples were above the critical level established for loblolly pine (1.2 percent) (Fowells and Krauss, 1959). Initial fertilization helped insure that nitrogen would not be limiting, but subsequent fertilization was avoided because of the reportedly adverse effect high nitrogen levels have on the colonization of roots by mycorrhizal fungi (Meyer, 1975).

Cations and pH

Exchangeable K, Ca, and Mg decreased with addition of sand (Table 5), but the levels were not reduced to the point of affecting seedling growth. Pritchett (1979) stated that southern pines could grow adequately in soil with exchangeable K of 0.25 umoles K g^{-1} soil (10 ppm). At present, there are no established critical levels for

exchangeable Ca and Mg. However, fine textured soils, such as the Cecil, are not usually deficient in Ca and Mg, and they may even have a considerable excess (Pritchett, 1979). No deficiency symptoms were observed for Ca or Mg in any seedling. Due to the nature of the tap water used to water the seedlings, Ca and Mg was continually added during the experiment.

The increased soil pH over the course of the experiment occurred because of the high concentration of calcium in tap water used to water the seedlings. The sand-amended soils had higher final pH levels than the unamended or fertilized soils (Table 6). In contrast, the clay component of the finer-textured soil treatments buffered the pH.

Phosphorus Fractionation

The major P fraction in the Cecil sandy clay loam is iron phosphate (Table 5). However, fertilization increased the relative amount of Al phosphate over the other P fractions. Aluminum phosphate is considered to be more plant available than Fe phosphate (Juo and Ellis, 1968). Fixation of P by Ca is thought to occur primarily on neutral or basic soils (Bonn et al., 1979), but some P was apparently fixed by Ca in this acid soil. This was evident by the levels of Ca phosphate in the unamended soil and the increased levels of

Ca phosphate after P fertilization. Calcium phosphate is considered to be more available to plants than Al, but high concentrations of Al with the Ca cause an unavialable Al-Ca phosphate mineral called crandalite to be formed. This mineral is partially soluble in the H_2SO_4 used to extract the Ca phosphate (Sample et al., 1976).

Water-soluble P is the most readily available form to plants. The 75 percent sand-amended soil had more water-soluble P than the 25 percent treatment or the unamended soil (Table 4). This was due to a higher Al and Fe content which quickly refixed the P released from the soil solution of these latter treatments (Figures 5 and 6). Two-thirds of the P was not accounted for in the fractionation. It was assumed that the majority was in the reductant and occluded fractions which were not analyzed. These fractions are considered unavailable to plants (Juo and Ellis, 1968).

With the addition of Ca with the tap water and the subsequent raise in pH, trivalent Al activity decreased. At pH levels above 4.5, trivalent Al forms low-density, diffuse polymers with a positive charge (Hsu, 1965). These polymers are not as important in P fixation as free Al. Due to the increased level of Ca, more crandalite may have formed. Also, hydroxyapatite may have formed, but the P associated with this mineral is thought to be totally plant available.

However, the naturally high concentrations of Al and Fe probably negated any change in P availability.

Changes in Soil Chemistry

Extractable Phosphorus

The differences in extractable Al, Fe, and P among mycorrhizae treatments shows the ability of each root system to change the soil chemical environment. Except for soil fertilized with 56 kg P ha^{-1}, extractable P was greater for the soils containing symbionts than for the control. It appeared that control seedlings did not have the root system to absorb the readily available P. Phosphorus uptake was influenced by the ability of the roots or mycorrhizae to extract P from the fixed iron and aluminum phosphates. Control roots could deplete the readily available P caused by fertilization. Fungi such as Sa were able to increase the extractable concentration probably by the addition of metabolites, probably organic acids, in the soil fertilized with 56 kg P ha^{-1}. These metabolites may have been produced in the mycorrhizosphere, by the roots through stimulation by the fungi, or by the fungi themselves (Slankis, 1974).

Pt and Tt are often pioneer species found on strip-mines, and Tt is commonly found in nurseries (Marx et al., 1978). However, the role of Pt and Tt are quite different. Marx et

al. (1978) demonstrated that Tt-colonized seedlings did as well as Pt-colonized seedlings at high levels of fertility. In this study, Pt-colonized seedlings increased shoot biomass and foliar P with the addition of 56 kg P ha^{-1} (Figures 8 and 9), and Tt-colonized seedlings responded to 112 kg P ha^{-1}. The changes in extractable P, Al, and Fe suggest that Tt requires the additional phosphorus to survive and colonize loblolly pine in the Cecil sandy clay loam (Figures 4-6). This was noted by the strong decrease in these elements in soil fertilized with 112 kg P ha^{-1}.

The results of this study have shown the ability of Sa to increase seedling growth in a Cecil fine sandy loam. Changes in soil extractable phosphorus, aluminum, and iron complement the growth of seedlings and the uptake of phosphorus. Henderson and Duff (1963) hypothesized that the the dissolution of phosphorus was carried out by organic acids produced by fungi. To produce these organic acids or support bacteria which do, the fungus must be physiologically active. If stress on the mycorrhizal system occurs, the production of organic acids would decrease, and the liberation of phosphorus would decrease. Low colonization was evidence that Pt and Tt were stressed in this system probably due to a sensitivity of some soil chemical factor, probably aluminum. aeration. This low

colonization resulted in less P being dissolved, taken up, and transferred to the seedling.

The decrease in extractable P levels in soil treatments containing Rr was similar to uncolonized soil levels (Figure 4). The notable exception was the soil fertilized with 112 kg P ha^{-1}; Rr seemed to have enough surface area to decrease the readily available P.

Extractable Iron and Aluminum

Changes asssociated with extractable P also existed for extractable Fe and Al (Figures 5 and 6). The changes in soil chemistry for Fe and Al were probably created by the same effects which changed extractable P. Organic acids or other metabolites released by the fungi to extract unavailable P would also react with Fe and Al. The effect of this action on the extractable Fe and Al is not known. Extractable Fe and Al increased in soils with control seedlings, but decreased in soils with the colonized treatments. This is additional evidence that the fungi are changing the soil environment in a way that renders Fe and Al inactive. However, roots of control plants seemed to increase the activity of Fe and Al. Another important point is that soil fertilized with 112 kg P ha^{-1} and containing Tt had a significant decrease of extractable Fe, Al, and P. The

increase of soil P apparently triggered some response in the fungus to produce substances to act on the soil chemical environment. Rr-colonized seedlings growing in soil fertilized with 112 kg P ha^{-1} changed the extractable levels of Fe and Al. Rr may produce compounds which change the extractable levels of these cations, but it did not accomplish the change as to benefit the seedling as Sa did.

Seedling Responses

Survival and Colonization

Tree survival was generally unaffected by mycorrhizal and soil treatments (Table 7). Similar patterns were evident in the number of individuals colonized (Table 8). However, there seems to be a chemical inhibitor for Tt in the unamended soil. Soil aluminum is toxic to agronomic crops in soils with 3.41 umoles Al g^{-1} (100 ppm) or less, and root growth is inhibited at 0.4 umoles Al g^{-1} (Foy, 1974). Pine trees may tolerate 300 to 400 ppm of Al in the soil without any effect (Foy, 1974). The levels of aluminum in the soil treatment was above what is considered toxic for most agronomic plants (Figure 6). Phosphorus applied to the soil can alleviate Al toxicity (Foy, 1974). Aluminum interferes with the phosphorylation processes, and increased phosphorus levels would stimulate these processes. In the 75 percent

sand-amended soil, the sand probably diluted the Al so that it no longer affected Tt. The high levels of soil P resulting from the addition of 112 kg P ha^{-1} probably compensated for the inhibitory aluminum levels. Survival and colonization of Sa-colonized seedlings seemed not to be affected by the soil chemical changes created by the soil amendments. Other colonized seedlings seemed not to be affected, but the variation in these treatments may mask any effect.

Shoot

Growth.

Shoot growth is dependent on the supply of P in situations where P is limiting (Barrow, 1977). Foliar P levels were significantly correlated with shoot growth (Table 7). Mycorrhizae supplied P to the seedlings in accordance with each symbionts ability to function in this particular soil environment. Bowen and Theodorou (1969) found that four different fungi differentially stimulated Pinus radiata seedlings fertilized with rock phosphate in a Mt. Burr sand. Macskaylo and Vozzo (1969) reported similar results with other fungi. In both cases, P addition did not compensate for the mycorrhizal effect.

Sa produced the largest shoot biomass of any mycorrhizal treatment. At low phosphorus concentrations seedlings colonized with Sa grew better than all other fungal treatments. The Scleroderma mycorrhizae stimulated growth in the unfertilized soil while phosphorus additon aided other colonized seedlings (Figure 8).

Phosphorus fertilizaton increased seedling growth in this experiment, but the fertilizer was thoroughly mixed in the soil and not applied from the top. If fertilizer was applied to the soil surface, it would probably not penetrate the soil more than 1 cm because the Al and Fe would strongly bind the P preventing further movement. Soil roots would not be able to extract the element due to that the majority of seedling roots were below this zone. Soil mixing is easily done in a pot study, but it is difficult in the field. Because phosphorus must be in close proximity to the roots to stimulate seedling growth, some form of soil incorporation would be necessary if P fertilization was being considered for Cecil soils.

Stem parenchyma are important reserves of carbonydrate (Kramer and Kozlowski, 1979). These reserves would be used in times of stress or growth. Control seedlings with smaller stems (Table 10) did not have the reserves that the colonized seedlings can utilize. A large stem biomass would

hold reserves for the spring growth flush. These reserves may also be used for root regeneration after planting.

Phosphorus uptake.

Mycorrhizal studies in a phosphorus fixing soil such as the Cecil are rare if nonexistent. Most studies are carried out in sand cultures or artifical media. In this study, it has been shown that mycorrhizae were able to take up P more efficiently than control roots in a P fixing soil. It was also shown that mycobionts differ in their ability to tolerate these conditions and to extract P from these iron and aluminum complexes.

Fertilization increased foliar P of all mycorrhizae treatments except Sa and Rr (Figure 9). This increased foliar P resulted in larger foliar and shoot biomass of all mycorrhizae treatments (Figures 7 and 8). This indicated that the soil was deficient in P for seedlings under these conditions.

Needles require P to convert light energy to chemical energy by cyclic, noncyclic, and pseudocyclic photophosphorylation (Salisbury and Ross, 1978). Any changes in the P nutrition of the plant would be manifested in the foliage. Foliar P concentration was influenced by the interaction of mycorrhizae and soil treatments (Figure 9). Control seedlings never reached the foliar P critical level

for optimum growth (32 umoles P g^{-1}) even at the highest level of fertilization, because they could not take up a significant amount of P from the soil. In all likelihood, this was due to the inability of the roots alone to solubilize the unavailable Fe and Al phosphates.

The presence of trivalent aluminum may have confounded the up take of P by fixing it in insoluble complexes and interfering with the physiology of the fungus. The Cecil series with kaolinite as the predominate clay mineral is considered to be high in aluminum (Bonn et al., 1979). Figure 6 shows the relativly high concentrations of extractable Al. An increase in soil aluminum concentration often decreases P uptake in agronomic plants and utilization even after P fixation has been taken into account (Foy, 1974). It is thought that Al interferes with the metabolism of P at the enzyme level during phosphorylation.

Tt may also be sensitive to aluminum, since it is a fungus common in soils where aluminum is not considered a problem, such as in nursery soils. Soils in which Tt predominates are usually higher in nutrients and pH. Unlike Pt-colonized seedlings the foliar P concentration of Tt-colonized seedlings did not quickly respond to phosphorus fertilization (Figure 9).

Studies have shown that Rr has a high phosphatase activity (Theodorou, 1971). Rr is a fungus found in pine stands with some organic matter development and uses the phosphatase to extract P from organic matter by cleaving the C-O-P bond. Fruiting bodies and associated mycorrhizae are found at the litter-soil interface. Therefore, this fungus also may not be able to tolerate low partial pressures of oxygen or efficiently extract fixed forms of inorganic phosphorus. Aluminum tolerance may also be a factor since this element is in low concentration in the litter.

Sa-colonized seedlings increased foliar P concentration with the decreasing sand amendments, peaked in the unamended soil, and decreased slightly in soil fertilized with P (Table 9). Sa seemed to extract soil phosphorus from these fixed inorganic forms more efficiently than any other fungi tested. This conclusion is supported by both foliar P and postharvest soil extractable P (Figure 4).

Control seedlings had less stem P than colonized seedlings (Table 9). Other differences may have been detected if the living tissue of the stem could have been separated from the dead tissue. Although there was a dilution due to the combination of dead and living tissues, differences between stems of colonized and uncolonized stems were evident. This shows that nonmycorrhizal (control)

seedlings were so deficient in P that even the small amount of living tissue in the stem was P deficient.

Seedling Roots

The root is a sensitive component in the mycorrhizal system. Hormones produced by the fungi act directly on the physiology and growth of roots (Slankis, 1973). In addition, increased nutrition induces the plant to produce a larger root system. Root length is more important than root biomass in phosphorus uptake. Lewis and Quirk (1967) stated that a large root system in terms of length was correlated with phosphorus uptake. Root length was significantly correlated with seedling growth and foliar phosphorus (Table 17). Sa-colonized roots were longer than roots of the other mycorrhizal treatments (Tables 11 and 12). These large roots were able to exploit more soil volume for phosphorus and create larger zones of depletion.

Roots are large carbohydrate reservoirs due to the large amounts of parenchyma (Kramer and Kozlowski, 1979). During times of stress or during spring growth flushes, the root's reserves are utilized. The larger root systems produced by Sa mycorrhizae would have the potential to supply a larger amount of carbohydrate to the expanding meristem.

Root phosphorus was not affected by mycorrhizal fungi because secondary growth diluted the P concentration in metabolically active tissue. The same was found for the woody stem. However, the presence of mycorrhizae did increase stem phosphorus over uncolonized seedlings. Fertilization did not affect root phosphorus probably for the same reason that the mycorrhizae treatments had no effect. The amount of woody tissue would dilute any difference in concentration of P in metabolically active tissue among treatments.

Mycorrhizae

Colonization.

Both length and amount of root colonized by mycorrhizae were closely related to seedling growth and P uptake (Table 17). Marx and others (1977a) demonstrated that root colonization (%) could be assessed subjectively. However, a more quantitative method for colonization is needed. A true indication of how well the fungi colonized the root systems was obtained by estimating the length colonized by mycorrhizae. Similar techniques have worked with vesicular-arbuscular mycorrhizae (Ambler and Young, 1977).

Contrary to findings of Marx and others (1977b), increased phosphorus fertility did not decrease mycorrhizal

infection in this study. They found that root sucrose concentration was negatively correlated with the amount of phosphorus in the growth media, and that the root sucrose was directly correlated with mycorrhizal infection. Sucrose is the main form of soluble carbohydrate in the root (Zimmermann and Brown, 1977), but the carbon source used by the fungus is glucose. It is presumed that separation of glucose and fructose in the sucrose is accomplished in the roots, and the glucose is transfered to the fungus (Marx et al., 1977b). However, neither P levels in the media nor amount of colonization were correlated with root glucose levels (Marx et al., 1977b). If P levels in the media are increased, sucrose is used for plant cell growth, and it is then not available as a carbon source for the fungus. As a result, colonization is decreased. It is thought that low levels of the enzyme which splits the sucrose molecule limits the conversion to sucrose to glucose. These low levels result in relatively constant glucose levels which do not change with external stimuli (Marx et al., 1977b). In this experiment, the amount of available phosphorus, even with the addition of 112 kg ha^{-1} of applied phosphorus, probably did not decrease root sucrose concentration enough to significantly affect colonization. The amount of roots colonized varied with the fungal species (Table 11). Roots

colonized with Sa had three times more total length covered with mycorrhizae as compared to roots colonized with the other fungi. This again demonstrated the ability of Sa to thrive in this experimental environment.

The length of roots not colonized by mycorrhizae was not significantly different for any of the five mycorrhizal treatments. The mycorrhizae apparently stimulated root growth over a defined growth potential, where growth potential of the root is defined as the length of a noncolonized root system. Chivers and Gust (1982) found that fungal propagules initiated a colonization sequence which resulted in an additional amount of growth. From this additional amount of growth, a mycorrhizal "colony" was formed which covered the slow growing, high order lateral roots. These colonies produced a pocket of roots which were heavily colonized. These "colonies" of mycorrhizal short roots were observed on loblolly pine in this study at the time of the harvest.

Research is needed to determine when to inoculate seedlings with different fungi, peak activities of propagules, and how to initiate colonization by these propagules. Some guideline is needed to determine when propagules are ready to initiate colonization. Also, more information is needed on the growth requirements of the

fungi in culture and on the seedling. Spores and resting structures may need some pretreatment such as stratification to begin the colonization process.

Biomass.

This study was unique in that mycorrhizae biomass was measured. Mycorrhizae biomass was significantly correlated with all growth measurements and foliar P concentration (Table 17). The correlation reflects the relationship of the phycobiont to the mycobiont. A large photosynthetic apparatus is required to support a large amount of mycorrhizae, and a large amount of mycorrhizae is required to supply the nutrients to an active plant. Mycorrhizae biomass differences among fungi followed the same trends as colonization rates (Table 12). Sa had produced a three-fold increase in mycorrhizae biomass over the other symbionts. This was another demonstration of the suitability of Sa to the Cecil soil.

Phosphorus.

Mycorrhizal P was not related to seedling growth or foliar P levels (Table 16). Other pot studies have demonstrated competition between the fungus and the higher plant for P if the nutrient becomes too limiting (Beckjord, 1978). The fungus and rhizosphere organisms have the first

opportunity to metabolize the P. As already stated, all nutrients reaching the root must pass through this mass of hyphae if the seedling is colonized. If the fungus' demand increases for P, less P is available to the seedling. Neither soil amendments nor species of fungi affected the P concentration in the mycorrhizae. This is evidence that the fungal demand for P was initally satisfied before any was transferred to the phycobiont.

Polyphosphates.

In this study, neither soil amendments nor the species of fungi affected insoluble polyphosphates. The amount of tissue used for analysis was marginal to detect differences among treatments for insoluble polyphosphates (Table 13), and accurate conclusions cannot be made for this experiment.

Acid soluble polyphosphates were more prevalent than insoluble polyphosphates. These are the most metabolically active forms, and are found in the vacuoles and cytoplasm of the fungi (Harold, 1966). Total and acid-soluble polyphosphate concentrations and responses to soil and mycorrhizal treatments were almost identical because over 90 percent of the total was composed of soluble polyphosphates (Figures 10 and 11). Tt mycorrhizae had more polypnosphates than Sa mycorrhizae until the available phosphorus concentration was increased with the addition of 112 kg P

ha^{-1} . This change in polyphosphate concentration was also accompanied by an increase in seedling growth, phosphorus uptake, a decrease in extractable Fe and Al, and a disproportionate change in extractable P. Polyphosphates in Sa mycorrhizae increased with an increase of available P (Figures 4-9). The fungal demand for polyphosphates was not as great for Sa as for Tt mycorrhizae. Tt may have accumulated polyphosphate in vacuoles to counteract the free Al in the cytoplasm taken up from the soil. In fungi and other microbes, polyphosphates hold and capture metallic and other cations by the numerous anionic charges. Sicko-Goad and Luzinski (1981) found heavy metals associated with polyphosphates in algae. Aluminum could be compartmentalized in the vacuoles away from the sites of metabolism by accumulating polyphosphates. Sa is probably not as affected by Al as Tt and would not need to compartmentalize it with the polyphosphates. This would mean more P would be transferred to the seedling or used for fungal growth. Trappe (1977) stated that insoluble oxalate salts were the anions responsible for compartmentalizing Al in vacuoles. It is quite possible that Sa may use the oxalate salts whereas Tt uses polyphosphates.

Total and acid-insoluble polyphosphates are also negatively correlated with seedling growth and foliar P

levels (Table 16). This suggests that the fungal demand for phosphorus preceded that of the seedling. Perhaps during periods of active accumulation of polyphosphates the benefit of mycorrhizae to the seedling may be compromised by the demand for energy and P by the fungi (Harley and McCready, 1981).

Total Plant

Nonmycorrhizal seedlings had a higher root to shoot ratio than mycorrhizal seedlings (Table 14). Control shoot biomass was smaller than the colonized seedlings, and it would appear that the additional portion of root biomass was produced to compensate for the lack of mycorrhizae. Sa-colonized seedlings had the largest shoot biomass and the lowest root to shoot ratios due to the apparent efficiency of the root-mycorrhizae system. This efficiency allowed more growth in the aerial portion of the seedling. (Bowen (1973), Bowen and Theodorou (1967), Hacskaylo and Vozzo (1967) have found similar comparisons of ratios in colonized and uncolonized seedlings.

Phosphorus uptake efficiency is based on the unit length of root. Since P uptake is a function of root length, the efficiency equation reflects current models of P uptake (Bieleski, 1973). Sa-colonized root systems were more

efficient than nonmycorrhizal roots (Table 15). There were no detectable differences in the other mycorrhizal systems and they also were less efficient than Sa-colonized roots. All colonized roots were more efficient than controls except those colonized with Rr. As stated, Rr may be hindered in P uptake by the presence of Fe and Al. However, the increased root length of Rr-colonized seedlings over the controls increased P uptake in soils amended with sand. Soil treatments did not affect uptake efficiency.

The mycorrhizal uptake efficiency was not affected by species of symbiont or soil amendment (Table 15). This would suggest that the mechanism of uptake is very similar, and that the difference in uptake was determined by the amount of root colonization. The colonization was a function of the ability of the fungi to grow compatibly in the soil environment and on the loblolly pine seedling root system. The relationship between growth and colonization was supported by significant correlations of percent mycorrhizae and length of colonized root with total biomass and foliar P levels (Table 17).

Adaptability and Selection of Fungi

Fungi in Piedmont Soils

Little is known about the response of mycorrhizal fungi to chemical stress. Many Piedmont soils have high aluminum concentrations which, in association with low soil pH, causes the trivalent form of aluminum to predominate (Bonn et al., 1979). Classical approaches to mycorrhizal studies involved pot cultures using sand or artifical media (Bowen, 1973). Very few studies have used soils that might induce chemical stress. Based on the results of this study, fungi vary in their ability to colonize seedlings, mediate growth, and take up phosphorus in these soils. This ability is a function of surviving reduced partial pressures of oxygen, tolerating high aluminum concentrations, and absorbing phosphorus from precipitated or adsorbed forms. Trappe (1977) concluded that serious efforts to establish ectomycorrhizae on problem soils must include careful selection of mycorrhizal fungi for adaptability. Pines are quite tolerant to soil aluminum (Foy, 1974), but their associated fungi may be sensitive. Trappe (1977) also concluded that it was desirable that the fungi function and grow rapidly in the soil to maximize benefit to the plant. Sa which intensively colonized loblolly pine roots in the Cecil soil stimulated biomass growth and P uptake over other colonized seedlings. It appears that this fungus is adapted

to the soil environment of the Cecil soil which contains high levels of unavailable P and trivalent Al.

The ecological adaptability of fungi depends on the metabolic pathways which tolerate adverse environments. However, genotype plays a role in the success of the fungus (Theodorou and Bowen, 1971; Gobl, 1975). Ecological studies of the fungi can produce useful clues to the identification of environmental tolerances and requirements. More research is needed in determining which fungal species colonize loblolly pine in Piedmont soils for specific age groups. With these results, proper selection of fungi through pot and field studies can be made provided that the fungi can be handled in culture.

Fine-textured soils such as the Cecil used in this study impede root and hyphal penetration due to small soil pore size. The surface area of clay soils, however, is several times greater than sandier soils. The number of nutrient exchange sites, therefore, would be greater in the finer textured soils. The fewer available exchange sites in the sandier soils are somewhat compensated by the ease of root and rhizomorph penetration. In this experiment, considerable hyphal penetration into the sandy clay loam was observed with pots containing Sa, but very little was observed with pots containing the other fungi. This increased penetration

allowed for greater exploitation of the soil which resulted in an overall increase in nutrient uptake.

Fungi and Fertilization in Piedmont Soils

This study demonstrated that different symbionts differentially increased early growth and P uptake of loblolly pine growing in a Piedmont soil. Available soil P was a limiting factor and proper selection of the fungi could increase the P supply to the young seedlings. Fertilization increased growth of all seedlings colonized with all fungi except Sa. If uncolonized seedlings are planted on a Piedmont site, the seedlings will grow slowly until a proper root system is established. If growth is too slow, the seedlings may be overtopped by competing vegetation and die. The same may happen to nursery seedlings colonized with fungi not adapted to the site unless fertilizer is added. The benefit of selecting fungi may offset the costs of site preparation, vegetation control, and fertilization, if the fungi can stimulate seedling growth enough to compete with other vegetation. The proper fungi might exploit the natural soil P pools and produce a seedling that would outgrow the flush of competing vegetation that occurs after reforestation.

The argument against the selection of fungi is that they are already present on the site and eventually the seedlings will become colonized. Pines are usually regenerated in one of the following environments: (1) a harvested pine site, (2) a converted hardwood site, or (3) an old field. Mycorrhizal fungi in young stands are not the same fungi which are present in older stands (Miller, 1982; Trappe and Fogel, 1977). It would take time for the proper fungi to enter the stand and colonize seedlings. Less efficient fungi would colonize the roots and compete for colonization sites that could be used for the more efficient symbionts. Fertilization of the stand would lead to a flush of competing vegetation and expensive control techniques would have to be implimented.

The mycorrhizae in hardwood stands probably would not be the same as those which are more efficient in colonizing pine. Also, a period of time would pass between harvesting and planting. During this time the number of natural, viable propagules would decrease.

Old fields do not have the propagules to properly infect the seedlings at the time of planting unless spores are disseminated from nearby stands. Even if this happens, the seedlings would still enter a period of suboptimal growth before the roots and mycorrhizae are established.

By using a fungus such as Sa on soils similar to a Cecil, growth may be significantly increased after the first year. This increase may be continued if the fungus is effective for longer periods.

The Need for Mycorrhizae

Ectomycorrhizae are important in the uptake of nutrients in Pinus spp. (Marx, 1980). This experiment is one of many which examined the benefits of ectomycorrhizae on the uptake of P (Bowen, 1973; Marx, 1980; Harley, 1978). Pirozynski and Malloc (1975) stated that land plants evolved because of mycotrophism, and P is the primary nutrient involved in the symbiosis. Several examples of co-evolution of mycobionts and trees have become evident through past research (Miller, 1981). Trees, such as loblolly pine, with a wide range of environmental tolerances must have either co-evolved with fungi which have a wide tolerance to soil factors, or co-evolved with several fungi which are site specific.

This experiment has shown that different mycobionts of loblolly pine are not equally effective in supplying P in the Cecil soil. Control seedlings could not uptake adequate P even from soil fertilized with 112 kg P ha^{-1}. Tt, Rr, and Pt-colonized seedlings each responded differently to the soil amendments. Sa-colonized seedlings did not respond to P

fertilization, but they were larger and contained more P than the other seedlings even after these other seedlings were fertilized. Sa seemed to be able to draw on the vast reservoir of unavailable P in the Cecil soil.

Polyphosphate levels provided some evidence about the mechanism of tolerance and accumulation of P. Tt appeared to accumulate polyphosphate in the presence of high aluminum levels, but Sa did not. More information on the role of polyphosphates in the symbiosis and the response to chemical stress is needed. This study has provided a base for further work in the area of chemical tolerances of mycobionts and selection of tolerant species. A more intensive effort is needed in both the laboratory and field before solid, scientific criteria are available for the selection of site specific fungi.

The Use of SCLERODERMA AURANTIUM in Forestation

The overall results of this study showed that of the four fungi studied, Sa was the best at mediating a positive growth response in loblolly pine seedlings grown in a Cecil soil. The mycorrhizae formed by Sa was apparently able to extract P from the Fe and Al complexes in the unamended soil as supported by the changes in extractable Fe, Al, and P. Seedlings colonized with Sa did not respond positively to

fertilization. However, they were larger and had higher foliar P levels than other colonized seedlings, whether or not they were fertilized. Scleroderma aurantium also demonstrated its compatibility with the soil environment and loblolly pine by colonizing more of the root system than the other fungi. Obviously, this fungus has a superior ability to take up P and hence aid seedling establishment and growth on Cecil and similar soils of the Piedmont.

In the past, cosmopolitan fungi such as Pt and Tt were assessed with mixed results as mycorrhizal symbionts in all types of environments. These organisms were used because of they were easily cultured and relatively abundant of these fungi on certain sites. Other potentially useful fungi, such as Sa were ignored. There are yet other mycobionts besides Sa that may prove to be beneficial on these soils.

Scleroderma aurantium was a relatively easy symbiont to culture compared to many other mycorrhizal fungi. Nutrient and growth requirements of the various mycobionts are not actually known, therefore limiting the number of symbionts that may be used. Research is needed to find ways to stimulate the growth in culture of Sa and other fungi so that more inocula may be produced, a greater number of symbionts may be used, and a greater number of seedlings can be tailored for specific sites.

Scleroderma aurantium stimulated seedling growth considerably more than Pt or Tt. An attempt by Abbott Laboratories Pt for nursery inoculation has not been successful. Pt may not be as adapted to a wide range of soil and site environments; therefore, if maximum economical and biological benefits are to be realized through inoculation programs, site-specific mycobionts should be used. The considerable increase in growth of seedlings colonized with Sa in this study supports the argument for careful selection of mycobionts.

The results of this study are promising in that a relatively unknown fungus significantly benefited loblolly pine seedlings over other commonly used symbionts. Based on this study, Sa should be used with loblolly pine in fine-textured Piedmont soils or other soils with a high P-fixation capacity associated with high levels of Fe and Al. Further mycorrhizal research efforts should consider Sa as an alternative to natural or other species of introduced mycobionts for use in these environments. In addition, field tests should be conducted on several Piedmont soils to verify these results. A rigorous selection scheme for mycobionts should be started under both field and laboratory conditions.

SUMMARY AND CONCLUSIONS

This study has demonstrated that different fungi differentially stimulated seedling growth and P uptake in loblolly pine seedlings in a Cecil soil. Scleroderma aurantium produced larger seedlings and mediated more phosphorus uptake than Thelephora terrestris, Rhizopogon roseolus, or Pisolithus tinctorius. Control seedlings never achieved the growth or foliar P levels of colonized seedlings even with the addition of 112 kg P ha^{-1}. Phosphorus uptake levels mediated by the fungi were a function of the physiological adaptations of each fungus to the soil environment. Scleroderma aurantium appeared to be adapted to the Cecil soil while the other fungi were not. Increased P by fertilization increased growth of seedlings colonized with other fungi. Seedlings colonized with S. aurantium did not respond to fertilization. Fungi depleted extractable phosphorus in the treatments where soil was amended with sand, but increased extractable phosphorus in the fertilized soils. Organic acids produced by the fungi may have mediated this change and the change in extractable iron and aluminum. Scleroderma aurantium also colonized

more of the root system than the other fungi and stimulated root growth. However, the length of uncolonized root was not significantly different for any of the mycorrhizal treatments.

Total mycorrhizal phosphorus concentration was not affected by soil phosphorus or species of fungi. Tt and Sa mycorrhizae responded differently to the amended soil. This provided some evidence that T. terrestris is sensitive to high Al levels in the soil. Polyphosphates were negatively correlated with seedling growth and phosphorus variables. These correlations and the lack of mycorrhizal phosphorus response to soil treatment were evidence that the fungal demand for phosphorus preceded the seedling demand.

The conclusions of this study are as follows:

1. All four fungi mediated seedling growth differently in the different soil amendments. These differences were manifested in the ability of each fungus to grow and survive in the soil environment. Scleroderma aurantium was better adapted to the soil enviroment than any of the other fungi as indicated by colonization and stimulation of seedling growth. The mycobionts stimulated root growth over a constant determined by the environment. This was indicated by the

nonsignificant effect mycorrhizal treatments had on noncolonized root length. The increased growth observed in the mycorrhizal seedlings was accompanied by increased phosphorus uptake.

2. Mycorrhizal phosphorus concentration was not correlated with seedling growth variables. The concentration also was not modified by increases in soil phosphorus. This demonstrates that the demand for phosphorus by the fungus precedes that of the seedling. Fungal polyphosphates were negatively correlated with seedling growth and phosphorus concentrations. Polyphosphate level also demonstrated the fungal demand for phosphorus. Acid-soluble polyphosphates of S. aurantium responded to increasing soil phosphorus created by the addition of 112 kg P ha^{-1}, but T. terrestris did not. Soluble polyphosphates in T. terrestris increased when the sand amendment was reduced from 75 percent to 25 percent, but remained at a constant level except in soil with 112 kg P ha^{-1}. At this level of applied phosphorus, there was a significant decrease in polyphosphate.

3. In general, all measured plant parts except the roots and mycorrhizae increased with increased levels of phosphorus in the tissue. Differences were not evident in the roots due to the amount of secondary growth. Stems were sensitive to phosphorus fertilization but not to differences among mycorrhizal fungi. Foliar phosphorus levels seemed to reflect the ability of the different fungi to thrive and function in the different soil amendments.

4. Extractable phosphorus, iron, and aluminum levels in the sand-amended soils was modified in pots which contained mycorrhizal seedlings. There appeared to be a decrease in the extractable levels of iron and aluminum. Extractable phosphorous decreased in the sand amended soils containing mycorrhizae, but increased in fertilized soils. Changes in the extractable elements suggested that compounds produced by the fungi were released into the soil and were dissolving the unavailable and otherwise insoluble aluminum and iron phosphates.

5. Scleroderma aurantium appears to be adapted to Piedmont soils such as the Cecil. It is able to extract more of the large amount of unavailable P present in these soils, and hence is able to stimulate growth and P levels in loblolly pine.

LITERATURE CITED

Alexander, M. 1977. Introduction to Soil Microbiology. John Wiley & Sons, New York. 467 pp.

Ambler, J.R. and J.L. Young. 1977. Techniques for determining root length infected by vesicular-arbuscular mycorrhizae. Soil Sci. Soc. Am. J. 41: 551-556.

Arrieta, L. and R. Grez. 1971. Solubilization of iron containing minerals by soil microorganisms. Appl. Microbiol. 22: 487-490.

Baker, A.L. and R.R. Schmidt. 1963. Intracellular distribution of phosphorus during synchronous growth of Chlorella pyrenoidosa. Biochim. Biophys. Acta 74: 75-73.

Barrow, N.J. 1977. Phosphorus uptake and utilization by tree seedlings. Aust. J. Bot. 25: 571-584.

Beckjord, P.J. 1978. The incidence of ectomycorrhizae by Pisolithus tinctorius on Quercus rubra seedlings fertilized with sodium nitrate and ammonium nitrate. Ph.D. dissertation, VPI & SU Dept of Forestry. 82 pp.

Bieleski, R.L. 1973. Phosphate pools, phosphate transport, and phosphate availability. Annu. Rev. Plant Physiol. 24: 225-252.

Bilan, M.V. 1967. Effect of low temperature on root elongation in loblolly pine seedlings. Int. Union. Forest Res. Organ., 14th, 23: 74-82.

Bjorkman, E. 1949. The ecological significance of some ectomycorrhizal associations in forest trees. Sv. Bot. Tidskr. 43: 223-264.

Bonn, H.L., B.L. McNeal, and G.A. O'Conner. 1979. Soil Chemistry. John Wiley and Sons, New York. 329 pp.

Bowen, G.D. 1973. Mineral nutrition of ectomycorrhizae. In Ectomycorrhizae: Their Ecology and Physiology. G.C. Marks and T.T. Kozlowski, eds., Academic Press, New York. 444 pp.

Bowen, G.D. and A.D. Roviria. 1966. Microbial factors in short term phosphate uptake studies with plant roots. Nature (London) 211: 665.

Bowen, G.D. and C. Theodorou. 1967. Studies in phosphate uptake by mycorrhizas. Proc. Int. Union Forest Res. Organ., 14 th, 14: 116-131.

Briscoe, C.B. 1959. Early results of mycorrhizal inoculation of pine in Puerto Rico. Carib. Forester. 20: 73-77.

Cole, C.V. and M.L. Jackson. 1951. Solubility equilibrium constant of dihydroxy aluminum phosphate relating to a mechanism of phosphate fixation in soils. Soil Sci. Soc. Amer. Proc. 15: 84-89.

Chang, S.C., and M.L. Jackson. 1957a. Solubility product of iron phosphate. Soil Sci. Soc. Amer. Proc. 21: 265-269.

Chang, S.C. and M.L. Jackson. 1957b. Fractionation of soil phosphorus. Soil Sci. 87: 133-144.

Chivers, G.A. and L.W. Gust. 1982. The development of mycorrhizal populations on pot-grown seedlings of Eucalyptus st-johnii R.T. Bak. New Phytol. 90: 677-699.

Clements, J.B. 1941. The introduction of pines into Nyasaland. Nyasald agric. q. J. 1: 5-15.

Cox, G., K.J. Moran, F.E. Sanders, C. Nickholds, and P.B. Tinker. 1980. Translocation and transfer of nutrients in vesicular-arbuscular mycorrhizas. III. Polyphosphate granules and phosphorus translocation. New Phytol. 84: 649-659.

Dorman, K.W. 1976. The Genetics and Breeding of Southern Pines. Agriculture Handbook No. 71. U.S.D.A. Forest Service. 407 pp.

Foster, R.C. 1981. Mycelial strands of Pinus radiata D. Don: ultrastructure and histochemistry. New Phytol. 88: 705-712.

Fowells, H.A. 1965. Silvics of Forest Trees of the United States. Agriculture Handbook No. 271. U.S.D.A. Forest Service. 762 pp.

Fowells, H.A. and R.W. Krauss. 1959. The inorganic nutrition of loblolly pine and Virginia pine with special references to nitrogen and phosphorus. Forest Sci. 5: 95-112.

Foy, C.D. 1974. Effects of aluminum on plant growth. In The Plant Root and Its Environment. E.W. Carson, ed., University Press of Virginia. 638 pp.

Giltrap, N.J. and D.H. Lewis. 1981. Inhibition of growth of ectomycorrhizal fungi in culture by phosphate. New Phytol. 87: 669-675.

Gobel, F. 1975. Erfahungen bei der Anzucht von Mykorrhiza Impfmaterial. Centralbl. Gesamte Forstwes 92: 227-237.

Hacskaylo, E. 1965. Thelephora terrestris and mycorrhizae of Virginia pine. For. Sci. 11:401-404.

Hacskaylo, E. and J.A. Vozzo. 1967. Inoculation of Pinus caribae with pure cultures of mycorrhizal fungi. pp. 139-148. XIV IUFRO Kongress, vol. 5, Muchen.

Hatch, A.B. 1936. The role of mycorrhizae in afforestation. J. For. 34: 22-29.

Harley, J.L. 1978. Ectomycorrhizas as nutrient absorbing organs. Proc. R. Soc. Lond. B. 203: 1-21.

Harley, J.L. 1969. The Biology of Mycorrhizae. Leonard Hill, London. 334 pp.

Harley, J.L. and B.C. Loughman. 1963. The uptake of phosphorus by excised mycorrhizal roots of beech. IX. New Phytol. 62: 350-359.

Harley, J.L. and C.C. McCready. 1981. The relationship between phosphate absorption and oxygen uptake in excised beech mycorrhizas. New Phytol. 88: 675-682.

Harley, J.L. and C.C. McCready. 1981. Phosphate accumulation in Fagus mycorrhizas. New Phytol. 89: 75-80.

Harley, J.L., C.C. McCready, and J.K. Brierly. 1953. The uptake of phosphate by excised mycorrhizal roots of beech. IV. New Phytol. 51: 342-348.

Harold, F.M. 1966. Inorganic polyphosphates in biology: structure, metabolism, and function. Bacteriol. Rev. 66: 772-794.

Harvey, A.E., M.F. Jurgensen, and M.J. Larsen. 1978. Seasonable distribution of ectomycorrhizae in a mature Douglas-fir/larch forest soil in western Montana. For. Sci. 24: 203-208.

Harvey, W.R. 1960. Least-squares analysis of data with unequal subclass numbers. Systems Application Division. Agricultural Research Service. U.S. Dept. of Agric. 157 pp.

Hendersen, M.E.K. and R.B. Duff. 1963. The release of metallic and silicate ions from minerals, rocks, and soils by fungal activity. J. Soil Sci.: 14:236-246.

Hsu, P.H. 1965. Fixation of phosphate by aluminum and iron in acidic soils. Soil Science 99: 398-502.

Jackson, M.L. 1958. Soil Chemical Analysis. Prentice-Hall, Englewood Cliffs, N.J. 496 pp.

Juo, A.S.R. and B.G. Ellis. 1968. Chemical and physical properties of iron and aluminum phosaphates and their relation to phosphorus availability. Soil Sci. Soc. Am. Proc. 32: 216-221.

Kessell, S.L. 1927. The dependence of pine on a biological soil factor. Emp. For. J. 6: 70-74.

Kormanik, P.P., W.C. Bryan, and R.C. Schultz. 1977. The role of mycorrhizae in plant growth and development. In Physiology of Root Microorganisms Associations. H.Max Vines, ed., Proc. of a Symp. of the South. Sect. Am. Plant Physiol.

Kramer, P.J. and T.T. Kozlowski. 1979. Physiology of Woody Plants. Academic Press, New York. 811 pp.

Kulaev, I.S. 1980. The Biochemistry of Inorganic Polyphosphates. John Wiley & Sons, New York. 255 pp.

Lewis, D.G. and J.L. Harley. 1965. Carbohydrate physiology of mycorrhizal roots of beech. III. New Phytol. 64: 256-269.

Lewis, D.G. and J.P. Quirk. 1967. Phosphate diffusion is soil and uptake by plants. IV. Plant and Soil 26: 454.

Lindsay, W.L. 1974. Role of chelation in micronutrient availability. In The Plant Root and Its Environment. E.W. Carson, ed., University Press of Virginia. 638 pp. bib. Lindsay, W.L. and E.C. Moreno. 1960. Phosphate phase equilibrium in the soil. Soil Sci. Soc. Am. Proc. 24: 177-182.

Ling-Lee, M., G.A. Chivers, and A.E. Ashford. 1975. Polyphosphate in three different kinds of tree mycorrhizae. New Phytol. 75: 551-554.

McComb, A.L. 1938. The relations between mycorrhizae and the development and nutrition absorption of pine seedlings in a prairie nursery. J. For. 36: 1148-1154.

Madu, M. 1967. The biology of ectotrophic mycorrhiza with reference to the growth of pines in Nigeria. Obeche, J. Tree Club, Univ. Ibanan. 1: 9-16.

Marks, G.C. and R.C. Foster. 1973. Structure, morphogenesis, and ultrastructure of ectomycorrhizae. In Ectomycorrhizae:Their Ecology and Physiology. G.C. Marks and T.T. Kozlowski, eds., Academic Press, New York. 444 pp.

Marx, D.H. 1980. Ectomycorrhizal fungus inoculations: a tool for improving forestation practices. In Tropical Mycorrhizal Research. P. Mikola, ed., Clarendon Press, Oxford. 270 pp.

Marx, D.H. 1977. Tree host range and world distribution of the ectomycorrhizal fungus Pisolithus tinctorius. Can. J. Bot. 33: 218-223.

Marx, D.H. 1972. Ectomycorrhizae as biological deterrents to pathogenic root infections. Annu. Rev. Phytopatn. 10: 459-464.

Marx, D.H. 1969. The influence of ectotropic mycorrhizal fungi on the resistance of pine root to pathogenic infection. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. Phytopath: 59: 153-163.

Marx, D.H. and W.C. Bryan. 1975. Growth and ectomycorrhizal development of loblolly pine seedlings in fumigated soil infested with the fungal symbiont Pisolithus tinctorius. For. Sci. 21: 245-254.

Marx, D.H., W.C. Bryan, and C.E. Cordell. 1977a. Growth and ectomycorrhizal development of pine seedlings in nursery soils infested with the fungal symbiont Pisolithus tinctorius. For. Sci. 22: 91-100.

Marx, D.H., A.B. Hatch, and J.F. Medicino. 1977b. High soil fertility decreases sucrose content and susceptibility of loblolly pine roots to ectomycorrhizal infection by Pisolithus tinctorius. Can. J. Bot. 55: 1569-1579.

Marx, D.H., W.G. Morris, and J.G. Mexal. 1978. Growth and ectomycorrhizal development of loblolly pine seedlings in fumigated and nonfumigated nursery soil infested with different symbionts. For. Sci. 24: 193-203.

Mason, P.A., F.T. Last, J. Pelham, and K. Ingleby. 1982. Ecology of some fungi associated with aging birches. Forest Ecology and Management. 4: 19-40.

Meyer, F.H. 1975. Physiology of mycorrhiza. Annu. Rev. Plant Physiol. 25: 567-586.

Mikola, P. 1973. Application of mycorrhizal symbiosis in forestry practice. In Ectomycorrhizae: Their Ecology and Physiology, G.C. Marks and T.T. Kozlowski, eds., Academic Press, New York. 444 pp.

Miller O.K., Jr. 1981. Taxonomy, morphology, and distribution of mycorrhizae. In Mycorrhizal Associations and Crop Production - Rhizosphere Research Group. R.F. Meyers, R.F. Bartha, and W. Busscher, eds., New Jersey Agricultural Experiment Station Research Report No. R04400-01-81.

Miller, S.L. 1982. Succession of ectomycorrhizal fungi associated with englemann spruce and subalpine fir in Wyoming. M.S. Thesis. VPI&SU. 93 pp.

Molina, R. 1977. Ectomycorrhizal fungi and forestry practice. pp. 147-161. In Mushrooms and Man, an Interdisciplinary Approach to Mycology Tony Walters, ed., U.S.D.A. Forest Service.

Moschler, W.W., G.D. Jones, and R.E. Adams. 1970. Effects of loblolly pine fertilization on a Piedmont Soil: Growth, foliar composition, and soil nutrients 10 years after establishment. Soil Sci. Soc. Am. Proc. 34: 683-685.

Moser, M. 1963. Die Bedeutung der Mycorrhiza bei Aufforstunger in der besunder Berucksichtigung von Hochlagen. pp. 407-424. In Mycorrhiza, W. Rawald and H. Lyr. Fischer, Jena.

Murphy, J. and J.P. Riley. 1962. A modified single solution determination of phosphorus in natural waters. Anal. Chim. Acta. 27: 31-36.

Nylund, J. 1980. Symplastic continuity during Hartig net formation in Norway spruce. New Phytol. 86: 373-378.

Oswald, C.T. and H.A. Ferchau. 1966. Bacterial associations of coniferous mycorrhizae. Plant and Soil 26:187.

Petersen, G.W. and R.B. Corey. 1966. A modified Chang and Jackson procedure for the routine fractionation of inorganic soil phosphates. Soil Sci. Soc. Am. Proc. 30: 563-565.

Pirozynski, K.A. and D.W. Malloch. 1975. The orgin of land plants: a matter for mycotrophism. Bio. Syst. 6: 153-164.

Pritchett, W.L. 1979. The Management and Properties of Forest Soils. John Wiley & Sons. New York. 500 pp.

Reuhle, J.L. and D.H. Marx. 1979. Fiber, food, fuel, and fungal symbionts. Science 206: 419-422.

Rosendahl, R.O. and S.A. Wilde. 1942. Occurence of ectotrophic mycorrhizal fungi in soils of cutover areas and sand cultures. Bull. ecol. Soc. Am. 23: 73-74.

Salisbury, F.B. and C.W. Ross. 1978. Plant Physiology. Second edition. Wadsworth Publishing Company, Belmont, California. 422 pp.

Sample, E.C., R.J. Soper, and G.C. Racz. 1976. Reactions of phosphate fertilizers in soil. pp. 263-310. In The Role of Phosphorus in Agriculture. American Society of Agronomy, Wadison, Wisconsin. 910 pp.

Sanders, F.E. and P.B. Tinker. 1973. Phosphate flow into mycorrhizal roots. Pestic. Sci. 4: 385-395.

Sas Institute. 1979. SAS 79.5. SAS Institute, Inc., Raleigh, North Carolina. 494 pp.

Schramm, J.R. 1966. Plant colonization studies in black wastes from anthracite mining in Pennsylvania. Tran. Amer. Philos. Soc. (Philadelphia) 56(1). 194 pp.

Shemakhanova, N.M. 1962. Mycotrophy of Woody Plants. U.S. Dept. of Commer. Transl. TT66-51073 (1967), Washington, D.C.

Sicko-Goad, L. and D. Lazinski. 1981. Accumulation and cellular effects of heavy metals in benthic and planktonic algae. Micron 12: 289-290.

Slankis, V. 1974. Soil factors influencing formation of mycorrhizae. Annu. Rev. Phytopath. 12: 437-457.

Slankis, V. 1973. Hormonal relationships in mycorrhizal development. In Ectomycorrhizae: Their Ecology and Physiology. G.C. Marks and T.T. Kozlowski, eds., Academic Press, New York. 444 pp.

Somers, L.E. and D.W. Nelson. 1972. Determination of total phosphorus in soils: a rapid perchloric acid digestion procedure. Soil Sci.Soc.Am.Proc. 36: 902-904.

Tan, K.H. 1980. The release of silicon, aluminum, and potassium during decomposition of soil minerals by humic acid. Soil Sci. 129: 5-11.

Tan, K.H., P. Sihanonth, and R.L. Todd. 1978. Formation of humic acid compounds by the ectomycorrhizal fungus, Pisolithus tinctorius. Soil Sci. Soc. Am. J. 42: 906-908.

Tennant, D. 1975. A test of a modified line intersect method of estimating root length. J. Ecol. 63: 995-1001.

Theodorou, C. 1971. The phytase activity of the mycorrhizal fungus, Rhizopogon luteolus. Soil Biol. and Biochem. 3: 89.

Theodorou, C. and G.D. Bowen. 1971. Influence of temperature on the mycorrhizal associations of Pinus radiata D. Don. Aust. J. Bot. 34: 13-20.

Thomas, G.W. 1974. Chemical reactions controlling soil solution electrolyte concentration. In The Plant Root and Its Environment, E.W. Carson, ed., The University of Virginia Press, Charlottesville. 691 pp.

Thomas, G.W. and D.E. Peaslee. 1973. Testing of soil phosphorus. pp. 115-132. In Soil Testing and Plant Analysis. C.W. Walsh and J.D. Beaton, eds., Am. Soc. of Agron., Madison, Wis.

Torbert, J.L. 1982. The fate of applied phosphorus and its effect on loblolly pine growth twenty years after application. M.S. Thesis, VPI & SU,

Trappe, J.M. 1977. Selection of fungi for ectomycorrhizal inoculation in nurseries. Annu. Rev. Phytopathol. 15: 203-222.

Trappe, J.M. and R.D. Fogel. 1977. Ecosystematics of mycorrhizae. In The belowground ecosystem: A sysnthesis of plant associated processes. pp. 205-214, Range Science Dept. Ser. No. 26, Colorado State Univ., Fort Collins.

van Suchtelen, M.J. 1962. Mycorrhiza bij Pinus spp. in de Tropen. Med. Landbouwhogesch. Opzoekingsstn. Staat Gent. 27: 1104-1106.

Vozzo, J.A. and E. Hacskaylo. 1971. Inoculation of Pinus caribae with ecotmycorrhizal fungi in Puerto Rico. For. Sci. 17: 239-245.

Wahlenberg, W.G. (ed) 1965. A Guide to Loblolly and Slash Pine Plantation Management in Southeastern U.S.A. Georgia Forest Research Council Report No. 14 Macon, Georgia. 360 pp.

Wells, C.G., D.M. Crutchfield, N.M. Berenyi, and C.B. Davey. 1973. Soil and foliar guidelines for phosphorus fertilization of loblolly pine. U.S.D.A. Forest Service Research Paper. SE-110. 15pp.

Went, F.W. and N. Stark. 1968. The biological and mechanical role of soil fungi. Proc. Nat. Acad. Sci. U.S. 61: 497.

White, D.P. 1941. Prairie soil as medium for tree growth. Ecology 22: 398-407.

Williamson, B. and I.S. Alexander. 1975. Acid phosphatase localised in the sheath of beech mycorrhiza. Soil Biol. Biochem. 7: 195-198.

Yuan, T.L., W.K. Robertson, and J.R. Neller. 1960. Forms of newly fixed P in three acid soils. Soil Sci. Soc. Am. Proc. 24: 447-450.

Zak, B. 1973. Classification of ectomycorrhizae. In Ectomycorrhizae: Their Ecology and Physiology. G.C. Marks and T.T. Kozlowski, eds., Academic Press, New York. 444 pp.

Zimmermann, M.H. and C.L. Brown. 1977. Trees:Structure and Function. Springer-Verlag, New York. 336 pp.

The two page vita has been removed from the scanned document. Page 1 of 2

The two page vita has been removed from the scanned document. Page 2 of 2