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# ENHANCED ECOLOGICAL SUCCESSION FOLLOWING PHOSPHATE MINING

Prepared by University of Florida, Center for Wetlands

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FLORIDA INSTITUTE OF PHOSPHATE RESEARCH

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**Florida Institute of Phosphate Research**
**1855 West Main Street**
**Bartow, Florida 33830**
**(863) 534-7160**

# ENHANCED ECOLOGICAL SUCCESSION FOLLOWING PHOSPHATE MINING

## FINAL REPORT

G. Ronnie Best, Peter M. Wallace, William J. Dunn, and H.T. Odum

CENTER FOR WETLANDS
PHELPS LAB
Gainesville, FL 32611

Prepared Under a Grant From the

FLORIDA INSTITUTE OF PHOSPHATE RESEARCH
1855 West Main Street
Bartow, Florida 33830

Contract Manager: David J. Robertson, Ph.D.

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# PERSPECTIVE

David J. Robertson, Ph.D.
Florida Institute of Phosphate Research Project Manager

## Historical Background

Upland habitat has been created in the wake of mining ever since the Florida phosphate industry abandoned river dredging in favor of exploitation of land pebble deposits. Advances in mining technology over the first four decades of this century have produced a variety of upland landforms, each with its own characteristics.

The first land-based operations used high-pressure water to remove the overburden and the phosphate matrix. Hydraulic mining thoroughly mixed the sand and clay components and threw them up in mounds surrounding the excavation. Since the spoils were composed largely of the sand and clay fractions of the matrix as well as significant quantities of overburden, hydraulic spoils were in many respects very similar to the native soils. In addition, most of the hydraulic operations were small and did not disrupt vast contiguous acreages (Layne, et al. 1977). Consequently, the spoil mounds rapidly revegetated from local seed sources and today support luxuriant growth typical of mesic hardwood hammocks (Schnoes and Humphrey 1980). Since the habitat that has redeveloped on the spoil mounds surrounding the hydraulic pits is essentially identical to undisturbed hammock habitat, these areas support virtually all of the aquatic and terrestrial wildlife normally associated with hardwood forests and lakes (Layne, et al. 1977).

Beginning in the second decade of this century, hydraulic mining was supplanted by the use of steam shovels, and later, by electric draglines. Although the method of obtaining the ore changed significantly with the introduction of mechanical excavators, phosphate operations were still limited to removing only the pebble-sized fraction as a product and discarding the clay, sand, and sand-sized phosphate. With the introduction of hydrocyclones, the phosphatic clays were segregated from the sand-sized particles and settled separately. The silica tailings - phosphatic sand mixture was returned to mining cuts as "debris."

In the 1940's, flotation technology was widely and universally adopted by the phosphate industry. Flotation allowed for the recovery of the sand-sized fine fraction ( 1mm) of the phosphate matrix that had formerly been discarded as debris. With the implementation of flotation, the sand tailings that were produced by flotation were used to fill the mine cuts just as debris had been used prior to the 1940's.

Shovel and dragline mining left a landscape of phosphatic clay settling areas and pits filled with overburden, tailings and debris. If the excavations were not backfilled, they rapidly collected water, producing a landscape of steep, narrow spoil windrows separated by canal-like "finger" lakes. Schnoes and Humphrey (1980), Wallace and Best (1983), and Kangas (1981,1983) provide excellent descriptions of succession on unreclaimed sites produced by mechanical excavation and overburden.

## Reclamation Research

Newly reclaimed areas are generally considered to be sterile because of the lack of classic soil zonation and the presence of mineral soil exposed at the surface. Since topsoil with a substantial organic content, inherent seed bank, and well-developed microbial community appears to be a beneficial, if not essential, component of natural ecosystems, the first impulse is to try to reestablish a fertile topsoil layer as a foundation for community development.

Topsoiling (mulching), which has been adopted as a technique for introducing native vegetation into newly reclaimed wetlands (mulching), has had limited application in upland revegetation. Preliminary studies have demonstrated that the technique is not as valuable on uplands as it is on wetlands (EcoImpact, Inc. 1980). Topsoiling generally is not a cost-effective technique; the topsoil is usually thin and heavily leached. However, a layer of toposil does increase infiltration, and selective topsoiling to introduce native species has been shown to be effective in xeric systems (Goodrich 1983). Additional research is needed for special applications.

Other soil amendments and dressings have been evaluated on a limited basis. For example, Occidental Chemical Company routinely mulches slopes with hay or straw to reduce erosion and stimulate more rapid growth. The value of this practice as a general procedure has not been thoroughly investigated in terms of value gained compared to costs incurred. However, Best, Dunn, and Wallace (1983) reported that straw mulching had the most significant positive effect on community development of all experimental treatments they tried including topsoil, gypsum, and phosphate-free fertilizer. In fact, in their experiments, gypsum and fertilizer exerted negative influences on community development. A variety of other amendments show promise for improving soil quality. For example, phosphatic clay and municipal sewage sludge could be disked into the upper layers of reclaimed soil to increase the soil's nutrient, organic content and water-holding characteristics. Inoculation of reclaimed soils with mycorrhizal fungi is another promising technique for improving the success of natural reclamation. This report presents the results of extensive research on the effects of inoculating vegetation with commercial and native strains of mycorrhizal fungi.

One aspect of upland reclamation, the actual planting of trees, has only recently begun to receive the attention it deserves. Trees are very visible and important components in upland reclamation both for

their aesthetic as well as functional values in the redevelopment of natural habitat. Research is currently underway on tree suitability for various reclaimed soil types. The Florida Division of Forestry recently completed a five year-project funded by the Institute of Phosphate Research entitled "Development of Techniques for the Use of Trees in the Reclamation of Phosphate Lands" (FIPR Publication #03-001-049). Five plots were established on reclaimed overburden throughout the central and north Florida phosphate districts to test planting stocks, planting techniques, and species differences. Nine additional plots were established on sand tailings, sand-clay mix and clay settling areas. The overall objective of the project was to develop criteria and guidelines for the use of trees to recreate wetland, island, upland, and aquatic habitat on mined areas (Wadsworth, 1983).

A principal deterrent to extensive tree planting on reclaimed areas is the cost of planting seedlings, which must be done by hand in many cases. Direct seeding would be far more cost-effective and would allow more extensive areas to be reforested at reasonable cost than currently envisioned. The Institute has funded two grants seeking to improve the success of direct seeding ventures. An initial attempt at direct seeding sand and slash pine on sand tailings conducted by the Division of Forestry was a failure, but the experiment was repeated a year later with some germination. As a part of the research documented in this report, researchers at the Center for Wetlands collected seeds from native sources and tested techniques in the laboratory and greenhouse to improve germination and survival of the seedlings. They also cultured organic soils to develop data on seed availability and viability in natural seed banks. Using the results of laboratory, greenhouse, and microplot experiments, the group established pilot-scale field tests on IMC and Agrico Mining Company sites.

Availability of suitable native plants for revegetation of mesic sites on overburden and xeric sites on sand tailings has been hampered by meager knowledge of techniques for propagating and outplanting appropriate desirable species. The primary goal of a project at the University of Florida being sponsored by the Institute, "Propagation and Establishment of Indigenous Florida Plants for Revegetation and Restoration of Phosphate Mining Sites" (FIPR #84-03-053R), is to develop information on selection, propagation and establishment of indigenous Florida plants to be used in reclamation efforts. The investigators identified species that are important components of their respective ecosystems from three sites in north and central Florida. These plants are being propagated by techniques requiring the least intensive effort, whether that be by seed, stem cutting, or in the case of plants very difficult to reproduce, by tissue culture techniques. Plants are then inoculated with native endomycorrhizal fungi species to improve their chances for survival and growth once they are planted on reclamation sites. Nursery and field trials have been established to compare the success of inoculated plants with that of other plants that have not been inoculated with fungi.

One upland community that has been singled out for particular attention is the sand pine scrub. Most of the scrub and other xeric communities in west and central Florida have been displaced by urban development, roads, and citrus groves. An opportunity exists to reestablish scrub on well-drained mined uplands. EcoImpact, Inc. (1981)

concluded that managed establishment of xeric ecosystems appears to be feasible and warrants further investigation. Sand pine scrub revegetation experiments are underway at three separate phosphate mine sites, all of which have incorporated topsoil mulch from native xeric communities. AMAX Phosphate (Sandrick and Crabill 1983) initially had very low survival on a 130 acre tailings area planted to turkey oak and sand live oak. A second planting of bare-root sand pine seedlings included topsoil, organic peat and sewage sludge as additives to the planting hole, and the site was stabilized with rye. Clewell and Poppleton (1983) concluded that scrub communities can be restored on reclaimed land assuming that soil moisture can be regulated and that the sites are mulched. Their conclusions are based on research conducted at Brewster Phosphates' Haynsworth Mine. IMC has had promising development of sand pine scrub understory vegetation on a settling area that had been capped with tailings and partially mulched with scrub topsoil (Goodrich 1983). All of these sites are fairly young and will require monitoring to document continued development over time. The Institute is currently providing support for a project to improve the survival of plants used in xeric systems reclamation through several techniques not currently incorporated into reclamation plans such as superabsorbant polymers, weed control and xeric soil analogs. The research, "Restoration Techniques for Sand Scrub" (FIPR #85-03-066R), is being conducted by Florida Southern College of Lakeland with the help of a local native plant nursery, The Natives, on property reclaimed by Brewster Phosphates in Hillsborough County, Florida.

Marion and O'Meara (1983) summarized the prospects for wildlife habitat development on reclaimed land by assessing the wildlife value of reclaimed parcels as proposed in the phosphate industry's conceptual reclamation plans and comparing them to pre-mining conditions. They found reduced acreage of native rangeland and upland forests in deference to increased agricultural lands, non-forested wetlands, and open water. However, there is an approximate 20% increase of both the number and length of edges , or interfaces between different habitat types (e.g. lake-forest, wetland-pasture, etc.). Changes in major edge types after reclamation include fewer involving rangeland and more involving wetlands and agriculture. Likely negative impacts derive from (1) a reduction in upland forest acreage, (2) increased agriculture, (3) fewer, larger blocks of habitat, and (4) diminished native rangeland. Positive changes include (1) increased numbers of edges, (2) reduced lengths of edges, and (3) larger acreage devoted to wetlands.

This report contains information from a diverse project encompassing three general approaches to improving reclamation. The thread that ties all of the approaches together is the emphasis on encouraging the natural organizing power embodied in succession to accelerate and improve the success of reclamation rather than investing heavily in human subsidy to revegetate disturbed areas.

Drs. Best and Odum, along with their graduate researchers, examined successional processes on mined land below, at, and above the soil surface as part of this research. They began with a study of the colonization, inoculation, and establishment of soil fungi which form mycorrhizal associations. Such symbiotic relationships are critical

to the health of nearly all terrestrial plants. They went further and inoculated hardwood seedlings with a variety of native and commercial fungi to assay the effect of these fungi on the survival and growth of hardwood trees.

The vegetative aspect of the project involved assessing the wealth of propagules deposited in the "bank" of wetland soil that can be used to introduce wetland vegetation onto reclaimed sites. Best and Odum investigated direct seeding as an alternative for introducing later successional trees and shrubs onto reclaimed land and tested techniques to enhance the success of direct seeding through the use of soil amendments and mycorrhizal fungi.

Once the plants are introduced, the progression from bare mineral soil to a mature, climax ecosystem is strongly controlled by interactions between the species present. The investigators concluded by evaluating the impact of competitive weedy species on the establishment and growth of more desirable plants on the site.

This project was among the first reclamation projects funded by the Institute. Between the time the research got underway and the publication of this report, similar research was initiated by the the Institute and the phosphate industry to refine the concepts tested herein. Nonetheless, this broad project developed a great deal of valuable information and should serve as a firm foundation for subsequent investigations.

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# EXECUTIVE SUMMARY

Preface--The Florida Institute of Phosphate Research funded a three-year research project to the Center for Wetlands, University of Florida, entitled "Enhanced Ecological Succession following Phosphate Mining." The primary goal of this research project was to accumulate data necessary to develop practical (e.g., cost effective and applicable on a large scale) methodology and technology for reclaiming phosphate surface-mined lands to native forested ecological systems as one of many viable reclamation alternatives. To achieve this goal, research was directed toward identifying components essential for enhancing development of native forest ecosystems. Certainly, it could correctly be argued that almost all components in ecosystems are essential. However, is it possible that there may be some components more important or essential for establishing ecosystems with the additional ecosystem complexity contributing to long-term ecosystem maintenance? Can we increase the rate at which ecosystems develop (i.e., enhance ecological succession) by actively introducing some of these "essential" ecosystem components? Research in this project focused on four "essential" components that may enhance reclamation to native forest ecosystems. The "essential" ecosystem components tested were (1) a multispecies mixture of seeds, (2) mycorrhizal fungi symbionts, (3) soil nutrients, and (4) an organic base (such as, straw or hay mulch, or wetland organic soil for wetland areas). These components were tested individually and/or in combination in greenhouse studies, microplot experiments, and at several reclaimed mined sites.

The ambitious ultimate goal of the project was to develop a field application method for direct seeding a multiple species seed mixture with a composite of beneficial mycorrhizal fungi. However, much preliminary research was needed to reach this ultimate goal. Therefore, several paths of research were conducted simultaneously. Studies were conducted on plant community succession and mycorrhizal colonization of unreclaimed sites of different ages to determine rate and success of natural invasion of woody plants and their mycorrhizal fungal symbionts to these areas. The effectiveness of using a "broad spectrum" mycorrhizal fungi and/or mycorrhizal fungi isolated from mined soils on growth of select woody plants was investigated. Seeds of native woody plants were collected and stored for use in greenhouse and field experiments. Field plots were studied to assess number and survival of directly seeded plants. A field application method of direct application of seeds and mycorrhizal fungi was also tested and refined.

Community Succession-The present study indicates that well-developed ecosystems have been established in a 60-year period following mining. Results obtained from this and other studies may tend to be misleading. Although 60-year-old sites somewhat resemble nondisturbed climax communities these areas were mined at a time when disturbance of vast areas was uncommon. Perhaps a better indicator of ecosystem direction and development can be attained by examining younger sites. A 17-year-old site studied was principally colonized by bird dis-

persed (e.g., wax myrtle, common guava, black cherry, blackberry) or windblown species (groundsel), which are capable of long distance dispersal. Seedlings of live oak, water oak, sweetgum, or pine species were not observed to be colonizing this site or similar aged sites within adjacent localities. If succession on these sites is to lead to a mature oak-dominated ecosystem (60-year-old site), then invasion of oaks must occur early in site development. Dominance of a plant species can only occur after initial invasion into a site and successful reproduction with time. Due to the vast areas of disturbed land currently present in these areas, seed sources for late successional species have essentially been restricted to floodplain forests. Removal of late successional seed sources would tend to insure the possibility of an arrested succession situation, i.e., sites will remain at some stage of ecosystem development which may possibly be similar to conditions observed at the 17-year-old site. It is only speculation, however, and further research must provide information on succession in these areas.

Mycorrhizal invasion into phosphate mined areas is extremely rapid. Within 3 years, the majority of invading plant species exhibit extensive mycorrhizal colonization. Infection levels in early sites have been shown to be comparatively higher than levels obtained in a mature ecosystem. Comparisons of mycorrhizal infectivity and occurrence between mined and undisturbed areas in Florida are difficult to perform. Disturbed lands in Florida may have originally been dominated by sandhill, oak shrub, pine flatwood, mesic hammocks, marsh, bayhead, or cypress dome type communities. Phosphate mining produces areas, characterized by soil conditions, that are totally different from areas not subjected to disturbance activities. Therefore, it is difficult to determine levels of infection which these mined areas may approach with time. Mycorrhizal species occurring on phosphate mined lands would also have to be adapted for survival in extremely high phosphorus soils. Phosphorus has repeatedly been shown to inhibit mycorrhizal colonization and performance. It is indeed an interesting question concerning the survival strategy that these fungi have adapted for these soils. Also, questions arise as to the extent to which mycorrhizae will promote a growth enhancement response in plants growing in phosphate mined soils. Data regarding such growth responses are not available; however, other research within the scope of this project is presently in progress to determine such effects.

The question of whether mycorrhizal inoculation can affect survival of plant species used in reclamation attempts has recently prompted considerable attention. Numerous questions exist regarding the possibility that field inoculation may enhance survival of naturally colonizing or planted individuals and whether or not large-scale inoculation can feasibly be performed. Data presented in this report may seem to question whether inoculation should be performed, since mycorrhizal invasion has been shown to be very rapid, and colonization occurs in the majority of species present. It is possible that mycorrhizal species occurring in early successional communities may, in fact, be adapted to biotrophic relationships with early colonizing grass and shrub species. Endomycorrhizae have generally been shown to be non-host specific. However, different growth responses and inoculation potential may be mediated by different species or different ecotypes. Hence, mycorrhizae which occur in early aged successional systems might not be as efficient in promoting growth in sweetgum, a later successional plant. Possibly during succession, mycorrhizal population changes (densities and species) occur in response to selection by changing host populations (or vice-versa?). This may account for the initially

high infectivity potential and root colonization occurring very rapidly in phosphate mine succession.

Another problem warranting investigation pertains to the use of indigenous versus introduced species for inoculation purposes. Variable results have been obtained from using indigenous versus introduced strains as mycorrhizal inoculum. The survival of an introduced fungus will be controlled by the ability to adapt to fertility, moisture, and temperature regimes present within the soil. Introduced mycorrhizal fungi species must be adapted to environmental and soil conditions to effect an advantage for plant growth enhancement and survival. Investigations in this area are urgently needed to determine if the indigenous mycorrhizal fungi species from phosphate mined systems can be increased in culture and used to enhance growth in reclamation attempts.

Mycorrhizal Fungi and Growth of Sweetgum-Data are abundant indicating the beneficial effects obtained from mycorrhizal inoculation of agricultural crops and important timber species. However, to date only limited information exists concerning plant response to mycorrhiza and fertilizer additions in overburden soils. The results presented here clearly indicate the dramatic effect which mycorrhizal fungi have on condition and growth of sweetgum. Nonmycorrhizal control plants without added nitrogen at all levels of other nutrient additions exhibited phosphorus deficiency symptoms. However, mycorrhizal inoculated plants at all nutrient levels appeared healthy with no nutrient deficiency symptoms. Increasing amount of fertilizer simply increased growth rate. Leaf area data indicated a significant growth response of plants inoculated with "CS8" mycorrhizal fungi (mycorrhizae isolated from plants growing in clay settling area CS8) over control plants at nutrient levels of 150 ppm-P and 150 ppm-N, indicating that these mycorrhizal plants were responding to both high phosphorus and nitrogen fertilization. Growth data also showed that with treatments in which macro- and micronutrients were added with no additional phosphorus or nitrogen, "CS8" inoculated plants increased in growth over Glomus macrocarpum inoculated plants 1.8 times and 30 times greater than nonmycorrhizal control plants. G. macrocarpum inoculated plants provided 17 times greater growth (leaf area) than control plants. Control plant biomass approached that of mycorrhizal plants only when relatively high concentrations of nitrogen and phosphorus were added. Although both mycorrhizal types used in the experiments resulted in greater growth of sweetgum, it is interesting to note the differences in the degree of response which were obtained. G. macrocarpum was originally selected because it is a readily obtainable native Florida species. Inoculum is also maintained by local mycorrhizal research labs and thus is a species which may be available for field inoculation in Florida. The "CS8" mycorrhizal fungi composite was utilized because it is an indiginous group occurring in the phosphate district and may possess adaptations to high soil phosphorus concentrations. Single species were not isolated from the composite simply because it was felt that if they existed together within the roots of field plants, then there may be some functional significance to the association. The overall greater enhancement effect of "CS8" over G. macrocarpum may be attributed to many factors. However without further investigations any attempt at explanation would merely be speculation. The "CS8" composite may in act be adapted to conditions of high soil phosphorus and function to supply other valuable nutrients needed in plant growth. G. macrocarpum may have been affected by factors such as pH. G. macrocarpum has-evolved in soil with comparatively lower phosphorus levels where its function is to supply phosphorus to the host plant. At high phosphorus soils the ability of G. macrocarpum to supply the plant with other essential

nutrients, e.g., nitrogen, may not be as efficient as that of "CS8". Another result which warrants specific consideration is the effect of nutrients on mycorrhizal root infection. It is not clear why such low (<1%) root infection levels were obtained for G. macrocarpum. Although a growth enhancement over control plants (at low nutrient levels was recognized, very little colonization was maintained by the plant at any of the various nutrient levels tested. "CS8" root colonization followed an extremely different pattern. High levels of colonization were maintained at all nutrient levels and no variation was associated with either increasing or decreasing levels of nitrogen or phosphorus. Generally it has been shown that increasing phosphorus concentrations results in subsequent reduction in mycorrhizal colonization. Effects of nitrogen on colonization have been less well documented; however, both decreases and increases in mycorrhizal colonization levels have been noted with increasing nitrogen concentrations. Other research has shown that the extent of depression in mycorrhizal colonization at high phosphate levels was dependent upon the ratio of nitrogen to phosphorus concentrations. Increasing amounts of nitrogen tended to increase root colonization at a given phosphate level. Results obtained in this study using the "CS8" mycorrhizal fungi composite significantly differ from those found in other studies.

Seed Banks-Seed bank samples from all but the youngest sites in the postmining landscape fall within or just below the range of densities and species diversities found in natural wetlands. Indications from results in this study are that it is possible for nature to reestablish a seed bank of approximately the same size and diversity as that occurring in some natural marshes. The time it takes for the seed bank to reach the point of being a "reasonable facsimile" to that of a natural marsh is an open question. If Clear Springs is any indication then modest sized seed banks with higher diversity can develop in 4 years with little actual marsh reclamation, and with some reclamation efforts seed banks that compare very favorably in size with natural marshes can develop in 5 years as demonstrated at Four Corners. It appears that in some cases the seed banks in some of the postmining wetlands are not all that different in size and species composition from the natural marshes sampled in this study, but the actual vegetation present is not always as diverse, dense, or well developed except in cases where "mulch" (topsoil) from a donor wetland was applied.

What are the implications of this consideration of successional status and regenerative strategy for vegetation management and wetlands restoration? We are trying to make the case for a closer melding of wetlands ecology and reclamation with the goal of restoring stable, self-maintaining marsh systems in the postmining landscape. Other studies of wetlands on unreclaimed lands have documented the paucity of the more "desirable climax" species in both marshes and swamps. These studies describe arrested succession in which the initial floristic composition of primary invading species is perpetuated. The question remains to be answered whether the succession is arrested due to the inability of the later successional species to (1) arrive on abandoned sites or (2) to arrive and find adequate resources available for germination, growth, and establishment. Initial colonizing species, once established, may be able to resist invasion by other species, but the ability of established plants to resist invasion may be independent of the position of species in the normal sequence of community development. Consequently, it may be feasible to establish self-maintaining, stable marsh communities dominated by desirable late successional species and able to resist invasion by aggressive weedy species. The key will be in creating wetlands well buffered against the disturbance regime typically

encountered in marshes-fire, flood, drought. As already mentioned, a well-buffered system has representatives of all successional stages, regenerative strategies, and life histories. For reclamation the emphasis should be on two goals: establishing those components that have been found to be lacking in unreclaimed systems-the late successional long-lived perennials-and control of aggressive weed species capable of arresting succession. Topsoiling may not be feasible in all cases due to quality of donor material or budgetary constraints of transporting the material. In such situations a combination of seed bank establishment through direct seeding coupled with transplant of an array of long-lived perennials might accomplish the two goals. In final analysis, seed banks are not a panacea for restoration of native marsh systems, but are critical components of stable, self-maintaining ecosystems. The persistent seed bank is one of the regenerative strategies by which wetlands respond to disturbance. This capacity needs to be established either through seeding or application of topsoil (peat mulch).

Microplot Experiments--Goals of this microplot experiment were to assess germination (propagule establishment), survivorship, growth, density, and species richness of tree seeds as a function of the following soil amendments on phosphate mined overburden: (1) vesicular-arbuscular fungi (Glomus mosseae and Glomus occultum) inoculum; (2) ectomycorrhizal fungi (Pisolithus tinctorius) inoculum; (3) fertilizer (15-0-15); (4) soil surface organic matter (straw mulch); (5) soil amendment (phosphogypsum); and (6) topsoil.

Tree seedlings establishment ranged from 7 to 9% during the first growing season and increased to about 15% during the second growing season. Mulch, topsoil, VA-mycorrhiza, and Pt-mycorrhiza had a significant positive effect on seedling density during initial establishment. Although 44% of the tree species planted established seedlings, the average for each treatment ranged from 15 to 20% during the first growing season and from 30 to 33% during the second growing season. During the initial establishment phase of the first growing season, development of the community in the macroplots was highest for the mulch, topsoil, and mycorrhiza treatments. A net decrease in community development resulted from treatment of Pt-mycorrhiza, fertilizer, and gypsum. Mulch, topsoil, and VA-mycorrhizal (endomycorrhizal) fungi significantly increased community development components such as growth, density, and species richness of tree seedlings. Differences between community development for each treatment were reduced during the second growing season partially as a result of the overriding dominance of the "pioneer species," silktree and catalpa.

Direct Seeding Experiments-The direct seeding field trial was a great success in terms of its primary goal of providing a field-scale demonstration of a mechanically direct seeded, multi-species assemblage of woody plants. The planting operation was successful in getting seeds and mycorrhizal fungi inoculum into the ground in a clean and efficient manner. The field trial represents the first of its kind in the industry. Another very positive result or aspect of the field-trial lies in the mechanical simplicity of it all. Easily available common planting equipment (corn planter, or unit planters) was used with only slight modification of the seed plates. Other advantages of the unit planters are their low cost and modular nature. Each planter produces a single row and several planters can be attached to a single tool bar at any one time. This flexibility allows for simultaneously planting seeds of different sizes, the simultaneous seeding of a cover crop along with woody plants, the addition of mycorrhizal inoculum directly into the seed furrow, and the ability to vary

the spacing between planters on the tool bar. Even though it is mechanically possible to do direct seeding and get the seeds into the ground, germination, growth and survival are functions of the quality of the seed used and the environmental conditions to which they are exposed. Seed quality of the trial demonstration was poor. It was learned after the field planting that the acorns used (water oak and laurel oak) were not viable when planted due to dehydration during storage. The same was also true for the two species of ash (white ash and popash). Finding the proper storage techniques (method) for each species is certainly an obstacle which can be hurdled and progress toward this was shown in subsequent field tests. The direct seeding test demonstrated a viable method for getting seeds into the soil. Until recently the industry has not had much success with getting the seeds to germinate and survive. In a second "pilot" study, hand seeded plots at Gardinier had 20% germination overall and 77% of the seedlings survived the first growing season. The Gardinier plots show that good results can be obtained in a direct-seeded, multi-species plot. The mechanical performance of the Agrico test and germination performance of the Gardinier test provide a more encouraging view for the future of direct seeding. Germination results and the accompanying survival rates in the field test plots highlight several points. First, there are wide differences in germination rates between species under field conditions as shown by the extremes of magnolia versus oaks. Survival of individuals germinated also exhibited interesting trends. The phenology, or pattern of germination and mortality also showed differences between species. Germination was non-existent-to-low for magnolia, low for hickory, hackberry, sweegtum and palmetto, and moderate for oak. Possible causes of the differences include: seed quality, germination requirements, seed predation and erosion. The hackberry, sweetgum, hickory, palmetto and magnolia seed had been collected in fall of 1982 and stored dry at 4°C, while acorns used were collected in November 1983 and stored out-of-doors in potting soil. The storage regime as well as the initial quality of the seed could have had an effect on germination potential. Many of the acorns were nearly germinating when planted. Also, since germination was determined only from two sampling periods in March and October, any seeds that germinated but did not survive for long would have been easily overlooked. This would have been more critical for hackberry and sweetgum since these species produce a rather delicate germling.

# CHAPTER 1
# ROLE OF MYCORRHIZAL FUNGI FOR ENHANCING ECOLOGICAL SUCCESSION OF RECLAIMED PHOSPHATE SURFACE MINED LANDS

## Introduction

The concept of enhanced ecological succession of devastated lands implies man's manipulation of natural processes. Designing reclamation techniques requires an understanding of both aboveground and belowground processes of ecosystem succession. In order to develop a holistic approach to natural ecosystem reconstruction, it must be emphasized that total reclamation involves more than simply revegetating the surface of the land. Reclamation of drastically disturbed lands (e.g., coal mining, oil spills, phosphate mining) in the United States is currently in the experimental stage. Critical parameters in natural ecosystem reconstruction need to be identified and analyzed to insure success of current reclamation efforts. One such parameter warranting special attention in reclamation studies is the potential role of mycorrhizal fungi. The function and importance of mycorrhizal infection in plant growth and survival are well documented (Gerdemann 1968; Mosse 1973); however, mycorrhizal population dynamics and function following disturbance have only received limited attention. It has been observed in several instances in the American Midwest that a majority of the invading species in disturbed areas are typically nonmycorrhizal (Allen and Allen 1980; Miller 1979; Reeves et al. 1979). Reeves et al. (1979) found that 99% of the plant species adjacent to a disturbed area (old roadbed) were mycorrhizal compared to only 1% in the disturbed area. Similar results were observed in strip mined areas of the Red Desert in Wyoming (Miller 1979). Land disturbance is accredited with reducing the availability of viable mycorrhizal propagules thus inhibiting the invasion of mycorrhizal plants. Although mycorrhizal infection may be mediated through spores, hyphal fragments, or infected plant roots, the most probable form of endomycorrhizal inoculum is via infected plant roots and not spores (Reeves et al. 1979). Therefore a reduction in host availability by drastic land alteration (i.e., mining) could subsequently limit invasion of disturbed areas by mycorrhiza-dependent species. This is very important when considering that a majority of naturally occurring late successional plants typically form mycorrhizal associations (see Gerdemann 1968; Marx 1975).

In contrast to these findings, investigations of coal spoils in Pennsylvania and Scotland showed a majority of colonizing plants to exhibit typical vesicular-arbuscular mycorrhizal infections (Daft 1974; Daft et al. 1975). It was suggested that mycorrhizae are necessary for plant survival on these harsh anthracite spoils. A similar study of bituminous coal mining wastes in New South Wales also found that colonizing species were generally endomycorrhizal (Khan 1978). It appears that in these areas plant colonization and subsequent survival may in fact be limited and controlled by mycorrhizal presence. Inva-

sion by nonmycorrhizal species is limited, which is in contrast to the previously cited examples.

To date no information exists concerning vesicular-arbuscular mycorrhizae dynamics following phosphate mining in Florida. During mining, nonweathered overburden is removed from various depths and deposited on the surface. This "abiotic" soil must be invaded and naturally colonized by native plant species. The degree or rate at which mycorrhizal reinvasion occurs could possibly determine rates of plant community establishment (Miller 1979; Janos 1980).

Several nonreclaimed mined sites in central Florida were selected to study belowground succession and endomycorrhizal dynamics following strip mining for phosphate. Site ages range from recently mined to 60 years postmining. These areas offer an excellent opportunity to study community succession following phosphate mining. In response to a need to develop reclamation techniques to establish natural ecosystems on mined lands, a study was initiated to investigate both aboveground and belowground changes during succession following phosphate mining disturbance. The study was divided into the major tasks of (1) assessing aboveground vegetative composition changes; (2) analyzing organic matter dynamics and soil nutrient parameters; (3) analyzing changes in root structure; and (4) assessing mycorrhizal population density changes through time and identifying indigenous mycorrhizae. Results indicate invasion of some mycorrhizal fungi species to mined areas is relatively rapid with most plants examined exhibiting mycorrhizal colonization. Root length and root biomass data indicate a continuing increase in belowground structure with time. Soil nutrient analyses revealed phosphorus concentrations ranged from 50 to 5528 mg/L (ppm). The importance of endomycorrhizal fungi to succession of native forest ecosystems on mined areas will be related to the potential use of mycorrhizal fungi inoculum as a reclamation practice. Emphasis is placed upon mycorrhizal dynamics during succession; however, numerous ecosystem parameters were examined to investigate total community changes influencing the rate and direction of succession in these disturbed areas.

## Literature Review

### Introduction

Ecosystem succession on mined land has been noted by several authors. Breedlove and Adams (1977) discussed the effectiveness of this process in revegetation of some mined lands. Humphrey (1979) and Kangas (1979) describe the aesthetically pleasing ecosystems that had developed on spoil mounds that were simply abandoned after mining. Although only 50 years had passed, the mounds resembled a mesic oak forest. However, that was when mined tracks of land were smaller, allowing for more rapid and complete encroachment of organisms from the surrounding natural communities. Natural encroachment of the necessary components of forest communities has probably been hampered by the greater distances involved in the current large-scale mining activities of the phosphate industry in Florida such that now there are problems with simply abandoning mined lands. One of these problems is time, On less-than-ideal sites and on sites where natural encroachment by forest plants is hampered by distance compounding the time problem, initial plant establishment can be slow, resulting in a low per-

cent cover even after several years. In some cases very persistant scrub communities, dominated by wax myrtle (Myrica cerifera) (Schnoes and Humphries 1979) or vines (McGee and Cooper 1975; Kangas 1979) gain a foothold during an early successional stage. Often these pioneer early-successional species become so well established before seeds from the later, more mature forest species (i.e., climax forest species) are disseminated to the area that germination, growth, and establishment of these climax species are greatly reduced.

There are several approaches to enhance establishing "desirable" woody shrubs and trees. A common silvicultural technique used in forestry is planting seedlings (Anon. 1980). This technique is generally adapted to planting monospecific forests for enhancing growth and production of wood resources. One current project in reclaiming phosphate mined lands has used this technique at the multispecies level (Gilbert et al. 1979). Another approach currently being tested for reclaiming wetlands uses surface litter and organic material from undisturbed or predisturbed areas for spreading over areas to be reclaimed (personal communication: Gary Uebelhoer, AMAX, Inc.; Uebelhoer 1979). This method not only helps to restore organic material to a generally depauperate site, but also provides a partial seed base coupled with an inoculum of forest floor biota. Direct seeding is also an alternative revegetation method (Abbott 1973; Brown 1973; Davidson 1980; Mann 1968; Plass 1976; and others). A seed mixture composed of several species has been used successfully in enhancing the reestablishment of natural communities in oil shale-mined test plots in Colorado (Braun and Best 1975). Direct seeding offers distinct advantages over other revegetation methods in that it is applicable to large-scale reclamation at reasonable costs and effort levels (Vogel 1980). One current disadvantage, at least in the Southeast, is seed source and availability. However, as has been demonstrated in central and western United States where state and federal regulations place an emphasis on restoring diverse natural communities, considerable attention is being paid to developing technology needed for adequately supplying a seed source for native plants. Johnson (1980), in his opening address to the symposium on "Trees for Reclamation" placed "increasing the production of high quality seed . . . with emphasis on native species" (p. 2) as an area where "research must be strengthened." The above mentioned revegetation methods coupled with additional methods--combinations and variations-are certainly functional and viable reclamation alternatives (Thronson 1975). Each revegetation alternative provides a unique set of advantages and disadvantages that can be assessed in developing the most functional reclamation plan for a specific area.

## Direct Seeding

"Direct seeding, an attractive alternative to planting, is not a simple method of reforestation" (Davidson 1980:93). Early reports of reclamation for forestation indicate that direct seeding has been attempted in many regions as an alternative to tree planting. However, most of the earlier attempts were direct seeding of a single species. Schavilji (1941) reported good first-year germination and survival of black walnut (Juglans nigra) with an average seedling height of 12 inches. However, others Davidson 1980; May et al. 1973; Vogel 1980) have reported only, moderate to poor success in direct seeding of single species generally for commercial purposes. Other recent experiments include direct seeding of loblolly (Pinus taeda), shortleaf (P. echinata), Virginia (P. virginiana), and white (P. strobus) pine (Zarger et al. 1973).

Seeds were broadcast by hand and helicopter. First-year results were moderately successful with some species performing better than other. White (1980), in one of the earlier tests on multispecies seeding, seeded about 6500 acres by helicopter. His varied mixture included the tree species of locust (Robinia), white birch (Betula sp.), and a mixture of Virginia, pitch (P. rigida), and white pine. He noted (p. 65), "Although it was several years before we could actually evaluate the effort, we were exceptionally pleased with the overall results." Plass (1976), in a test using multispecies seed mixtures, was able to select a combination of several compatable species of herbs and trees for direct seeding. Identification of the promising species resulted from a species evaluation trial in which 34 species of trees, shrubs, and herbaceous plants were hydroseeded on five sites. Braun and Best (1975) initiated a field experiment on strip-mined oil shale lands in Colorado in 1976 in which a 24-species seed mixture consisting of grasses, herbaceous plants, shrubs, and trees was planted in test plots where fertilizer rate and timing, and mulch type were varied. Initial (first and second year) results were promising with moderate to good germination success for most species (personal communication: Constance Braun 1978).

Acquiring sufficient seed quanities of native species is a current problem in the direct seeding alternative. Johnson (1980) expressed that a high research priority should be placed on developing the technology for increasing the availability of seeds of native plants. Most of the seed for our experiment were collected from extant ecosystems, although the more popular tree species were purchased from commercial or governmental agencies complemented with field collection trials. Excellent information on collection of seed from many common tree species is available in Seeds of Woody Plants in the United States (Forest Service 1974). This volume also includes excellent information on storage and planting. Natural ecosystems in the area were analyzed for details, such as time of ripening, seed drop, and local seed predators. Many actual collection methods are illustrated and described in Matusz (1964). Safe and efficient methods appropriate to local conditions and species were selected. An example of the detailed type of information that was developed is given by Bonner (1973), a study of the timing of seed collection for green ash, Fraxinus pennsylvanica.

Exact seeding time was a compromise based on requirements of individual species, seasonal weather patterns, and mining activities. Mann (1968) notes the importance of seeding close to the normal germination time of the species, with being a little early preferable to being late. Davidson (1980) lists seeding guidelines, including a freshly prepared seedbed. Vogel (1980) suggests seeding trees one year and herbaceous the next to avoid some problems discussed below. This would probably not satisfy the legal cover requirement. A possible problem with planting late in the year is the effect of high soil temperatures on germination (McGee 1976). However, Marx (1975) has shown that the ectomycorrhizal fungus, Pisolithus tinctorius, increases plant tolerance to higher soil temperatures.

Suggested seeding methods range from helicopter broadcasting (White 1980) to traditional broadcasting, drilling, or hydroseeding. Cook (1976) notes the advantages of drilling, which is the method of choice with expensive or hard to obtain seed. However, adaptations in drilling may be necessary when seeding mixtures containing seeds of varying sizes are seeded. The Fish and Wildlife Service (1978) illustrates and describes the equipment involved in the various methods. Mann (1968) recommends sowing adequate quantities of seed to insure

sufficient stocking in the event of unusually dry weather. If herbaceous species are planted a lower seeding rate is recommended to reduce over competition with tree and shrub species (Vogel 1980).

## Role of Mycorrhizae in Reclamation

The concept of enhanced ecological succession of devasted lands implies man's manipulation of natural processes. Ashby et al. (1980) noted that some formerly mined lands in Illinois, though planted successfully with several species of trees 30 years earlier, had a greater number of pioneer trees than planted trees suggesting that total reclamation involves more than simply revegetating the surface (a problem being addressed within the goals of this research project). In order to develop a "holistic approach to natural ecosystem reconstruction" an understanding of both macrocomponents and microcomponents is mandatory (Richardson 1980). Reclamation of drastically disturbed lands (e.g., coal mining, oil spills, phosphate mining) in the United States is currently in experimental stages. Critical parameters in natural ecosystem reconstruction need to be identified and analyzed. Most recent studies of revegetation have examined only aboveground components of the plant community while completely ignoring belowground microbial participation (e.g., Jones et al. 1975; Cook 1976). Reclamation studies on phosphate mined lands in Florida have previously concentrated on aboveground biomass production (Mislevy and Blue 1980a, 1980b). One very important microbial entity warranting special attention is mycorrhizal fungi. Termed by Hacskaylo (1967) as "indispensable invasions by fungi," mycorrhizae have been proven to be essential for plant survival in stressed environments. In 1973 Hacskaylo and Tomkins indicated that greater than 3000 papers had been published concerning mycorrhizal fungi. Review of recent literature indicates that this number has substantially increased since their survey, especially in ecologically applied areas. Studies of mycorrhizae importance in reclamation of phosphate mined lands in Florida are relatively nonexistant although the significance of mycorrhizae in revegetation of disturbed lands is well documented in other regions (Daft et al. 1975; Marx 1975, 1977a). Reeves et al. (1979) stated that "the reestablishment and maintenance of the mycorrhizal fungus component is vital in producing stable plant ecosystems on disturbed areas" (in Marx 1980). It is imperative that successful reclamation studies include examinations of the mycorrhizal fungi that could possibly be utilized for inoculation of native invading species.

Mycorrhizae have been demonstrated to enhance revegetation of disturbed lands extending from tropical to artic ecosystems. In Virginia, black willow, Salix nigra, colonizing abandoned railroad sites was found to be heavily infected with endomycorrhizae (Antibus et al. 1980). It was suggested that this symbiosis is necessary for successful colonization survival of willow invading these disturbed sites. Invasion of anthracite coal wastes in Pennsylvania was accomplished primarily by tree species that develop ectomycorrhizal associations (Schramm 1966). Nonmycorrhizal plants that survived on these wastes did not exhibit normal growth and displayed symptoms of severe nutrient deficiency. Antibus and Linkins (1978) reported the importance of the ectomycorrhizal fungus Cenococcum graniforme (Sow.) Ferdand Winge. in the survival of Salix rotundifolia Trautv. inhabiting oil spills sites in northwest Alaska. They proposed the possible utilization and emphasized the importance of C. granifomre in reclamation of oil spill sites in the artic ecosystems. Mycorrhizae furthermore have been used for reforestation practices in regions of Puerto Rico (Hacskaylo

1967). Considerable difficulty was encountered when efforts were made to establish slash pine stands in the Puerto Rican mountains. Early attempts were unsuccessful with plants exhibiting evidence of phosphorus deficiency. These observations eventually led to utilization of mycorrhizal inoculum from pine stands located in the southeastern United States. The results enforce the hypothesis of mycorrhizal-enhanced growth. During the first three years following inoculation, noninoculated plants grew less than 12 inches tall while inoculated trees reached heights of 8 feet and displayed no signs of nutrient deficiency. Harris and Jurgensen (1977) demonstrated the necessity of mycorrhizae development to survival of Salix and Populus trees colonizing metallic mine tailings (Fe and Cu) located on the upper Michigan Peninsula. Poor growth was exhibited by nonmycorrhizal trees on copper tailings while trees on iron tailings developed extensive mycorrhizae and displayed good survival and growth.

The mechanisms by which mycorrhizae enhance plant survival are not clearly understood and have recently been the subject of considerable research (e.g., Antibus et al. 1980; Ho and Trappe 1980; Linkens et al. 1978; Marx et al. 1977; Melhuish and Hacskaylo 1980). Primarily mycorrhizae function to increase the root surface area available for water, nutrient, and mineral absorption. Safir et al. (1971, 1972) demonstrated the enhancement of plant water uptake and transport by mycorrhizal infection. By augmenting absorption processes mycorrhizae assume important roles in nutrient regimes hence the restoration of soil fertility and productivity (Parkinson 1976). Mycorrhizae have been shown to enhance symbiotic nitrogen fixation of legumes infected with Rhizobium (Daft and El-Giahmi 1975) thus increasing both available plant and soil nitrogen.

In the previous cited studies of mycorrhizal involvement in reclamation processes, it is important to emphasize that in each case a different mechanism for survival was contributed by the mycorrhizal association. In the Alaskan study (Antibus and Linkins 1978; Linkins et al. 1978), the selective ability of the fungus Cenococcum graniforme to function in water-stressed systems was responsible for the survival of the plant. The hydrophobic nature of oil spill soils induced dessication of many colonizing plants and therefore selected for the stress-tolerant mycorrhizae-plant association. Schramm's (1966) results on anthracite mine spoils suggested nitrogen as the limiting factor inhibiting revegetation and causing mortality of the invading species; however, fertilizer utilization only allowed for temporary growth. Schramm indicated that nitrogen reaching the soil via precipitation is in sufficient quantities to support plant growth. Nitrogen uptake was thus facilitated by the mycorrhizal infection therefore increasing critical concentrations available for plant use. Phosphorus absorption by mycorrhizae has probably received the greatest attention in recent research (e.g., Lambert and Cole 1980). The ability to adequately absorb phosphorus was responsible for the survival of slash pine in the Puerto Rican study with nonmycorrhizal trees displaying stunted growth and reduced survival.

One aspect of mycorrhizal function that deserves more attention is the extensive development of root systems resulting from mycorrhizal infection. Mislevy and Blue (1980a) (Florida) studied the yield's of tropical forage grasses on four amended quartz sand tailing treatments (Amendments: 1) sand tailing control (SC); 2) collodial phosphate (CP); 3) CP and sewage sludge; 4) CP and topsoil: All plots were fertilized). Total yields of control plots and amended plots showed significant differences during the first two years; however, no significant difference could be discerned during the third year. They attributed these results to the more extensive root system development after the ini-

tial two-year period. Roots were deeper and encompassed more total soil volume. The greater root biomass was thus responsible for increasing the total area available for nutrient and water uptake. In this study mycorrhizal associations were not studied. However it is probable that mycorrhizal inoculation could accelerate the extensive root development needed for survival and thus significantly reduce the need of fertilizer addition in phosphate mining reclamation practices. Unless mycorrhizal inoculum is introduced by man in reclamation studies, it is unlikely that adequate viable mycorrhizae are present in the mined soils (Rives et al. 1980) such as overburden or quartz sand tailings. Invasion of endomycorrhizae such as those that enhance survival of grasses (as used in Mislevy and Blue 1980a) into disturbed areas is generally very slow due to their mechanisms for dissemination (Marx 1975, 1976a, 1977a; Reeves et al. 1979).

The occurrence of mycorrhizal associations in all ecosystems is more the rule than the exception (Hacskaylo 1967). The discovery of the importance of mycorrhizae infection to plant survival in recent years has led to new concepts involving plant communities in ecosystem succession. It has been observed in several instances that the majority of invading species on disturbed areas are typically nonmycorrhizal (Allen and Allen 1980; Miller 1978; Reeves et al. 1979; Schramm 1966). Reeves et al. (1979) found that 9% of plant species adjacent to a disturbed area (old roadside) to be mycorrhizal however only 1% of invading species on the disturbed area were mycorrhizal. Similar results were observed in natural and disturbed habitats of the Red Desert of Wyoming (Miller 1978). Both studies indicate that mycorrhizal symbiosis is a stress-tolerant association characteristic of competitive natural communities. Land disturbance is accredited with reducing the availability of viable mycorrhizae propagules thus inhibiting the invasion of mycorrhizal plants (Allen and Allen 1980; Miller 1978; Reeves et al. 1979). Read et al. (1976) indicated that the most probable form of endomycorrhizal inoculum is via infected plant roots and not spores. Therefore a reduction in host availability by land devastation (mining) could subsequently limit invasion of disturbed areas by mycorrhizae dependent species. The invading species that are characterized by extremely low densities in natural communities have adapted weedy strategies, relinquishing stress-tolerant abilities needed for later successional communities, i.e., mycorrhizal symbiotic relationships (Miller 1978). Miller (1978) found that the invading species of Halogeton actually inhibit mycorrhizae infection of plants. The resulting consequence is the inhibition of the system to rapidly establish a stress-tolerant community.

Kangas (1979) demonstrated that phosphate mined spoil mounds in Florida will eventually produce productive natural communities; however, the time span is much too slow to resolve current reclamation needs especially now that the larger mine size compounds the time factor. The question of whether this natural process could be accelerated will demand research in areas such as mycorrhizae inoculation to provide insight into productive revegetation strategies. Due to the severe perturbation of phosphate mined lands and the nature of the spoil material it is probable that severe disturbances occur within microbial communities during mining, resulting in the arrested restoration of the soil environment.

Reeves et al. (1979) proposed a list of several working hypotheses warranting special attention for the continual study of rehabilitation of oil shale

lands. The ideas are very general in nature and should be adapted for any attempts of revegetation of disturbed lands:

1) Disturbance of soil leads to reduction and possible elimination of propagules of mycorrhizal fungi (because host plants are reduced in numbers).
2) Reduced numbers of propagules leads to a lower potential for infection of new host plants.
3) Nonmycorrhizal species become established because normally mycorrhizal plants die in the seedling stage (for lack of mycorrhizal fungi).
4) Success of nonmycorrhizal species further reduced the propagules of mycorrhizal fungi since the fungi are obligate symbionts.
5) Total elimination obviates competition by mycorrhizal higher plants.
6) Succession is slowed because of the lack of potential mycorrhizal fungi (these fungi may be slow invaders).
7) The harsher the site the greater the potential for elimination of mycorrhizal propagules and, therefore, a longer time is required for reestablishment of mycorrhizal vegetation.

It is important to emphasize that many variables, e.g., presence of phytotoxic substances, are responsible for successful reclamation of devastated lands to a natural state. Increasing evidence indicates that mycorrhizae are very important in establishing a stable natural ecosystem on surface mined areas. Future reclamation research cannot ignore the importance of these microbial interactions in ecosystem survival. To do so will severely hinder any advancement possible in our knowledge of reclamation strategy.

## Selection of Mycorrhizae

The selection of fungi to be utilized in inoculation experiments emphasizes the application of both endomycorrhizal and ectomycorrhizal symbionts. Generally (but not always) most early to mid-successional tree species tend to form ectomycorrhizal associations while herbaceous plants, some aquatic plants, shrubs, and late-successional trees tend to be endomycorrhizal (Aldon 1975; Daft and Hacskaylo 1977; Daft et al. 1975; Søndergaard and Laegaard 1977). Numerous climax or mature forest trees are both endomycorrhizal and ectomycorrhizal. In particular *Pisolithus tinctorius* (ectomycorrhizal) and *Glomus mossae* were employed because of their widespread occurrence and previous successful use in revegetation experiments. Each is capable of being produced in large quantities facilitating use in large-scale, economic inoculation studies.

The selection of *P. tinctorius* has been based upon several factors. D. H. Marx of the USDA Forest Service has given four basic reasons justifying the. selection of *P. tinctorius* in revegetation efforts (in Marx 1977b).

1) The availability of practical techniques for artificially introducing it into nursery soils.
2) Its ability to improve tree survival and growth in the nursery and in the field.
3) Its near worldwide distribution on a variety of soils.

4) Its broad host range encompassing many of the world's most important tree species.

All of these factors have recently been proven valid in reclamation research. Research concerning P. tinctorius has recently had considerable impact in prompting reforestation and reclamation practices in both natural and devastated ecosystems.

P. tinctorius has been reported to occur in 33 countries on 6 continents and 38 states in the United States (Marx 1977b). It has widespread distribution in Florida with most reported collections being from the central areas of the state (Grand 1976). Marx (1977b) catalogued the occurrence of P. tinctorius and its host associates. It has definitely been shown to form ectomycorrhizal associations with 46 species of trees comprising many genera including Abies, Betula, Carya, Eucalyptus, Pinus, Pseudotsuga, Quercus, and Tsuga. Another 25 species exist as probable hosts but only field associations have been made. Marx (1977b) suggests that possibly P. tinctorius has the ability to form ectomycorrhizae with most of the worlds tree species that normally form ectomycorrhizae. He further states that no ectomycorrhizal tree species that he has tested has failed to form an association with P. tinctorius under controlled conditions. These results have recently been supported by mycorrhizae synthesis in Taiwan of P. tinctorius and Taiwan red pine, Pinus taiwanensis (Hung and Chien 1979). Although the fungus has not been reported associated with the pine, pure-culture synthesis of ectomycorrhizae were successful.

An important criteria for selection of P. tinctorius is the readily available, recent literature concerning inoculation and growth enhancement studies. Recently formulations of inoculum have been studied in over 40 nurseries in 33 states (including Florida: Marx et al. 1976) and Canada (Marx 1980). Inoculum may be in the form of basidiospores or vegetative mycelium (Marx 1976b; Marx et al. 1979; Molina 1979) with each proving very successful in augmenting seedling survival. Each method is capable of being utilized on large-scale operations at low cost. Commercial vegetative inoculum was available from Abbot Laboratories in early 1981 (Marx 1980). Marx and Bryan (cited in Marx 1976b) collected more than 1300 grams of P. tinctorius basidiospores in less than 3 hours on a stripmined coal spoil in Alabama. They determined that approximately 1.1 billion basidiospores are contained in 1 gram. These results indicate the practicality of utilization of this fungus for large-scale inoculation studies.

In all reforestation studies utilizing P. tinctorius mycorrhiza, favorable results of seedling survival have been found. Inoculated seedlings with P. tinctorius have shown greater survival than noninoculated seedlings in both greenhouse and field studies (Marx 1976b). Tree species with P. tinctorius have been shown to outgrow and have greater survival on adverse sites than trees-with naturally occurring mycorrhizae (Marx 1980). Schramm (1966) indicated that P. tinctorius was the dominant fungus associated with invading species and associated it with the healthiest tree seedlings. Survival has in some instances been attributed to the greater ability of P. tinctorius to survive at high temperatures (Hung and Chien 1979; Marx 1976a) (characteristic of nonvegetated surfaces) however the controlling mechanism enhancing plant survival is not yet known.

Glomus mosseae has also been implicated in enhanced survival in reclamation and reforestation practices (Aldon 1975; Kormanik et al. 1977). Marx (1976a) suggests G. mosseae as a possible endomycorrhizal fungus being adapted for sur-

viva1 on adverse sites exhibiting temperature tolerance greater than those of other endomycorrhizal fungi. Daniels and Graham (1976) in conducting nutrition and germination studies of G. mosseae suggest, "G. mosseae may be adapted to a niche in which competition is essential. It would appear that only when food becomes a limiting factor to most other organisms do the spores of G. mosseae germinate." Considering the poor nutrient availability in phosphate mined soils (e.g., sand tailings) (Hortenstine and Rothwell 1972) the utilization of this fungus could substantially improve survival of seeded plants.

Literature concerning G. mosseae is not as abundant as that of P. tinctorius but success in its utilization concerning plant survival is well-documented (Aldon 1975; Marx n.d.). Best (1976) in an experiment using sweet gums, Liquidambar styraciflua, grown with and without inoculations of G. mosseae realized a 24 times increase in total biomass in nine-month-old inoculated plants. Not only was there an increase in total plant biomass, but Ca, K, and Mg accumulations were also 29, 25, and 20 times, respectively, greater than that accumulated by noninfected plants. Kormanik et al. (1977) realized both greater survivorship and growth of sweet gum seedlings inoculated with G. mosseae. Endomycorrhizae are considerably more difficult to culture than ectomycorrhizae; however, adequate seed inoculum may be obtained by host plants grown in greenhouse conditions. Best (1976) successfully used this technique to acquire a large amount of G. mosseae inoculum for experimental purposes.

## Site Selection

Six different aged non-reclaimed phosphate mined areas in southwest Polk County in central Florida were selected for study (Table 1, Figure 1). Sites selected included an area presently being mined (0 years) and areas mined 3, 8, 17, 43, and 60 years ago. Criteria used for site selection were (1) that sites had not been subjected to any reclamation procedures following mining and (2) that sites were sufficiently isolated so that natural vegetation was undisturbed and not subjected to any interfering activities (e.g., cultivation, pasturing, construction, etc.). Study sites were originally selected using aerial photographs to eliminate any bias towards community composition. The site selected within each representative age group was considered to be typical of areas mined within the designated time frame. Each site selected represents typical areas strip mined for phosphate characterized by a mosaic of overburden mounds having extremely steep slopes and generally surrounded by deep water-filled channels.

### Sampling Technique

Three 5 x 50-m quadrats were established on all sites (Figure 2). Quadrat locations were positioned as follows: quadrat 1 was located on the crest (ridge) of the overburden mounds; quadrat 2 corresponded to the midslope region of the overburden mounds; quadrat 3 was located at the base of overburden piles adjacent to the water's edge. Quadrats were placed in this stratified-random fashion because during initial observations of these sites it was determined that vegetation differences were prominent between the more hydric area at the base and the somewhat xeric situation that exists at the crest of the overburden piles. Therefore to determine if intrasite location was a significant variable in determining community response with time, quadrat locations were established

Table 1. Succession study sites: locations for different aged phosphate mines located in southwest Polk County in south-central Florida.

| Property Owners | Access Road | Location | Date Mined | Age Group |
|---|---|---|---|---|
| Agrico | District Line | S27,34, T32S, R24E | 1920–25 | 60 |
| Agrico | Rt. 630 | S36, T31S, R24E | 1937–42 | 43 |
| IMC | Rt. 640 | S7, T31S, R25E | 1965 | 17 |
| IMC | Rt. 640 | S12, T31S, R24E | 1975 | 8 |
| IMC | Rt. 640 | S12, T31S, R24E | 1979 | 3 |
| IMC | Rt. 640 | Kingsford Mine | 1982 | 0 |

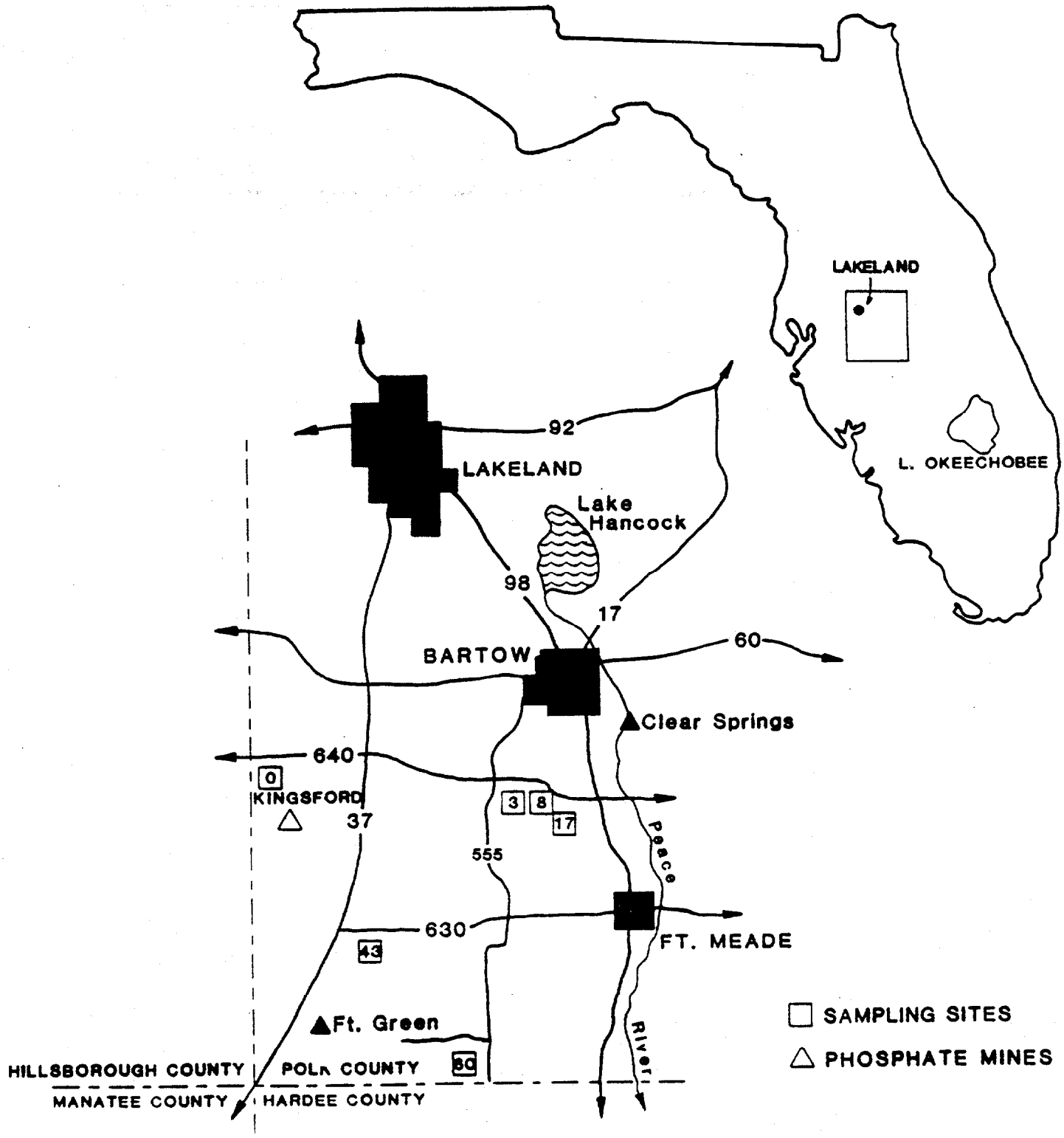



Figure 1. Phosphate mined succession study sites located in southwest Polk County in central Florida.

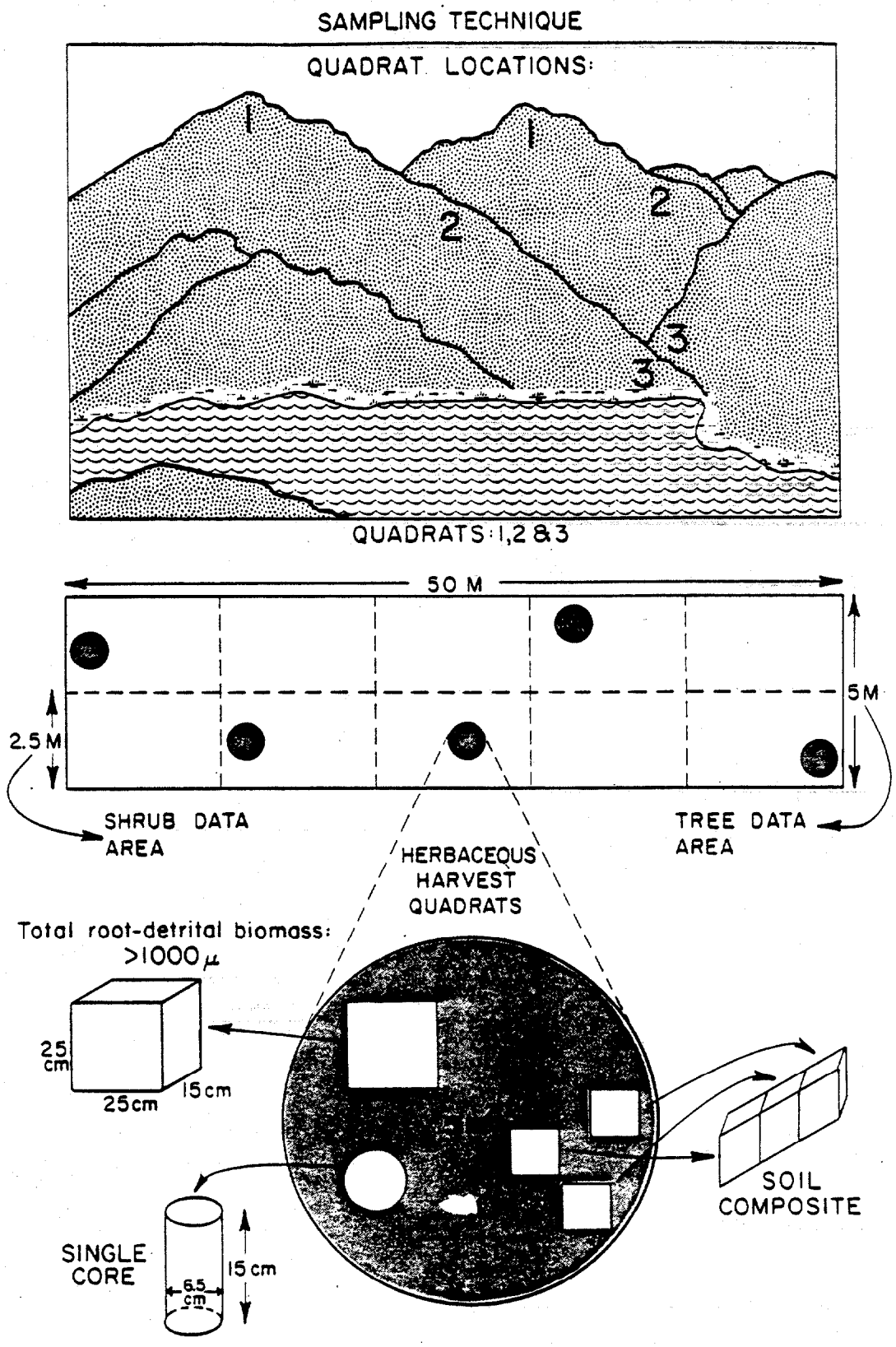



Figure 2. Sampling methodology for both aboveground and belowground components of different aged phosphate mined sites.

within these three zones. Sampling in this manner attempted to delineate belowground differences which might occur in response to the obviously different patterns exhibited by plant colonization and growth. Maximum width of quadrats was established at 5 m because distance from base (water's edge) to crest of spoil mounds seldom exceeds 20 m.

### Aboveground Biomass

Within each quadrat, aboveground biomass estimates were made for all tree, shrub, and herbaceous species (Figure 2). Total tree basal area estimates were made for all species having a diameter at breast height (dbh [dbh = 1.3 m above the ground]) measurement greater than 10 cm. Tree species were sampled within the entire area (250 $m^2$) of each quadrat. Shrub biomass estimates were determined for all species where dbh was less than 10 cm and height was at least 1.3 m. Due to the high densities of shrub individuals sampling area was limited to only one-half the area utilized for tree sized components (125 $m^2$), Herbaceous plots were 1 $m^2$ and were located at 10-m intervals within the quadrat. For each quadrat five herbaceous plots were harvested, totaling 15 plots per site (this particular sample group will be referred to as herbaceous biomass). Herbaceous biomass was separated by species and oven dried at 70°C for 48 hours, and dry weights were determined. Following harvest of all live herbaceous material within a given plot, all litter was completely removed exposing the mineral soil. Litter was oven dried at 70°C for 48 hours and total dry weights were determined.

## Belowground Components

Several categories of belowground parameters were examined. All belowground data were collected directly below the areas in which herbaceous plants were harvested (Figure 2). Sample categories, techniques, and processing are explained below.

### Soil Organic Matter (SOM)

Soil organic matter consists of total root biomass and detrital components located within the soil column. Three size fractions were analyzed to best determine organic matter dynamics as affected by time.

SOM>1000µ. Soil cores averaging 25 x 25 x 15 cm (9375 $cm^3$) were excised from within the herbaceous sample areas. Samples were weighed and subsequently placed in 5 gallon containers of water. Repeated stirring and agitation separated the organic matter from the mineral soil via flotation. The supernatant containing the particulate organic matter was sieved through 1000µ screens. This process was repeated several times to insure that the majority of organic matter had been removed from the soil. All organic material retained on the screen was oven dried at 70°C for 48 hours and weighed. These samples generally consisted of all live and dead root biomass and large particulate detritus. Initially, efforts were made to separate root from detrital material; however,

this technique proved to be much too time consuming for such a large sample size.

SOM ≤ 600µ and SOM 600µ < x < 1000µ. To separate and analyze the small and intermediate soil organic matter size fractions, 6.5 cm dia. x 15 cm deep (500 $cm^3$) cores were removed using a soil auger. Samples were air dried and passed through consecutive 1000µ and 600µ sieves. Material that passed through the 1000µ sieve, but was retained on the 600µ sieve was collected and processed as an independent size fraction. Material passing through the 600µ sieve was also collected and analyzed. Organic matter within these size fractions was determined by the Walkley-Black wet combustion technique (Allison 1965). Percentage of carbon oxidized was assumed to be 75% of the total present and the conventional Van Bemmelen factor of 1.724 was used to convert percent carbon to percent organic matter. This procedure assumes that organic matter is composed of 58% carbon. Values are reported as grams organic matter per kilogram soil.

## Soil Chemical Analysis

Three soil cores measuring 6.5 cm. diameter x 15 cm deep (500 $cm^3$) were removed from each herbaceous plot and composited, and an aliquot (1000 g) was removed for chemical analysis. Samples were dried at 70°C for 48 hours and subsequently passed through a 2-mm sieve. A dilute double-acid solution (Mehlich 1953) was used to determine the extractable levels of calcium, magnesium, manganese, potassium, and sodium at each study site. Soils from the 3 and 8 year old sites were also extracted with 1N neutral ammonium acetate (Gaines and Mitchell 1979) to determine levels of exchangeable cations for comparison purposes. Five grams of soil was shaken with 20 ml of extracting solution for 5 minutes in 50 ml polycarbonate centrifuge tubes. Samples were subsequently centrifuged for 2 minutes and the supernatant filtered through No. 42 Whatman filter paper (ashless). The filtrates were then refrigerated at 4°C until, dilutions were made prior to analysis.

Extractable and exchangeable levels of calcium, magnesium, manganese, potassium and sodium were then determined by atomic absorption -- emission on a Perkin-Elmer model 5000 employing standard operating techniques. One milliliter of a 10,000 ppm (1%) Lanthanum chloride ($LaCl_3$) solution was added to each dilution series of the extract which resulted in a 1000 ppm solution (.1%) in each analysis sample. This procedure is necessary to control for interferences by silicon, aluminum, phosphate and sulfate which depress sensitivity in these cation analyses. Equal amounts of lanthanum were also added to standards and controls prior to analysis.

Several extracting solutions were utilized to better delineate phosphorus availability within these soils. Dilute double acid extractions (0.025 N $H_2SO_4$ + 0.050 N HCl) were performed on soils at each study site. Double-acid was originally employed because it is the primary procedure used for Florida and southeastern sandy soils. In order to facilitate comparisons with other research three additional extraction techniques were performed. These are as follows: (1) Bray 1 - 0.03N $NH_4F$ in 0.025 N HC1 (2) Bray 2 - 0.03 N $NH_4F$ in 0.10 N HC1 and (3) Olsen - 0.5 M $NaHCO_3$ buffered to pH 8. All extractions used 5 g soil to 20 ml of extraction solutions and samples were processed as previously described. Extraction times for each extractant was 5 minutes except with 0.5 N $NaHCO_3$ in which both a 5 minute and 30 minute extraction period was

utilized. Phosphorus analyses were determined colorometrically by ascorbic acid reduction of an ammonium molybdiphosphate complex in the presence of antimony (Wantanabe and Olsen 1965; Murphy and Riley 1962). The blue color produced has generally been considered to be stable for 24 hours. However, all samples that were performed were read no sooner than 2 hours or greater than 3.5 hours after initial color development. Color development is extremely sensitive to solution acidity, therefore the procedure utilized was adjusted to 0.45 N $H_2SO_4$ to achieve optimum values as indicated by John (1970).

## Root Length

Single cores (6.5 cm dia. x 15 cm deep: 500 $cm^3$) were removed from herbaceous plots to determine total root length. Sample weights and volumes were determined prior to sieving through a 600μ sieve. All material retained by the sieve was collected and placed in a fomalin-acetic acid-alcohol (FAA--5:5:90) preservative. Total root length measurements were determined using a grid line intercept method (Newman 1966; Bohm 1979; Marsh 1971; GouBran and Richards 1979). This method is based upon a formula which relates the number of intersections of a root mass to root length when randomly placed upon a grid of lines of known dimensions. The formula which relates the relationship of intersections with root length is as follows:

$$R = \frac{\pi A \times N}{2H}$$

in which R = the total length of root, A = the area in which the grid lines are distributed, N = the number of intersections, and H = the total length of the lines within the grid area. Marsh (1971) found that when the grid has squares in which sides equal 14/11 of the desired measuring unit then $\pi A = 2H$, hence, the number of intersections equals root length in the desired units. For example, a grid composed of squares with sides equalling 1/2 inch gives root length estimates directly in centimeters: i.e., 1/2 in = 14/11 cm.

The grid presently used was rectangular with sides equalling 11 and 6 inches. The total line length within the grid equaled 264 inches (H). Therefore,

$$R = \frac{\pi(66 in^2)}{2(264\ in)} \quad (N)$$

$$R = \frac{207.345\ in^2}{528\ in} \quad (N)$$

$$R = .392699\ in\ (N)$$

or

$$R = .392699\ (in) \times (2.54\ \tfrac{cm}{in})\ (N)$$

$$R = 1.00\ cm\ (N).$$

## Mycorrhizal Infectivity

A multiple dilution series technique was employed to determine the "most probable number" of mycorrhizal propagules in a given volume of soil (Daniels and Skipper 1982; Porter 1979; Powell 1980; Maloy and Alexander 1957). Soil samples used in these analyses were composites of five samples collected from the herbaceous plots in each quadrat. Therefore an infectivity value was obtained for each independent quadrat. A fourfold dilution series was prepared using mixtures of test soil and autoclaved test soil. Fifty grams of soil was placed in five replicate vials of each dilution and planted with seeds of Cassia obtusifolia, a naturally occurring legume common in central Florida. Plants were grown for 45 days, and roots were removed, cleared and stained, and examined for mycorrhizal infection. Any form of colonization, regardless of intensity, was denoted as positive. The "most probable number" of mycorrhizal propagules was determined from tables presented in Fisher and Yates (1963, see Table $VIII_2$).

## Mycorrhizal Colonization of Plant Species

The mycorrhizal status of several plants species was examined at each site. Roots of commonly occurring herbaceous, shrub, and tree species were carefully excavated, sealed in plastic containers, and refrigerated. Generally three members of each species were harvested from different areas within each study site; however, no reference is made to specific quadrat location. Prior to examining, roots were cleared in 10-20% potassium hydroxide, bleached in alkaline ammonium hydroxide, and stained with trypan blue or acid fuchsin (Phillips and Hayman 1970; Daniels and Skipper 1982). Percent root colonization was quantified using a modified grid-line intercept method (Giovannetti and Mosse 1980). Criteria used for infection assessment were the presence of intercellular or intracellular hyphae, arbuscles, vesicles, or spores within the root cortex. Infection is reported as percentage of total root length which is colonized and assesses comparative root colonization levels of plant species occurring on all study sites.

## Mycorrhizal Colonization of Random Root Samples

Single cores (6.5 cm dia. x 15 cm deep: 500 $cm^3$) were removed from herbaceous plots to determine total root length. Sample weights and volumes were determined prior to flotation and sieving through a 600µ sieve. All material retained by the sieve was collected and placed in a formalin-acetic acid-alcohol (FAA-5:5:90) preservative. Total root length was determined as previously described (see Methods: Root Length). Following root length analyses, large root aliquots were randomly selected for observation of mycorrhizal colonization. Roots were cleared in 10-20% potassium hydroxide, bleached in alkaline ammonium hydroxide and stained with 0.05% trypan blue. Percent of root length examined which exhibited mycorrhizal colonization was determined by a modified gridline intercept method (Giovannetti and Mosse 1980). Percent colonization values together with values for sample root lengths have been used to calculate the total root length colonized by mycorrhizae in each sample.

## Results

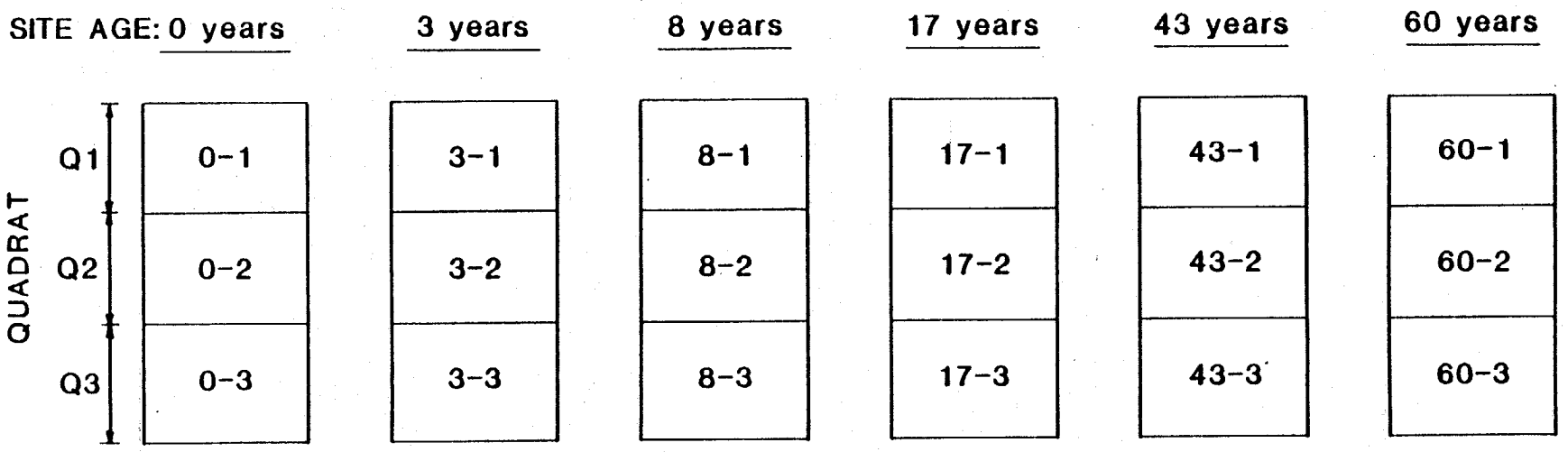
### Method of Analysis

Analysis of variance procedures have been used to delineate differences which occur in all dependent variables between the different aged study sites. As was previously described., sites were divided into 3 sample quadrat regions due to observed intrasite heterogeneity of vegetation distribution. To analyze whether significant differences occur in aboveground and belowground components, three separate statistical models were employed (Figure 3). Each model uses a different set of independent variables to compare responses of the dependent variables (litter, SOM, etc.) which were sampled. Each analysis is explained with respect to independent variables, as follows:

1) INDEPENDENT VARIABLE - Site Age - Site age initially represented the most obvious method for comparison of dependent variables. On most studies involving successional response to disturbance, comparisons between different aged sites has been the method most utilized. For example, Kangas (1982) in comparisons of phosphate mine recovery and succession utilized site age (time) as the dominant factor controlling ecosystem recovery. This method primarily assumes that age (time) is the most important function which controls successional dynamics following disturbance. Hence, physical and biological constraints are generally presumed to be controlled or modified as time proceeds. Analysis within this procedure was performed using site age (time) i.e. 0, 3, 8, 17, 43, 60 years post mining, as the method of comparison testing differences among dependent variables.

2) INDEPENDENT VARIABLE - Quadrat Location - Data have been analyzed in which the different quadrat locations were compared without respect to site age. For example, all samples which were taken from quadrat 1 on all sites were combined into a data set and compared to all samples (over all sites) from quadrat 2 and quadrat 3. This method of analysis is intended to determine if processes (e.g. biomass accumulation) which occur during succession are controlled primarily by quadrat location. Initial observation indicated that possible gradients (physical differences) exist from crest to base of overburden mounds in which gradients of temperature, moisture, erosion, etc. may exist. Analysis of quadrat location is intended to determine differences which may result from these gradient differences, regardless of the time since disturbance.

3) INDEPENDENT VARIABLE: SITE AGE - QUADRAT LOCATION. This method of analysis separated each site into 3 distinct sample groups corresponding to quadrat location.- These groups (site age - quadrat location) were treated as separate entitites and comparisons were made with all other entities at all sites. For example, samples from quadrat 3 on the 8. year old study site were compared with samples from Quadrat 2 on the 60 year old site. All possible comparisons (i.e. 6 sites times 3 quadrat locations = 18 possibilities or treatments) of site-quadrat combinations were analyzed. The procedure was analyzed in this manner to determine if succession, i.e. soil - organic matter or root biomass accumulation, with time, is a response not only to time but also to physical-chemical constraints or controls which vary from different locations within the




MODEL ANALYSIS

| INDEPENDENT VARIABLE | COMPARISON VARIABLE | CONDITIONS |
|---|---|---|
| 1 Site Age | 0,3,8,17,43,60 | Disregards Intrasite Location |
| 2 Quadrat | Q1,Q2,Q3 | Disregards Site Age |
| 3 Site-Age:Quadrat Location | 0-1,0-2,0-3,...60-3 | Incorporates both Age and Location |

Figure 3. Description of three statistical models used in analysis of aboveground and belowground succession data.

site. Hence, the community within quadrat 3 - site 8 may exhibit similar organic matter storages of the much older area located within quadrat 2 - site 60.

## Aboveground Biomass

Aboveground biomass data are presented in several general categories as follows. Occurrence of herbaceous, shrub, and tree species populations are discussed for each site and associated dynamics through time. Litter dynamics are treated as an aboveground component, and, in addition, litter and herbaceous data have been combined into a total aboveground biomass category for analysis. Vegetation data are tabulated so that total biomass (in kg/ha) and importance values (IV) are provided for herbaceous species (Table 2), and basal area and importance values are are given for tree and shrub species (Table 3). Herbaceous, shrub, and tree measurement data are presented in Appendix A, Tables A1-A16. Importance values have been calculated for all species that were sampled to simplify review and discussion of data. These importance values serve as a measure of the significance of each species as a structural component of the plant community. Herbaceous importance values were calculated using relative frequency and relative biomass data. Shrub and tree importance values are a combination of relative density and relative dominance (basal area) determinations. Importance values (IV) as presented may be interpreted to be a percentage of the total importance of all species and hence may be more accurately referred to as importance value percentage. The combination of information on occurrence, density, dominance, and frequency into importance value indices is a simple and ideal method for characterizing the plant population structure of the communities that occur during succession of these sites. Standard methods for calculation of density, dominance, frequency, and importance value have been used (Smith 1974).

Site 0 (recently mined site). Eupatorium compositifolium, Digitaria sanguinalis, and Sesbania macrocarpa were randomly colonizing this area. Salix caroliniana was also present but individuals occurred as resprouts from preexisting rhizomes redistributed at various levels during mining. S. caroliniana seedlings were not observed to be colonizing the site at the time sampling was performed. Representative members of the species encountered were not found to occur within the herbaceous sample area; therefore, zero values exist for all herbaceous harvest data at this site. The site can generally be characterized as having barren mineral soil with no litter deposition or accumulation and extremely limited herbaceous colonization.

Three-year-old mine. Herbaceous vegetation is very abundant within this area, and substantial plant cover has been established since mining was discontinued. Indigofera hirsuta is the most important plant species present (IV = 46.5) with Cynodon dactylon (IV = 14.7) and Digitaria ciliaris (IV = 11.8) being very abundant also. It is necessary to emphasize that even though I. hirsuta is very common within the sample area, dominance by this species does not seem to be prevalent on all sites within this age classification. Observation of overburden mounds within the general locality adjacent to the sample site revealed that I. hirsuta may vary from very dense to very sparse populations. Although several site edaphic factors may be involved, dominance on any one site is probably a result of the mining period being incidental with species seed dispersal. Dominance by any one species should not be interpreted as absolute. Shrub

Table 2. Herbaceous harvest biomass data (kg/ha) and calculated importance values (IV) obtained from phosphate mine succession study.

| Plant Species | Site Age (Years) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 3 | | 8 | | 17 | | 43 | | 60 | |
| | | BIO | IV | BIO | IV | BIO | IV | BIO | IV | BIO | IV |
| GRASSES | | | | | | | | | | | |
| Cynodon dactylon | - | 1030.2/ | 14.7 | - | | - | | - | | 0.3/ | 0.8 |
| Cyperus sp. | - | 4.7/ | 1.0 | - | | - | | 12.3/ | 3.1 | - | |
| Digitaria ciliaris | - | 218.2/ | 11.8 | - | | - | | - | | - | |
| Paspalum urvillei | - | 230.1/ | 5.8 | - | | - | | - | | - | |
| Rhynchelytrum repens | - | 18.8/ | 2.8 | 2141.8/ | 39.3 | 1457.8/ | 23.7 | 1004.3/ | 32.5 | - | |
| Setaria geniculata | - | 4.8/ | 1.0 | - | | - | | 4.4/ | 1.6 | - | |
| Grass species | - | 2.4/ | 1.0 | 79.5/ | 5.0 | - | | 0.7/ | 1.6 | - | |
| Andropogon virginicus | - | - | | 317.0/ | 8.7 | 57.4/ | 1.7 | - | | - | |
| Juncus sp. | - | - | | 8.3/ | 1.1 | - | | - | | - | |
| Panicum hemitomon | - | - | | 69.4/ | 2.8 | - | | - | | - | |
| Panicum sp. | - | - | | 4.4/ | 1.1 | - | | - | | 55.7/ | 6.1 |
| Andropogon sp. | - | - | | - | | - | | 140.1/ | 5.2 | - | |
| Panicum commutatum | - | - | | - | | - | | 0.8/ | 1.6 | 5.6/ | 4.6 |
| Paspalum sp. | - | - | | - | | - | | - | | 0.4/ | 0.8 |
| Oplismenus setarius | - | - | | - | | - | | - | | 9.8/ | 2.9 |
| HERBACEOUS | | | | | | | | | | | |
| Ambrosia artemisiifolia | - | 247.3/ | 5.9 | - | | - | | - | | - | |
| Chenopodium ambrosioides | - | 0.5/ | 1.0 | 24.8/ | 2.3 | - | | - | | - | |
| Crotolaria spectabalis | - | 125.1/ | 3.5 | - | | - | | - | | - | |
| Eupatorium capillifolium | - | 15.5/ | 1.8 | 102.8/ | 4.2 | - | | 11.7/ | 1.8 | - | |
| Eupatorium compositifolium | - | 23.5/ | 1.1 | - | | - | | 8.5/ | 1.7 | - | |
| Euphorbia maculata | - | 0.5/ | 1.0 | - | | - | | - | | - | |
| Indigofera hirsuta | - | 5493.9/ | 46.5 | - | | 1493.8/ | 21.7 | - | | - | |
| Conyza canadensis | - | - | | 1.3/ | 1.1 | - | | - | | - | |
| Momordica charantia | - | - | | 95.9/ | 7.2 | 1.6/ | 1.3 | - | | - | |
| Solidago fistulosa | - | - | | 1146.0/ | 20.5 | - | | 20.7/ | 3.4 | - | |
| Aeschynomene americana | - | - | | - | | 1.2/ | 2.3 | - | | - | |
| Drymaria cordata | - | - | | - | | 157.4/ | 9.6 | - | | 0.1/ | 0.8 |
| Passiflora pectinata | - | - | | - | | 2.7/ | 1.3 | 19.5/ | 3.3 | - | |
| Lamiaceae sp. | - | - | | - | | 14.0/ | 1.4 | - | | - | |
| Asplenium heteorchorum | - | - | | - | | - | | 1.3/ | 2.9 | - | |
| Cassia fasciculata | - | - | | - | | - | | 119.3/ | 9.0 | - | |
| Trifolium sp. | - | - | | - | | - | | 2.4/ | 1.6 | - | |
| Unknown composite | - | - | | - | | - | | 16.3/ | 3.2 | 13.9/ | 1.9 |
| Ampelopsis arborea | - | - | | - | | - | | - | | 0.8/ | 0.8 |
| Bidens bipinnata | - | - | | - | | - | | - | | 6.4/ | 2.6 |
| Blechnum serrulatum | - | - | | - | | - | | - | | 8.1/ | 1.4 |
| Desmodium sp. | - | - | | - | | - | | - | | 0.5/ | 0.8 |
| Tilandsia unesioides | - | - | | - | | - | | - | | 40.8/ | 11.6 |
| Toxicodendron radicans | - | - | | - | | - | | - | | 2.0/ | 1.5 |

Table 2. (continued)

| Plant Species | Site Age (Years) 0 | 3 | | 8 | | 17 | | 43 | | 60 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | BIO | IV | BIO | IV | BIO | IV | BIO | IV | BIO | IV |
| SHRUBS | | | | | | | | | | | |
| Baccharis halimifolia | - | 2.9/ | 1.8 | 162.3/ | 4.8 | 1369.2/ | 14.7 | 71.6/ | 3.4 | - | |
| Lantana camara | - | - | | - | | 276.1/ | 7.2 | 75.7/ | 3.5 | - | |
| Rubus sp. | - | - | | 74.6/ | 2.0 | 189.8/ | 5.2 | - | | 7.0/ | 2.6 |
| Urena lobata | - | - | | - | | 501.1/ | 10.3 | 74.7/ | 3.5 | 12.5/ | 3.1 |
| Psidium guajava | - | - | | - | | - | | 231.5/ | 7.6 | - | |
| Smilax bononox | - | - | | - | | - | | 10.6/ | 1.8 | 166.7/ | 21.8 |
| Vitis rotundifolia | - | - | | - | | - | | 32.6/ | 5.2 | 38.0/ | 6.0 |
| Callicarpa americana | - | - | | - | | - | | - | | 10.3/ | 1.6 |
| Gelsimium sempervirens | - | - | | - | | - | | - | | 55.6/ | 11.5 |
| TREES | | | | | | | | | | | |
| Quercus virginiana | - | - | | - | | - | | 48.6/ | 2.8 | 1.2/ | 0.8 |
| Liquidambar styraciflua | - | - | | - | | - | | - | | 22.4/ | 3.3 |
| Quercus nigra | - | - | | - | | - | | - | | 118.5/ | 13.0 |
| TOTAL | 0 | 7418.2/100.7 | | 4228.2/100.2 | | 5522.2/100.4 | | 1907.7/100.3 | | 577.2/100.4 | |

Table 3. Shrub (<10 cm dbh) and tree (>10 cm dbh) biomass data obtained from succession study sites: Density (stems/ha), basal area ($m^2$/ha), and importance values (%) are reported for all species having a dbh at 1.3 m (dbh = diameter at breast height). Note: the younger aged sites (0 and 3 years) did not have shrub or tree size class individuals.

| | SITE AGE, YEARS | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 8 | | | 17 | | | 43 | | | 60 | | |
| Species | Density | Basal Area | I.V. | Density | Basal Area | I.V. | Density | Basal Area | I.V. | Density | Basal Area | I.V. |
| **SHRUB** | | | | | | | | | | | | |
| Baccharis halimifolia | 2400 | 0.824 | 44.0 | 5295 | 1.143 | 28.0 | 107 | 0.003 | 1.1 | 27 | 0.005 | 0.1 |
| Psidium guajava | 31 | 0.011 | 0.5 | 533 | 0.121 | 2.9 | 80 | 0.001 | 0.8 | 1867 | 0.074 | 12.2 |
| Salix carolinIana | 1323 | 2.410 | 54.3 | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| Schinus terebinthifolius | 62 | 0.037 | 1.3 | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| Myrica cerifera | -- | -- | -- | 381 | 0.381 | 6.8 | 1787 | 0.473 | 33.4 | 373 | 0.077 | 4.6 |
| Urena lobata | -- | -- | -- | 14436 | 0.591 | 36.2 | -- | -- | -- | 160 | 0.001 | 0.9 |
| Lantana camara | -- | -- | -- | 6323 | 0.903 | 26.1 | 2080 | 0.037 | 20.9 | -- | -- | -- |
| Serenoa repens | -- | -- | -- | -- | -- | -- | 320 | 0.130 | 7.6 | -- | -- | -- |
| Prunus serotina | -- | -- | -- | -- | -- | -- | 267 | 0.115 | 6.6 | 613 | 0.365 | 15.6 |
| Quercus virginiana | -- | -- | -- | -- | -- | -- | 426 | 0.552 | 23.4 | 160 | 0.003 | 1.0 |
| Quercus laurifolia | -- | -- | -- | -- | -- | -- | 133 | 0.098 | 4.7 | 107 | 0.023 | 1.4 |
| Persea palustris | -- | -- | -- | -- | -- | -- | 80 | 0.019 | 1.4 | -- | -- | -- |
| Callicarpa americana | -- | -- | -- | -- | -- | -- | -- | -- | -- | 1867 | 0.097 | 13.0 |
| Smilax bononox | -- | -- | -- | -- | -- | -- | -- | -- | -- | 880 | 0.059 | 6.6 |
| Vitis rotundifolia | -- | -- | -- | -- | -- | -- | -- | -- | -- | 107 | 0.014 | 1.0 |
| Quercus nigra | -- | -- | -- | -- | -- | -- | -- | -- | -- | 3334 | 0.585 | 37.2 |
| Liquidambar styraciflua | -- | -- | -- | -- | -- | -- | -- | -- | -- | 133 | 0.170 | 6.5 |
| TOTAL | 3816 | 3.282 | 100.1 | 26968 | 3.139 | 100.0 | 5280 | 1.428 | 99.9 | 9628 | 1.473 | 100.1 |
| **TREE** | | | | | | | | | | | | |
| Quercus virginiana | -- | -- | -- | -- | -- | -- | 13 | 0.138 | 6.2 | 147 | 11.810 | 45.2 |
| Quercus laurifolia | -- | -- | -- | -- | -- | -- | 27 | 0.479 | 14.6 | -- | -- | -- |
| Pinus elliottii | -- | -- | -- | -- | -- | -- | 80 | 3.567 | 62.9 | 80 | 2.612 | 17.2 |
| Pinus palustris | -- | -- | -- | -- | -- | -- | 13 | 1.246 | 16.4 | -- | -- | -- |
| Prunus serotina | -- | -- | -- | -- | -- | -- | -- | -- | -- | 40 | 1.678 | 9.3 |
| Quercus nigra | -- | -- | -- | -- | -- | -- | -- | -- | -- | 53 | 6.268 | 20.3 |
| Liquidambar styraciflua | -- | -- | -- | -- | -- | -- | -- | -- | -- | 13 | 3.053 | 8.0 |
| TOTAL | | | | | | | 133 | 5.430 | 100.1 | 333 | 25.421 | 100.0 |

species were not encountered within the sample quadrats although Salix caroliniana individuals were becoming established at the water's edge.

Eight-year-old site. In addition to numerous herbaceous and grass species, several shrub species were sampled at this site. S. caroliniana (IV = 54.3) and Baccharis halimifolia (IV = 44.0) were the two most important shrub species encountered. These species, although not attaining tree size (>10 cm dbh), were very large shrubs forming a low, closed canopy in the most basal areas of quadrat 3, generally near the water's edge. Rhynchelytrum repens (IV = 39.3) and Solidaqo fistulosa (IV = 20.5) were the most important herbaceous species; however, occurrence was restricted to different areas within quadrats. R. repens was dominant in areas of quadrats 1 and 2 in which full intensity sunlight occurred. S. fistulosa occurred in the shaded, closed canopy areas in which substantial litter accumulation has occurred. Imperata cylindrica is commonly found forming large monospecific stands in many areas within this age group; however, it did not occur within sample quadrats.

Seventeen-year-old site. The 17-year-old mine represented a stage of transition from herbaceous- to shrub-dominated communities. Unlike the 8-year-old mine, shrubs were generally not restricted to slope bases but were also distributed throughout the ridge and midslope regions of the site profile. Baccharis halimifolia (IV = 14.7) and Urena lobata (IV = 10.3) were very implant components within the herbaceous harvest samples. R. repens was the most important herbaceous species, comprising 23.5% of the total importance value. In addition, in the shrub layer B. halimifolia (IV = 28.0), and U. lobata (IV = 36.2) along with Lantana camara (IV = 26.1) attained densities of approximately 26,000 stems/ha (note: dbh <10 cm; height $\geq$ 1.3 m). Also observed in areas adjacent to the site were monospecific stands of I. cylindrica. Psidium quajava, Shinus terebinthifolius, and Myrica cerifera were commonly encountered although at very low densities.. Prunus serotina was the only tree species observed within this areas or on other overburden mounds in the general vicinity.

Forty-three-year-old mine. R. repens was the most important herbaceous species sampled. Although numerous tree and shrub species occur on this site, occurrence is generally restricted to areas located within quadrats 2 and 3. Quadrat 1, which is located on ridge areas of the overburden mounds, is generally restricted to colonization by grasses and forbs. R. repens is common in this area where intense sunlight and low moisture levels prevail. Ridge areas occurring at this mine are very similar in appearance to ridge areas of younger mines with the exception of oak species, which occasionally appear. Pinus elliottii (IV = 62.9) and Pinus palustris (IV = 16.4) are the most important species encountered and are the largest individuals occurring on the site. Quercus virginiana and Quercus laurifolia are important components of both tree and shrub size class categories; however, tree size does not approach that attained by pines within this area. Although several tree and shrub species occur within the sample site, closed canopy conditions do not exist. Tree density estimations indicate that approximately 133 tree size individuals occur per hectare, illustrating that tree occurrence is somewhat patchy. It is interesting to note that even though pines are the dominant individuals (total basal area), seedlings were not observed or sampled within herbaceous plots or shrub transects.

Sixty-year-old mine. The 60-year-old site differs somewhat from the younger mines previously described. This area was mined before flotation methods were used to remove small particulate phosphate. In this location, large

debris piles surround somewhat rectangular-shaped dredge pits as contrasted to the ridge and furrows characteristic of present day mining. Vegetation sampling revealed these areas to resemble mesic hammock community types commonly found in Florida.

Quercus virginiana (IV = 45.2) and Quercus nigra (IV = 20.3) were the dominant tree species observed on the site. Quercus virginiana attained very large dimensions with many exhibiting dbh measurements greater than 1 m (not located in sample area). Most individuals of Q. nigra, which were extremely large, were infected with heart rot; however, no quantitative data were taken. Q. nigra individuals were by far the most dominant individuals in the shrub size class and represented 13% of the importance value comprising the herbaceous harvest, At this site, the greatest total number of individuals (species number) were encountered in all vegetation categories. Several species were noted that had not occurred at any previous sites. Gelsimium sempervirens, Oplismenus setarius, and Callicarpa americana were prevalent and are generally considered late successional species and indicators of mature climatic conditions (Dunn 1982). Unlike the 43-year-old mine this area is characterized by a closed canopy forming a completely shaded community.

## Floristic Similiarity of Sites

In an effort to determine which sites were more similiar with respect to floristic composition, similarity indices were calculated. Percent floristic similarity was determined using Czekanowski's index in which 1.0 represents complete similarity and 0.0 represents absence of similarity. The index uses binary data (presence - absence) and is defined as follows,

$$\text{Czekanowski's Index} = \frac{2a}{2a+b+c}$$

in which a equals species common to both sites being compared, b equals species found at site 1 but not site 2 and c equals species found at site 2 but-not site 1. Czekanowski's index is unique in that conjoint absences (d) are neglected in analysis however concurrences (a) carry double weight. The index is commonly used and is essentially the equivalent of the Bray-Curtis measure for continuous and meristic data. Entities utilized for basis of comparisons were site age quadrat location. This was done in a effort to delineate if vegetation composition varied not only with respect to site age but also to physical location within the site. The index was calculated based upon the plant species occurring within each site. No separation was made with regard to herbaceous, shrub or tree categories. All species encountered were used regardless of size class designation. The results of similarity indices calculations (Table 4) indicate that with the exception of the 43-year-old site, all quadrats within any one site of a specific age showed greater similarity to each other than to quadrats of a different aged site. At no time did percent similarity of quadrats from different aged sites exceed 50%. The 43-year-old site exhibited a somewhat different trend than the other sites. Quadrats within this site showed greater similarity with other sites than within themselves. However, none of the three quadrats exhibited a similarity coefficient of greater than 36 with any other quadrat which can be considered to be in a moderately low similarity range.

TABLE 4. Summary matrix of percent floristic similiarity between entities classified as site age - quadrat location. Percentage floristic similarity was determined using Czekanowski's index in which 1.0 represents complete similarity and 0.0 represents absence of similarity.

Czekanowski's Index = 2a/2a + b + c

| | 3-1 | 3-2 | 3-3 | 8-1 | 8-2 | 8-3 | 17-1 | 17-2 | 17-3 | 1,1 a 43-1 | 1,0 b 43-2 | 0,1 c 43-3 | 0,0 d 60-1 | 60-2 | 60-3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3-1 | - | .55 | .75 | .15 | .13 | .10 | .27 | .29 | .27 | .27 | 0 | .10 | .07 | 0 | 0 |
| 3-2 | .55 | - | .70 | .24 | .11 | .25 | .21 | .22 | 0 | .24 | .10 | .17 | .12 | 0 | 0 |
| 3-3 | .75 | .70 | - | .40 | .24 | .27 | .35 | .38 | .13 | .27 | .11 | .09 | .13 | 0 | 0 |
| 8-1 | .15 | .24 | .40 | - | .57 | .53 | .44 | .46 | .17 | .33 | .13 | .11 | .15 | 0 | 0 |
| 8-2 | .13 | .11 | .24 | .57 | - | .70 | .38 | .29 | .14 | .29 | .35 | .0 | .07 | 0 | 0 |
| 8-3 | .10 | .25 | .27 | .53 | .70 | - | .38 | .30 | .21 | .32 | .27 | .08 | .16 | .14 | .13 |
| 17-1 | .27 | .21 | .35 | .44 | .38 | .38 | - | .66 | .57 | .14 | .24 | .10 | .07 | .17 | .15 |
| 17-2 | .29 | .22 | .38 | .46 | .29 | .30 | .66 | - | .57 | .15 | .25 | .10 | .14 | .09 | .09 |
| 17-3 | .27 | 0 | .13 | .17 | .14 | .21 | .57 | .57 | - | .17 | .13 | .21 | .07 | .18 | .16 |
| 43-1 | .27 | .24 | .27 | .33 | .29 | .32 | .14 | .15 | .17 | - | .27 | .21 | .07 | .09 | .08 |
| 43-2 | 0 | .10 | .11 | .13 | .35 | .27 | .24 | .25 | .13 | .27 | - | .18 | .32 | .36 | .36 |
| 43-3 | .10 | .17 | .09 | .11 | 0 | .08 | .10 | .10 | .21 | .21 | .18 | - | .29 | .28 | .31 |
| 60-1 | .07 | .12 | .13 | .15 | .07 | .16 | .07 | .14 | .07 | .07 | .32 | .29 | - | .68 | .73 |
| 60-2 | 0 | 0 | 0 | 0 | 0 | .14 | .17 | .09 | .18 | .09 | .36 | .28 | .68 | - | .86 |
| 60-3 | 0 | 0 | 0 | 0 | 0 | .13 | .15 | .09 | .16 | .08 | .36 | .31 | .73 | .86 | - |

The results indicate that vegetation on the different aged sites is highly variable. Vegetation of quadrats within each site is more similar than vegetation of quadrats within different aged groups. However, there is still substantial variability within each site dependent on quadrat location. With the exception of the 60-year-old site similarity values obtained from intrasite quadrats ranged from .18 to .75. This indicates, based on a complete similarity value of 1.0, that even within sites vegetation is variable from the hydric to the xeric locations.

These results indicate that the vegetation within a site probably occurs in response to the availability of seed source in adjacent areas at the time of mining. Distribution of vegetation within a site is controlled by the seed source and physical-chemical factors which influence survival, germination and growth of successful invaders. This is evident by the less than perfect simi larity of vegetation which occurs between quadrats of a given aged site.

The 43-year-old site displayed the lowest intrasite similarity. This is in part due to the fact that tree and shrub species were generally restricted to Quadrats 2 and 3. Also, herbaceous species were more dominant on quadrat 3 with grass species being most prevalent on quadrat 1. This segregation of species resulted in low intrasite similarity of quadrats with the quadrat 1 location being most similar (although moderately low similarity) to the younger 3 and 8 year sites while quadrats two and three showed greater similarity to the 60 year old site due to the presence of the tree size class individuals. The 60 year site showed the greatest intrasite similarity between quadrats. Moderately high to high similarity coefficients were obtained between quadrats within the site. Nil similarity values were obtained between the 60 year old site and the 3 year old site and quadrats 1 and 2 of the 8 year old site. Closed canopy conditions existing at the 60 year site inhibit the establishment and maintenance of a prolific sun-loving grass-herbaceous community characteristic of the earlier aged sites. Vine and shrub species are the principle dominant subcanopy species of the older site.

These results indicate that vegetation on these areas is heterogenous with respect to species composition and distribution appears somewhat stratified with regard to intrasite location. Characterization of the community in future ecological studies should therefore reflect this distribution pattern.

## Herbaceous and Litter Biomass

Herbaceous biomass data were subjected to statistical analyses using methods previously described and presented in Figure 3. Intersite comparisons of mean values for herbaceous biomass (Table 5) indicate the highest value obtained ($742 g/m^2$) occurred at the 3 year old study site. Zero values were obtained from the most recently disturbed site as might be expected. Although substantial differences appear to occur between mean values, variance between samples was extremely high (see Appendix A) and significant differences (Duncan's multiple range test - a = 0.05) were difficult to ascertain. However, the young sites' (ages 3, 8, 17 years) contained greater herbaceous biomass in square meter samples than the 43 and 60 year old sites.

Analyzing the data differently using quadrat location (Table 6) and site age-quadrat location (Table 7) as independent variables rendered different

TABLE 5. Mean values for all parameters studied at 6 different aged surface mine sites. Each mean is based upon 15 samples taken from each site. Anova comparisons used site age as the basis for dependent variable comparisons. Means with the same letter are not significantly different as determined by Duncan's multiple range test (a=0.05).

| Site Age, Years | Herbaceous Biomass, grams/$m^2$ | Litter grams/$m^2$ | Total Cover Litter and Herbaceous grams/$m^2$ | Root Length cm/kg Soil | Soil Organic Matter grams/kg Soil | | |
|---|---|---|---|---|---|---|---|
| | | | | | $\leq 600\mu$ | $600 < SOM \leq 1000\mu$ | $>1000\mu$ |
| 0 | 0 C | 0 C | 0 B | 53 D | 4.79 D | 5.89 B | 0.40 B |
| 3 | 742 A | 257 B,C | 999 A | 658 D | 4.28 D | 10.91 B | 0.76 B |
| 8 | 422 A,B,C | 889 A | 1312 A | 3346 C | 8.92 C,D | 22.98 B | 3.36 B |
| 17 | 552 A,B | 653 A,B | 1205 A | 3140 C | 11.46 C | 19.77 B | 3.43 B |
| 43 | 193 B,C | 568 A,B | 761 A | 5798 B | 16.16 B | 59.30 A | 9.04 A |
| 60 | 58 C | 896 A | 954 A | 8310 A | 26.74 A | 75.07 A | 11.07 A |

TABLE 6. Mean values for all parameters studied at 6 different aged surface mine sites. Anova procedure utilized quadrat location as independent variable. Each mean is based upon 30 observations ie. samples per quadrat at each site. Means with the same letter are not significantly different as determined by Duncan's Multiple Range Test (a=0.05).

| Quadrat Location | Herbaceous Biomass grams/$m^2$ | Litter grams/$m^2$ | Total Cover Litter and Herbaceous grams/$m^2$ | Root Length cm/kg Soil | Soil Organic Matter grams/kg Soil | | |
|---|---|---|---|---|---|---|---|
| | | | | | SOM≤600μ | 600<SOM≤1000μ | SOM>1000μ |
| 1 | 277 A | 352 B | 630 B | 3178 A | 7.64 B | 11.48 C | 2.54 B |
| 2 | 340 A | 459 B | 799 A,B | 3881 A | 13.38 A | 32.24 B | 4.45 A,B |
| 3 | 366 A | 820 A | 1186 A | 3593 A | 15.16 A | 53.23 A | 7.03 A |

Table 7. Mean values for all parameters studied at the 6 different aged surface mine sites. Anova comparisons used site age - quadrat location as independent variables. All values represent means of 5 samples. Means with the same letter are not significantly different as determined by Duncan's multiple range test ($\alpha$=0.05).

| Herbaceous Biomass grams/$m^2$ | | | Litter grams/$m^2$ | | | Total Cover grams/$m^2$ | | | Root Length cm/kg Soil | | | Soil Organic Matter grams/kg Soil ≤600 | | | 600<SOM≤1000 | | | >1000 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Site | Quadrat | Mean | Site | Quadrat | Mean | Site | Quadrat | Mean | Site | Quadrat | Mean | Site | Quadrat | Mean | Site | Quadrat | Mean | Site | Quadrat | Mean |
| 3-3 | 1662 | A | 8-3 | 1318 | A | 3-3 | 2260 | A | 60-3 | 10494 | A | 60-3 | 32.86 | A | 60-3 | 163.30 | A | 43-3 | 16.26 | A |
| 17-2 | 922 | B | 60-3 | 1245 | AB | 8-3 | 1506 | AB | 60-2 | 8352 | AB | 60-2 | 31.88 | A | 43-2 | 81.44 | B | 60-3 | 15.18 | AB |
| 8-1 | 574 | BC | 43-3 | 1086 | ABC | 17-1 | 1423 | AB | 43-2 | 7863 | ABC | 43-3 | 18.94 | B | 43-3 | 81.17 | B | 60-2 | 11.42 | BC |
| 17-1 | 549 | BC | 60-2 | 991 | ABC | 17-2 | 1334 | ABC | 60-1 | 6083 | BCD | 43-2 | 18.26 | BC | 60-2 | 46.36 | C | 43-2 | 7.81 | CD |
| 8-2 | 505 | BC | 17-1 | 873 | ABC | 60-3 | 1327 | ABC | 43-3 | 5654 | BCDE | 60-1 | 15.48 | BCD | 17-3 | 28.64 | CD | 60-1 | 6.61 | DE |
| 3-2 | 383 | BC | 8-2 | 703 | ABCD | 8-1 | 1221 | ABC | 8-1 | 5208 | BCDE | 17-3 | 15.12 | BCDE | 8-3 | 28.45 | CD | 8-3 | 5.28 | DEF |
| 43-1 | 322 | BC | 17-3 | 673 | ABCDE | 8-2 | 1208 | ABC | 17-2 | 4310 | CDEF | 8-3 | 12.41 | BCDEF | 8-2 | 22.84 | CD | 17-2 | 4.24 | DEF |
| 8-3 | 188 | BC | 8-1 | 647 | ABCDE | 43-3 | 1166 | ABC | 43-1 | 3877 | DEFG | 43-1 | 11.29 | CDEF | 17-2 | 20.09 | CD | 17-3 | 3.86 | DEF |
| 17-3 | 185 | BC | 3-3 | 598 | BCDE | 60-2 | 1044 | BCD | 17-1 | 3131 | DEFG | 17-2 | 11.22 | CDEF | 8-1 | 17.64 | CD | 43-1 | 3.04 | DEF |
| 3-1 | 180 | BC | 43-2 | 545 | CDE | 17-3 | 858 | BCD | 8-3 | 2670 | DEFG | 8-2 | 8.49 | DEFG | 3-2 | 16.73 | CD | 8-1 | 2.49 | EF |
| 43-2 | 177 | BC | 60-1 | 451 | CDE | 43-2 | 722 | BCD | 8-2 | 2159 | EFG | 17-1 | 8.04 | EFG | 60-1 | 15.55 | CD | 8-2 | 2.29 | EF |
| 60-3 | 82 | BC | 17-2 | 412 | CDE | 60-1 | 490 | BCD | 17-3 | 1977 | EFG | 0-3 | 6.30 | FG | 43-1 | 15.30 | CD | 17-1 | 2.17 | EF |
| 43-3 | 79 | BC | 3-2 | 106 | DE | 3-2 | 489 | BCD | 3-1 | 744 | FG | 8-1 | 5.86 | FG | 3-3 | 11.39 | CD | 3-3 | 0.99 | F |
| 60-2 | 53 | BC | 43-1 | 74 | DE | 43-1 | 396 | BCD | 3-3 | 689 | FG | 3-2 | 5.43 | FG | 17-1 | 10.58 | CD | 3-1 | 0.72 | F |
| 60-1 | 38 | BC | 3-1 | 68 | DE | 3-1 | 248 | CD | 3-2 | 542 | FG | 3-3 | 5.34 | FG | 0-3 | 6.42 | D | 0-3 | 0.64 | F |
| 0-1 | 0 | C | 0-1 | 0 | E | 0-1 | 0 | D | 0-3 | 72 | G | 0-2 | 4.99 | FG | 0-2 | 6.00 | D | 3-2 | 0.57 | F |
| 0-2 | 0 | C | 0-2 | 0 | E | 0-2 | 0 | D | 0-2 | 60 | G | 0-1 | 3.10 | G | 0-1 | 5.23 | D | 0-2 | 0.35 | F |
| 0-3 | 0 | C | 0-3 | 0 | E | 0-3 | 0 | D | 0-1 | 28 | G | 3-1 | 2.07 | G | 3-1 | 4.61 | D | 0-1 | 0.21 | F |

results. The best or most simplistic way in which to contrast analysis techniques is by comparison of statistical model R-square values obtained from ANOVA procedures (Table 8). ANOVA tables for all parameters are given in Appendix B, Table B1-B8. R-square is a measure of the variance attributed to differences in treatment compared to total variance obtained (i.e., sum of squares for treatments plus sum of squares for error). Sum of squared errors is a measure of the variability of the observations within each treatment. Increasing the value of R-square by a difference in analysis would indicate a more satisfactory grouping of observations with. respect to treatment. Using quadrat location as a treatment substantially reduced the R-square value which indicates that variance among samples within treatments was higher. Hence, herbaceous biomass occurrence may be more influenced by site age than quadrat location (ignoring age as a variable). Mean values obtained for quadrat locations (Table 6) were not found to be significantly different ($\alpha = 0.05$) however values do increase from crest to base in the overburden sites.

Using site age-quadrat location as a method of comparison more than doubled the R-square value, indicating a more appropriate grouping of treatments. Mean values for site age-quadrat location treatments (Table 7) however do not indicate that significant differences do occur between the majority of locations. Significant differences were obtained only between the quadrats of site 0 in which no biomass was sampled and the area of site 3-Q3 and site 17-Q2. These results indicate herbaceous biomass accumulation is highly variable and is more accurately defined in relation to both site age and quadrat location.

Definite trends were also seen to occur in the distribution of forbs, grasses, shrubs, and tree species in the herbaceous sample classes (Figure 4). Distribution of the vegetation components have been grouped and analyzed on a quadrat basis and definite trends are shown to occur, Species in the shrub size class did not occur in sample quadrats of the 0 or 3 year sites. However, shrub species were sampled at the 8 year site in the midslope and basal quadrats but were not noted in the crest area. Greatest shrub biomass was encountered in both herbaceous size classes (Table A14) and shrub size classes (Table 3) at the 17 year site which is consistent with observations of other sites within the area. The 60 year site is characterized by dominance of shrub and tree species and the relative disappearance of the herbaceous and grass components. At all sites grass biomass was greater than forb biomass in the quadrat 1 area. These xeric regions were generally dominated by Rhynchelytrum repens and Cynodon dactylon. The large forb component obtained at site 3-Q3 was attributed to an extremely dense stand of Indigophera hirsuta which had colonized the area. Forb biomass characteristically surpassed grass biomass at all sites in quadrat locations 2 and 3 (Figure 4).

In summary, the colonization of plant species on mined areas is rapid with maximum mean biomass values developing within three years. Herbaceous biomass reduces with time in response to development of a shrub layer canopy which reduces the herbaceous component below. With increased development of the canopy of the older sites, shaded conditions promote the development of a sparse herbaceous layer usually dominated by vines, shrubs and shade tolerant herbaceous species.

Mean values for litter accumulation and statistical analysis for each site are presented in Table 5. The greatest litter biomass occurs at the 60 and 8 year sites. The youngest site (site 0) displayed no Titter accumulation either

Table 8. R-square values obtained from three different analysis methods for community succession study.

| | Independent Variable | | |
|---|---|---|---|
| | Site Age | Quadrat Location | Site Age Quadrat Location |
| DEPENDENT VARIABLE | | | |
| Herbaceous Biomass | 0.16 | 0.003 | 0.38 |
| Litter | 0.29 | 0.11 | 0.52 |
| Total Biomass | 0.22 | 0.06 | 0.44 |
| Root Length | 0.53 | 0.01 | 0.63 |
| SOM $\leq 600\mu$ | 0.62 | 0.11 | 0.78 |
| $600 < SOM \leq 1000\mu$ | 0.32 | 0.14 | 0.74 |
| SOM $> 1000\mu$ | 0.47 | 0.10 | 0.69 |

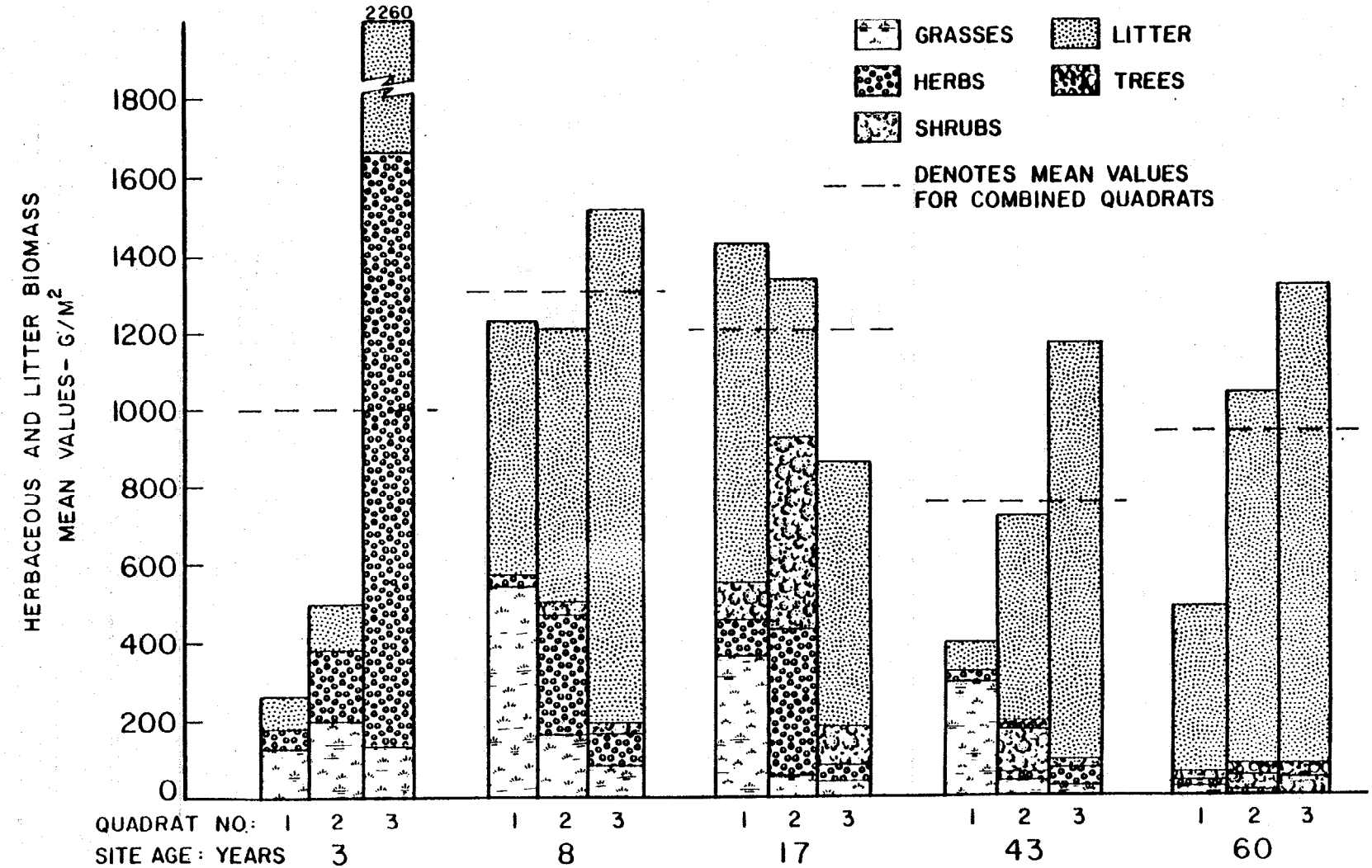



Figure 4. Cummulative mean values for herbaceous and litter biomass (total aboveground biomass). Data are combined and presented for each quadrat. Herbaceous data have been divided into grasses, herbs, shrubs and tree categories to allow for intrasite and intersite comparisons.

from new growth or residual litter which had originated prior to mining. Generally, no significant differences appear in litter biomass between site ages 8 to 60 years. Litter is rapidly established and maintained at fairly constant levels as time proceeds.

An evident predictable pattern arises between herbaceous vegetation colonization and litter accumulation. As evidenced by the proportion of litter to herbaceous biomass there is a lag between herbaceous colonization and litter accumulation (Figure 4). Beginning at eight years there is an increase in the ratio of litter to herbaceous biomass, until at sixty years all samples (n=l5) contained greater than 90% of biomass in the form of litter. The exception to the pattern is the area of quadrat 1 - Site 43. This location was most floristically similar to area 8-1 and displayed litter accumulation very similar to that of area 3-l. The 43 year old site presented the greatest visual contrast with respect to vegetation quantity, species composition and litter accumulation from crest to base of all study sites.

Analyzing the litter data using quadrat location - site as independent variables indicated that although no significant differences were obvious from between ages 8 and 60, evident differences occurred between quadrat location within the individual sites in most all cases (except site 17). Litter accumulation increased from crest to basal locations within the site (Figure 4, Table 7). Reorganizing data for analysis in this respect resulted in a substantial decrease in within treatment variability, i.e., R-square = 0.52 in comparison with using site age as the independent variable in which R-square = 0.29 (Table 8).

## Soil Organic Matter

Soil organic matter values and results of statistical analyses are presented in Figure 5 (see Table 5). SOM values in the >1000µ size fraction ranged from 0.40 g/kg soil at site 0 to 11.7 g/kg at the 60 year old mine. Significant differences were obtained between the two oldest sites and the remaining four younger sites indicating there is a definite trend for accumulation with time. At all sites SOM within this size designation represented the smallest storage sampled within a 15-cm depth from the surface. There was a noticeable absence of large root material within all samples. Roots from large woody species appear to occur deeper than the top 15-cm horizon, which is supported by these results.

SOM values for the intermediate size fraction range from 5.89 g/kg soil at the most recently mined area to 75.07 g/kg soil at the 60 year old mine. The intermediate fraction represented the greatest storage component of SOM displaying significantly higher values at all sites (except Site 0) than with the smaller and larger size category (Figure 5, Table 9).

SOM values in the <6OOµ size class ranged from 4.28 g/kg soil at the three year site to 26.74 g/kg soil at the 60 year old site. SOM in this size category was at all times higher than that in the >1000µ class; however, significant differences were not obtained between values (a = 0.05). These results indicate that a substantial organic matter storage within the soil probably occurs in a relatively small refractory particulate fraction (<l000µ) in the top 15-cm soil horizon.

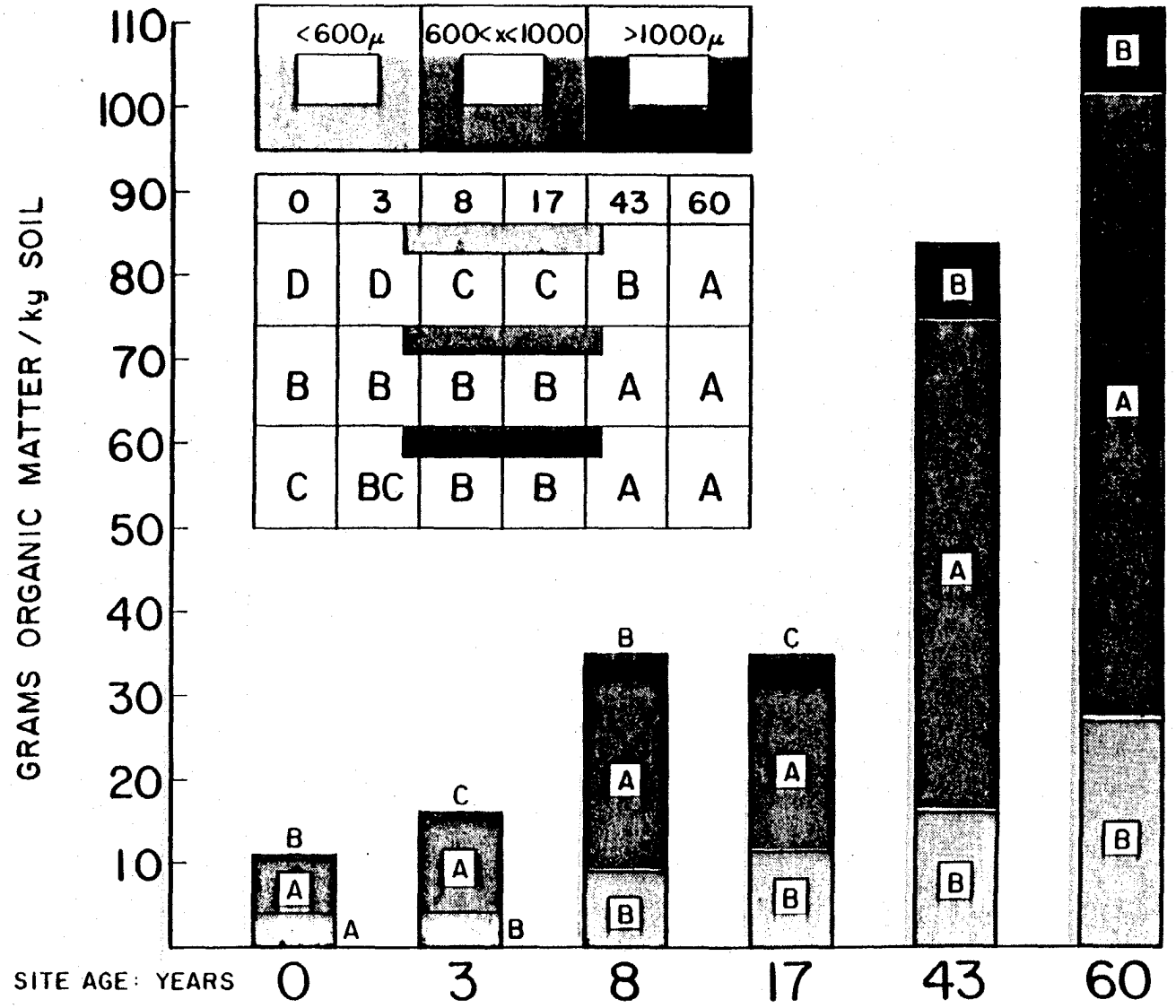



Figure 5. Mean value for soil organic matter concentrations (g/Kg soil) are given for each particulate size fraction. Results of statistical analysis (Duncan's, l=0.10) are given for both intersite (table values) and intrasite (bar values) comparison. Means with the same letter were not significantly different.

TABLE 9. Intrasite comparisons of three organic matter size fractions. Mean values for SOM≤600, 600<SOM≤1000μ, and SOM>1000μ are given by site. Means with the same letter are not significantly different as determined by Duncan's multiple range test ($\alpha$=.05).

| Soil Organic Matter Size Fraction | Site Age: Years | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 3 | 8 | 17 | 43 | 60 |
| SOM≤600μ | 4.79 A | 4.28 B | 8.92 B | 11.46 AB | 16.16 B | 26.74 B |
| 600<SOM≤1000μ | 5.89 A | 10.91 A | 22.98 A | 19.77 A | 59.30 A | 75.09 A |
| SOM>1000μ | 0.40 B | 0.76 B | 3.36 B | 3.43 B | 9.04 B | 11.07 B |

Trends are pronounced, which indicates that there is a definite increase in SOM in overburden soils of all the size fractions in response to time. Ignoring time (site age) as a forcing function and analyzing data with regard to quadrat location only (Table 6) also yielded interesting results. In all three size class designations mean values for the three respective quadrats increased from crest to base of the spoil mounds (Table 6). Significant differences ($a = 0.05$) were obtained for the intermediate-size class between the three locations; however, separation of the remaining size class was not nearly as distinct between locations of quadrats 2 and 3. Quadrat 3 was always signficantly different than quadrat 1. These results indicate that SOM accumulation is significantly affected by physical location within the site even if age is not considered.

Separating data and analyzing with respect to site age-quadrat location improved model R-square values (reduced within treatment variability) of each size class especially for the intermediate fraction (Table 8). The data indicate that in each organic matter category there is a tendency for means to increase in each site from crest to base (Figures 6-7). In addition, mean values at each quadrat increase with age although at different rates. Hence, SOM accumulation is affected by site age and quadrat location. This can be seen by comparison of the slopes of the responses surfaces obtained within each size class designation (Figures 6-7).

Root length. Values for root length determinations and results of statistical analyses for intersite comparisons are given in Table 5. Root length mean values range from 53 cm/kg soil at the recent mine to 8310 cm/kg soil at the 60-year-old mine. Values as high as 20520 cm/kg soil were obtained from samples at the most mature site. Data indicate that significant differences between means occur at 60 years, 43 years, 8 and 17 years, and 3 and 0 years (ANOVA results: Appendix B, Table B8). Root length continually increases with ecosystem development. It cannot be determined from the present results if the increasing trend would continue toward some maximum value beyond 60 years, or if this is the highest value maintained. Root length measurements determined by these methods (sieving and flotation) probably result in underestimations of actual values. Loss of very small root fragments is impossible to avoid during sample processing, and counting is a very tedious and laborious process. However, comparative estimates obtained between these different aged sites prove to be a valuable tool in determining and predicting levels of community maturity. Root length as a measure of accumulated belowground structure may prove to be an important community development indicator.

Analyzing root length with respect to quadrat location resulted in a poor model R-square value in comparison to using site age vs. independent variable (Table 8). Root length was the only parameter sampled which clearly did not show an increase from crest to base when quadrat location was analyzed over all site ages. Using site age-quadrat location for comparison of data once again decreased intratreatment variability. Unlike the results obtained from SOM, root length only showed a consistent increase from crest to base at sites 43 and 60. Values obtained at sites 17, 8, 3, and 0 show no consistent pattern between the quadrat locations (Table 7, Figure 8).

Mycorrhizal colonization of plant species. Results obtained from observing mycorrhizal colonization of commonly occurring plant species at each mine site are presented in Tables 10-15. The data indicate that mycorrhizal invasion is

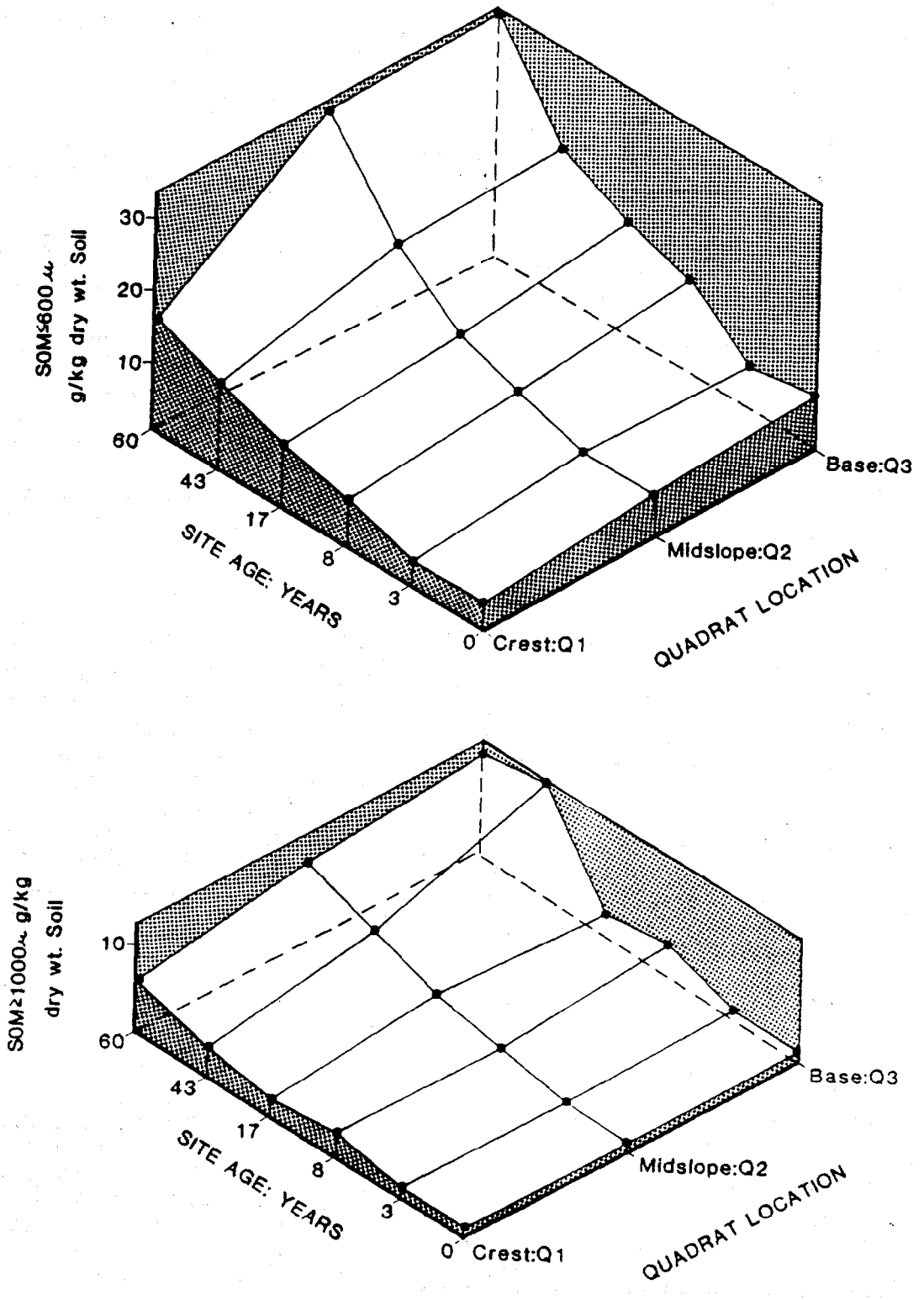



Figure 6. Response surface curves indicating organic matter accumulation rates based on site quadrat location. Values for SOM≤600μ and SOM≥1000μ size classes are presented. All values equal g dry wt. SOM per Kg dry wt. of soil.

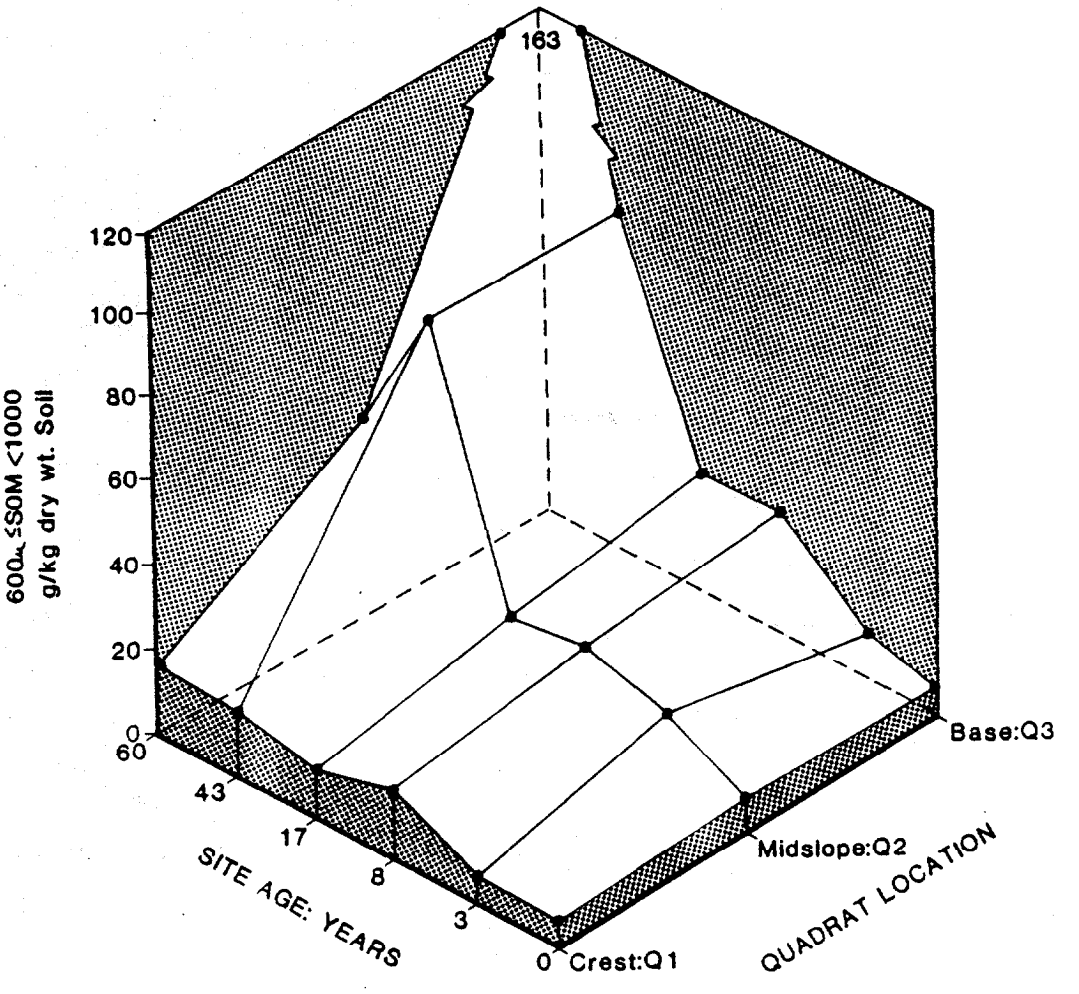



**Figure 7.** Response surface curves indicating organic matter accumulation rates based on site age and quadrat location. Values for the 600µ ≤ SOM<1000 size class, are presented and equal g dry wt. SOM per kg dry wt. of soil.

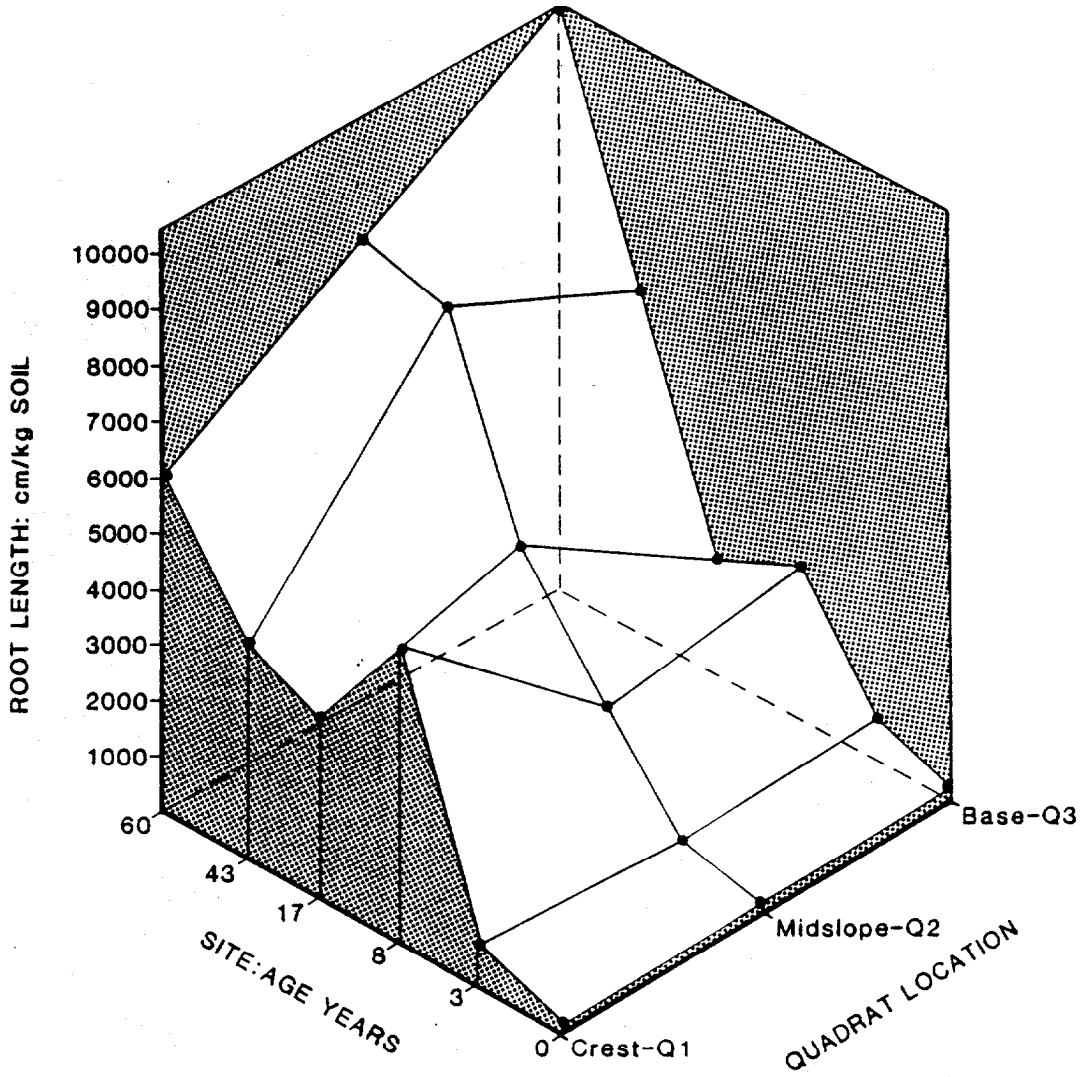



**Figure 8.** Mean values for total root length (cm/kg dry wt. soil) are presented in relation to both site age and quadrat location.

Table 10. Mycorrhizal colonization of commonly occurring plant species for the most recently mined site. Values represent percent mycorrhizal colonization of root length examined.

| Plant Species | Percent Colonization | | |
|---|---|---|---|
| | Sample 1 | Sample 2 | Sample 3 |
| Digitaria sanguinalis | NEG | NEG | NEG |
| Eupatorium compositifolium | 2 | -- | -- |
| Salix caroliniana | 5 | -- | -- |

-- Indicates no sample
NEG = No mycorrhizae seen

Table 11. Mycorrhizal colonization of commonly occurring plant species for the 3-year-old mine. Values represent percentage of root length examined that was colonized by mycorrhizal fungi.

| Plant Species | Percent Colonization | | |
|---|---|---|---|
| | Sample 1 | Sample 2 | Sample 3 |
| Ambrosia artemisiifolia | 19 | 28 | 26 |
| Baccharis halimifolia | 32 | 16 | 52 |
| Crotolaria spectabilis | 49 | POS | POS |
| Cynodon dactylon | 13 | 15 | 4 |
| Cyperus sp. | 3 | 7 | 20 |
| Eupatorium capillifolium | 25 | 29 | 25 |
| Heterotheca subaxillaris | 41 | 74 | 75 |
| Indigofera hirsuta | 11 | POS | 83 |
| Lactuca floridana | 39 | 49 | 45 |
| Lepidium virginicum | 27 | NEG | NEG |
| Ludwigia leptocarpa | 19 | -- | -- |
| Ludwigia peruviana | 19 | -- | -- |
| Paspalum urvillei | 49 | 79 | 74 |
| Paspalum notatum | 32 | 90 | 78 |
| Paspalum repens | NEG | NEG | NEG |
| Polygonum punctatum | NEG | NEG | NEG |
| Rhynchelytrum repens | NEG | 10 | POS |
| Salix caroliniana | 17 | 87 | 2 |
| Setaria geniculata | 82 | 75 | 45 |

-- Indicates no sample.
NEG = No mycorrhizae seen.
POS = Sample showed positive mycorrhizal infection; however, due to staining difficulties infection percentage could not be assessed.

Table 12. Mycorrhizal colonization of commonly occurring plant species for the 8-year-old mine. Values represent percentage of root length examined that was colonized by mycorrhizal fungi.

| Plant Species | Percent Colonization | | |
|---|---|---|---|
| | Sample 1 | Sample 2 | Sample 3 |
| Ambrosia artemisiifolia | 4 | 98 | 13 |
| Baccharis halimifolia | 91 | 47 | 75 |
| Chenopodium ambrosioides | NEG | NEG | NEG |
| Eupatorium capillifolium | 39 | 85 | NEG |
| Eupatorium compositifolium | 82 | 64 | 96 |
| Gnaphalium obtusifolia | 69 | 83 | 91 |
| Heterotheca subaxillaris | 86 | 79 | 81 |
| Imperata cylindrica | 8 | 24 | 50 |
| Lactuca floridana | 30 | 26 | 70 |
| Paspalum urvillei | 53 | 50 | 74 |
| Polygonum punctatum | NEG | 4 | 5 |
| Psidium guajava | POS | POS | POS |
| Rhynchelytrum repens | 5 | 48 | 44 |
| Salix caroliniana | 76 | 61 | 72 |
| Solidago fistulosa | 18 | 68 | -- |
| Thelypteris normalis | POS | POS | POS |

NEG = No mycorrhizae seen.
POS = Sample showed positive mycorrhizal infection; however, due to staining difficulties infection percentage could not be assessed.

Table 13. Mycorrhizal colonization of commonly occurring plant species for the 17-year-old mine. Values represent percentage of root length examined that was colonized by mycorrhizal fungi.

| Plant Species | Percent Colonization | | |
|---|---|---|---|
| | Sample 1 | Sample 2 | Sample 3 |
| Baccharis halimifolia | 34 | 4 | 41 |
| Drymaria cordata | 13 | POS | NEG |
| Imperata cylindrica | 62 | -- | -- |
| Lantana camara | 67 | 45 | 92 |
| Myrica cerifera | NEG | 1 | -- |
| Psidium guajava | 69 | 50 | 71 |
| Rhynchelytrum repens | 20 | 48 | 20 |
| Rubus sp. | 67 | 52 | -- |
| Schinus terebinthifolius | 86 | -- | -- |
| Thelypteris normalis | NEG | 20 | 8 |
| Urena lobata | 84 | 95 | 71 |

-- Indicates no sample.
NEG = No mycorrhizae seen.
POS = Sample showed positive mycorrhizal infection; however, due to staining difficulties infection percentage could not be assessed.

Table 14. Mycorrhizal colonization of commonly occurring plant species for the 43-year-old mine. Values represent percentages of root length examined that was colonized by mycorrhizal fungi.

| Plant Species | Percent Colonization | | |
|---|---|---|---|
| | Sample 1 | Sample 2 | Sample 3 |
| Ampelopsis arborea | 44 | 81 | 81 |
| Ambrosia artemisiifolia | 2 | 25 | 16 |
| Andropogon sp. | NEG | 3 | 17 |
| Asplenium heterochorum | NEG | NEG | NEG |
| Bidens bipinnata | 10 | 58 | 22 |
| Cassia fasiculata | 60 | 60 | 33 |
| Chrysopsis graminifolia | 50 | 43 | 75 |
| Eupatorium capillifolium | 100 | 79 | 51 |
| Euthamia minor | 34 | 33 | 52 |
| Indigofera hirsuta | NEG | 7 | 47 |
| Lantana camara | 65 | 68 | 26 |
| Liatris sp. | 36 | 8 | 11 |
| Phlebodium aureum | NEG | NEG | NEG |
| Psidium guajava | 63 | 40 | 50 |
| Rhynchelytrum repens | 48 | 25 | 18 |
| Solidago fistulosa | NEG | 56 | 1 |
| Urena lobata | 86 | 44 | 81 |
| Vitis rotundifolia | 85 | 98 | -- |

NEG = No mycorrhizae seen.

Table 15. Mycorrhizal colonization of commonly occurring plant species for the 60-year-old mine. Values represent percentage of root length examined that was colonized by mycorrhizal fungi.

| Plant Species | Percent Colonization | | |
|---|---|---|---|
| | Sample 1 | Sample 2 | Sample 3 |
| Bidens bipinnata | 44 | 11 | 29 |
| Callicarpa americana | POS | 72 | 23 |
| Gelsimium sempervirens | POS | 1 | NEG |
| Liquidambar styraciflua | POS | -- | -- |
| Myrica cerifera | NEG | NEG | NEG |
| Oplismenus setarius | NEG | POS | NEG |
| Panicum commutatum | 14 | 12 | 15 |
| Prunus serotina | NEG | NEG | NEG |
| Psidium guajava | NEG | 14 | 22 |
| Psychotria nervosa | 24 | 2 | 23 |
| Quercus laurifolia | ECTO | ECTO | ECTO |
| Quercus nigra | ECTO | ECTO | ECTO |
| Quercus virginiana | ECTO | ECTO | ECTO |
| Smilax bononox | POS | 43 | 13 |
| Solanum sp. | 60 | 28 | 35 |
| Tilandsia utriculata | NEG | NEG | NEG |
| Urena lobata | 35 | 60 | 39 |
| Vitis rotundifolia | NEG | NEG | 1 |

NEG = No mycorrhizae seen.
POS = Sample showed positive mycorrhizal infection; however, due to staining difficulties infection percentage could not be assessed.

rapid into the areas following termination of mining activity. Of the species sampled on the most recent site, Eupatorium compositifolium and Salix caroliniana exhibited colonization, however, at very low levels. Species sampling at the 3-year-old site revealed a majority of species exhibiting mycorrhizal colonization with percent root colonization ranging from 2% in Salix caroliniana to 90% in Paspalum notatum. Inconsistent or negative results were obtained from Lepidium virginicum, Polygonum punctatum, and Paspalum repens. L. virginicum and P. punctatum are members of the families Brassicaceae and Polygonaceae, respectively, which are known to be typically non-mycorrhizal families (Gerdemann 1968). P. repens is generally an aquatic species, which may account for its non-mycorrhizal status. Aquatic species are believed to not typically form mycorrhizae; however, only very limited research has been completed in this area (Sondergaard and Laegaard 1977; Bagyaraj et al. 1979; Keeley 1980). Due to staining difficulties of several species possessing very coarse, dense roots, percent infection could not be assessed accurately. Therefore, results were given as positive if mycorrhizal structures were observed.

Results obtained from the 8-year-old mine were generally the same as the 3-year-old site. The majority of plants exhibited typical endomycorrhizal infection. Colonization percentages were very high in a number of species (e.g., Heterotheca subaxillaris, Salix caroliniana). Several species gave highly variable results. with individuals exhibiting both very high and very low root colonization (e.g., R. repens, A. artemisiifolia). Variation between individuals of the same species may be due to location, plant age or vigor, or any number of factors; however, this analysis intends only to determine general occurrence of mycorrhizae with different species. Chenopodium ambrosioides was the only species encountered not to exhibit colonization. Typically members of the family Chenopodiaceae do not form mycorrhizal associations (Gerdemann 1968).

A greater proportion of shrub species was sampled at the 17-year-old site than at the more recent mines. All species that were sampled exhibited endomycorrhizal colonization; however, Myrica cerifera infection levels were extremely low. Urena lobata and Lantana camara, which were the most dominant shrubs on the site were extensively colonized by mycorrhizal endophytes. Extremely dense stands of these two species occurred within the site. Such high colonization percentages may indicate these species are maintaining a large mycorrhizal propagule reservoir within the soil. Thelypteris normalis, a common fern species in these areas, exhibited low levels of colonization; however, samples from many areas in Florida have revealed extensive colonization within roots of this species (Wallace unpub. data).

Plant species sampled on the 43-year-old mine also exhibited generally high colonization percentages. Endophytes were typically found in all plant growth forms examined, i.e., vines, grasses, herbs, and shrubs. Vitis rotundifolia and Ampelopsis arborea, which are two common vine species, exhibited extremely high levels of colonization. Lantana camara and Urena. lobata were extensively infected as was also the case noted on the 17-year-old mine. Asplenium heterochorum and Phlebodium aureum (ferns) were the only species consistently found to be non-mycorrhizal. Members of the genus Asplenium in Florida (e.g., A. platyneuron) have been found to be mycorrhizal (Wallace unpub. data); however, at this site all samples were negative. Phlebodium aureum (golden polypody), which is typically found growing epiphytically on decaying stumps, was also found to be non-mycorrhizal. Although ectomycorrhizal species compose a significant com-

ponent of the vegetation on this site, reductions in occurrence in endomycorrhizal colonization were not noted.

The 60-year-old mine yielded the most highly variable results of mycorrhizal occurrence within plant roots. Tilandsia utriculata, Prunus serotina, Vitis rotundifolia, and Myrica cerifera were all found to be typically non-mycorrhizal in this area. Results similar to the 18-year-old site were obtained for M. cerifera; however, it is surprising that V. rotundifolia was not colonized since extremely high levels of infection were noted at the 43-year-old site. Generally Prunus species form endomycorrhizal associations (Marx 1975). The absence of infection in P. serotina indicates that sample technique may possibly be a factor. Only saplings of this species were sampled due to the difficulty of excising roots from deep within the soil profile. Small primary roots were extremely difficult to find; hence, a good representative sample was difficult to obtain. Endomycorrhizal infection of species at these sites were substantially reduced compared to all younger sites (except site 0). Ectomycorrhizal species comprise a significant proportion of the aboveground biomass, which may tend to decrease endomycorrhizal occurrence due to host availability. Also the typically endomycorrhizal herbaceous component is a very sparse component of the aboveground vegetation. All individuals of Q. nigra, Q. virginiana, and Q. laurifolia that were sampled exhibited ectomycorrhizal infection characterized by fungal mantle formation on simple, unforked feeder roots.

## Dilution Series: "Most Probable Number" (MPN) Determinations of Mycorrhizal Propagules

"Most Probable Number" determinations of mycorrhizal propagules in the different soils were obtained for each individual quadrat within all sample sites (Table 16). The initial experiment for the youngest site was destroyed during a freeze so the soil was retested again; however, only total site composites were used. Therefore individual quadrat determinations for this site are not available.

Mean MPN values have been calculated for each site (Table 16) to facilitate comparisons of inoculation potential between the different areas. The 3-year-old site had the highest mean value estimate of 0.52 propagules per gram. The results indicate a trend of general reduction of mycorrhizal infectivity with time. The lowest mean value obtained was 0.11 propagules per gram which occurred at the 60 year mine. It is quite surprising that the highest values were obtained at a very young site. Several possible explanations exist for this observation. Mycorrhizal invasion is extremely rapid and a high level of inoculum source is maintained to increase inoculum potential for invading plant species. Also, it is possible that the fungal species present had a greater affinity for the test plant used than species occurring in the later aged sites. In the MPN analyses, difference in host plant infection responses should be minimized, since colonization is simply based on positive or negative results and not colonization intensity analyses (Daniels and Skipper 1982).

Mycorrhizal occurrence in random root samples. Mean values for sample root lengths and mean mycorrhizal colonization percentages (Figure 9) have been used to calculate mean colonized root length. Mean colonized root lengths allows for a method of mycorrhizal occurrence comparisons between different communities. Mean percent values for root colonization are shown to substantially decrease

Table 16. Estimates of the dilution series results of the "Most probable number" (MPN) of mycorrhizal fungi propagules at the different aged mine spoils. Values represent propagule number per gram of soil.

| Site Age Years | Quadrat Location | Mycorrhizal Propagule Density (#/g soil) | |
|---|---|---|---|
| 3 | 1 | 0.83 | |
| | 2 | 0.31 | $\bar{x}$=.52 |
| | 3 | 0.42 | |
| 8 | 1 | 0.23 | |
| | 2 | 0.17 | $\bar{x}$=.17 |
| | 3 | 0.12 | |
| 17 | 1 | 0.42 | |
| | 2 | 0.17 | $\bar{x}$=.27 |
| | 3 | 0.23 | |
| 43 | 1 | 0.23 | |
| | 2 | 0.12 | $\bar{x}$=.16 |
| | 3 | 0.12 | |
| 60 | 1 | 0.07 | |
| | 2 | 0.17 | $\bar{x}$=.11 |
| | 3 | 0.09 | |

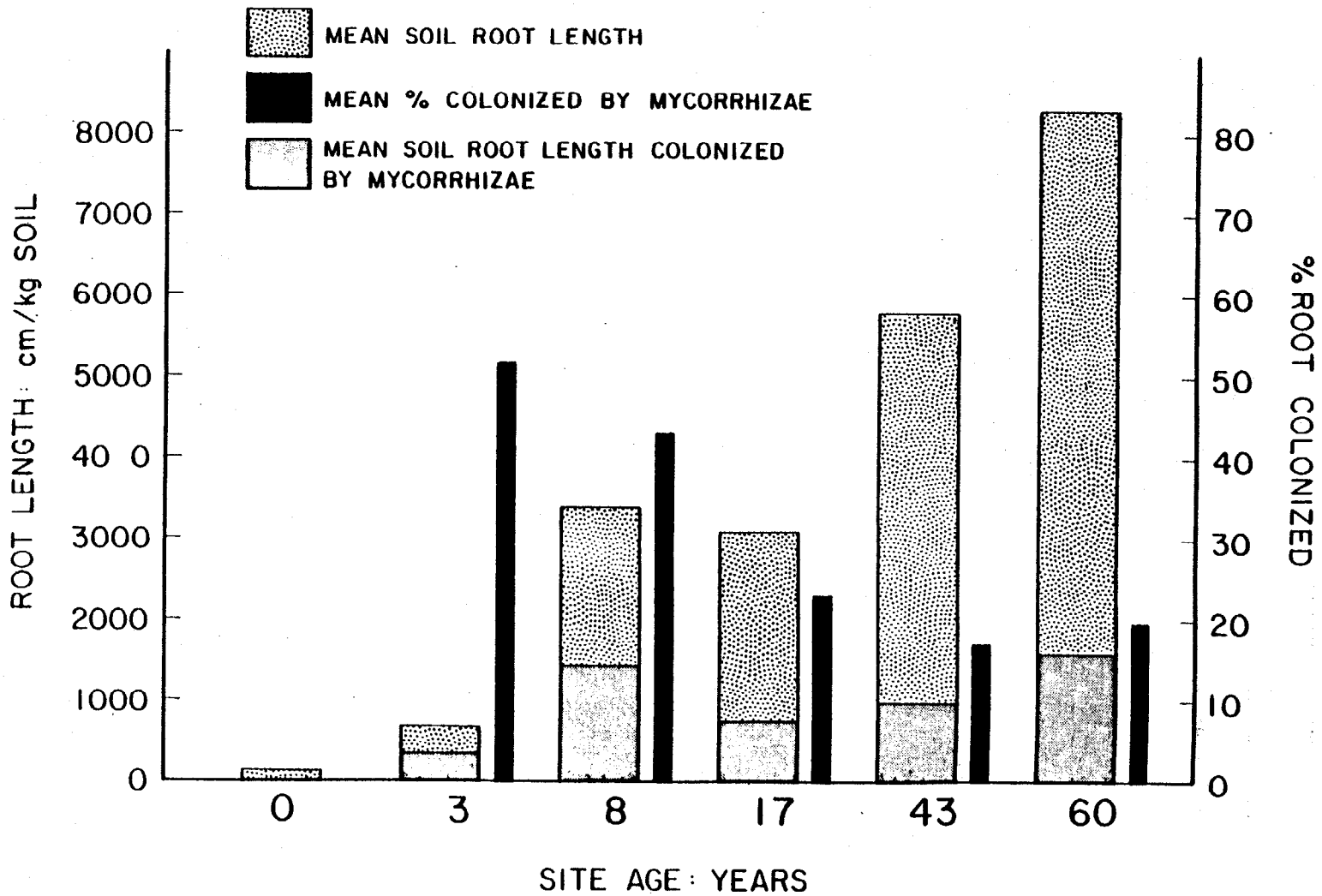



Figure 9. Mean values for total root length (cm/kg soil) and percent mycorrhizae colonization of root length are given. Mean root length colonized by mycorrhizae is presented as the product of mean root length x percent colonization.

with time, from a maximum value of 51.5% at 3 years to a minimum value of 16.8% and 19.5% obtained at the 43- and 60-year-old sites, respectively. Percent colonization values, however, are misleading when interpreted independently of total root length measurements obtained within the sample. Total estimated colonized root length (e.g., root length mean x percent colonization mean) exhibits a somewhat different distribution. Actual total mycorrhizal occurrence within a given soil core indicates minimum values at the 3-year-old site (339 cm/kg soil colonized) with maximum values obtained at the 60-year-old site (1620 cm/kg soil colonized). Although colonization percentage of any random root length within a sample may be minimal, when total root length is considered fungal biomass may be quite high.

These results indicate that when determining percent colonization of roots obtained from random soil cores, values for total root length (or biomass) must be given to accurately represent estimates of mycorrhizal occurrence within a given soil column.

Comparisons between percent mycorrhizal root colonization and "most probable number" determinations. Comparisons between the different methods for assessing mycorrhizal populations previously described are difficult. In review, the dilution series indicated highest infection potential at the 3 year site with the minimum value obtained at the 60 year site. This general trend corresponds well when compared to mean percent infection of root lengths; however, comparisons with total colonized root length (Figure 9) indicate negative correlations. The differences obtained cannot readily be explained. Cassia obtusifolia, which was used in the dilution series test procedure is an opportunistic colonizer of disturbed habitats, roadsides, ditchbanks, etc. occurring in Florida. The plant may be more readily colonized by mycorrhizal species occurring in the herbaceous dominated recent mines as opposed to the mature ecosystems. This, however, infers that host specificity may be a factor, although this is not typical of endomycorrhizal associations. Another explanation may be offered when considering root infection intensity. It was noted that the roots sampled from the younger sites were more intensively colonized than roots from the more mature sites (site ages 43 and 60). Root cortical areas were massively infected in both random root samples and species samples obtained from the earlier sites. In contrast infection noted in later sites was often restricted to single hyphal lengths with sporadic vesicle production. Similar observations were also noted in roots observed from the dilution series experiments; The most intense infections on Cassia obtusifolia were noted in samples from sites 3 and 8 years old. Extensive infection was observed extending through the cortex from the initial penetration area. In contrast, the 60-year-old site plants were characterized by very little proliferation of cortical infection extending from the initial penetration point. The grid-line intercept method (Giovannetti and Mosse 1980) does not distinguish between intensities of infection (e.g., 5 hyphal filaments/cm or 1 hyphal filament/cm) only that colonization occurs within a given root section. Intensity of root infection may be the factor determining inoculum potential and not total root length infected.

## Soil Chemical Analyses

Soil chemical analyses were performed in an effort to provide baseline data for plant growth-soil nutrient requirements in overburden soils. It was originally proposed that soil nutrient data might provide information as to succes-

sional status of the overburden sites, i.e., nutrient changes could be detected with time in response to plant uptake and leaching from these soils. Initital determination of both major macronutrients and micronutrients indicated that due to intrasite and intersite variability these types of analyses were virtually impossible. Sodium was used as a control in that it is not used by plants and possibily not affected by plant growth dynamics.

Results of double acid extractable cations and phosphorus for all sites have been summarized in Table 17. Calcium, phosphorus, and sodium levels exhibit moderately high to high concentrations with manganese, magnesium, and potassium being in a somewhat low range (based on fertilizer recommendations for agricultural crops.). It is difficult to delineate any trend in soil analyses which may be a function of site age or quadrat location. Analysis of phosphorus and the major cations all revealed extremely variable data. For example calcium analysis of soil from the 8 year old site ranged from a minimum of 40 µg/g soil to 13,300 µg/g soil. Phosphorus exhibited the same trend with minimum values of 131 µg/g to maximum values of 5269 µg/g. Only by obtaining an extremely large sample set could any conclusions be drawn concerning changes in elemental concentrations during succession.

In addition to the double acid extraction method a second method was employed to determine exchangeable cations in the soil. 1N $NH_4OAC$ was used as an extracting solution. The use of acid extracting solutions sometimes will give (especially for calcium and magnesium) higher values due to the dissolution of insoluable forms of a base containing compound as in carbonate compounds. Therefore exchangeable and hence more available amounts of compounds may more adequately be determined by the ammonium acetate extraction. Comparisons of extracting solutions were performed on samples taken from the 3 year old site. Results (Table 18) indicate that, with the exception of potassium, double acid extracted substantially higher concentrations of cations than did ammonium acetate with means of $Ca^{++}$ concentrations being greater than 10 times that found in $NH_4OAC$ solutions. This is due to the large amount of acid soluable calcium carbonates which occur in the soil. Double acid extraction resulted in higher concentrations of magnesium, manganese, and sodium than ammonium acetate; however, lower levels of potassium were extracted.

## Discussion

### Community Succession

Aboveground structure. The present study indicates that well-developed ecosystems have been established in a 60-year period following mining. Kangas (1981) in a similar study stated that "within a period of approximately 50 years barren, undifferentiated mounds develop into a forest ecosystem with fertile soil." Results obtained from these studies may tend to be misleading. Although 60-year-old sites somewhat resemble nondisturbed climatic communities (Kangas 1981) these areas were mined at a tine when disturbance of vast areas was uncommon. Perhaps a better indicator of ecosystem direction and development can be attained by examining the 17-year-old site. These areas are principally colonized by bird dispersed (e.g., Myrica cerifera, Psidium guajava, Prunus serotina, Rubus sp.) or windblown species (Baccharis halimifolia which are

Table 17. Summary data (n=15) of soil chemical concentrations (PPM:mg/L) obtained from the 0, 3, 8, 17, 43, and 60 year old mine sites. Cation and phosphorus analysis were performed on dilute double acid extractions. pH determinations were performed on 1:2 of soil:0.01 M $CaCl_2$ suspensions.

| | SITE AGE (YEARS) | | | | | |
|---|---|---|---|---|---|---|
| ANALYSIS | 0 | 3 | 8 | 17 | 43 | 60 |
| CALCIUM: MEAN ± SE | 6511 ± 1169 | 1698 ± 378 | 4456 ± 1116 | 6020 ± 655 | 540 ± 70 | 9916 ± 1213 |
| RANGE | 450 - 12180 | 320 - 4800 | 40 - 13300 | 1900 - 8900 | 76 - 1195 | 640 - 14500 |
| PHOSPHORUS: MEAN ± SE | 2397 ± 454 | 901 ± 215 | 1572 ± 381 | 2555 ± 333 | 229 ± 28 | 3875 ± 480 |
| RANGE | 131 - 5269 | 71 - 2810 | 50 - 5340 | 700 - 5340 | 52 - 413 | 251 - 5528 |
| MAGNESIUM: MEAN ± SE | 146 ± 37 | 93 ± 10 | 126 ± 24 | 343 ± 31 | 29 ± 6 | 96 ± 18 |
| RANGE | 3 - 427 | 2 - 45 | 46 - 390 | 200 - 650 | 8 - 45 | 34 - 229 |
| POTASSIUM: MEAN ± SE | 39 ± 9 | 16 ± 3 | 72 ± 7 | 127 ± 6 | 22 ± 2 | 27 ± 4 |
| RANGE | 3 - 122 | 2 - 45 | 43 - 120 | 87 - 169 | 11 - 47 | 12 - 63 |
| MANGANESE: MEAN ± SE | 13.1 ± 3.0 | 2.2 ± 0.5 | 5.6 ± 1.3 | 4.1 ± 0.6 | 4.0 ± 0.7 | 17.5 ± 2.7 |
| RANGE | 1.1 - 35 .2 | 0.6 - 7.1 | 0.7 - 16.0 | 1.1 - 11.2 | 1.1 - 11.0 | 6.6 - 44.0 |
| SODIUM: MEAN ± SE | 61 ± 9 | 41 ± 5 | 50 ± 8 | 71 ± 7 | 28 ± 2 | 132 ± 17 |
| RANGE | 22 - 140 | 19 - 80 | 21 - 120 | 41 - 146 | 21 - 40 | 28 - 210 |
| pH: RANGE | 4.2 - 5.3 | 4.0 - 5.7 | 4.2 - 6.0 | 4.8 - 5.5 | 3.9 - 4.6 | 4.4 - 5.7 |

Table 18. Soil chemistry results obtained from comparison of double acid and ammonium acetate extracting solutions on soils of the 3 year site. All concentrations are expressed as ug/g soil.

| | Calcium | | Magnesium | | Manganese | | Potassium | | Sodium | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $NH_4OAC$ | DA | $NH_4OAC$ | DA | $NH_4OAC$ | DA | $NH_4OAC$ | DA | $NH_4OAC$ | DA |
| 1 | 301 | 12040 | 89 | 215 | 0.6 | 5.6 | 299 | 51 | 40 | 193 |
| 2 | 302 | 10980 | 87 | 211 | 0.4 | 6.1 | 86 | 69 | 25 | 212 |
| 3 | 305 | 420 | 85 | 72 | 0.2 | 3.8 | 59 | 15 | 27 | 37 |
| 4 | 300 | 10770 | 86 | 140 | 0.5 | 12.6 | 26 | 25 | 22 | 146 |
| 5 | 301 | 4040 | 80 | 201 | 0.1 | 4.4 | 21 | 20 | 19 | 78 |
| 6 | 300 | 3510 | 112 | 147 | 0.1 | 3.9 | 77 | 36 | 20 | 67 |
| 7 | 401 | 2470 | 135 | 97 | 0.2 | 3.8 | 50 | 30 | 28 | 57 |
| 8 | 330 | 1440 | 124 | 113 | 0.5 | 1.2 | 170 | 18 | 32 | 53 |
| 9 | 360 | 2600 | 130 | 125 | 0.2 | 1.5 | 90 | 30 | 35 | 64 |
| 10 | 330 | 15000 | 112 | 269 | 0.5 | 6.9 | 44 | 58 | 35 | 201 |
| 11 | 300 | 5450 | 113 | 272 | 0.2 | 5.9 | 30 | 32 | 33 | 77 |
| 12 | 320 | 780 | 127 | 46 | 0.2 | 1.6 | 26 | 14 | 34 | 24 |
| 13 | 250 | 890 | 116 | 162 | 0.2 | 2.1 | 25 | 12 | 20 | 23 |
| 14 | 270 | 880 | 130 | 50 | 0.2 | 2.4 | 30 | 31 | 17 | 22 |
| 15 | 240 | 1800 | 111 | 67 | 0.4 | 1.6 | 24 | 16 | 16 | 47 |
| MEAN | 307 | 4871 | 109 | 146 | 0.3 | 4.3 | 70 | 30 | 27 | 85 |
| RANGE | | | | | | | | | | |
| MIN | 240 | 420 | 80 | 46 | 0.1 | 1.2 | 21 | 12 | 16 | 22 |
| MAX | 401 | 15000 | 135 | 272 | 0.6 | 12.6 | 299 | 69 | 40 | 201 |

capable of long distance dispersal. Seedlings of Q. virginiana, Q. nigra, Liquidambar styraciflua, or Pinus species were not observed to be colonizing this site or similar aged sites within the adjacent localities. If succession on these sites is to lead to a mature oak-dominated ecosystem (60-year-old site), then invasion of oaks must occur early in site development. Dominance of a plant species can only occur after initial invasion into a site and successful reproduction with time. Due to the vast areas of disturbed land currently present in these areas, seed sources for late successional species have essentially been restricted to floodplain forests. Removal of late successional seed sources would tend to insure the possibility of an arrested succession Situation (for discussion see Lambert et al. 1985), i.e., sites will remain at some stage of ecosystem development which may possibly be similar to conditions observed at the 17-year-old site. It is only speculation, however, and further research must provide information on succession in these areas. This may prove to be a very difficult task, since succession analysis requires static analysis of dynamic processes. It implies that information can be obtained from areas disturbed at different times, which have developed under conditions that are constantly changing with time. The example previously described--seed source availability changes that occur from 60 years ago to the present--adequately illustrates this point.

Belowground structure. Research of belowground processes during succession of phosphate mined lands has been limited; therefore, comparison of the present data with previous findings is difficult. The extent to which vegetation modifies the soil environment and how these modifications affect successive plant communities is generally unknown. During succession from barren areas to climatic communities, numerous processes occur (see Cromack 1981). Belowground structure is established through production of roots and organic matter. Roots function to actively remove nutrients from the mineral soil for use in establishing plant biomass. Plant biomass eventually becomes soil detritus, in which nutrients are stored-and slowly released during decomposition. Accumulation of soil organic matter is a very important factor in succession of phosphate mined areas. During mining, the original surface soil is removed and large quantities of unweathered mineral material are exposed. Production of organic matter by plants conserves nutrients released by weathering, prevents leaching from the profile and establishes a readily available nutrient pool (Witkamp 1971). Whereas nutrients are continually cycled within a community, and conservation is mediated by organic matter, organic matter itself represents net additions to the system, hence, development of ecosystem structure. Data obtained from the study indicate that a continual increase in both root biomass and soil organic matter occurs through time. It appears that accumulation is a constant process that has not attained maximum limits within the time span studied (i.e., values in Table 6 have not yet begun to plateau). An intriguing question occurs concerning whether below ground succession is a time controlled or community controlled phenomenon. In the case of arrested succession, will belowground parameters (such as root biomass or organic size fractions) of a persistent shrub community (developing, for instance, on the 17-year-old site) approach during the same period of time a pattern similar to a mature hammock community (for example, the 60-year-old site) if the younger site continues to retain its present aboveground characteristics (that is, a persistent shrub dominated community) until it reaches a similar age of 60 years. In simpler terms, which influences belowground parameters more, succession time or community structure?

## Mycorrhizal Succession

Mycorrhizal invasion into phosphate mined areas is extremely rapid. Within 3 years, the majority of invading plant species exhibit extensive mycorrhizal colonization. Infection levels in early sites have been shown to be comparatively higher than levels obtained in a mature ecosystem. Allen and Allen (1980) demonstrated that mycorrhizal occurrence in reclaimed coal strip mined areas in Wyoming attained levels of 50% of undisturbed-values within 2-3 years. Comparisons of mycorrhizal infectivity and occurrence between mined and undisturbed areas in Florida is difficult to perform. Disturbed lands in Florida may have originally been dominated by sandhill, oak shrub, pine flatwood, mesic hammocks, marsh, bayhead, or cypress dome type communities. Phosphate mining produces areas, characterized by soil conditions, that are totally different from areas not subjected to disturbance activities. Therefore, it is difficult to determine levels of infection which these mined areas may approach with time. Mycorrhizal species occurring on phosphate mined lands would also have to be adapted for survival in extremely high phosphorus soils. Phosphorus has repeatedly been shown to inhibit mycorrhizal colonization and performance. It is indeed an interesting question concerning the survival strategy that these fungi have adapted for these soils. Also, questions arise as to the extent to which mycorrhizae will promote a growth enhancement response in plants growing in phosphate mined soils. Data regarding such growth responses are not available; however, other research within the scope of this project is presently in progress to determine such effects.

The question of whether mycorrhizal inoculation can affect survival of plant species used in reclamation attempts has recently prompted considerable attention. Numerous questions exist regarding the possibility that field inoculation may enhance survival of naturally colonizing or planted individuals and whether or not large-scale inoculation can feasibly be performed. Data presented in this report may seem to question whether inoculation should be performed, since mycorrhizal invasion has been shown to be very rapid, and colonization occurs in the majority of species present. It is possible that mycorrhizal species occurring in early successional communities may, in fact, be adapted to biotrophic relationships with early colonizing grass and shrub species. Endomycorrhizae have generally been shown to be non-host specific. However, different growth responses and inoculation potential may be mediated by different species or different ecotypes (Lambert et al. 1980; Rangeley et al. 1982). Hence, mycorrhizae, which occur in early aged successional systems, might not be as efficient in promoting growth in L. styraciflua, a later successional plant. Schenck and Kinloch (1980) reported that yearly changes did occur in incidence of root colonization and spore occurrence in six monoculture crops in-a recently cleared woodland area.. Species type and spore numbers were noticeably affected by the plant host species. They concluded that mycorrhizae occurrence changes were the result of host species interaction and not site edaphic factors. Crush (1978) demonstrated differences in the abilities of endomycorrhizal populations originating from different stages of pasture development to enhance growth of white clover. Endophytes from improved pasture more efficiently elicited a growth response than those obtained from systems characteristic of earlier stages of pasture development. Crush (1978) indicated that these results may have been attributed to cultivar origin, which had been selected from a high fertility pasture. It is apparent from these studies that host-endophyte interactions are very important in deter-mining mycorrhizal occurrence in the soil. Crush (1978) discusses the possibility that through host selection, from an initially heterogeneous mycorrhizal soil population, certain fungal species may eventually dominate. Possibly during succession,

mycorrhizal population changes (densities and species) occur in response to selection by changing host populations (or vice-versa ). This may account for the initially high infectivity potential and root colonization occurring very rapidly in phosphate mine succession.

Another problem warranting investigation pertains to the use of indigenous versus introduced species for inoculation purposes. Variable results have been obtained from using indigenous versus introduced strains as mycorrhizal inoculum (Lambert et al. 1980; Khan 1981; Powell 1976). The survival of an introduced fungus will be controlled by the ability to adapt to fertility, moisture, and temperature regimes present within the soil. Lambert et al. (1980) suggests that introduced species generally would not improve plant yield in soils with indigenous fungi. They indicate these conditions may change if vegetation, pH, and fertility factors to which the indigenous fungi have adapted are altered. This view is also expressed by Crush (1978) pertaining to inoculation of New Zealand pasture soils. Introduced mycorrhizal fungi species must be adapted to environmental and soil conditions to effect an advantage for plant growth enhancement and survival. Investigations in this area are urgently needed to determine if the indigenous mycorrhizal fungi species from phosphate mined systems can be increased in culture and used to enhance growth in reclamation attempts.

# CHAPTER 2
# MYCORRHIZAE ENHANCE GROWTH OF SWEETGUM IN PHOSPHATE MINED OVERBURDEN SOILS

## Introduction

Reclamation of mined lands in Florida presents several unique and interesting problems with regard to community development. During mining, nonweathered overburden material is removed from varying depths and deposited on the surface, creating a mosaic of steep-sided hills surrounded by deep water-filled canals. Reclamation of mined lands has been mandatory since the mid-1970's, requiring a recontouring of the mined areas and restoration to useful systems. Generally reclamation has been to pastured ecosystems, or pasture and lake systems. Since Florida law now requires that a portion of the disturbed area must be reclaimed to natural ecosystems problems have developed which were not en-. countered in traditional pastureland restoration situations. Two avenues exist by which these areas may be returned to natural systems. The method of natural system reclamation used requires that extensive labor be used in replanting. An alternative to this approach is natural ecosystem reconstruction via succession (Best et al. 1983). Can nature restore a mature self-maintaining system in a time frame which is acceptable ? Although these approaches are vastly different, success of either may be affected in part and limited by common components such as soil, organic matter, nitrogen, mycorrhizae and/or others. Premining ecosystems in Florida may range from xeric communities dominated by oaks (Quercus spp.) to less well drained pine flatwood to hydric bottomland hardwood or cypress-dominated communities. Although many systems of varying hydrologic regimes are mined, restoration efforts more commonly produce mesic to xeric upland habitats. In addition, post-mining soils characteristically possess very different physical and chemical properties compared with their undisturbed counterparts (see Hawkins 1983 for general description). Typically, mined soils have higher concentrations of major nutrients, e.g. Ca, P, K, Mg, than premined soils, and contain considerably greater proportions of clay to sand. Mined soils, however, contain extremely low concentrations of organic matter and nitrogen. Tine creation of these new habitats proposes the question as to whether locally occurring native plant species which have evolved in generally acid, low clay, low nutrient sandy soils can be expected to invade, survive, and successfully regenerate on these newly created soil systems.

Success of reclamation, whether through the intervention of man or natural ecosystem regeneration, involves an understanding of both macrocomponents and microcomponents of the developing ecosystems. Whether seedlings are planted or occurrence is through natural invasion, success will be determined through interaction with abiotic and biotic environmental parameters. One such parameter warranting special attention is the potential role of mycorrhizal fungi. During the process of mining, overburden soil is removed from depths ranging

from 15 to 80 feet. This soil which can generally be assumed to be abiotic, thus becomes the site of not only aboveground biomass invasion but also of microbial recolonization, i.e. mycorrhizal fungi. Successful inhabitation by planted or invaded species may in fact be governed by the degree and rate at which mycorrhizal populations become reestablished within the disturbed area.

Presently little information exists regarding mycorrhiza occurrence in surface mined lands in Florida or the potential role and function of mycorrhizae in these high phosphorus overburden soils. The consideration of mycorrhizal inoculation for enhanced plant growth for reclamation activities presents several difficult but intriguing questions. The source of endophyte inoculum should be an important consideration. High soil phosphate levels have generally been shown to substantially reduce mycorrhizal colonization (Mosse 1973; Sanders 1975) thus decreasing or alleviating plant dependence on the symbiosis. Presently mycorrhizae that can be used for inoculum purposes do not originate from phosphate mined areas and these ecotypes may not be adapted to soil conditions such as high phosphate which would be encountered in this area. Indigenous mycorrhizae from previously mined areas may in fact be adapted to these conditions which have been shown to be antagonistic to plant mycorrhiza interactions, hence, offering the most viable alternative. Although plant mycorrhizal interactions involving phosphate absorption are well known, information regarding interactions with other nutrients is generally inadequate. However, it is reasonable to assume that in situations of high phosphorus availability, mycorrhizae may enhance uptake of nutrients which exist at suboptimal concentrations. On phosphate overburden soil nitrogen occurs in very low concentrations and is thus considered the major nutrient limiting growth of invading plant species. A growth experiment was designed to investigate factors which affect growth of sweetgum (Liquidambar styraciflua) in overburden soils. Sweetgum was used because of its often poor performance when planted in overburden soils, although it is a common species in adjacent unmined systems. Mycorrhizal additions were evaluated and growth responses determined in relation to both increasing nitrogen and phosphorus additions.

## Materials and Methods

### Experimental Design

Factorial experiments were utilized to assess the effects of nutrient and mycorrhiza additions on growth of sweetgum in phosphate overburden soil.

A 5x3x3 factorial design experiment was utilized in which five levels of phosphorus (0, 12.5, 25, 75, 150 ppm - P), three levels of nitrogen (0, 50, 150 ppm - N) and endomycorrhiza additions were evaluated. This design allowed for forty-five separate treatments to be analyzed (Table 19). In this experiment treatments encompassing all levels of N, P, and mycorrhiza received an additional standard micronutrient and macronutrient solution supplement.

A concurrent 5x3x2 factorial design was also used in assessing the effects of micronutrient, macronutrient-micronutrient combinations, and a third treatment with no additional micro-macronutrient fertilizer. The design allowed for multiple comparisons of 5 levels of phosphorus and 3 micro-macronutrient combin-

Table 19. Experimental design for the 5 x 3 x 3 and 5 x 3 x 2 growth experiments. Numbers within matrix indicate treatment number. All treatments were composed of 5 replicates.

5 x 3 x 3 FACTORIAL DESIGN

| | FACTOR = CS 8 PHOSPHORUS (ppm) | | | | | FACTOR = G. MACROCARPUM PHOSPHORUS (ppm) | | | | | FACTOR = CONTROL PHOSPHORUS (ppm) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NITROGEN (ppm) | 0 | 12.5 | 25 | 75 | 150 | 0 | 12.5 | 25 | 75 | 150 | 0 | 12.5 | 25 | 75 | 150 |
| 0 | 1 | 2 | 3 | 4 | 5 | 16 | 17 | 18 | 19 | 20 | 31 | 32 | 33 | 34 | 35 |
| 50 | 6 | 7 | 8 | 9 | 10 | 21 | 22 | 23 | 24 | 25 | 36 | 37 | 38 | 39 | 40 |
| 150 | 11 | 12 | 13 | 14 | 15 | 26 | 27 | 28 | 29 | 30 | 41 | 42 | 43 | 44 | 45 |

5 x 3 x 2 FACTORIAL DESIGN

| | FACTOR = CS 8 PHOSPHORUS (ppm) | | | | | FACTOR = CONTROL PHOSPHORUS (ppm) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| NUTRIENT | 0 | 12.5 | 25 | 75 | 150 | 0 | 12.5 | 25 | 75 | 150 |
| NONE | 1 | 2 | 3 | 4 | 5 | 16 | 17 | 18 | 19 | 20 |
| MICRO | 6 | 7 | 8 | 9 | 10 | 21 | 22 | 23 | 24 | 25 |
| MICRO + MACRO | 11 | 12 | 13 | 14 | 15 | 26 | 27 | 28 | 29 | 30 |

ations to be made within 2 mycorrhizal blocks. Nitrogen was not utilized as a treatment in this design. Data from the 5x3x3 design in which N=0 was used as a component of the second 5x3x2 design. All treatments within both experiments were composed of 5 replicates. These relatively complicated designs were used because they allow for multiple factors to be examined concurrently. To date, there is essentially no information regarding tree growth responses within these mined soils. Therefore a factorial design provided the best tool for initial experimentation. Data obtained were analyzed using analysis of variance techniques contained within the Statistical Analysis System (SAS).

## Endomycorrhiza Inoculum Preparation

Endomycorrhizal treatments for the 5x3x3 design consisted of control, Glomus macrocarpum and a composite sample (CS8) of Glomus intraradices (personal communication: Dr. N. C. Schneck, University of Florida) and other unidentified species isolated from a phosphatic clay settling area. G. macrocarpum (a commonly occurring Florida species) was obtained from Dr. PI. C. Schenck, University of Florida in the form of soil and infected plant roots. This inoculum was increased by placing in large 1x2 ft. flats containing sterilized native Florida soil and planted with bahia grass (Paspalum notatum) and soybean (Glycine max).

These pot cultures were allowed to grow for four months, at which time roots and soil were separated by sieving and stored in plastic containers at approximately 5% soil moisture until initiation of the experiment. The CS8 mycorrhiza was obtained from Salix caroliniana roots collected from a partially inundated eight month old clay settling area in southwest Polk County (International Minerals and Chemical Corporation; Clear Springs Mine clay settling area no. 8). Roots were placed in large 1x2 ft. flats containing phosphate overburden soil and inoculum prepared as previously described.

The 5x3x2 design (Nitrogen = 0) incorporated only two mycorrhizal treatments, CS8 and Control. Due to limitations of time and greenhouse space it was decided that G. macrocarpum treatments should be deleted from this design.

## Test Plant Inoculation

Seeds of sweetgum (Liquidambar styraciflua) were collected from Polk County in central Florida. Surface sterilized seeds were placed in germination flats containing autoclaved vermiculite. Two weeks following germination seedlings were removed and transferred to pots containing 1100 grams of 1:1 phosphate overburden - sand tailings mix. The sand tailing-overburden mix was used in order to save considerable time and space. Ideally, studies in each individual soil type would provide important information concerning growth response. However, during reclamation these two components are not generally encountered as separate entities but mixed to various degrees. The processes of mixing changes the physical characteristics of the soil e.g. sand, salt and clay ratios, however has very little effect on the macro-nutrient composition, especially phosphorus, calcium, or nitrogen concentrations.

Mycorrhizal inoculum consisting of 0.5 grams sieved roots and 5 grams soil was added directly below the seedling prior to planting. The inoculum used con-

sisted of infected root fragments, mineral soil, fungal hyphae, mycorrhizal spores, and rhizosphere microflora.

In order to standardize microflora for all plants a rhizosphere microflora inoculum was prepared from both inoculum types by washing roots and soil in deionized water. The soil and root wash was subsequently sieved through 45 and 42 µ soil sieves followed by filtration through Whatman No. 4 filter paper (20-25µ). The filtration process eliminates mycorrhizal propagules (spores) which are typically greater than 20µ. However, beneficial soil biota such as bacteria and algae components remain in the inoculum solution. This procedure also does not eliminate propagules of potentially harmful parasitic fungi, It was determined from previous experimentation that this procedure eliminated the possibility of mycorrhizal contamination in the rhizosphere - microflora inoculum. The microflora inoculum was added to all plants including controls. Control and CS8 plants in addition received 5 grams of sterilized G. macrocarpum inoculum soil because it was chemically different than the phosphate overburden matrix. Control plants also received 0.5 g of sterilized root fragments.

## Fertilizer Addition

Phosphorus treatments were performed by incorporating 20% superphosphate fertilizer into the entire contents of each pot at the designated concentration. All phosphorus treatments were based upon concentrations (parts per million: ppm) of elemental phosphorus. Nitrogen fertilization was performed biweekly for 16 weeks by adding 6.25 mg-l and 18.25 mg-l as ammonium nitrate in 20 ml deionized water. This resulted in the total addition of 50 ppm-N (100 lbs./acre) and 150 ppm-N (300 lbs./acre) respectively.

All treatments-within the 5x3x3 design received an additional standard nutrient solution (from Hepper 1983) described as follows:
$CaCl_2 \cdot 2H_2O$, 294; $K_2SO_4$ , 174; $MgSO_4 \cdot 7H_2O$, 184; $MnSO_4 \cdot 4H_2O$, 0.23; $ZnSO_4 \cdot 7H_2O$, 0.03; $CuSO_4 \cdot 5H_2O$, 0.024; $FeSO_4 \cdot 7H_2O$, 1.10; $(NH_4) Mo_7O_{24} \cdot 4H_2O$, 0.0035.
All plants received 40 ml of nutrient solution biweekly for 16 weeks. After the final nutrient addition plants were allowed to grow four weeks before harvesting was performed. Treatments within the 5x3x2 design (Nitrogen = 0) received separate micronutrient and macronutrient solutions for the respective entitites. All plants were fertilized as previously described.

## Data Collection and Harvest

Seedling height was recorded biweekly for the first eight weeks, then monthly until harvest to allow for construction of growth curves. Initially it was believed that to best determine grow response for individual treatments both leaf area and root length should be quantified for each plant. However, several parameters were monitored in order to determine statistically which entity displayed the greatest differential response to the individual treatments. Also it could be determined if different parameters responded differently to nutrient fertilization. Parameters examined included root fresh weight (RFW), leaf fresh weight (LFW), change in height (final height - initial height), leaf area (LA) and root length (RL), growth response variables, calculated response variables.

At harvest stems were clipped at the soil surface and leaves pressed in paper bags and stored at 4°C for no more than 48 hrs. Leaf area was determined with a Hayashi Denko Automatic Area Meter, model AAM-5. Roots were removed from the soil by washing and sieving and stored in a 90:5:5 Alcohol-Acetic Acid - Formalin (FAA) mixture until further processing. Total root length was determined using a grid-line intercept method (Marsh 1971; Giovannetti and Flosse 1980). Following total root length analysis smaller aliquots were removed for determination of the percent of root length colonized by mycorrhizal fungi. Roots were cleared in 10% potassium hydroxide for 1 hour, bleached 15 minutes in alkaline $H_2O_2$ solution and subsequently stained in 0.05% trypan blue staining solution. Percent mycorrhizal colonization was obtained by using the grid line intercept method. Having two measurements of root length i.e. total root length and percent colonized of the aliquot, allowed for a conversion of total root length colonized by mycorrhizae. Root length was considered a better indicator of plant response than root biomass because it gives a better estimate of root absorptive surface. Large diameter root segments can contain a proportionately large biomass although possess only very little nutrient exploiting capacity (surface area) and significantly bias results. However, both variables were utilized in analysis of variance procedures.

## Soil Nutrient Analysis

Soil phosphorus determinations were performed on both the 1:1 overburden-sand tailing mix (test soil) and each of the individual components (Table 20). Although double acid extracts are commonly employed in Southeast sandy soils, two additional extracts were analyzed to facilitate comparisons with other research. Double acid extractions were performed on both sterilized and unsterilized test soil. P analyses were performed by the Environmental Science and Engineering Laboratory, Gainesville, Florida.

# Results

## 5 x 3 x 3 Factorial Design: Nitrogen, Phosphorus, Mycorrhizae

Several growth response variables were monitored throughout the experiment to more adequately and accurately define the effects of different treatments. Analysis of variance results for all dependent variables examined are given in Table 21. It is obvious from comparison of ANOVA R-square values that different degrees of variability were obtained from different observations. In general, root measurements displayed greater within-treatment variability (lower R-square value) than did foliar measurements. For simplicity, discussions of results will deal primarily with observed response differences obtained from leaf area and root length data.

Results of analysis of variance (ANOVA) for both root length and leaf area growth responses are given in Table 22. Comparisons of model R-square (0.87/0.67) values indicate that leaf area measurements produced less variability among samples within each individual treatment than did root length. Leaf area as a measure of photosynthetic area and root length as an indicator of nutrient absorptive surface are both significant indicators of plant vigor. However,

Table 20. Comparisons of extractable phosphorus concentrations (ppm: mg/1) obtained using various common extracting solutions.

| 1:1 Overburden/Sandtailings Mix - Sterilized | | | 1:1 OB/ST Mix Nonsterilized |
|---|---|---|---|
| Double Acid* | Bray 1** | Bray 2*** | Double Acid |
| 2592 | 400 | 2313 | 2404 |

| Overburden Nonsterilized | Sandtailings Nonsterilized |
|---|---|
| 2408 | 2068 |

*Double Acid - (0.025 N $H_2SO_4$ + 0.050 N HCl)
**Bray 1 - (0.030 N $NH_4F$ H 0.025 N HCl)
***Bray 2 - (0.030 N $NH_4F$ H 0.100 N HCl)

Table 21. Analysis of variance results for dependent variables examined in Sweetgum growth experiments. R-square values indicate ratio of model sum of the squares to total sum of squares.

| 5 x 3 x 3 DESIGN | | | | 5 x 3 x 2 DESIGN | | | |
|---|---|---|---|---|---|---|---|
| VARIABLE | F-STATISTIC | PR>F | R-SQUARE | VARIABLE | F-STATISTIC | PR>F | R-SQUARE |
| LEAF AREA (LA - $cm^2$) | 21.06 | 0.0001 | 0.870 | LEAF AREA (LA - $cm^2$) | 32.79 | 0.0001 | 0.914 |
| LEAF FRESH WEIGHT (LDW-g) | 24.26 | 0.0001 | 0.888 | LEAF FRESH WEIGHT (LDW-g) | 29.41 | 0.0001 | 0.905 |
| ROOT LENGTH (RL - cm) | 8.24 | 0.0001 | 0.670 | ROOT LENGTH (RL - cm) | 13.69 | 0.0001 | 0.768 |
| ROOT FRESH WEIGHT (RFW - g) | 17.19 | 0.0001 | 0.808 | ROOT FRESH WEIGHT (RFW - g) | 35.51 | 0.0001 | 0.896 |
| CHANGE IN HEIGHT ( H - cm) | 12.81 | 0.0001 | 0.758 | CHANGE IN HEIGHT ( H - cm) | 16.71 | 0.001 | 0.802 |
| BIORATIO $\frac{(SFW + LFW)}{(RFW)}$ | 5.00 | 0.0001 | 0.619 | BIORATIO $\frac{(SFW + LFW)}{(RFW)}$ | 8.88 | 0.0001 | 0.741 |
| RATIO (LA/RL : CM) | 6.32 | 0.0001 | 0.673 | RATIO (LA/RL : CM) | 11.74 | 0.0001 | 0.791 |

Table 22. Analysis of variance of effects of phosphorus (P), nitrogen (N), and Mycorrhiza type (Myc) on root length and leaf area growth responses.

Growth Response Variable: Root Length (cm)

ANOVA

| SOURCE | DF | SS | F | PR>F | $R^2$ |
|---|---|---|---|---|---|
| Model | 44 | 79329167 | 8.24 | 0.0001 | 0.67 |
| Error | 180 | 39392395 | | | |
| TOTAL | 224 | 118721562 | | | |

| | DF | F VALUE | PR>F |
|---|---|---|---|
| P | 4 | 6.15 | 0.0001 |
| N | 2 | 113.09 | 0.0001 |
| P x N | 8 | 1.55 | 0.1431 |
| Myc | 2 | 17.15 | 0.0001 |

Growth Response Variable: Leaf Area ($m^2$)

ANOVA

| SOURCE | DF | SS | F | PR>F | $R^2$ |
|---|---|---|---|---|---|
| Model | 44 | 27477331081 | 21.06 | 0.0001 | 0.87 |
| Error | 135 | 4003226878 | | | |
| TOTAL | 179 | 31480557959 | | | |

| | DF | F VALUE | PR>F |
|---|---|---|---|
| P | 4 | 7.24 | 0.0001 |
| N | 2 | 250.40 | 0.001 |
| P x N | 8 | 1.83 | 0.0774 |
| Myc | 2 | 116.35 | 0.0001 |

interpretations of leaf area data may offer the most simplistic and precise interpretation of growth response to the various treatments. Due to the difficulty associated with determination of root length, variability may in fact be largely a reflection of error in sample processing rather than actual response differences. However, because utilization of both response variables results in slightly different interpretation of experimental outcome, each response will be briefly discussed. The leaf area F statistic indicates that all main effects of P, N, and Myc (Mycorrhiza) are highly significant. However, growth response to N and mycorrhiza additions were far greater than those associated with P. To facilitate interpretation of these results response surface curves (Figure 10) have been constructed so that differential responses of each mycorrhizal treatment to P and N fertilization may be compared and optimum levels of nutrients and mycorrhizal type may be seen.

Generally, responses of CS8 mycorrhiza and control plants followed the same trend. Addition of increasing amounts of N at each P level resulted in substantial growth enhancement. Responses to increased P concentration at each N level also resulted in greater growth however not of the magnitude that was noted in the previous situation. In both cases it is evident from the response surfaces that maximum production was obtained at levels of 150 ppm - N and 150 ppm - P indicating that both control and mycorrhizal plants were positively responding to fertilizer additions. Comparisons between CS8 and control plants indicate that at all nutrient levels leaf area of CS8 plants were higher than those of corresponding control plants. At levels of 150 ppm - P and 150 ppm - N CS8 plants possessed significantly greater leaf area (446.7 $cm^2$/352.2 $cm^2$: a = .05) than control plants, indicating mycorrhizal-enhanced growth. In the treatment in which no additional P or N was added CS8 resulted in a 30 times (113.78 $cm^2$/3.79 $cm^2$) increase in leaf area over control.

Response of G. macrocarpum plants to the various treatments offers somewhat of a variable and difficult pattern to analyze. Response curves indicate that maximum mean values for both root length and leaf area were obtained when nutrient levels equaled 150 ppm - N and 75 ppm - P. Variable results were obtained when P levels were elevated to 150 ppm. Intratreatment variability of growth responses was greater for G. macrocarpum plants than either control or CS8 plants making interpretation of response exceedingly difficult. However, at nutrient levels in which no concurrent additions of P or N were made, G. macrocarpum resulted in greater growth than control treatments, yet significantly less than CS8 inoculated individuals. Combinations of added P and N levels resulted in highly variable results. G. macrocarpum plants with no additional P or N possessed 17 times (65.02/3.79 $cm^2$) more leaf area than control plants but produced only 0.57 (113.78/65.02 $cm^2$) the area produced by CS8.

At the onset of the experiment it was believed that not only would mycorrhizal/nonmycorrhizal plants respond differently to fertilizer treatments but in addition different responses might be noted between belowground or aboveground plant structures. For this reason a variety of growth response variables were monitored and used for interpretation of plant response. The differences that were observed between response variables can cause very different conclusions to be drawn when analyzing the data. For example when interpreting the ANOVA tables (Table 22) for leaf area and root length, at the a = 0.05 level very different responses are noted between interaction effects. Root length indicated that the P X N, N x Myc and P x N x Myc interactions were all insignificant while only the P x N interaction resulted in an insignificant interaction

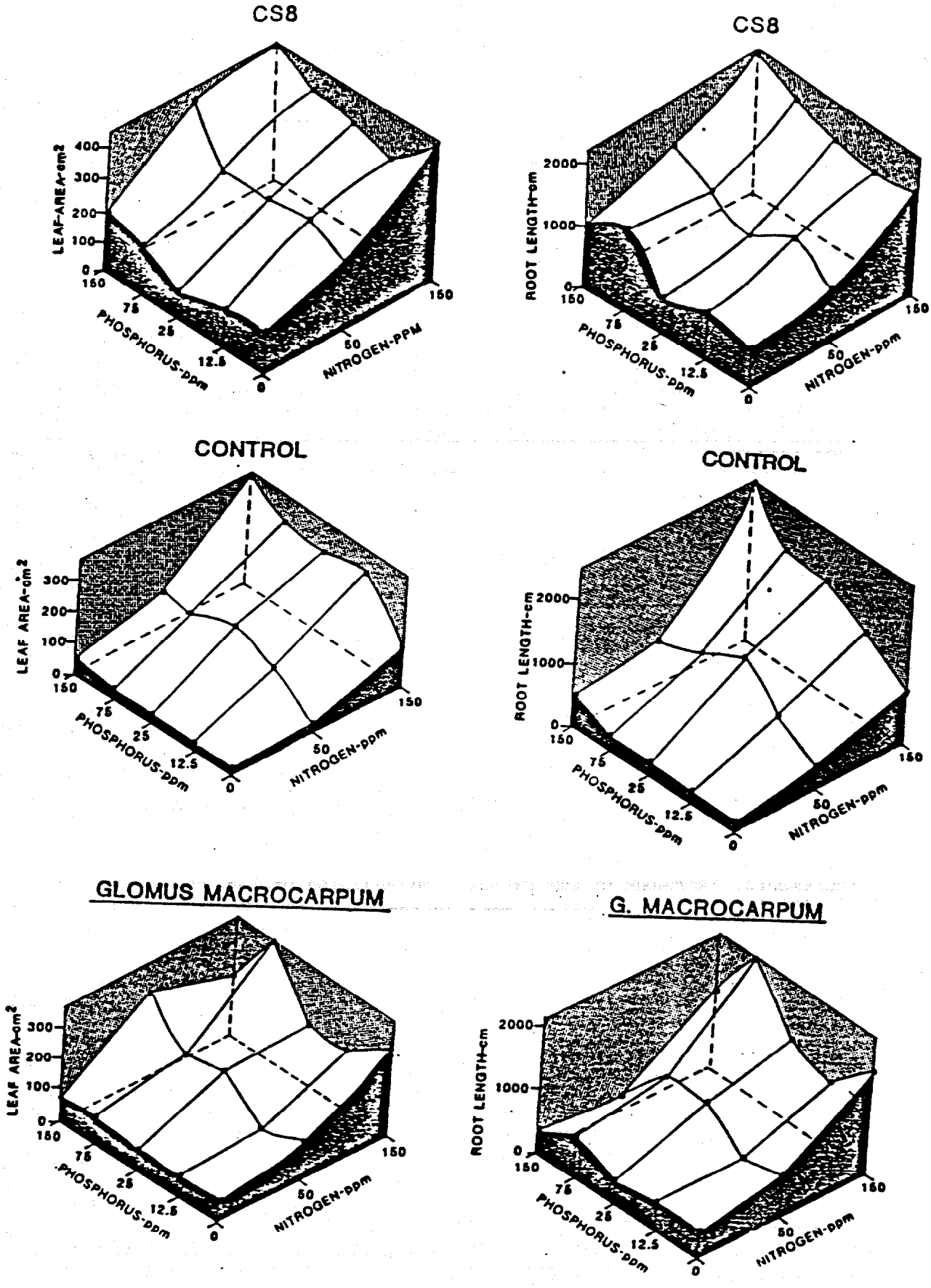



Figure 10. Response surface curves for the root length and leaf area obtained from the various nutrient treatments within each mycorrhizal group.

as determined by leaf area. The significant (P x Myc) (N x Myc) interaction of the leaf area data indicate that the reaction to N and P addition is different within each mycorrhizal group; however, the responses to N fertilization at each P level (or vice-versa) are predictable within all mycorrhizal groups. Interpretation of the root length ANOVA is somewhat different with the only significant interaction expressed being first order between P and Myc. This indicates that each mycorrhizal group (CS8, MAC, CON) reacted differently, e.g., growth responses were of a significantly different magnitude when subjected to phosphorus fertilization.

The most simplistic method in which to analyze these data may be to momentarily ignore interactive effects and just examine the main effects of the three factors within the experiment, i.e. nitrogen, phosphorus and mycorrhizae. The mean values obtained for all growth response variables examined are presented in Table 23. These values represent means of the main effects of all levels of each factor over all levels of the remaining two factors. For example, mean values are given for 150 ppm - P obtained for all levels of mycorrhizae and nitrogen. Briefly, results indicate that a significant growth response was obtained when P concentrations were elevated to 75 ppm or more. In addition, increasing the amount of nitrogen resulted in a doubling of the leaf area response (i.e., leaf area, root fresh weight, etc.) of that obtained at the next lower level. For all responses measured, CS8 mycorrhizae exhibited significantly greater growth than that obtained by both control and G. macrocarpum when means were averaged over all nutrient treatments whereas no significant differences appeared between the average responses of G. macrocarpum and controls.

A calculated variable was also used for comparison of responses at the different treatments. The variable, BIORATIO, represents the ratio of aboveground biomass to belowground biomass. Typically it has been found that mycorrhizae function to increase this ratio in inoculated plants vs. uninoculated plants. The analysis of main effects (Table 23) indicates that over all levels of Myc and N, increasing the P concentration essentially has no effect on the aboveground-belowground biomass ratio. The main effects of nitrogen indicate all levels to be significantly different with increasing rates causing an incremental increase in the ratio. The addition of CS8 over all P and N levels resulted in a significant increase in the ratio of the CON and MAC treatments which were not significantly different from one another.

Response surfaces have been generated (Figure 11) to illustrate the individual effects on the aboveground-belowground biomass ratio of the various fertilizer rates. within each mycorrhizal treatment. The ratio is shown to be very much greater for the CS8 plants than for control plants. Increasing nitrogen results in a much greater ratio increase in the control versus the CS8 plants; however, at all nutrient levels CS8 plants maintained a greater aboveground biomass per unit of belowground biomass than did control. This indicates that the mycorrhizal root system supports a larger amount of photosynthetic biomass per unit of plant root biomass than the nonmycorrhizal root system of control plants in this experiment. This is generally believed to be possible due to the high nutrient absorptive capacity of the extensive fungal mycelial system associated with mycorrhizal roots. In all but one treatment (P = 12.5 and N = 150) control plant aboveground biomass was less than belowground biomass (BIORATIO < 1.00). In treatments in which no additional nitrogen was added control belowground biomass was greater than that of aboveground biomass.

Table 23. Analysis of variance of main effects of phosphorus, nitrogen, and mycorrhiza type on dependent variables for the 5x3x3 experimental design.

| | MYCORRHIZA | | | NITROGEN (PPM) | | | PHOSPHORUS (PPM) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| VARIABLE | CSB | CON | MAC | 0 | 50 | 150 | 0 | 12.5 | 25 | 75 | 150 |
| CALCULATED RESPONSE VARIABLE: | | | | | | | | | | | |
| BIORATIO $\frac{(SFW + LFW)}{RFW}$ | 0.93 | 0.70 | 0.67 | 0.61 | 0.78 | 0.90 | 0.76 | 0.79 | 0.75 | 0.79 | 0.72 |
| | A | B | B | C | B | A | A | A | A | A | A |
| RATIO (cm) $LA(cm^2)/RL(cm)$ | 0.23 | 0.13 | 0.20 | 0.14 | 0.20 | 0.22 | 0.21 | 0.17 | 0.16 | 0.17 | 0.21 |
| | A | C | B | B | A | A | A | AB | B | AB | A |
| GROWTH RESPONSE VARIABLE: | | | | | | | | | | | |
| CHANGE IN HEIGHT (cm) | 18.67 | 10.71 | 12.32 | 6.55 | 13.32 | 21.83 | 10.60 | 11.63 | 13.84 | 16.96 | 16.47 |
| | A | B | B | C | B | A | C | B,C | B | A | A |
| ROOT FRESH WEIGHT (g) | 7.33 | 4.45 | 4.68 | 2.29 | 4.78 | 9.39 | 4.40 | 4.50 | 5.08 | 6.72 | 6.72 |
| | A | B | B | C | B | A | B | B | B | A | A |
| LEAF FRESH WEIGHT (g) | 3.85 | 1.69 | 1.66 | 0.89 | 2.17 | 4.14 | 2.09 | 2.16 | 2.17 | 2.79 | 2.78 |
| | A | B | B | C | B | A | B | B | B | A | A |
| LEAF AREA $(cm^2)$ | 256.72 | 118.47 | 113.60 | 66.13 | 155.40 | 287.26 | 151.70 | 148.31 | 156.07 | 191.48 | 200.42 |
| | A | B | B | C | B | A | B | B | B | A | A |
| ROOT LENGTH (cm) | 1237.00 | 847.00 | 854.00 | 491.00 | 835.00 | 1612.00 | 767.00 | 853.00 | 981.00 | 1174.00 | 1123.00 |
| | A | B | B | C | B | A | C | B,C | A,B | A | A |

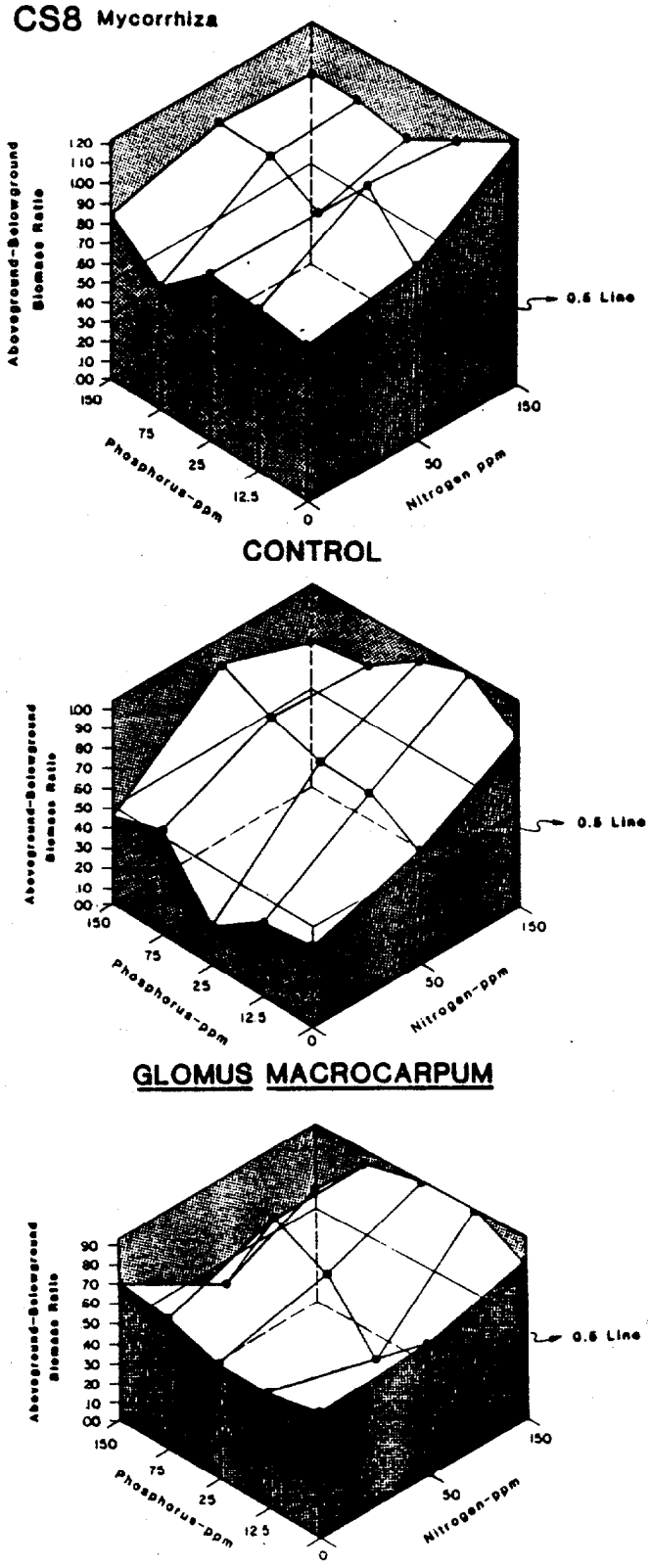



Figure 11. Response surfaces illustrating changes in the aboveground/belowground biomass ratio of the different mycorrhizal groups to N and P fertilization.

Possibly the most interesting experimental result is the observed response of CS8 root infection to the various levels of phosphorus and nitrogen. The percentage of root length colonized of randomly selected aliquots was determined and is presented in Figure 12. The values represent the percentage of the roots which were observed that possessed some form of mycorrhizal structure. The response surface indicates that very little change in the percent of root colonized occurred with respect to treatment and no trends were associated with increasing either nitrogen or phosphorus supply. Infection percentage remained high for all treatments with values ranging from 56.83 where P = 0 and N = 0 to 75.2% where P = 75 and N = 150. Infections recorded in G. macrocarpum roots were significantly lower with generally all values being less than 1%. Although spores were seen in soil and attached to roots, large areas of infected root length were not detected.

Observations of percent mycorrhizal colonization of random root lengths is one method of assessing the amount of concentration of mycorrhizae within a given sample. However, when expressed in this fashion, there is. no indication of the mycorrhizal biomass which may be present in the total root system of a given plant. Therefore in addition to percent colonization, the total length of the samples which were viewed were determined. Hence, the total plant root length colonized by mycorrhizae could be assessed. Results (Figure 13) of total mycorrhizal root length indicate that both increasing nitrogen and phosphorus concentrations resulted in a greater root length being colonized by mycorrhizae. Although the response surface essentially parallels the response surface of root length (Figure 10), it indicates that total mycorrhizal biomass was not reduced by increasing the amount of available nutrients. Infection, by contrast, remained at a constant percentage of the total plant root length which is generally contrary to case observed in most experimental results.

## Discussion

Data are abundant indicating the beneficial effects obtained from mycorrhizal inoculation of agricultural crops and important timber species. However, to date only limited information exists concerning plant response to mycorrhiza and fertilizer additions in overburden soils. The results presented here clearly indicate the dramatic effect which mycorrhizal fungi have on growth of sweetgum. Control plant biomass approached that of mycorrhizal plants only when relatively high concentrations of nitrogen and phosphorus were added. Although both mycorrhizal types resulted in greater growth of sweetgum, it is interesting to note the differences in the degree of response which was obtained. G. macrocarpum was originally selected because it is a readily obtainable native Florida species. Inoculum is also maintained by local mycorrhizal research labs and thus is a species which may be available for field inoculation in Florida. CS8 was utilized because it is an indiginous group occurring in the phosphate district and may possess adaptations to high soil phosphorus concentrations. Single species were not isolated from the composite simply because it was felt that if they existed together within the roots of field plants, then there may be some functional significance to the association. Inoculation with individual species components could have dramatically affected experimental outcome. The overall greater enchancement effect of CS8 over G. macrocarpum may be attributed to many factors. However without further investigations any attempt at explana-

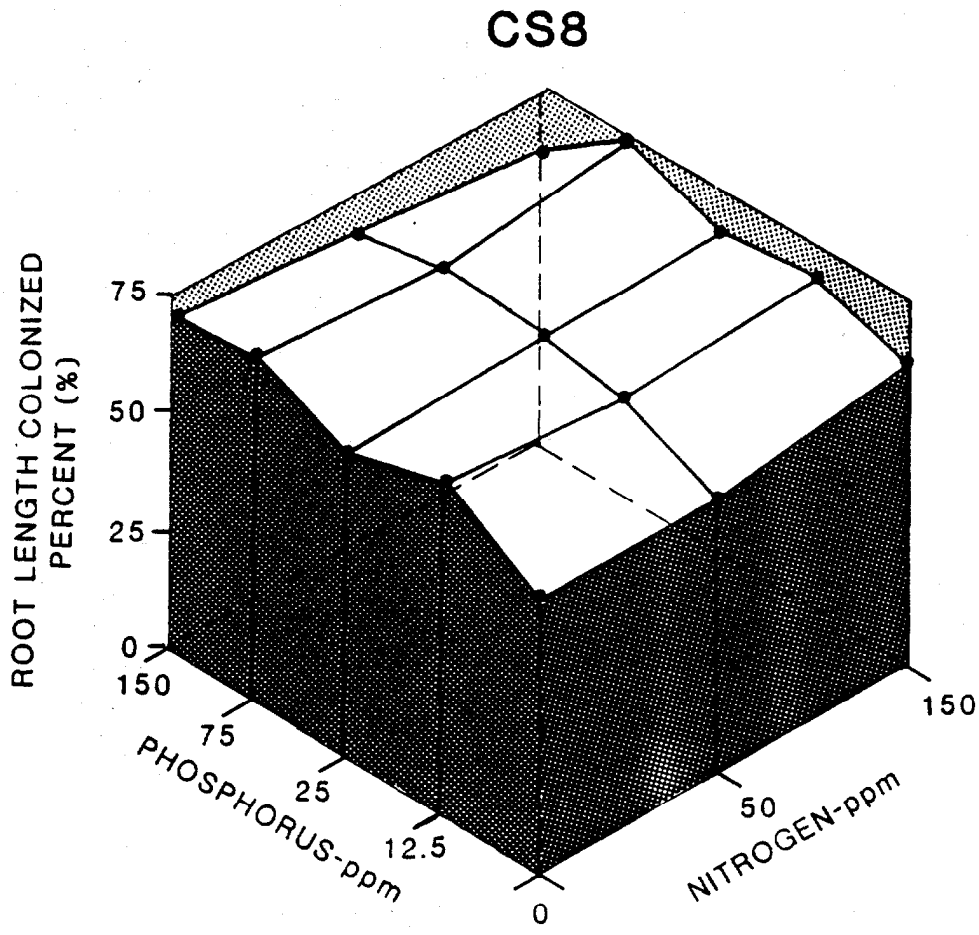



**Figure 12.** **Responses of CS8 mycorrhizae percent root infection to increasing nitrogen and phosphorus concentrations.**

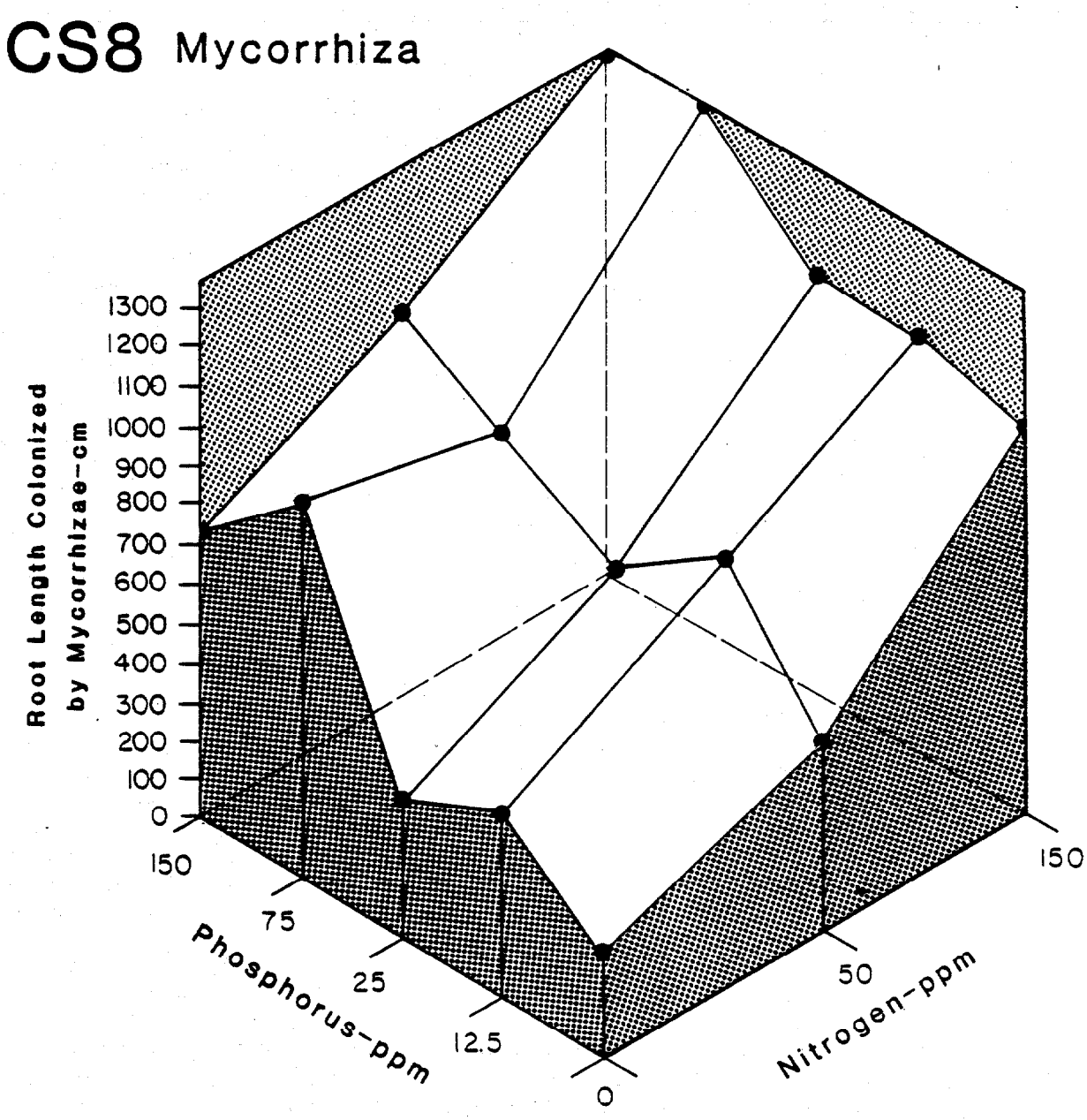



**Figure 13.** Response of CS8 mycorrhizae colonized root length to N and P fertilization. Percent colonization values have been used to calculate total root length colonized in samples of known root length.

tion would merely be speculation. CS8 may in fact be adapted to conditions of high soil phosphorus and function to supply other valuable nutrients needed in plant growth. G. macrocarpum may have been affected by factors such as pH. G. macrocarpum has evolved in soil with comparatively lower phosphorus levels where its function is to supply phosphorus to the host plant. At high P soils the ability of G. macrocarpum to supply the plant with other essential nutrients, e.g., nitrogen, may not be as efficient as that of CS8.

Multispecies inoculation may offer several advantages over use of single species inoculum. First, multiple species being present allows the plants to select for the symbiont or quite probably allow for multiple symbiosis to occur. Numerous studies have shown that a plant may form symbiotic associations with many mycorrhizal species at the same time (Molina et al. 1978). The presence of several different endophytes may be advantageous because each may have variable importance at different life cycles or times during the year (Daft 1983; Daft et al. 1982). Daft (1983) recently offered several criteria describing the properties an ideal endophyte would have to possess to ensure successful field inoculation. These are abilities to (1) infect plants early in the growth period, (2) efficiently exploit the soil, (3) transfer nutrients readily to the host, (4) spread and multiply, (5) compete effectively and (6) infect a wide range of plants under variable environment conditions. He suggests all these characteristics could possibly not be found in a single endophyte, however a multispecies inoculum may be the answer. Utilizing a single species inoculum requires that the researcher, rather than "mother nature," make the selection. Possible drawbacks for this approach are numerous , especially if an indigenous source is desired,. Selecting endophytes from field samples may result in a biased selection of the more common sporulating and/or large-spored species because of ease of isolating. Non-sporulating species may not be seen even though they may possibly be the most abundant and efficient at enhancing plant growth.

Variable results have been obtained from using indigenous versus introduced strains as mycorrhizal inoculum (Khan 1981; Lambert et al. 1980; Powell 1977). The survival of an introduced fungus will be controlled by the ability to adapt to fertility, moisture, and temperature regimes present within the soil. Lambert et al. (1985) suggest that generally introduced species would not improve plant yield in soils with indigenous fungi. They indicate these conditions may change if vegetation, pH, and fertility factors to which the indigenous fungi have adapted are altered. This view is also expressed by Crush (1978) pertaining to inoculation of New Zealand pasture soils. Introduced mycorrhizal fungi species must be adapted to environmental and soil conditions to effect an advantage for plant growth enhancement and survival.

Another result which warrants specific consideration is the effect of nutrients on mycorrhizal root infection. It is not clear why such low (<1%) root infection levels were obtained for G. macrocarpum. Although a growth enhancement over control plants (at low nutrient levels) was recognized, very little colonization was maintained by the plant at any of the various nutrient levels tested. CS8 root colonization followed an extremely different pattern. High levels of colonization were maintained at all nutrient levels and no variation was associated with either increasing or decreasing levels of N or P. Generally it has been shown that increasing P concentrations results in subsequent reduction in mycorrhizal colonization (Mosse 1973; Sanders 1975; Mosse 1973b). Effects of nitrogen on colonization have been less well documented however both decreases (Buwalda and Goh 1981) and increases (Brown et al. 1975) in

mycorrhizal colonization levels have been noted with increasing nitrogen concentrations. Hepper (1983) showed that the extent of the depression in mycorrhizal' colonization at high phosphate levels was dependent upon the ratio of nitrogen to phosphorus concentrations. Increasing amounts of nitrogen tended to increase root colonization at a given phosphate level. Results obtained in this study using the CS8 composite, significantly differ from those found in the above studies.

# CHAPTER 3
# SEED BANKS IN WETLANDS

## Introduction

Reclamation of "high quality" wetland ecosystems is a desirable if not defineable goal of land reclamation efforts. in the phosphate mining industry. It is a problem companies must often wrestle with under the varied and conflicting input from regulatory agencies, consultants, and university researchers. All involved agree with the goal, though few agree on what the best method of reclamation is. Reclamation is a type of ecosystem engineering and should be done using ecological principles. One goal of reclamation should be. to design self-maintaining ecosystems in concert with nature, especially native ecosystems. Ecosystems have evolved mechanisms that allow them to survive naturally occurring disturbances such as fire, flood, and drought. Reclamation goals will be best met if through a better and clearer understanding of recovery and maintenance processes in ecosystems we can enhance reestablishment of nature's self-maintaining systems.

The establishment of vegetation is one of the first stages in primary succession on an abiotic substrate whether it results from geologic uplift, glacial retreat, volcanic lava flow, landslide, or strip mining. The question arises, How can humans best use their energies to enhance the recovery of vegetation? For wetland systems lessons may be learned from the disturbance recovery processes observed in established communities. In several cases, to be discussed below, stored viable seed in wetland soils plays a critical role in the recovery the soil is known as the seed bank.

The functional significance of seed banks lies in providing the plant community with an in situ means of regenerating from naturally occurring disturbances (Grime 1978). Van der Valk (1981) and van der Valk and Davis (1976, 1978) have aptly documented and demonstrated the role seed banks play in the vegetation dynamics of prairie glacial marshes that undergo cyclic patterns of flooding-drawdown-drought. In prairie glacial marshes and other marsh systems (Keddy and Reznicek 1982; Leck and Graveline 1979), seeds remain dormant, yet viable, in the seed bank during periods in which environmental conditions. are unfavorable for germination, growth, and development of the population. Seed banks provide a mechanism for rapid recovery from catastrophic mortality due to fire (Johnson 1975), clear-cutting (Marks 1974), and drought (Myers 1383). Marks (1974) has shown that the rapid response of pin cherry to clear-cutting in the Hubbard Brook ecosystem helped minimize the effect of canopy removal on nutrient losses from the ecosystem.

As an initial step in understanding and using ecological principles to design self-maintaining ecosystems, this study was undertaken to examine seed

bank dynamics in marsh ecosystems. The objectives of the study were (1) to assess the size and species composition of seed banks in selected marsh ecosystems from natural and postmining landscapes, (2) to elucidate ecological role and significance of seed banks in marsh community dynamics, and (3) to evaluate the feasibility of establishing marsh ecosystems by mimicking natural recovery processes such as those known to occur with seed banks.

## Materials and Methods

Seven wetland sites were sampled in the present study (Table 24, Figure 14). At each site one to several major vegetation zones were sampled if the water was not more than 1 m deep, assuring that samples came from the shallow littoral zone-the area of greatest water level fluctuation. Samples were taken with a 5-cm-diameter hand core sampler which was pushed into the substrate to the mineral soil layer. The depth of any overlying organic layer was noted, and only the upper 10 cm of the core was retained. Four individual cores were combined to yield a sample with three samples taken in each vegetation zone selected. Sampling took place over a 3-week period from October 28 to November 18. Samples were stored in sealed plastic bags at 4°C until they were processed in late November.

All live plant material was removed from the samples to prevent any vegetative regeneration from confusing the seed germination results. Once the plant material was removed, the samples were placed in wooden flats (25 cm x 25 cm) containing approximately 4 an of sterilized gravel mixed with tailings sand; the samples were approximately 2 cm deep when spread out evenly in the flats. The flats were then placed outdoors in large plastic tubs containing sufficient water to maintain a saturated soil condition.

Seedling emergence by species was monitored through time. Unidentified seedlings were counted, transplanted to flower pots, and allowed to mature until they could be identified.

## Results

Information gathered from a survey of native and postmining wetlands in central Florida is presented in Tables 25a and 25b. The information contained in these tables is further summarized in Tables 26 and 27. The results are presented in four general areas: seed bank density, species importance values, floristic similarity between samples, and species diversity of samples.

### Seed Bank Densities

Table 26 summarizes the results of seed bank samples and includes values from other seed bank studies from Florida, Iowa, New Jersey, and Ontario. The overall range of seed bank size (density) covers three orders of magnitude with the lowest density of 1877/$m^2$ in the topsoiled (peat mulched), unvegetated

Table 24. Sample locations and site characteristics.

| Site | County | Vegetation Zone | Substrate |
|---|---|---|---|
| *Unreclaimed Mine Area* | | | |
| Sanlan (Clay Settling Area) (30 yr old) | Polk | *Juncus-Polygonum* | Clay |
| | | *Eichhornia* | Clay |
| *Reclaimed Mined Areas* | | | |
| Clear Springs (4 yr old) | Polk | *Polygonum-Ludwigia* | Clay-overburden |
| Fort Green (2 yr old) | Polk | *Pontederia* (Mulched) | Organic muck-overburden |
| | | Open water (Mulched) | Organic muck-overburden |
| | | Open water (Unmulched) | Overburden |
| Four Corners (5 yr old) | Manatee | *Pontederia* (Mulched) | Organic muck-sand |
| | | *Pontederia* (Planted) | Some organic matter-sand |
| | | *Eleocharis* (Control) | Sand |
| | | *Polygonum-Ludwigia* (Swamp) | Some organic matter-sand |
| *Natural Wetlands* | | | |
| Pasture marsh | Polk | *Juncus-Pontederia* | Muck-sand |
| Peace River bayhead | Polk | *Saururus* | Muck-peat-sand |
| Lake Kanapaha* | Alachua | *Amaranthus* | Muck-sand |
| | | *Echinochloa* | Muck-sand |
| | | Open water | Muck-sand |
| Four Corners marsh | Manatee | *Pontederia-Juncus* | Muck-sand |

*Lake Kanapaha not sampled in this study, but results of previous sampling was graciously provided by Dr. Ron Myers.

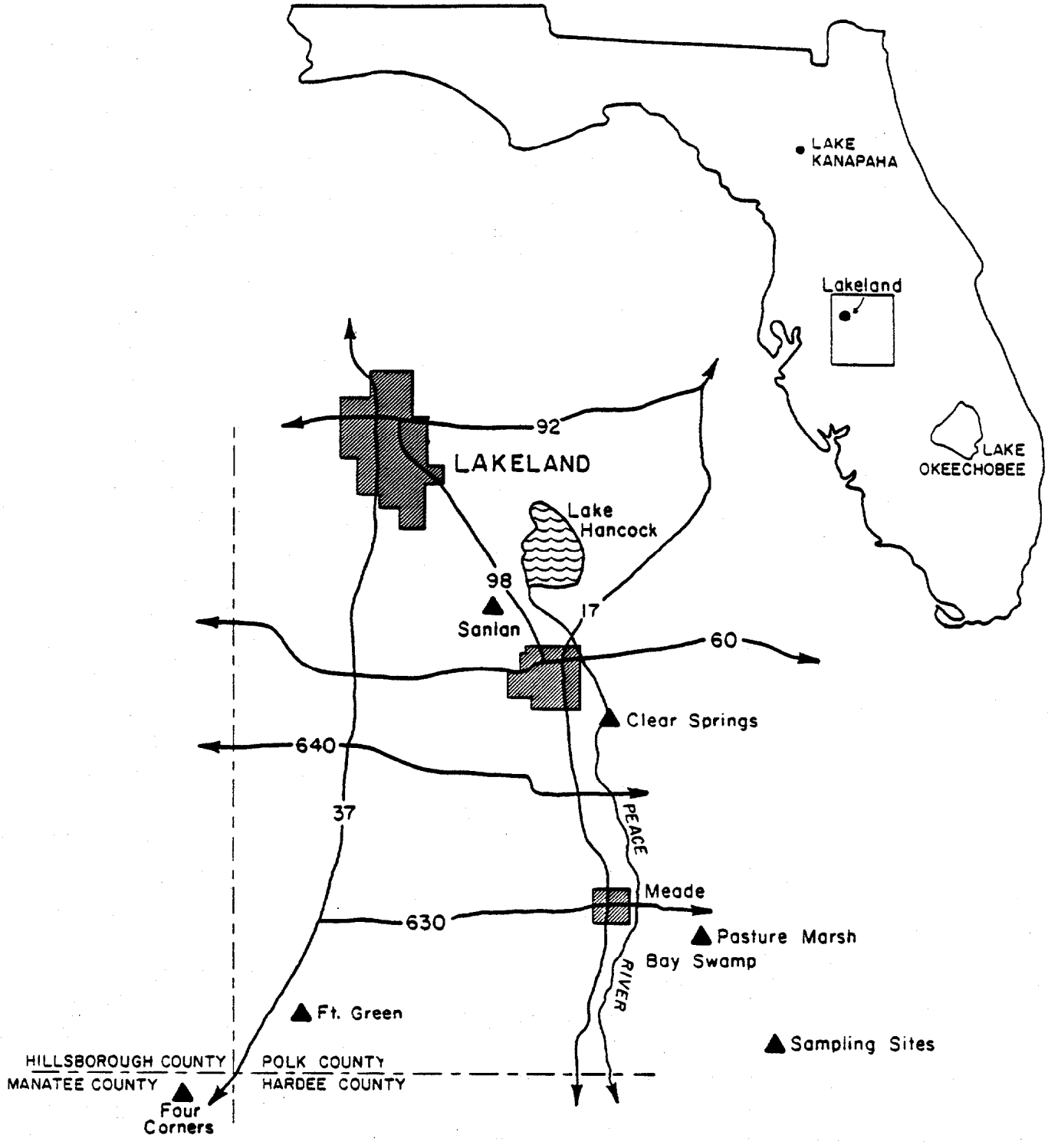



Figure 14. Sampling site location map.

Table 25. Mean number of germinating seeds per $m^2$* by species for natural wetlands and reclaimed and unreclaimed marshes in central Florida phosphate district.

| | Bay Swamp | Four Corners Natural Marsh | Pasture Marsh | Sanlan | | Four Corners | | | | Clear Springs | | | Fort Green | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Juncus-Polygonum | Eichhornia | Topsoiled Marsh | Planted Marsh | Control Marsh | Planted Swamp | South Basin #1 | South Basin #2 | North Basin | Topsoiled, Vegetated | Topsoiled, Unvegetated | Unmulched |
| Aster subulata | --- | --- | --- | --- | --- | --- | --- | --- | --- | 416 | 500 | 210 | --- | --- | --- |
| Baccharis halimifolia | --- | --- | --- | --- | --- | --- | --- | --- | --- | 125 | 42 | 292 | --- | --- | --- |
| Carex sp. | --- | --- | --- | --- | 125 | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Cyperus brevifolius | --- | --- | --- | --- | 416 | --- | --- | --- | --- | 84 | --- | --- | --- | --- | --- |
| Cyperus sp. | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | 915 | 334 | 960 |
| Cyperus rotundus | 84 | --- | --- | 416 | 3,750 | 166 | --- | --- | 500 | 1,666 | 4,125 | 1,500 | --- | --- | --- |
| Cyperaceae ? | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | 84 | --- | --- | --- |
| Echinochloa walteri | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | 125 |
| Eclipta alba | --- | --- | --- | 125 | --- | --- | --- | --- | --- | 250 | 541 | 42 | --- | --- | --- |
| Eupatorium compositifolium | 84 | --- | --- | 541 | --- | --- | --- | 125 | 46 | 125 | 250 | 375 | --- | 42 | 84 |
| Graphalium obtusifolium | --- | --- | --- | --- | --- | --- | --- | --- | --- | 84 | --- | --- | --- | --- | --- |
| Grasses, unknown #1 | --- | --- | 42 | --- | --- | --- | --- | --- | 125 | --- | --- | --- | --- | --- | --- |
| #2 | --- | --- | --- | --- | --- | --- | --- | --- | 42 | --- | --- | --- | --- | --- | --- |
| #3 | --- | --- | --- | --- | --- | --- | 84 | --- | --- | --- | --- | --- | --- | --- | --- |
| #4 | 42 | --- | --- | --- | --- | --- | --- | --- | --- | --- | 250 | 125 | --- | --- | --- |
| #5 | --- | --- | --- | --- | --- | --- | --- | --- | --- | 1,625 | --- | --- | --- | --- | --- |
| #6 | --- | 42 | 84 | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Hydrocotyle verticillata | 42 | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Hypericum mutilum | 42 | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | 166 | --- | --- | --- |
| Juncus effusus | 292 | 67,210 | 40,832 | 58,625 | 7,625 | 32,750 | 31,416 | 1,500 | 10,625 | 1,875 | 1,916 | 1,460 | 209 | 42 | 1,210 |
| Juncus bufonius | 125 | --- | --- | 42 | --- | 42 | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Ludwigia virgata | 292 | --- | --- | --- | --- | --- | --- | --- | --- | 500 | 416 | 2,666 | 1,834 | 1,375 | 1,416 |
| Ludwigia palustris | 334 | --- | --- | --- | --- | --- | --- | --- | --- | 42 | --- | --- | --- | --- | --- |
| Ludwigia leptocarpa | --- | --- | --- | --- | --- | --- | --- | --- | --- | 84 | 210 | --- | --- | --- | --- |
| Polygonum punctatum | --- | 5,250 | 292 | 2,460 | 125 | 42 | 126 | --- | 42 | 42 | 125 | 84 | --- | --- | --- |
| Ptilimnium capillaceum | --- | --- | --- | --- | --- | --- | --- | --- | --- | 42 | 1,042 | 84 | --- | --- | --- |
| Rumex verticillatus | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | 42 | --- | --- | --- | --- |
| Samolus parviflorus | 250 | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | 42 | --- | --- | --- |
| Scrophulariaceae ? | --- | --- | --- | --- | --- | --- | --- | --- | --- | 42 | --- | --- | --- | --- | --- |
| Stellaria media | --- | --- | --- | --- | --- | --- | --- | 334 | --- | --- | --- | --- | --- | --- | --- |
| Unknown species #1 | --- | --- | --- | --- | --- | --- | --- | --- | --- | 375 | 1,834 | 2,750 | 375 | --- | --- |
| #2 | --- | --- | --- | --- | --- | --- | 84 | 250 | 42 | --- | --- | --- | --- | --- | --- |
| #3 | 2,541 | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | 84 | 125 |
| Mean # seeds/m2 | 4,125 | 72,502 | 41,250 | 62,250 | 12,040 | 33,000 | 31,710 | 2,210 | 11,460 | 7,375 | 11,300 | 9,880 | 3,334 | 1,877 | 3,920 |
| # species | 11 | 3 | 4 | 6 | 5 | 4 | 4 | 4 | 7 | 16 | 13 | 14 | 4 | 5 | 6 |

*Results from core samples 10 cm deep with actual area sampled corrected to standard reference area of 1 $m^2$.

Table 25b. Seed bank density data from Table 25a summarized across sites for species totals of density, relative density, frequency, relative frequency, and importance value.

| | Density Total (mean #/$m^2$) | % Relative Density | Sampling Site Frequency | % Relative Frequency | Importance Value |
|---|---|---|---|---|---|
| Aster subulata | 1,126 | 0.36 | 0.20 | 2.80 | 3.16 |
| Baccharis halimifolia | 459 | 0.15 | 0.20 | 2.80 | 2.95 |
| Carex sp. | 125 | 0.04 | 0.07 | 0.95 | 0.99 |
| Cyperus brevifolius | 500 | 0.16 | 0.13 | 1.90 | 2.06 |
| Cyperus sp. | 2,209 | 0.70 | 0.20 | 2.80 | 3.50 |
| Cyperus rotundus | 12,207 | 4.00 | 0.53 | 7.50 | 11.50 |
| Cyperaceae ? | 84 | 0.03 | 0.07 | 0.95 | 0.98 |
| Echinochloa walteri | 125 | 0.04 | 0.07 | 0.95 | 0.99 |
| Eclipta alba | 958 | 0.30 | 0.27 | 3.80 | 4.10 |
| Eupatorium compositifolium | 1,672 | 0.50 | 0.60 | 8.50 | 9.00 |
| Graphalium obtusifolium | 84 | 0.03 | 0.07 | 0.95 | 0.98 |
| Grasses, unknown #1 | 167 | 0.05 | 0.13 | 1.90 | 1.95 |
| Grasses, unknown #2 | 42 | 0.02 | 0.07 | 0.95 | 0.97 |
| Grasses, unknown #3 | 84 | 0.03 | 0.07 | 0.95 | 0.98 |
| Grasses, unknown #4 | 417 | 0.13 | 0.20 | 2.80 | 2.93 |
| Grasses, unknown #5 | 1,625 | 0.50 | 0.07 | 0.95 | 1.45 |
| Grasses, unknown #6 | 126 | 0.04 | 0.13 | 1.90 | 1.94 |
| Hydrocotyle verticillata | 42 | 0.02 | 0.07 | 0.95 | 0.96 |
| Hypericum mutilum | 206 | 0.07 | 0.13 | 1.90 | 1.97 |
| Juncus effusus | 257,587 | 84.00 | 1.00 | 14.00 | 98.00 |
| Juncus bufonius | 209 | 0.07 | 0.20 | 2.80 | 2.87 |
| Ludwigia virgata | 8,499 | 3.00 | 0.47 | 6.60 | 9.60 |
| Ludwigia palustris | 376 | 0.12 | 0.13 | 1.90 | 2.00 |
| Ludwigia leptocarpa | 294 | 0.09 | 0.13 | 1.90 | 2.00 |
| Polygonum punctatum | 8,588 | 3.00 | 0.67 | 9.50 | 12.50 |
| Ptilimnium capillaceum | 1,168 | 0.40 | 0.20 | 2.80 | 3.20 |
| Rumex verticillatus | 42 | 0.02 | 0.07 | 0.95 | 0.97 |
| Samolus parviflorus | 292 | 0.09 | 0.13 | 1.90 | 2.00 |
| Scrophulariaceae ? | 42 | 0.02 | 0.07 | 0.95 | 0.95 |
| Stellaria media | 334 | 0.10 | 0.07 | 0.95 | 1.00 |
| Unknown species #1 | 5,334 | 1.70 | 0.27 | 4.00 | 5.50 |
| #2 | 376 | 0.10 | 0.20 | 2.80 | 2.90 |
| #3 | 2,750 | 0.90 | 0.20 | 2.80 | 3.70 |
| Column total | 308,000 | 100.00 | 7.07 | 100.00 | 200.00 |

Table 26. Seed bank densities, species richness, and Shannon-Weaver diversity index from Florida wetlands and selected marsh studies from temperate North America.

| | Mean # seeds/$m^2$ | Number of Species | Shannon-Weaver Diversity, H' | Source |
|---|---|---|---|---|
| Natural Systems, Florida | | | | |
| Bay Swamp | 4,125 | 12 | 1.45 | This study |
| Lake Kanapaha | | | | |
| *Sacciolepis* zone | 156,000 | 38 | 2.64 | (11) |
| *Amaranthus* zone | 28,000 | 17 | 1.72 | (11) |
| *Echinochloa* zone | 30,000 | 13 | 0.98 | (11) |
| Pond zone | 9,000 | 8 | 1.17 | (11) |
| Four Corners Marsh | | | | |
| *Juncus-Pontederia* zone | 72,502 | 3 | 0.06 | This study |
| Pasture Marsh | | | | |
| *Juncus-Pontederia* zone | 41,250 | 4 | 0.26 | This study |
| Unreclaimed Systems | | | | |
| Sanlan | | | | |
| *Juncus-Polygonum* Marsh | 62,250 | 6 | 0.30 | This study |
| *Eichhornia* Marsh | 12,040 | 5 | 0.86 | This study |
| Reclaimed Systems | | | | |
| Four Corners Reclamation Project | | | | |
| Mulched plot | 33,000 | 4 | 0.05 | This study |
| Planted plot | 31,710 | 4 | 0.05 | This study |
| Control plot | 2,210 | 4 | 0.95 | This study |
| Planted swamp plot | 11,460 | 4 | 0.30 | This study |
| Clear Springs Reclamation Project | | | | |
| South Basin #1 | 7,375 | 16 | 2.03 | This study |
| South Basin #2 | 11,300 | 13 | 1.92 | This study |
| North Basin | 9,880 | 14 | 1.88 | This study |
| Fort Green Reclamation Project | | | | |
| Mulched, vegetated | 3,334 | 4 | 1.11 | This study |
| Mulched, unvegetated | 1,877 | 5 | 0.84 | This study |
| Unmulched | 3,920 | 6 | 1.38 | This study |
| Other Natural Systems | | | | |
| Iowa, Prairie glacial marsh | 20–40,000 | 7–16 | Not calculated | (3, 5) |
| Ontario, Lakeshore marsh | 9–20,000 | 31 | Not calculated | (7) |
| New Jersey, Freshwater tidal marsh | 6–32,000 | 12–20 | Not calculated | (8) |

Table 27. Twenty species with highest imortance values along with the two components used to calculate the IV. All data taken from Tables 25a and 25b.

| Species | % Relative Density | % Relative Frequency | Importance Value |
|---|---|---|---|
| Juncus effusus | 84.00 | 14.00 | 98.00 |
| Polygonum punctatum | 3.00 | 9.50 | 12.50 |
| Cyperus rotundus | 4.00 | 7.50 | 11.50 |
| Ludwigia virgata | 3.00 | 6.60 | 9.60 |
| Eupatorium compositifolium | 0.50 | 8.50 | 9.00 |
| Unknown 1 | 1.70 | 3.80 | 5.50 |
| Eclipta alba | 0.30 | 3.80 | 4.10 |
| Unknown 3 | 0.90 | 2.80 | 3.70 |
| Cyperus sp. | 0.70 | 2.80 | 3.50 |
| Ptilimnium capillaceum | 0.40 | 2.80 | 3.20 |
| Aster subulata | 0.36 | 2.80 | 3.16 |
| Baccharis halimifolia | 0.15 | 2.80 | 2.95 |
| Grass 4 | 0.13 | 2.80 | 2.93 |
| Unknown 2 | 0.10 | 2.80 | 2.90 |
| Juncus bufonius | 0.07 | 2.80 | 2.87 |
| Cyperus brevifolius | 0.16 | 1.90 | 2.06 |
| Ludwigia palustris | 0.12 | 1.90 | 2.02 |
| Ludwigia leptocarpa | 0.09 | 1.90 | 1.99 |
| Samolus parviflorus | 0.09 | 1.90 | 1.99 |
| Hypericum mutilum | 0.07 | 1.90 | 1.97 |
| | | | 185.00 |

sample from Fort Green project and the high value of 156,000/$m^2$ from the Sacciolepis striata zone at Lake Kanapaha, Florida.

The range for natural wetlands samples is 4,000-156,000 seeds/$m^2$ with the lowest value caning from bay swamp (incidentally the only forested wetland sample) and the high value again for the Sacciolepis zone at Lake Kanapaha. A trend evident in the results from studies at Lake Kanapaha is that the species richness and size of the seed bank appears to decrease as the water depth increases in the Sacciolepis zone-Amaranthus zone-Echinochloa zone-Pond zone. For the wetland samples cited from outside Florida the densities range from 6,000 to 40,000, and for the three natural systems sampled in this study the range of densities is 8,000-72,000. The two marsh samples (Four Corners natural marsh and pasture marsh) had densities of 41,000/$m^2$ and 72,500/$m^2$, respectively.

The unreclaimed wetland sampled-the Sanlan sample-had densities of 12,000/$m^2$ and 62,000/$m^2$ from the Eichhornia and Juncus marshes, respectively. Interestingly, here as with the Kanapaha samples seed bank size apparently decreases with depth, the water depth being over a meter in the Eichhornia marsh.

The Sanlan samples fall in the range of the natural wetlands already discussed, especially the 62,000/$m^2$, which represents one of the higher densities encountered and indicates that sizeable seed banks can develop in absence of any reclamation efforts in postmining wetlands.

Wetland samples from reclaimed mine lands had a range of 1,800 to 33,000/$m^2$, which is low to moderate by comparison to natural wetland systems Samples from the three basins at Clear Springs ranged from 7,000 to 11,000/$m^2$, while at Four Corners project the range was 2,200-33,000. More specifically the treated plots had densities well within the range of the natural systems already discussed; topsoiled (peat) marsh plot (33,000), planted marsh plot (31,000), and swamp planted plot (11,300). The lowest density found at the Four Corners project came from the control plot (2,200), indicating that seed bank establishment is facilitated by reclamation efforts.

Finally, samples from the Fort Green project had the lowest and narrowest range of densities 1,800-3,900/$m^2$, but it should be remembered that this project is only in its second growing season. Surprisingly the lowest density value from Fort Green and for all samples, came from an unvegetated topsoiled (peat) area with open water. It may be due to the vagaries of sampling, or it may be that the seed bank in the peat at this spot is either dominated by (1) short-lived seeds or species that only germinate under flooded conditions (which were not duplicated in this study), or (2) that the topsoil material (peat) had been stockpiled (as is known to have occurred with some peat material at this site).

## Species Importance Values

As an estimate of the overall influence or importance of the species in the seed bank survey, a modified importance value was calculated using the density and frequency totals for each species (Table 25b). The importance value is

calculated by adding relative density and relative frequency for each species, where relative density is defined as the density of the species divided by the sum of all densities and where relative frequency is defined as the frequency of occurrence of the species divided by the sum of all species frequencies, Both relative density and frequency were connected. When calculated this way both relative frequency and relative density are constrained to values between 0 and 100% and therefore the importance value of each species takes on a value in the range 0 to 200. The most striking aspect of all these calculations is the numerical dominance of Juncus effusus, which accounted for 84% of germinating seeds in this study, and in addition to its numerical dominance it was the only species found in all samples, yielding an absolute frequency of 1.0.

The 20 species of highest importance value are listed in Table 27; the 20 account for 185 out of the total importance value of 200. In fact, the four species of highest importance value (Juncus effusus, Polygonum punctatum, Cyperus rotundus, and Ludwigia virgata) account for 94% of the relative density and 132 of the total importance value. These four species can be considered the dominant species so far in this study and can serve in general to characterize the seed banks sampled from central Florida.

## Floristic Similarity

A measure of floristic similarity of seed bank samples was calculated using the similarity index of Czekanowski for binary data. The index is defined as follows:

$$\text{Czekanowski's index} = 2a/(2a + b + c)$$

where a = species common to sites 1 and 2, b = species found at site 1 but absent at site 2, and c = species found at site 2 but absent at site 1. The index has a range of 0-1.0, where 0 represents no similarity and 1.0 represents complete similarity.

There were few cases of high floristic similarity (Figure 15). One was a comparison between the two natural marshes sampled, another was the comparison between the Sanlan-Juncus marsh and the Four Corners mulched plot. These two cases are both comparisons of samples with low species richness. The other cases of high floristic similarity are within site sample comparisons, one from Clear Springs and one from Fort Green.

The Clear Springs samples had the largest number of species and had moderately high to high within-site floristic similarity. The species assemblage at Clear Springs had several unique or less frequently encountered species including: Aster subulata, Baccharis halimifolia, Eclipta alba, and Ptilimnium capillaceum. The samples from Fort Green also exhibited moderately high to high within-site floristic similarity, largely due to three species (Juncus effusus, Ludwigia virgata, and a species of Cyperus).

Figure 2 also shows many comparisons of low to moderate similarity largely due to the near ubiquity of Juncus effusus and Polygonum punctatum in all samples.

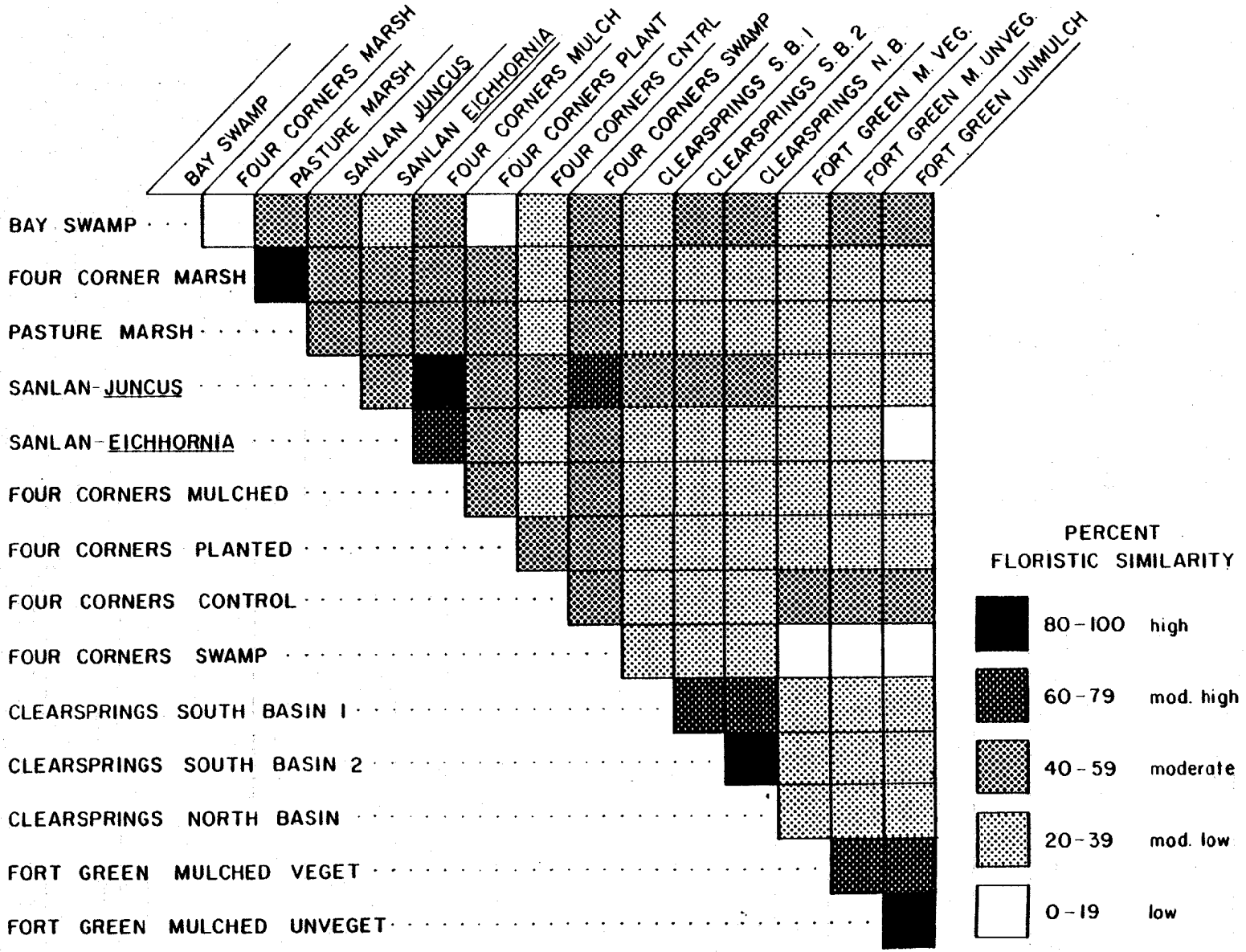



Figure 89. Summary matrix of floristic similarity.

## Species Diversity

Species richness and species diversity were compiled for data from this study and Lake Kanapaha (Table 26). Diversity was calculated using the Shannon-Weaver diversity index, given as H' and defined as

$$H' = \sum - P_i \ln P_i$$

where Pi is the ratio of number of individuals of the ith species divided by the total number of individuals in the sample. The value of H' is influenced by two factors: the number of species, known as species richness, and the equitability with which the individuals of the population are apportioned among the species. The greater the species richness and/or the equitability the greater the value of H'.

The overall range of H' values was 0.05-2.64, the lower value is from the mulched, vegetated plot at Fort Green while the highest value was from the Sacciolepis-zone at Lake Kanapaha. This Kanapaha sample also had the highest seed density (156,000/ $m^2$) and the highest species richness (38). As can be seen in Table 26, the samples with the greater number of species typically had H' values in the upper range. The most diverse samples from natural wetlands came from Lake Kanapaha and the bay swamp, while the highest diversity in the mined wetlands group was in the Clear Springs samples. In several cases (Four Corners natural marsh, pasture marsh, Four Corners mulched plot, Four Corners planted plot, and Sanlan-Juncus-zone) the seed bank had relatively few species and was dominated numerically by one species in particular-Juncus effusus. This situation more or less defined the low end of the H' range.

## Discussion

Seed bank samples from all but the youngest sites in the postmining landscape fall within or just below the range of densities and species diversity found in natural wetlands of Florida, Iowa, New Jersey, and Ontario. The indications from the results in this study are that it is possible for nature to reestablish a seed bank of approximately the same size and diversity as that occurring in some natural marshes such as with the Juncus-Polygonum marsh at Sanlan (30 year old). The time it takes for the seed bank to reach the point of being a "reasonable facsimile" to that of a natural marsh is an open question. If Clear Springs is any indication then modest sized seed banks with higher diversity can develop in 4 years with little actual marsh reclamation, and with some reclamation efforts seed banks that compare very favorably in size with natural marshes can develop in 5 years as demonstrated at Four Corners.

It appears that in some cases the seed banks in some of the postmining wetlands are not all that different in size and species composition from the natural marshes sampled in this study, but the actual vegetation present is not always as diverse, dense, or well developed except in cases where "mulch" (topsoil) from a donor wetland was applied. As an example, in line intercept transects run by the authors in mulched and unmulched areas of the marsh at Fort Green it was found that the mulched areas had almost 100% cover, while the unmulched areas had less than 30% cover. Scanning the list of species in Table

25 it is striking to see the kinds of species represented in these seed bank samples. They are a mixture of annuals and perennials that are for the most part found on exposed mudflats, wetland transition zones, and shallow littoral zones. The list is notably bereft of emergent macrophytes, submergent macrophytes, and free-floating aquatic species. This may in part be due to the germination conditions, which were similar to that of an exposed mudflat. The species present are largely the wetland ruderal species characteristic of environments subject to disturbance such as water level fluctuations. They are the annuals and short-lived perennials of mudflats and gravel bars (Grime and Hunt 1975).

This begs the question that if Pontederia cordata, Sagittaria lancifolia, Nuphar luteum, and Nymphaea odorata were present in at least a few of the wetlands sampled, why were none of their seeds detected in this assay? These species are the long-lived perennials of freshwater marshes-the late successional marsh species. It may be beneficial to examine the species present versus those conspicuous by their absence in terms of successional status and life history characteristics of the adult established phase and juvenile regenerative phase. Persistent seed banks represent only one of three regenerative strategies used by wetland plants. The other two are vegetative expansion and production of numerous wind-dispersed seeds. It may be that regenerative strategies are related to successional status of the individual species. Generally speaking vegetative expansion is successful in situations where the adult plant is already established especially in the case of long-lived perennials where vegetative expansion allows for rapid colonization of open space under conditions unconducive to seed germination. In habitats of chronic but unpredictable disturbance (fire, flood, drought) where most of the vegetative structure is destroyed, the persistent seed bank is the primary regenerative mechanism. The third regenerative strategy employed by wetland plants is the production of numerous wind-dispersed seeds (Typha, Salix, and Baccharis). Ultimately the key is seed source, even for species whose primary regenerative strategy is vegetative, the production of viable seed represents a critically essential component of the total regenerative strategy.

The pertinence of considering life history characteristics and regenerative strategies is supported by a qualitative model of freshwater marsh succession proposed by van der Valk (1981) in which the plant community response to climatic cycles of flood and drought is analyzed throuqh the life history characteristics of the species found to reside in the seed bank. The model depicts the plant community as the result of prevailing environmental conditions "screening" species from the seed bank. The life history characteristics considered in the model include: propagule longevity (short-lived dispersing or long-lived seed bank forming), life span of the established plant (annual, perennial, etc.), propagule establishment requirements (germination under drawdown or flooded conditions). It is the contention of van der Valk (1981) that once the composition of the seed bank is known the course of successional response to environmental changes, such as water level fluctuations, can be predicted by a knowledge of the species-specific life history characteristics. A wet land whose seed bank contains species representative of all regenerative strategies and life history characteristics is well buffered against all disturbance regimes. It is this type of wetland we should try to establish in the postmining landscape through the use of seeding, mulching, and planting.

What are the implications of this consideration of successional status and regenerative strategy for vegetation management and wetlands restoration? We are trying to make the case for a closer melding of wetlands ecology and reclamation with the goal of restoring stable, self-maintaining marsh systems in the post-mining landscape. Studies of wetlands on unreclaimed lands have documented the paucity of the more "desirable climax" species in both marshes (Clewell 1981) and swamps (Clewell 1981; Rushton 1983). These studies describe arrested succession in which the initial floristic composition, sensu Egler (1954), of primary invading species is perpetuated. The question remains to be answered whether the succession is arrested due to the inability of the later successional species to (1) arrive on abandoned sites or (2) to arrive and find adequate resources available for germination, growth, and establishment. Initial colonizing species, once established, may be able to resist invasion by other species, but as suggested by Egler (1954) the ability of established plants to resist invasion may be independent of the position of species in the normal sequence of community development. Consequently, it may be feasible to establish self-maintaining, stable marsh communities dominated by desirable late successional species and able to resist invasion by aggressive weedy species such as Typha. The key will be in creating wetlands well buffered against the disturbance regime typically encountered in marshes--fire, flood, drought. As already mentioned, a well-buffered system has representatives of all successional stages, regenerative strategies, and life histories. For reclamation the emphasis should be on two goals: establishing those components that have been found to be lacking in unreclaimed systems--the late successional long-lived perennials--and control of aggressive weed species capable of arresting succession such as Typha. (To date topsoiling [peat mulching] experiments using soil/sediment from donor marshes has been successful in both of these areas.) Topsoiling, may not be feasible in all cases due to quality of donor material or budgetary constraints of transporting the material. In such situations a combination of seed bank establishment through direct seeding coupled with transplant of an array of long-lived perennials might accomplish the two goals.

In final analysis, seed banks are not a panacea for restoration of native marsh systems, but are critical components of stable, self-maintaining ecosystems. The persistent seed bank is one of the regenerative strategies by which wetlands respond to disturbance. This capacity needs to be established either through seeding or application of topsoil (peat mulch).

# CHAPTER 4
# GROWTH, DENSITY, AND SPECIES RICHNESS OF FOREST COMMUNITY MICROPLOTS ESTABLISHED FROM SEED ON AMENDED OVERBURDEN SOILS

## Introduction

The primary goal of this research project is to accumulate data necessary to develop practical (e.g., cost effective and applicable on a large scale) methodology and technology for reclaiming phosphate mined lands to native forested ecological systems as one viable reclamation alternative. The main thrust of the project is towards whole ecosystem reconstruction--that is, reconstruction of wetland, transitional, and terrestrial plant communities--through enhancing ecological succession (Dunn and Best 1983a, 1983b; Wallace and Best 1983a, 1983b; Best et al. 1983). To achieve this goal research is directed towards identifying components essential for enhancing development of native forest ecosystems. Certainly, it could correctly be argued that almost all components in ecosystems are essential. However, is it possible that there may be some components more important or essential in the actual establishment phase of ecosystems with the additional components primarily functioning in long-term ecosystem maintenance? Research focused on four "essential" components that may enhance reclamation to native forest ecosystems. They were (1) seeds (multi-species mixture), (2) mycorrhizal fungi symbionts, (3) soil nutrients, and (4) organic matter. Techniques for enhancing plant community succession tested through this project and related research included direct seeding of late-successional and climax trees coupled with establishing the symbiotic micro-floral component (mycorrhizal fungi) of the soil ecosystem necessary for more rapid growth and development of woody plants.

Direct seeding offers several distinct advantages over conventional means of tree establishment. Capital and energy invested in reclamation through direct seeding should be less than for more conventional revegetation approaches, albeit at the expense, perhaps, of a greater investment of seed resources (especially since the success of seed-to-seedling establishment will probably be less in direct seeding). In multispecies seeding several plant species have the opportunity to exploit the environmental conditions of the planting zone. In other words, the microenvironment of the planted zone coupled with the environmental tolerances of the germinating seedling of each species determines initial establishment and ultimate survivorship of plants. Conversely, when seedlings are direct planted, the reclamation planner and ultimately the person planting the seedlings, are predetermining the microenvironmental setting under which the species and individual plant are expected to survive or not survive. Disadvantages to direct seeding do exist. Seed use is less efficient since the number of propagules established is generally lower in direct seeding than in seed-to-seedling development in a nursery operation. Plus the technology presently does not exist to efficiently and economically plant seed mixtures with widely vary-

ing seed sizes, especially seed planting concomitant with mycorrhizal fungi inoculation. However, the technology is currently being developed (Best and Odum 1980). Finally, large quantities of seed necessary for large-scale reclamation are currently not available. Will the seed producing technology develop if the technology is developed for efficiently planting and establishing multispecies forest through direct seeding?

Goals of this microplot experiment were to assess germination (propagule establishment), survivorship, growth, density, and species richness of tree seeds as a function of the following soil amendments on phosphate mined overburden: (1) vesicular-arbuscular fungi (Glomus mosseae and Glomus occultum) inoculum; (2) ectomycorrhizal fungi (Pisolithus tinctorius) inoculum; fertilizer (15-0-15); (4) soil surface organic straw mulch); (5) soil amendment (phosphogypsum); and (6) topsoil.

## Literature Review

Ecosystem succession on mined land has been noted by several authors. Breedlove and Adams (1977) discussed the effectiveness of the successional process in revegetation of some mined lands. Humphrey (1979) and Kangas (1983) describe the aesthetically pleasing ecosystems that had developed on spoil mounds that were simply abandoned after mining. Although only 50-60 yr had passed, the mounds resembled a mesic oak forest. However, that was when mined tracks of land were smaller, allowing for more rapid and complete encroachment of organisms from the surrounding natural communities. Natural encroachment of the necessary components of forest communities has probably been hampered by the greater distances involved in the current large-scale mining activities of the phosphate industry in Florida such that now there are problems with simply abandoning mined lands. One of these problems is time. On less-than-ideal sites and sites where natural encroachment by forest plants is hampered by distance, compounding the time problem, initial plant establishment can be slow, resulting in a low percent cover even after several years. In some cases very persistent scrub communities, dominated by wax myrtle (Myrica cerifera) (Schnoes and Humphrey 1980) or vines (Kangas 1983; McGee and Cooper 1979) gain a foothold during an early successional stage. Often these pioneer early-successional species become so well established before seeds from the later, more mature forest species (i.e., climax forest species) are disseminated to the area that germination, growth, and establishment of these climax species are greatly reduced.

There are several approaches to enhancing establishment of "desirable" woody shrubs and trees. A common silvicultural technique used in forestry is planting seedlings (Anon. 1980). This technique is generally adapted to planting monospecific forests for enhancing growth and production of wood resources. One current project in reclaiming phosphate mined lands has used this technique at the multispecies level (Gilbert et al. 1979). Direct seeding is also an alternative revegetation method (Abbott 1973; Brown 1973; Davidson 1980; Mann 1968; Plass 1976). A seed mixture composed of several species has been used successfully in enhancing the reestablishment of natural communities in oil shale-mined test plots in Colorado (Braun and Best 1975). Direct seeding offers distinct advantages over other revegetation methods in that it is applicable to

large-scale reclamation at reasonable costs and effort levels (Vogel 1980). One current disadvantage, at least in the Southeast, is seed source and availability. However, as has been demonstrated in central and western United States where state and federal regulations place an emphasis on restoring diverse natural communities, considerable attention is being paid to developing technology needed for adequately supplying a seed source for native plants. Johnson (1980), in his opening address to the symposium on "Trees for Reclamation" placed "increasing the production of high quality seed . . . with emphasis on native species" (p. 2) as an area where "research must 'be strengthened." The above mentioned revegetation methods coupled with additional methods--combinations and variations-are certainly functional and viable reclamation alternatives (Thronson 1975). Each revegetation alternative provides a unique set of advantages and disadvantages that can be assessed in developing the most functional reclamation plan for a specific area.

"Direct seeding, an attractive alternative to planting, is not a simple method of reforestation" (Davidson 1980). Early reports of reclamation to forest indicate that direct seeding has been attempted in many regions as an alternative to tree planting. However, most of the earlier attempts were direct seeding of a single species. Schavilji (1941) reported good first-year germination and survival of black walnut (Juglans nigra) with an average seedling height of 12 inches. However, others Davidson 1980; Vogel 1980; May et al. 1973) have reported only moderate to poor success in direct seeding of single species. Other recent experiments include direct seeding of loblolly (Pinus taeda) shortleaf (P. echinata), Virginia (P. virginiana), and white (P. strobus) pine (Zarger et al. 1973). First-year results were moderately successful with some species performing better than others. Plass (1976), in a test using multispecies seed mixtures, was able to select a combination of several compatible species of herbs and trees for direct seeding. Identification of the promising species resulted from a species evaluation trial in which 34 species of trees, shrubs, and herbaceous plants were hydroseeded on five sites. Others have developed seed handling methods (Forest Service 1974), collection techniques (Matusz 1964), and planting strategies for specific environmental conditions (Fish and Wildlife Service 1978).

The concept of enhanced ecological succession of devastated lands implies man's manipulation of natural processes. Ashby et al. (1980) noted that some formerly mined lands in Illinois, though planted successfully with several species of trees 30 years earlier, had a greater number of pioneer trees than planted trees suggesting that total reclamation involves more than simply revegetating the surface (a problem being addressed within the goals of this research project). In order to develop a "holistic approach to natural ecosystem reconstruction" an understanding of both macrocomponents and microcomponents is mandatory (Richardson 1980). Most recent studies of revegetation have examined only aboveground components of the plant community while completely ignoring belowground microbial participation. One very important microbial entity warranting special attention is mycorrhizal fungi. Termed by Hacskaylo (1967) as "indispensable invasions by fungi," mycorrhizae have been proven to be essential for survival of most plants. Few studies of the importance of mycorrhizae in reclamation of phosphate mined lands in Florida have been conducted although the significance of mycorrhizae in revegetation of disturbed lands is well documented in other regions (Daft and Hacskaylo 1977; Marx 1975; Monk 1977). Reeves et al. (1979) stated that "the reestablishment and maintenance of the mycorrhizal fungus component is vital in producing stable plant ecosystems on

disturbed areas." It is imperative that successful reclamation studies include examinations of the mycorrhizal fungi that could possibly be used for inoculation of native forest species. (For a more complete discussion on the role of mycorrhizae in reclamation see Wallace and Best [1983b].)

## Methods

The statistical design for the microplot experiment is a $2^6$ factorial design, resulting in 64 different treatment cells. This design is excellent-when the goal is to screen direct and possible interactive effects of a large number of parameters. The six treatments were (1) inoculation with an endomycorrhizal fungus; (2) inoculation with an ectomycorrhizal fungus; (3) application of straw mulch; (4) fertilizing; (5) soil amendment with gypsum; and (6) topsoiling. Application rates for the treatments are presented in Table 1. The "topsoil treatment" serves a dual purpose. First, topsoil is a reclamation alternative worth testing in its own right. Second, the topsoil treatments can serve as a control to which the other soil amendment parameters can be compared.

The microplots are 50 x 50-cm cells 75 cm deep constructed from plywood. Prior to planting microplots were filled with strip mined overburden soil supplied by IMC and AMAX. The two supplies of overburden soil were thoroughly homogenized in a 50/50 mixture using a large cement mixer. Overburden-filled microplots were sterilized with methyl bromide and covered with plastic for 48 hours in order to standardize all microplots with regard to soil microflora and fauna. The plastic was removed to allow the microplots to "air out" for 21 days to insure complete dissipation of the methyl bromide gas.

A week prior to experiment set up, surface soil (for topsoiling) was collected from several upland forest areas in north-central Florida that were being prepared for mining by Occidental. The area was intermixed with predominantly young pine and a few hardwoods. Saw palmetto (Serenoa repens) was a significant component of the ground cover. Nonsterilized surface soil topsoil) was added to appropriate cells to simulate the practice of topsoiling the top 2-3 inches of a reclaimed area.

Inoculation material of the vesicular-arbuscular (VA) mycorrhizal fungi (endomycorrhizal fungi) Glomus mosseae and Glomus occultum was cultured in a soil matrix planted to bahia grass (Paspalum notatum) and soybean (Glycine max) as host plants. The cultures were maintained in a greenhouse until prepared for use. The fresh inoculum actually used consisted of ground-up soil matrix that included mineral soil, plant roots, fungal hyphae, and spores. Microplots receiving the endomycorrhizal treatment were treated with 500 grams of the VA-mycorrhizal inoculum. All other microplots (those not receiving VA-mycorrhiza) were treated with an equivalent amount of the same material that had first been sterilized through autoclaving (in other words, a nonmycorrhizal equivalent). The material was worked into each seed planting row to simulate actual field planting conditions.

Table 28. Essential components, possible techniques for providing "essential" components, and experimental treatments and application rates for field microplot experiments.

| Essential Components | Possible Technique | Method Used and Application Rate | Materials |
|---|---|---|---|
| Seed Bank | Multiple species seeding | Direct seeding | 25 species of trees (see Table 29). |
| Soil Microflora | Inoculation with mycorrhizae | VA-mycorrhizae (endomycorrhizae) *Glomus mosseae* & *occultum* | Greenhouse inoculum cultures consisting of soil, plant roots, fungal hyphae and spores. |
| | | Pt-mycorrhizae (ectomycorrhizae) *Pisolithus tinctorius* | Vegetative hyphae mycorrhizal inoculum in vermiculite carrier (purchased from Abbott Labs). |
| Organic Matter | Mulching (straw, woodchips, etc.) | Straw (4500 kg/ha; 2 tons/acre) | Commercially available material. |
| Nutrients | Fertilizer, sludge amendments | 15-0-15 (320 kg/ha; 285 lb/ac) | Commercially available Marico brand. |
| Others | Soil structure amendments | Gypsum (1750 kg/ha; 1500 lb/ac) | Phosphogypsum from a chemical plant's gyspum stack. |
| All of above | Topsoiling....? | Premined surface soil (6–8 cm; 2–3 in) | Surface soil from upland forested area that was being site prepped for mining |

The ectomycorrhizal fungus (Pisolithus tinctorius [Pt]) inoculum material was purchased from Abbott Labs.* The material consists of mycorrhizal vegetative hyphae inoculum in a vermiculite carrier. Microplots receiving the ectomycorrhizal inoculum were treated with 75 grams of Pt-inoculum. A "nonmycorrhizal" equivalent of the Pt-inoculum was prepared using sterilized vermiculite and nutrient broth matrix minus the Pt-fungi. The nutrient broth was subsequently leached from the inoculum to simulate actual preparation conditions.

Nutrient rich soils, especially soils high in available phosphate, generally reduce mycorrhizal fungi colonization on host plants. In addition, high levels of fertilizer application tend to favor growth and development of the more rapidly growing and aggressive weedy plants. Therefore, the fertilizer treatment was limited to a one-time, low-level application of a nonphosphate fertilizer, hopefully at a level sufficient to stimulate growth of the planted species without encouraging over competition of weedy plants. The application rate was 8 grams/microplot (equivalent to 320 kg/ha or 285 lbs/acre) of 15-0-15 (N, P, K).

Phosphogypsum, a by-product of chemical processing of phosphate ore, is occasionally used as an agricultural soil amendment (Baird and Kamprath 1980). The phosphogypsum for the experiment was obtained from Occidental's Suwannee River chemical plant. Phosphogypsum, added at a rate of 1750 kg/ha (1500 lbs/acre), was worked into the surface soil of the respective microplot.

Straw mulch was applied to appropriate treatment plots at a rate equivalent to 4500 kg/ha (2 tons/acre). To prevent introduction of extraneous microbes in the experiment the straw mulch was autoclaved prior to use.

The field microplot experiments were planted on 17 July 1982. All microplots were planted with a seed mix of 25 tree species (Table 29) using 5 seeds of each species (125 seeds/microplot). The species chosen represent a wide variety of trees typically found in natural systems in Florida, and/or are either early-, mid-, or late-successional woody plants. Seeds were collected fall 1981 and spring 1982 and were stored in a large walk-in cooler at $4^0$C until 2 weeks prior to use.

*The Pt-inoculum is being developed as part of an experiment to produce commercially available ectomycorrhizal fungi for nursery operations. For further information contact: Dr. Donal Marx, Institute of Mycorrhizal Research and Development, U.S. Forest Service, Carlton Street, Athens, Georgia 30602 (404-546-2435).

Table 29. Tree species used in the microplot experiments. A seeding rate of five individuals of each species was used in the microplot experiments.

| | |
|---|---|
| Acer rubrum | Nyssa ogeche |
| Albizzia julibrissins | Persea palustris |
| Catalpa bignoniodes | Pinus elliottii |
| Celtis laevigata | Platanus occidentalis |
| Cercis canadensis | Prunus angustifolia |
| Chamaecyparis thyoides | Prunus serotina |
| Cornus florida | Quercus laurifolia |
| Diospyros virginiana | Quercus virginiana |
| Fraxinus americana | Sabal palmetto |
| Fraxinus caroliniana | Sambucus canadensis |
| Gordonia lasianthus | Taxodium distichum |
| Juniperus silicicola | Taxodium ascendens |
| Liquidambar styraciflua | |

The microplots were sampled at the end of 3 months (end of first growing season) and 12 months for number and height of seedlings by species. The 3-month sampling was done in October 1982 just prior to the onset of winter dormancy. The 12-month sampling was done in July 1983.

Data were analyzed using analysis of variance to test significant main and interactive effects, Student-Newman-Kuels (SNK) test for differences between means of treatments, and the Student's t-test for comparison of "with treatment" and "without treatment" means. Differences were tested at $a = 0.05$ level.

## Results

Growth. During the first growing season, the presence of mulch, topsoil, and VA-mycorrhiza significantly increased growth of tree seedlings over plots without these treatments, whereas Pt-mycorrhiza, gypsum, and fertilizer significantly decreased growth. When treatment effects alone are considered, mulch and topsoil had a significantly higher mean growth than the other treatments (Table 30a). However, during the second growing season differences between treatments were significantly reduced (Table 30a). In fact, only the VA-mycorrhiza and fertilizer treatments differed significantly.

Density. Initially, surviving propagules ranged from a low of 7% for microplots without mulch to a high of 9% for cells with mulch. Considering that seeds of most tree species generally have better germination after overwintering, the 7-to-9% range in propagule survivorship represented a reasonable level of germination. After overwintering the number of surviving propagules increased to 14 to 15%.

During the first 3 months, mulch, topsoil, and VA- and Pt-mycorrhizae had a significant positive effect on tree seedling density whereas fertilizer and

**Table 30. Average values for (a) height (in cm), (b) number of individuals (density) and (c) species richness for treatments, Similar means are connected by the bar. The vertical "*" presented for the first growing season separates those treatment means where the "without treatment" mean was significantly (a + 0.05) higher than the "with treatment" means. "With treatment" and "without treatment" means were not significantly different during the second growing season.**

a. Mean height (cm) of tree species

| Growing Season | Mulch | Topsoil | VA-mycorrhizae | * | Pt-mycorrhizae | Gypsum | Fertilizer |
|---|---|---|---|---|---|---|---|
| First | 13.71 | 12.86 | 11.08 | * | 10.86 | 10.53 | 10.21 |
| Second | 23.03 | 22.91 | 22.14 | | 22.51 | 22.49 | 23.29 |

b. Mean density of tree species

| Growing Season | Mulch | Topsoil | VA-mycorrhizae | Pt-mycorrhizae | * | Fertilizer | Gypsu |
|---|---|---|---|---|---|---|---|
| First | 11.16 | 10.38 | 10.30 | 9.93 | * | 9.63 | 9.40 |
| Second | 18.91 | 19.09 | 17.41 | 18.19 | | 17.69 | 18.56 |

c. Mean tree species richness (mean number of tree species)

| Growing Season | Mulch | Topsoil | VA-mycorrhizae | * | Pt-mycorrhizae | Fertilizer | * | Gypsum |
|---|---|---|---|---|---|---|---|---|
| First | 4.79 | 4.62 | 4.28 | * | 4.21 | 3.97 | | 3.91 |
| Second | 8.25 | 8.12 | 7.62 | | 7.69 | 7.47 | | 7.69 |

gypsum significantly reduced tree seedling density (Table 30b). There were no significant differences between the effects of VA- and Pt-mycorrhizae on tree density. The second growing season yielded different results. There were no significant differences between treatments on density.

Species richness. Species richness, a measure of the number of species having seedlings established, ranged from a mean low of 15% for gypsum-treated plots to a mean high of 20% for mulch-treated plots at the end of the first growing season. The grand mean for all treatments was 17%; that is, out of 25 tree species planted an average of 4.24 species established seedlings. Eleven species (44%) of the 25 tree species planted had at least one individual seedling established after only 3 months. This trend changed after overwintering. During the second growing season species richness almost doubled, ranging from a mean of 30 to 33%. In other words of the 25 species planted at least 7 to 8 species were established per treatment.

Initially, mulch, topsoil, and VA-mycorrhiza had a significant positive influence on species richness, whereas Pt-mycorrhiza, fertilizer, and gypsum produced significantly lower species richness (Table 30c). There were no significant differences between treatments at the end of the third growing season.

Community development index. Numerous factors contribute to community structure of an ecosystem. Total biomass production (growth), whether by a few or many individuals; total number of individuals (density), whether of one or many species; and the number of species (species richness) are all significant components of community structure. The "community development index" (CDI) was developed as a cumulative measure of the relative contribution of growth, density, and species richness to community structure. Mean growth for all individuals in each microplot was normalized to the maximum growth. Density was normalized by dividing the number of individuals per microplot by 125 (total number of seeds planted per microplot). Species richness was normalized by dividing the number of species per microplot by 25 (number of species planted). Normalized growth, density, and species richness were summed to yield the CDI (Table 31). Hopefully, the CDI or some variant of the CDI will have application to experimental field revegetation programs.

During the first growing season, mulch, topsoil, and VA-mycorrhiza soil amendments had significantly higher CDI's than did Pt-mycorrhiza, fertilizer, and gypsum. In fact, the ranked order of means for treatments in Tables 30 and 31 are almost identical. The only anomaly occurs in the reversed ranking of treatment means for gypsum and fertilizer treatments on plant growth (Table 30a). This trend shifted during the second growing season where there were no treatment effects on the CDI.

## Discussion

During initial establishment in the first growing season, the straw mulch treatment had the most significant positive effect on all aspects of community development in the microplots. There are several positive aspects of mulched surface soils such as reducing eroding impact of raindrops, binding soil against wind erosion, etc. (Anon. 1975). But perhaps the most important func-

**Table 31. Mean community development index (CDI), The CDI is the sum of normalized values of growth, density, and species richness. The maximum possible CDI value is 3 and would represent maximum means and equal contributions of all three parameters. Similar means are connected by the bar. The vertical "*" presented for the first growing season separates those treatment means where the "without treatment" mean was significantly (a + 0.05) higher than the "with treatment" means. "With treatment" and "without treatment" means were not significantly different during the second growing season.**

| Growing Season | Mulch | Topsoil | VA-mycorrhizae | * | Pt-mycorrhizae | Fertilizer | Gypsum |
|---|---|---|---|---|---|---|---|
| First | 1.98 | 1.87 | 1.73 | * | 1.70 | 1.62 | 1.61 |
| Second | 2.12 | 2.11 | 1.99 | | 2.03 | 2.02 | 2.04 |

tion of mulching is through its effect on decreasing both surface soil moisture loss and soil crusting. The importance of preventing surface soil crusting was realized about 2 weeks after the microplots were planted. During the 2-week period after planting several afternoon thundershowers had occurred. The observation had been made that overburden-only soils had crusted to a very hard surface, whereas straw mulched or topsoil treated microplots had not crusted as hard. About 2 weeks after planting, the microplots were deluged with an intense afternoon thundershower. All microplots containing only overburden soil as the surface soil (i.e., no mulch or topsoil) were overflowing the walls of the microplot (> 5-7 cm of standing water). All microplots treated with topsoil but without mulch had 2-3 cm of standing water but were not overflowing. However, none of the microplots treated with straw mulch, whether directly over overburden or topsoil, had standing water. The straw mulch, by preventing surface crusting, had apparently increased the rate of infiltration. Therefore, the straw mulch, by increasing water infiltration and decreasing soil moisture loss, increased water availability and, in turn, propagule survivorship and growth. However, as plants became established during the first growing season, the significance of mulch, albeit still important, was reduced. Perhaps by the second growing season the "role" mulch originally played was replaced by standing plant biomass and leaf litter produced during the first growing season.

Initially, topsoil also had a positive effect on community development. Although this trend carried over. into the second growing season, topsoiling as. a treatment was not significantly different from any of the other treatments. In fact, when one considers the overall negative economic, energy and environmental effects of topsoiling (indirectly resulting from increased fuel/energy use), the potential benefits that may be realized from topsoiling must be closely weighed with the associated negative impacts of topsoiling.

During the first growing season, inoculating the microplots with VA-mycorrhiza had a slightly positive, significant effect on the community development parameters of growth, density, and species richness. Conversely, the Pt-mycorrhizal inoculum appeared to have little or no influence on community development. This result was not unexpected since the Pt-mycorrhizal inoculum is known to stimulate growth of only one of the species planted-pine. Although oaks are also ectomycorrhizal it is not know if they are colonized by Pt. The importance of mycorrhizae did not carry over into the second growing season. Careful examination of the species list (Table 29) reveals that several of the species are "pioneer invaders" (e.g., Albizzia, Catalpa, etc.). True to their descriptive namesake, these "pioneer invaders" became dominant features of the microplots after the first growing season, masking, both in means as well as through actual competition, the establishment of the more climax species. In fact, when the experiments were terminated in the third growing season, the combined biomass of Albizzia and Catalpa alone accounted for over 90% of the total biomass for each plot. Often these pioneer species are facultative-mycorrhizal; therefore, the reduced significance of the mycorrhizal treatment is not unexpected since pioneer species were sown in equal proportion to other climax species.

## Summary

1. Tree seedlings establishment ranged from 7 to 9% during the first growing season and increased to about 15% during the second growing season. Mulch,

topsoil, VA-mycorrhiza, and Pt-mycorrhiza had a significant positive effect on seedling density during initial establishment.

2. Although 44% of the tree species planted established seedlings, the average for each treatment ranged from 15 to 20% during the first growing season and from 30 to 33% during the second growing season.

3. During the initial establishment phase of the first growing season, the community development index (CDI) was highest for the mulch, topsoil, and VA-mycorrhiza treatments. A net decrease in the mean CDI resulted from treatment of Pt-mycorrhiza, fertilizer, and gypsum. Mulch, topsoil, and VA-mycorrhizal (endomycorrhizal) fungi significantly increased community development components such as growth, density, and species richness of tree seedlings. Differences between CDI's for each treatment were reduced during the second growing season partially as a result of the overriding dominance of the "pioneer species," Albizzia and Catalpa.

4. Pt-mycorrhizal fungi slightly decreased average growth and species richness and slightly increased density of tree seedlings. However, this may partially be due to the fact that only a few of the tree species planted may be potential ectomycorrhizal hosts.

# CHAPTER 5
# FIELD MACROPLOT EXPERIMENT TESTING MECHANICAL PLANTING OF DIVERSE SEED MIXTURE AND MYCORRHIZAL INOCULUM

## Introduction

Natural revegetation of minelands has generally been deemed an unsuccessful alternative. In many instances in which unreclaimed phosphate surface-mined areas have been subjected only to natural succession, an arrested state of community development occurs (Clewell 1981, 1983; Robertson 1983; Rushton 1983; Florida Bureau of Geology 1980). Arrested succession is probably mediated through a variety of mechanisms, but seed source availability is a primary causal agent. Due to the vast scale of present surface-mining activities, seed sources have generally been restricted to floodplain areas. Mined areas in close proximity to wetland areas may receive sufficient seed rain to establish woody vegetation; however, due to the nature of later successional tree dispersal, distance from source to sink is a limiting factor, i.e., mines which are fairly distant from floodplains have limited probability for seed introduction and definitely not at levels adequate enough to insure survival.

A direct seeding field experiment was carried out in the final project year. A field trial conducted with the cooperation of Agrico Mining Company at their Fort Green reclamation area (Agrico Swamp) was designed to test the feasibility of using conventional agricultural planters in direct seeding of woody species. The primary goal of the experiment was to work out details of and demonstrate that direct seeding of a mixture of tree and shrub seeds of different sizes concomitant with mycorrhiza inoculum was possible using presently available seed drilling equipment.

## Literature Review

Direct seeding has recently been proposed as a possible mechanism for reestablishment of woody vegetation. Direct seeding would introduce a multispecies mixture of seeds indigenous to native Florida communities. The pro-, cedure would allow for selection of species on a site-specific basis, i.e., the survivors are adapted for the area where they are found. Presently, direct seeding has received only limited utilization by the Florida phosphate industry and has generally been restricted to small field test plots using broadcasting methods (Clewell 1983). Wadsworth (1983) reports on the development of techniques for direct seeding sand pine and slash pine on tailings sand using a hand held broadcaster.

Tourney and Korstian published the second edition of their book Seeding and Planting in the Practice of Forestry in 1942. The book synthesized the literature dating back to the turn of the century and served as the state of the art handbook on seeding in the practice of reforestation. Their treatment of the subject remains a classic. In spite of the research carried out over the last 40 years on direct seeding the success of a seeding still depends on factors discussed by Tourney and Korstian. These chief factors include:

1. quality of seed used;
2. species selected for use;
3. vegetation cover present on site;
4. condition of surface soil;
5. freedom of site from seed-eating birds or rodents;
6. quantity of seed sown per unit area;
7. time of sowing; and
8. depth of covering of seeds.

Direct seeding has been a reforestation tool in use for some time. The use of seeding in reclamation has a shorter and much less successful history. Early experiences in both forestry and reclamation were discouraging due to inconsistent results and complete failures in many cases. Direct seeding has been used extensively by the forest industry in the Southeastern United States for the regeneration of clear-cut pine forests. On a less widespread basis it has been used for the establishment of hardwoods in uplands and bottomlands (Klawitter 1959; McKnight 1965; McElwee 1965). Interest in direct seeding has been as an alternative to stand establishment by planting seedlings, which until recently has been a labor-intensive operation. Advantages offered by direct seeding potentially include: large acreages can be seeded in a relatively short time, operation can be less labor intensive than planting seedlings, seeding can be done at times when planting isn't feasible, seeding can be done in areas where planting is difficult, and it is possible to establish woody species and herbaceous cover crop in one operation. The major disadvantage to seeding is low germination levels.

Direct seeding in the practice of forestry has had many successes and many failures. The same is true for direct seeding in reclamation of mined lands. Strip mined land presents the seed with an environment that is harsher and more stressful than that found in forestry situations. Mine spoils differ considerably from an old field or the soil of a cut-over forest. Erosion potential on spoils is higher and drought stress and nutrient deficiencies are also common impediments.

Various species of oaks have been successfully seeded on mined lands. Tackett and Graves (1983) manually seeded test plots on strip mine coal spoils in Kentucky. Five species were used, two of which were exotics and the other three were oaks (northern red oak, pin oak, fur oak). Germination and sur-, vival of the oaks under various experimental treatments ranged from 36 to 50% at the end of the first growing seasc. At the end of the fifth growing season survival ranged from 43 to 68%.

The use of direct seeding in the reclamation of coal strip mined lands has a longer history to draw on than the phosphate industry. Among the earliest attempts was in Illinois and involved the seeding of black walnut (Schavilje 1941). Good first-year germination and survival were reported. In contrast,

Medvick (1973) reports that black walnut was planted for reclamation in Indiana between 1930 and 1940. The seeding was unsatisfactory and later efforts concentrated on plantings.

Black locust has been widely seeded for reclamation in West Virginia since the 1940's and field tests showed that green ash, Virginia pine, shortleaf pine and loblolly pine could also be seeded for reclamation (Plass 1976):

## Methods

The direct seeding experiment was conducted in March 1983. Seeds were mechanically planted on a l-acre plot at Agrico's Fort Green reclamation area. The plot straddled a transition zone from upland to wetland. The planting apparatus consisted of a tractor with two corn planters (John Deere 23-C) mounted on a tool bar behind the tractor. The seed plates used in the planters were modified at a machine shop to allow for larger seeds such as acorns to be planted. Two seed batches were used: one containing the majority of the large-seeded species and the other with the small seeded species (Table 32). The corn planters are designed to be used on tilled ground so the tool bar had to be fitted with point plows mounted in front of each planter to open a furrow in the overburden soil.

Prior to planting, seed drop calibration pointed out several obstacles that needed attention. First, the multi-species seed mixes had seeds of varying size, shape, and density, while the holes in the seed plates were of uniform size. Therefore, some seeds, especially small round seeds, fell through preferentially. Second, if the seed bin on the planter contained only seeds, it then gave a seeding rate that was too high. To get around both of these problems calibrations were next run with various filler materials. The choice of filler materials was constrained by the remote nature of the field site. The filler materials evaluated were: sawdust, tailings-sand, screened endo-mycorrhizal soil inoculum, and several combinations of these three. The various fillers did aid in lowering the seeding rates and helped with the problems created by the differences in size; shape and density between species, but they also created a few other problems. The moisture level of the filler greatly affected the fall rate in the seed bins. For example, very dry tailings-sand fell too fast, while damp sawdust hardly fell at all. Finally, as a compromise tailings-sand was used as the filler for uninoculated seed rows and the screened endo/soil was used as filler for the inoculated seed rows. It was later noted during the actual planting that the tailings-sand fell at a faster rate than the endo/soil. The filler seed dilution factors used were, on a volume basis, 1:1 for the large seed mix, and 3:1 for the small seed mix.

### Endomycorrhizal Inoculum

The endomycorrhizal inoculum used in the direct seeding field trial consisted of soil containing spores of Glomus. The soil came from microplot inoculum cultures in which primary inocula were propagated by the culture of soybeans and bahia grass. This spore-laden soil was then sifted through a coarse

Table 32. List of species used in direct seeding experiment at Agrico Fort Green reclamation area.

| Small Seed Batch | Large Seed Batch |
|---|---|
| Aralia spinosa (devil's walking-stick) | Catalpa bignonioides (catalpa) |
| Callicarpa americana (American beauty-berry) | Celtis laevigata (sugar berry) |
| Cephalanthus occidentalis (buttonbush) | Diospyros virginiana (persimmon) |
| Chamaecyparis thyoides (Atlantic white cedar) | Fraxinus caroliniana (pop ash) |
| Cornus florida (flowering dogwood) | Magnolia grandiflora (southern magnolia) |
| Fraxinus americana (white ash) | Persea palustris (swamp redbay) |
| Gordonia lasianthus (loblolly bay) | Prunus angustifolia (chickasaw plum) |
| Ilex cassine (dahoon holly) | Prunus serotina (black cherry) |
| Juniperus silicicola (southern red cedar) | Quercus laurifolia (laurel oak) |
| Liquidambar styraciflua (sweetgum) | Quercus nigra (water oak) |
| Magnolia virginiana (sweetbay) | Sabal palmetto (cabbage palm) |
| Nyssa sylvatica var. biflora (swamp blackgum) | Taxodium ascendens (pond cypress) |
| Platanus occidentalis (sycamore) | Taxodium distichum (bald cypress) |
| Sambucus canadensis (elderberry) | |
| Ulmus alata (winged elm) | |

mesh screen to remove clods and stored in closed containers at 4°C until needed.

## Seeding Rates

For each of the two treatments, inoculated and uninoculated, 700 seeds/species/treatment for all 28 Species (Table 32) were counted out. This amounted to (28 x 700) 19,600 seeds/treatment. During the actual planting though only approximately half of the large-seeded batch was used in each treatment, thereby reducing the seeding level to 15,000 seeds/treatment. Each treatment was apportioned over 10 paired rows with one row containing the small- seeded batch and the other the large-seeded batch. Each row was 200 ft in length, and so the seeding rate for the small-seeded batch was 21,000 seeds/2,000 ft = 10.5 seeds/ft or about one seed/in. The seeding rate for the large-seeded batch likewise was 9,100 seeds/2,000 ft = 4.5 seeds/ft or about one seed every 2-3 in.

The direct seeding field plot was a 200 ft x 200 ft plot sloping from upland down to wetland edge. As just described, each treatment, inoculated vs. uninoculated, consisted of 10 paired rows giving a total of 20 such rows each 200 ft in length. The paired-rows were randomly assigned to 10-ft intervals across the plot, and to prevent cross contamination of treatments all the uninoculated rows were seeded prior to any inoculated rows. In planting each paired-row the tractor began at the lower end of the plot in wet soil and moved upslope for 200 ft. The ends of each paired-row were marked with PVC stakes. The two planters were mounted 50 cm apart, therefore each paired-row was the same distance apart. After the completion of the planting operation straw mulch was applied at a rate of two bales per 200 ft paired-row.

The study area was fenced off with hog wire after planting because of the known presence of feral hogs on the site and adjacent floodplain of Payne Creek.

## Results

The slightly modified corn seed drill was found to be an extremely useful tool for direct seeding of mixed size seeds. An increase in control of seed drop rate/proportion could be achieved by pre-selecting seeds at least the size of grass seeds (i.e., large and small seed sizes) and then sowing the seeds from separate seed drills. Spacing between seeds averaging 4-6 inches regardless of size. Although mycorrhizal inoculum could also be dispensed via the seed portal, it was difficult to regulate the rate mycorrhiza drops relative to seed drop.

The field plot was checked qualitatively several times for the presence of seedlings. Diospyros virginiana, Celtis laevigata, Liquidambar styraciflua and Prunus angustifolia were present in low numbers in both treatments (inoculated vs. uninoculated) during the first growing season. A quantitative survey of surviving seedlings was made at the end of the second growing season. The

results of the survey were extremely disappointing in that only four surviving seedlings could be located - all four were persimmon (Diospyros).

## Discussion of the Agrico Test Plots

The field trial was a great success in terms of its primary goal of providing a field-scale demonstration of a mechanically direct seeded, multi-species assemblage of woody plants. The planting operation was very successful in getting seeds and inoculum into the ground in a clean and efficient manner. The field trial represents the first of its kind in the industry.

Another very positive result or aspect of the field-trial lies in the mechanical simplicity of it all. Easily available common planting equipment was used with only slight modification, which amounted to nothing more than a simple re-grinding of seed plates. The corn planters, or unit planters, proved to be easy to use even for the novice. Other advantages of the unit planters are their low cost and modular nature. Each planter produces a single row and several planters can be attached to a single tool bar at any one time. This flexibility allows for simultaneously planting seeds of different sizes, the simultaneous seeding of a cover crop along with woody plants, the addition of mycorrhizal inoculum directly into the seed furrow, and the ability to vary the spacing between planters on the tool bar.

The failure of the ancillary goal of the direct seeding field-trial is discouraging yet very instructive. Even though it is mechanically possible to do direct seeding and get the seeds into the ground, germination, growth and survival are functions of the quality of the seed used and the environmental conditions to which they are exposed. Seed quality obviously has a great bearing on the success of this type of revegetation. It was learned after the field planting that the acorns used (water oak and laurel oak) were not viable when planted due to dehydration during storage, the same was also true for the two species of ash (white ash and popash). Finding the proper storage techniques (method) for each species is certainly an obstacle which can be hurdled and progress toward this was shown in field tests conducted at Gardinier Parcel discussed in a following section.

The ideal environmental milieu for successful germination, growth and survival of an individual plant is a harder factor to control than the mechanics of planting and proper storage of seeds. Seed germination involves information processing on part of the seed. Germination will not occur unless the proper cueing is provided by the environment. Seeds can receive and process cues dealing with light levels, temperature, moisture levels, oxygen levels, mechanical scarification.

Overburden soils present seeds with an extremely harsh micro-environment lacking in the buffering provided by shading, soil organic matter etc. Some mulch was hand applied to specific rows to provide the ameliorations of shade and improved moisture retention. It may well have been that too much straw was applied and delicate young seedling were inhibited by the thick cover and eventually succumbed due to an inability to reach light. Another critical factor affecting the outcome of any direct seeding operation is the soil moisture status. The root zone of a newly germinated seedling is only the upper few centimeters of the soil profile, and gravity and solar insolation largely dic-

tate that this horizon is also the driest portion of the profile. The problem was probably especially acute for the wetland species. The plot as a whole was not in a wetland. The lowest portion of the plot received some seepage, but for the most part the soil moisture conditions would be termed mesic to xeric.

No seedlings of any true wetland species were ever observed on the plots. The results of the second year inventory showed that even if germination of wetland species occurred none survived.

Since the moisture level of the surface horizon is largely a function of precipitation, the amount and frequency of rainfall become critical factors in the success of a seeding operation. Rainfall patterns are more critical in seeding than in transplanting because of the greater susceptibility of the germling to mortality induced by environmental stress.

The direct seeding test has demonstrated a viable method for getting seeds into the soil. Until recently the industry has not had much success with getting the seeds to germinate and survive. Wadsworth (1983) reports that winter time direct seeding trial of sand pine and slash pine on tailings sand in central Florida had no germination in spite of eight acres being seeding. In a second "pilot" study, hand seeded plots at Gardinier had 20% germination overall and 77% of the seedlings survived the first growing season. The Gardinier plots show that good results can be obtained in a direct-seeded, multi-species plot. The mechanical performance of the Agrico test and germination performance of the Gardinier test provide a more encouraging view for the future of direct seeding.

# CHAPTER 6
# ROLE OF INTERACTIONS BETWEEN EARLY AND LATE SUCCESSIONAL PLANTS ON ENHANCING ECOLOGICAL SUCCESSION

## Introduction

This study was undertaken to examine the relationships between early successional plants and late successional trees. The colonizing suite of annuals, biennials, perennials and low shrubs, the old field denizens, are designated the early successional species. The interactions between early and late species were to be examined in terms of several competing paradigms (models) of ecosystem development. Field plot experiments using species removals and additions were designated to determine the affect, if any, of the colonizing vegetation on tree species.

Three paradigms of succession tested in field plot experiments are facilitation, tolerance and inhibition. A brief statement of each follows:

> Facilitation - presence of later successional climax species is dependent upon early ones preparing a favorable environment for them . . . implies a high degree of organization in the ecological community.
>
> Tolerance - succession leads to a community composed of those species most efficient in exploiting resources.
>
> Inhibition - no species necessarily has a competitive superiority over another; whichever colonizes the site first holds it against all comers. Once available space is filled invasion is only possible if the new colonist brings along its own resources (large seed to sustain early growth), or if a gap is created in the existing vegetation by some disturbance.

Other paradigms of succession exist and may be found in the literature. The choice of these three is due to the fact that they encompass most of the long debated issues concerning ecosystem development and they can be tested in field experiments.

The field experiments examined the growth of late successional tree species under four treatments. The treatments were selected to determine what effect if any colonizing early successional species have on tree seedling growth and survivorship. Of the four treatments three have various arrays of colonizing species added and/or allowed to colonize. In one of these three the natural colonization process is allowed to proceed and so this treatment is designated the colonized treatment. The addition of seeds of several legumes constitutes the legumed treatment. An enhanced colonizer treatment received seeds of several common old field weeds. In both the legumed and enhanced treatments the natural colonization process was allowed to occur also. The final treatment

involved a periodic weeding to maintain the plots free of any colonizing vegetation.

The decision criteria for accepting or rejecting the competing paradigms is given below.

- If seedlings grow better with either the enhanced, colonized or legumed treatments, then reject the tolerance and inhibition models in favor of the facilitation model.
- If seedlings grow better in weeded plots, then reject tolerance and facilitation models in favor of the inhibition model.
- If seedlings grow about the same in the weeded plot as the other treatment plots then reject the facilitation and inhibition models in favor of the tolerance model.

## Literature Review

The pattern and process of ecosystem development is still much debated in the ecological literature, especially the interactions between early colonizing plants and the later arriving climax tree species, and the interactions between producers and consumers. The post mining landscape provides an exceedingly rich milieu for testing models and hypotheses concerning the pattern and process of succession.

Clements (1916) described succession as a universal, orderly process of progressive change. He asserted that the community developed from diverse pioneer stages to converge on a single, stable, mesophytic community (monoclimax) under the control of the regional climate. He held that the community repeated in its development the sequence of stages of development of an individual organism from birth through death and was orderly and predictable in its development in the same sense as the development of an individual organism. As succession proceeded, the community came increasingly to control its own environment and barring disturbance became self-perpetuating or climax.

E. P. Odum (1969) provided one of the best parallels to the Clement's views of succession. Odum noted the similarity of succession to the development of individual organisms and converged with Clement's description of succession as: (1) an orderly process that is reasonably directional and therefore predictable; (2) resulting from modification of the physical environment by the community (autogenic); culminating in a stabilized (climax, mature) ecosystem with homeostatic properties.

To many ecologists the community was less well defined and unitary, and succession less orderly, than Clements suggested. Alternative models of succession advocate a Gleasonian population-based approach emphasizing life history attributes of organisms, the consequence of natural succession, as the essential basis of a modern theory of succession. Gleason (1926) described his individualistic concept of the plant association in which he asserted that the associstion, or community, is a coincidence rather than an entity unto itself. Gleason

felt each species was a law unto itself, its distribution in space depends upon its individual peculiarities of migration and environmental requirements. On a long time scale the dissemules of a species migrate everywhere, but grow only where environmental conditions are favorable. The species disappears from areas where environmental conditions are no longer favorable. It grows in the company of any other species with similar requirements. In the eyes of Gleason the plant association was an artifact depending solely on the grouping of species with overlapping environmental requirements.

There are numerous modern proponents of the Gleasonian view (McCormick 1968; Drury and Nisbet 1971, 1973; Horn 1971, 1974, 1975; Pickett 1976; Connell and Slatyer 1977). These widely cited studies find the classical Clementsian succession and the Odum-Margalef representation quite unpalatible. McIntosh (1982) points out that these articles share at least three characteristics:

1. They are commonly cited in recent discussion of succession as providing new insights for successional theory, although there has been little critical analysis of the substance of their interpretations, or the alternatives they offer.

2. They are explicitly critical of Clements' holistic, organism theory of succession and of what they interpret as the successional theory of the organismic, holistic, ecosystem ecology expressed by ecosystem ecologists.

3. The alternative models of succession proposed advocate a population-based approach emphasizing life-history attributes of organisms, the consequence of natural selection, as the essential basis of a modern theory of succession.

Connell and Slatyer (1977) described three models by which species may replace each other in a successional sequence. The three models were designated the facilitation, tolerance, and inhibition models. The facilitation is essentially the traditional view of Clements. The tolerance model is based on species differing in adaptation for resource exploitation and succession proceeds as slow growing tolerant species invade and mature in the presence of -earlier, faster growing, less tolerant species. The inhibition model is based on the resistance of earlier species to invasion. Succession occurs only with replacement due to disturbance or death.

Connell and Slatyer (1977) also offered suggestions on how models might be tested in field experiments involving the removal of selected species from successional communities.

Several previous studies have examined the effects of species removals on the course of old field succession. McCormick (1968). removed annual plants from some section of a first year old field in Pennsylvania, but allowed them to grow elsewhere. The experiment was designed to test whether annual plants were needed to "prepare the way" for perennial plants. The results showed that the biomass of individual perennial plants on the annual-free areas were many (15 to 82) times as great as those on areas with annuals. Pinder (1974) studied the effects of the presence of the dominant grasses on the productivity of subordinate forbs within a perennial-grass old field community. He found that

removing the dominants increased the net productivity of subordinate species. The increased net productivity was due to increased productivity by almost all subordinate species.

Hils and Vankat (1983) used the species removal approach to test Connell and Slatyer's (1977) models in the first year of old field succession. Their experimental treatments included removals of annuals, annuals and biennials, and perennials. Results from the first growing season favored the acceptance of the tolerance model over the inhibition model. The facilitation model was rejected as inapplicable to the community investigated. The authors cautioned that more than one model of succession may apply in the same field at the same time reflecting the spatial heterogeneity of the old field community. The same old field plots were further studied by Zimmerman and Vankat (1984). The species removal treatments were maintained in the second year and for the next three years the developing community was studied. The tolerance model was again supported at the end of five years because the authors found no statistically significant differences between the biomass of perennials when grown with annuals and biennials as opposed to when grown alone. Succession resulted in the development of nearly identical communities in the two treatments. Their two observations imply that the early-successional species had little affect on later successional species.

Like species removals the addition of species is another tool available to the ecologist in studying the process of ecosystem development. Brown (1973) used propagule additions as one treatment in the study of the development and design of tropical agroecosystems. The selective enrichment of certain species allows for another level of manipulation in tests of species interactions and community development.

## Methods

An upland area on the west side of Parcel 6 was cleared in late October 1983. Cleared area was 63 m x 63 m with a gentle slope from the south to the north. Plots were staked out on December 11 and 12, 1983. Two experiments were set up adjacent to each other (Figure 16). The experimental design was a nested analysis of variance with four experimental treatments: colonizing species allowed, colonizers weeded out, colonizers added, and legumes added. Within each of the two experiments there were four replicates for each treatment, therefore there were 4 x 4 = 16 plots in each for 32 plots overall. In each case the treatments were randomly assigned to plots.

The experimental treatments used in both the seedling plots and direct seeded plots were the addition of seed of four colonizing species, the addition of seed of four legume species, the removal of all colonizing species through weeding, and finally a natural invasion of colonizing species. The first two treatments involved the application of seed and this was completed just prior to planting the tree seeds or seedlings. Due to the initial site clearing in October all plots were free of any colonizing species at planting time.

The enhanced colonizer treatment consisted of four of the most common species found on old fields and abandoned mine lands in central Florida: natal

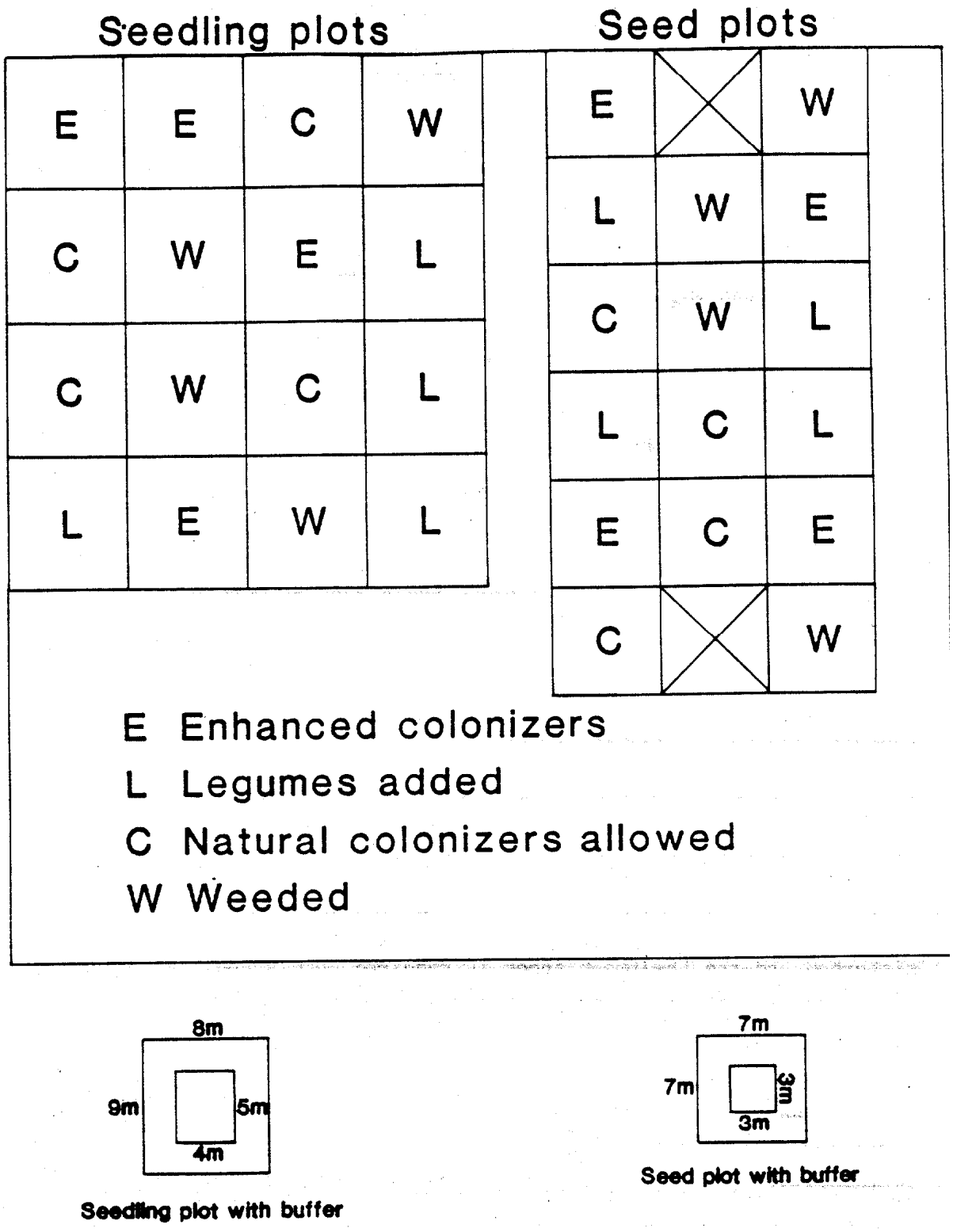



Figure 16. Layout of seed and seedling plots.

grass (Rhynchelytrum repens), groundsel (Baccharis halimifolia), dogfennel (Eupatorium capillifolium), and broomsedge (Andropogon virginicus). Seeds of each species were collected in October and November.

Four species were added in the legume treatment Cassia obtusifolia (sicklepod), Sesbania macrocarpa, Sesbania punicea, and Sesbania vesicaria (bladderpod). In the seeded plots 50 seeds of each legume was added totaling 200 seeds/plot, while 110 seeds per legume totaling 440 seeds/plot were added to seedling plots receiving this treatment. In both cases the seeding rate gave a density of 22 legume seeds per square meter.

The application of treatment seeds for the enhanced colonizer treatment and the legume treatment was accomplished by mixing the seeds with some overburden soil from the plot and then hand broadcasting this onto the plots.

In order to incorporate the non-legume colonizers the soil was disturbed by hand with rakes and cultivators. This was deemed necessary due to the effects wind would have on surface spread seeds of these wind dispersed species. Therefore, all plots were subsequently disturbed as part of a preplanting treatment. Planting and preplanting treatments were carried out on December 17 and 18, 1983.

## Seed Plots

In the direct seeding test plots seven species were utilized: Liquidambar styraciflua (Sweet gum), Sabal palmetto (Cabbage palm), Quercus virginiana (Live oak), Quercus laurifolia (Laurel oak), Magnolia grandiflora (Magnolia), Celtis laevigata (Sugar berry), and Carya glabra (Pignut hickory).

These seven taxa were selected as being representative of common mesic hardwood species of central Florida.

Seeding rates per plot as 50 seeds/species yielding (7 x 50) 350 seeds per plot. Each plot was 9 $m^2$ (3 m x 3 m) resulting in a density of 39 seeds/$m^2$.

Plots were arranged in 6 x 3 grid with two of the plots remaining unused (Figure 16). Treatments were assigned randomly to the plot grid. Individual plots were 7 x 7 m which allowed for a 2-m buffer all around a planting area of 3 x 3 m. The seed mix was hand broadcast onto the plots after the soil was disturbed and the treatment seeds if required were added. Finally the plots were raked lightly to incorporate the seed into the substrate.

## Seedling Plots

In the seedling test plots three mesic hardwood species were used: sweetgum (Liquidambar styraciflua), live oak (Quercus virginiana) and cabbage palm (Sabal palmetto). The sweetgum and cabbage palm planting stock was 8-mo old containerized seedlings grown in overburden soil. The oak seedlings were 1-mo old bare root seedlings.

Ten individuals of each species were used in each of the sixteen plots, giving 30 trees per plot and (16 x 30) 480 seedlings total. Tree seedlings were

planted after the soil was disturbed and the treatment seeds if any were added. The 30 trees were randomly assigned to grid to fit into the plots, and the same planting schematic was used in all the plots (see Figure 17).

Plots were 8 x 9 m overall allowing for a 2-m buffer around an actual planted area of 4 x 5 m. Seedlings were planted on approximate l-m centers.

Heavy rains in the first month after planting initially caused erosion problems on the study plots. Erosion rills quickly developed with many of them running through plots. In order to avoid the cross contamination of seeds washing out of one plot and into another hay was spread between all plots that had downslope neighbors. Hay was secured in place with overburden soil. All major erosion rills on the site were mapped as an aid to interpreting results. In addition, several rills that had developed through the seed plots were diverted to interplot areas with a shallow, shovel-dug diversion trench. Once colonizing vegetation began to appear in early spring of 1984 it afforded a modest degree of soil stabilization and the severity of the erosion problem diminished considerably.

The extremely severe winter freezes of December 1983, and January, February 1984 took their toll on planted seedlings at the Gardinier plot. Since the freezes occurred before any possible treatment effects could have been exerted all of the freeze-killed seedlings were replanted on March 27, 1984. This date was used as the starting date for growth measurements on the seedling plots. The plots were measured again on September 1984. At time of measurement the height of each seedling and growth condition were recorded.

The seed plots were measured in March and October 1984. In each plot the species, height, and growth condition of each seedling was recorded. In addition the location of each seedling was recorded so the fate of individuals could be followed.

The weeding treatment was administered four times in March, May, June and September for both the transplanted and direct seeded plots. All colonizing plants were hand-weeded and removed from the plots.

## Results

### Seed Plot Percent Germination

The experiment was designed as nested analysis of variance with a balanced design (i.e., equal replications for each treatment). A sampling mistake during the second weeding event changed this. The second time the plots were weeded (May)' plot 15 was weeded instead of plot 18. The error wasn't discovered until the third weeding (June) at which time it was decided to continue weeding plot 15. This change created some problems as to the treatment status of plots 15 and 18. The statistical analysis for the seed plot data was handled two ways in order to incorporate plots 15 and 18 into the analysis. Each ANOVA was carried out using two different assignments for the plots: 15 assigned to weeded treatment and 18 dropped from the analysis and both plots dropped from the analysis.

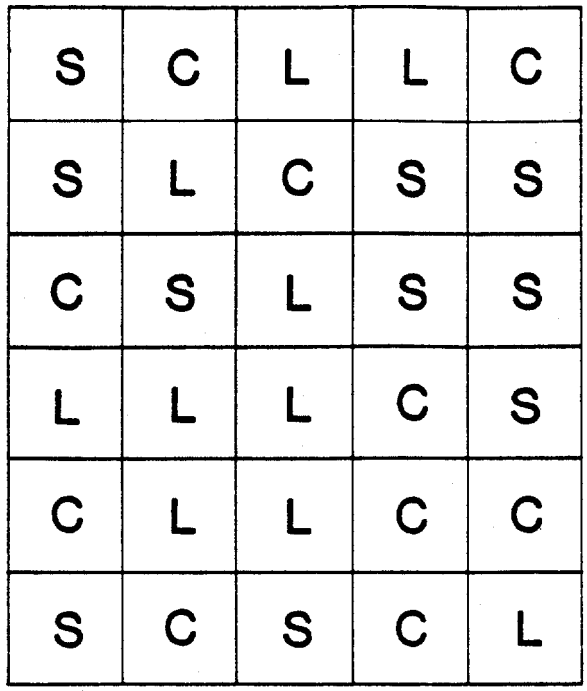



**Figure 17. Schematic. for seedlings planted at Gardinier test plots,**

The seed plots yielded information on germination, the survival of the germinating seeds, and the growth of the surviving seedlings. Total germination was estimated with data from the two sampling periods in March and October. Since the location of each seedling in the plot was recorded at time of sampling the fate of any given seedling could then be followed through time. Using this method the mortality of germinating seedlings could be estimated and some idea of the phenology of germination could be seen (Figure 18). The germination and survival rate of germinating seeds is given in Table 33a and summarized by species and treatment in Table 33b.

Overall, 20% of the seed planted did germinate and 78% of those germlings survived through the first growing season. The individual species showed a full range of response in both germination and survival. Magnolia was unique since no seeds germinated regardless of plot or treatment. Liquidambar had only 6% germination and only 33% of those survived. Celtis had 19% germination. but only 30% survival. The other three taxa had much higher survival rates. Carya had 10% germination with 75% survival. Germination in Sabal was 13% with 100% survival. Quercus had both high germination (46%) and highsurvival (87%). Due to the difficulty in accurately distinguishing between young laurel oak seedlings (Q. laurifolia) and young live oak seedlings (Q. virginiana) the two are lumped together as a single taxon.

The species showed interesting combinations of germination phenology and survival. Celtis and Liquidambar had the majority of their germination occurring prior to the March sampling. Additional seeds germinated between March and October, but mortality in both cases was high so the total number of live seedlings was lower in October than in March (Figure 18). Carya and Quercus had germination spread out over the growing season with a larger number of seeds germinating after March. For these two taxa, the germination phenology combined with low mortality resulted in a greater number of live seedlings at the second sampling period relative to the first. Finally, Sabal demonstrated a different germination pattern with all germination occurring after March (Figure 18).

Another way of handling the seed germination and survival data is to look at the end of season survivors as a proportion of the seeds planted. The data on seedling survival from the experimental plots was converted to percentages for analysis. It is known from statistical theory that proportions or percentages have binomal distribution, rather than a normal distribution, and the deviation from normality is greatest for small and large values. To obtain a distribution that approximates normal the data can be transformed. Zar (1974) recommends that the square root of each percentage be transformed to its arcsine to give an underlying distribution that is nearly normal. Following this advice the percentages (Table 33) were transformed prior to conducting the ANOVA.

For the case where the data are summed over species within each plot the results of the ANOVA depend on how plots 15 and 18 are treated (Table 34a). In the first case when data from plots 15 and 18 are neglected in the analysis the means for the weeded, colonized and legumed treatments are not significantly different from each other, but the enhanced treatment mean is lower and significantly different from the other three. In the second case when plot 18 is ignored and plot 15 is assigned to the weeded treatment the result is the same - the enhanced treatment mean is lower and significantly different from the other three treatment means which are not significantly different from each other.

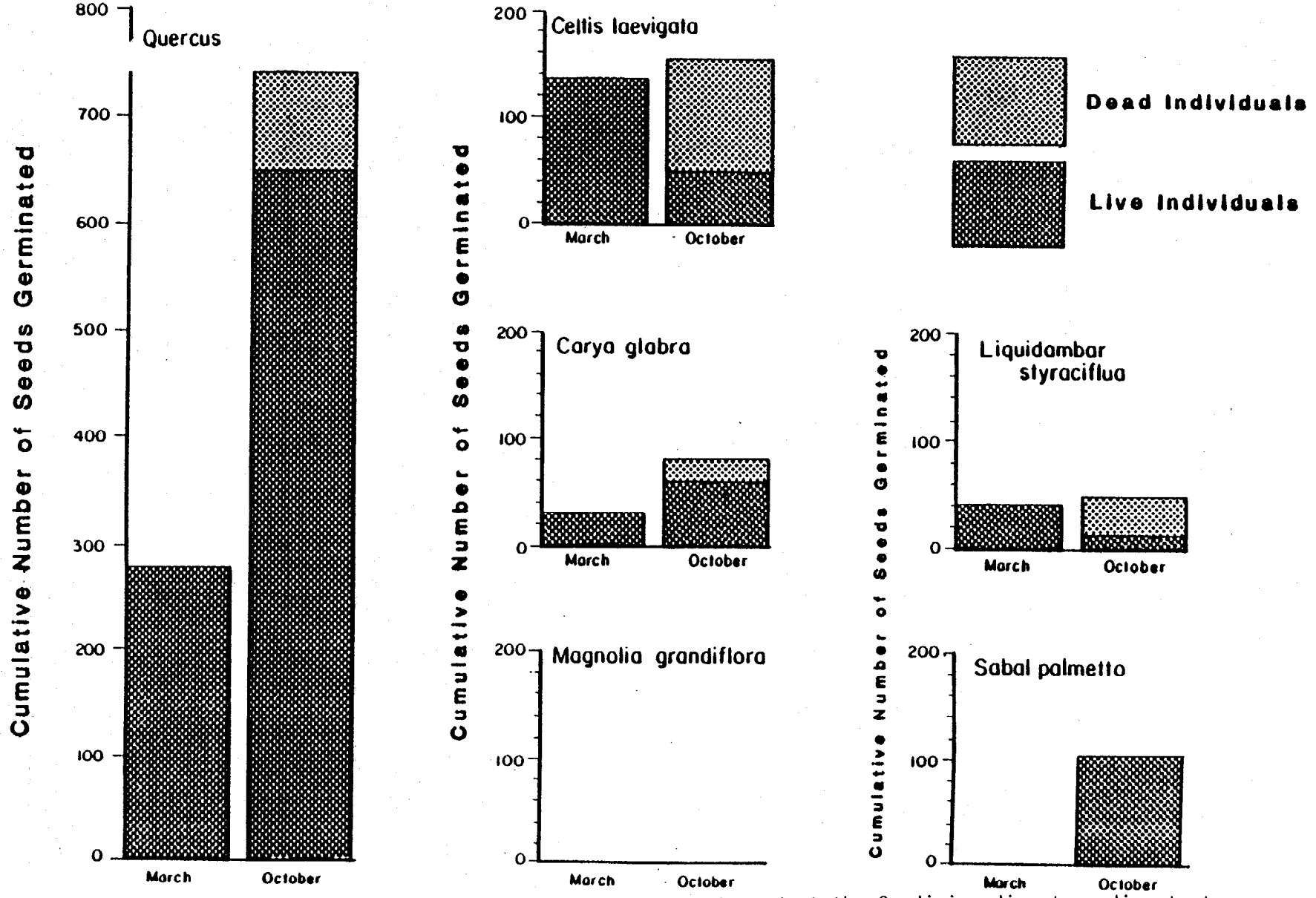



Figure 18. Number of germinated seeds of select tree species planted at the Gardinier direct seeding test plots. Seeds were planted fall 1983 and plots sampled spring and fall 1984.

Table 33a. Seed germination in Gardinier seed plots as measured by seedling counts in March and October 1984 along with the total cumulative germination. Data given by species and treatment.

| | Celtis laevigata | | | Liquidambar styraciflua | | | Carya glabra | | | Sabal palmetto | | | Quercus | | | Magnolia grandiflora | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment and plot | March | Oct | Cumulative total | March | Oct | Cumulative total | March | Oct | Cumulative total | March | Oct | Cumulative total | March | Oct | Cumulative total | March | Oct | Cumulative total | March | Oct | Cumulative total |
| Enhanced | | | | | | | | | | | | | | | | | | | | | |
| 1 | 9 | 1 | 9 | 4 | 0 | 4 | 1 | 1 | 2 | 0 | 3 | 3 | 6 | 23 | 23 | 0 | 0 | 0 | 20 | 28 | 41 |
| 6 | 6 | 1 | 6 | 5 | 2 | 7 | 0 | 4 | 4 | 0 | 6 | 6 | 16 | 37 | 39 | 0 | 0 | 0 | 27 | 50 | 62 |
| 13 | 14 | 0 | 14 | 2 | 0 | 2 | 6 | 1 | 6 | 0 | 0 | 0 | 14 | 34 | 47 | 0 | 0 | 0 | 36 | 35 | 69 |
| 15 | 10 | 6 | 13 | 0 | 0 | 0 | 1 | 4 | 4 | 0 | 13 | 13 | 25 | 49 | 55 | 0 | 0 | 0 | 36 | 72 | 85 |
| | 39 | 8 | 42 | 11 | 2 | 13 | 8 | 10 | 16 | 0 | 22 | 22 | 61 | 143 | 164 | 0 | 0 | 0 | 119 | 185 | 257 |
| Colonized | | | | | | | | | | | | | | | | | | | | | |
| 7 | 11 | 1 | 11 | 4 | 2 | 5 | 2 | 6 | 7 | 0 | 5 | 5 | 15 | 44 | 47 | 0 | 0 | 0 | 32 | 58 | 75 |
| 11 | 8 | 1 | 8 | 1 | 1 | 1 | 3 | 4 | 7 | 0 | 5 | 5 | 22 | 49 | 57 | 0 | 0 | 0 | 34 | 58 | 76 |
| 14 | 5 | 1 | 6 | 1 | 1 | 2 | 3 | 4 | 6 | 0 | 8 | 8 | 13 | 37 | 43 | 0 | 0 | 0 | 22 | 51 | 65 |
| 16 | 5 | 2 | 7 | 4 | 4 | 8 | 0 | 4 | 4 | 0 | 4 | 4 | 29 | 41 | 58 | 0 | 0 | 0 | 38 | 55 | 81 |
| | 29 | 5 | 32 | 10 | 8 | 16 | 8 | 18 | 24 | 0 | 20 | 20 | 79 | 171 | 205 | 0 | 0 | 0 | 136 | 222 | 297 |
| Weeded | | | | | | | | | | | | | | | | | | | | | |
| 3 | 6 | 3 | 7 | 2 | 0 | 2 | 1 | 2 | 3 | 0 | 6 | 6 | 13 | 47 | 50 | 0 | 0 | 0 | 22 | 51 | 68 |
| 5 | 7 | 5 | 7 | 5 | 5 | 5 | 2 | 4 | 4 | 0 | 10 | 10 | 15 | 40 | 45 | 0 | 0 | 0 | 27 | 62 | 71 |
| 8 | 3 | 6 | 6 | 4 | 1 | 4 | 4 | 6 | 7 | 0 | 13 | 13 | 17 | 42 | 47 | 0 | 0 | 0 | 28 | 68 | 77 |
| 18 | 15 | 7 | 16 | 2 | 1 | 3 | 3 | 8 | 9 | 0 | 11 | 11 | 21 | 38 | 38 | 0 | 0 | 0 | 41 | 65 | 77 |
| | 31 | 21 | 36 | 11 | 5 | 14 | 10 | 20 | 23 | 0 | 40 | 40 | 66 | 167 | 180 | 0 | 0 | 0 | 118 | 246 | 293 |
| Legumed | | | | | | | | | | | | | | | | | | | | | |
| 4 | 7 | 0 | 7 | 2 | 1 | 3 | 1 | 3 | 4 | 0 | 5 | 5 | 16 | 35 | 42 | 0 | 0 | 0 | 26 | 44 | 61 |
| 9 | 9 | 5 | 11 | 0 | 0 | 0 | 1 | 4 | 4 | 0 | 7 | 7 | 18 | 41 | 49 | 0 | 0 | 0 | 28 | 57 | 71 |
| 10 | 6 | 6 | 12 | 0 | 0 | 0 | 1 | 5 | 6 | 0 | 3 | 3 | 11 | 42 | 48 | 0 | 0 | 0 | 18 | 56 | 69 |
| 12 | 14 | 2 | 14 | 3 | 0 | 3 | 3 | 1 | 4 | 0 | 8 | 8 | 26 | 48 | 51 | 0 | 0 | 0 | 46 | 59 | 80 |
| | 36 | 13 | 44 | 5 | 1 | 6 | 6 | 13 | 18 | 0 | 23 | 23 | 71 | 166 | 190 | 0 | 0 | 0 | 118 | 216 | 281 |
| | 135 | 47 | 154 | 37 | 16 | 49 | 32 | 61 | 81 | 0 | 105 | 105 | 277 | 647 | 739 | 0 | 0 | 0 | 491 | 869 | 1128 |

Table 33b. Summary of germination and survival results from Gardiner seed plots. The percentage survival is here defined as the percentage of all seeds that germinated which survived to the end of the growing season.

| Treatment | Celtis | | | | Liquidambar | | | | Carya | | | | Sabal | | | | Magnolia | | | | Quercus | | | | Total | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cumulative germination | | Survival | | Cumulative germination | | Survival | | Cumulative germination | | Survival | | Cumulative germination | | Survival | | Cumulative germination | | Survival | | Cumulative germination | | Survival | | Cumulative germination | | Survival | |
| | # | (%) | # | (%) | # | (%) | # | (%) | # | (%) | # | (%) | # | (%) | # | (%) | # | (%) | # | (%) | # | (%) | # | (%) | # | (%) | # | (%) |
| Enhanced | 42 | (21) | 8 | (19) | 13 | (6.5) | 2 | (15) | 16 | ( 8) | 10 | (62) | 22 | (11) | 22 | (100) | 0 | | 0 | | 164 | (91) | 143 | (87) | 257 | | 185 | (72) |
| Colonized | 32 | (16) | 5 | (16) | 16 | (8) | 8 | (50) | 24 | (12) | 18 | (75) | 20 | (10) | 20 | (100) | 0 | | 0 | | 205 | (51) | 171 | (83) | 297 | | 222 | (75) |
| Weeded | 36 | (18) | 21 | (58) | 14 | (7) | 5 | (36) | 23 | (11.5) | 20 | (87) | 40 | (20) | 40 | (100) | 0 | | 0 | | 180 | (45) | 167 | (93) | 293 | | 246 | (84) |
| Legumed | 44 | (22) | 13 | (29) | 6 | (3) | 1 | (17) | 18 | ( 9) | 13 | (72) | 23 | (11.5) | 23 | (100) | 0 | | 0 | | 190 | (47) | 166 | (87) | 281 | | 216 | (77) |
| | 154 | (19) | 47 | (30) | 49 | (6) | 16 | (33) | 81 | (10) | 61 | (75) | 105 | (13) | 105 | (100) | 0 | | 0 | | 739 | (46) | 647 | (87) | 1128 | (20) | 869 | (77) |

The plot by plot survival results can also be examined on an individual species level. In order for this to be done in a statistically valid manner it is necessary to assume that there are no interactions between species. Since it is impossible to make this assumption in multi-species seed mixes any inferences about the ANOVA results will lack some degree of statistical rigor. In spite of this inability to meet all assumptions required by theory the analysis on a species by species basis may provide valuable insight pertinent to the hypotheses being tested which might otherwise be ignored. To-this end an ANOVA was run on the transformed survival data for each species. Maqnolia grandiflora was not included in the analysis since none germinated in any plots. The results are again presented in two ways depending on how plots 15 and 18 were assigned.

The five taxa showed varying results under the four analysis regimes. Carya glabra (Table 34b) and Liquidambar styraciflua (Table 34d) represent the simplest cases with no significant differences between any of the treatment means in either of the two different ways plots 15 and 18-were assigned.

Celtis laevigata (Table 34c) and Quercus (Q. virginiana and Q. laurifolia summed) (Table 34e) showed similiar patterns in response to two different ANOVA situations. Both had significant differences in at least some of the treatment means for the two analysis situations. When plots 15 and 18 were neglected two taxa exhibited slightly differing results. For Celtis the weeded, legumed and colonized means were not significantly different and the legumed, colonized and enhanced means were not significantly different, but weeded and enhanced means are significantly different. For Quercus the weeded, colonized and legumed means were not significantly different from each other, but all three are different from the enhanced mean which is also lower.

In the second ANOVA configuration in which plot 15 was assigned to the weeded treatment and plot 18 was ignored Celtis and Quercus showed similar but not identical patterns. The means were not significantly different for Celtis for the weeded and legumed treatments and neither were they different between the legumed, colonized and enhanced, but once again the weeded and enhanced means were different-with the enhanced value being the lower of the two. In the case of Quercus the weeded, colonized and legumed treatment means were not different from each other and all were significantly different from and higher than enhanced treatment mean.

Sabal palmetto exhibited significant differences between some treatment means for both ANOVA configurations (Table 34f). In the case where both plots 15 and 18 were neglected in the analysis the means for the weeded and colonized treatments were not significantly different, and the colonized, enhanced and legumed means were not significantly different, but the weeded mean was different from and higher than the legumed mean. With plot 18 neglected and plot 15 assigned to the weeded treatment the results were exactly the same as in the preceding analyses.

## Seed Plot Height Growth

Height growth data from the seed plots was analyzed in the same manner as the survival data. The plot assignments, and therefore the statistical analysis, was handled two ways for each individual species and for the sum of all species.

Table 34. Comparison of mean percent germination/survival for four experimental treatments (weeded, colonized, legumed, enhanced) using transformed data (arsine-square transformation). Means compared, following an ANOVA, using Duncan's multiple range test. Means with the same letter are not significantly different ($\alpha$ =0.05).

Table 34-a. All Species Summed.

| Plot Assignments for ANOVA | PR>F-value (ANOVA) | TREATMENT MEANS Weeded | Colonized | Legumed | Enhanced |
|---|---|---|---|---|---|
| #15 & #18 Neglected | .0079 | 25.017 A | 23.567 A | 22.857 A | 19.027 B |
| #15 = Weeded #18 Neglected | .0031 | 25.510 A | 23.567 A B | 22.857 B | 19.027 C |

Table 34-b. *Carya glabra*.

| Plot Assignments for ANOVA | PR>F-value (ANOVA) | TREATMENT MEANS Weeded | Colonized | Legumed | Enhanced |
|---|---|---|---|---|---|
| #15 & #18 Neglected | N.S. | 16.080 A | 17.390 A | 14.292 A | 10.897 A |
| #15 = Weeded #18 Neglected | N.S. | 16.167 A | 17.390 A | 14.292 A | 10.897 A |

Table 34-c. Celtis laevigata.

| Plot Assignments for ANOVA | PR>F-value (ANOVA) | TREATMENT MEANS Weeded | Colonized | Legumed | Enhanced |
|---|---|---|---|---|---|
| #15 & #18 | .0772 | 17.693 | 8.982 | 12.420 | 5.847 |
| Neglected | | A | A<br>B | A<br>B | B |
| #15 = Weeded | .0297 | 18.387 | 8.982 | 12.420 | 5.847 |
| #18 Neglected | | A | B | A<br>B | B |

Table 34-d. *Liquidambar styraciflua*.

| Plot Assignments for ANOVA | PR>F-value (ANOVA) | TREATMENT MEANS Weeded | Colonized | Legumed | Enhanced |
|---|---|---|---|---|---|
| #15 & #18 Neglected | N.S. | 7.863 A | 11.057 A | 2.992 A | 4.7 A |
| #15 = Weeded #18 Neglected | N.S. | 6.217 A | 11.057 A | 2.992 A | 4.7 A |

Table 34-e. Quercus.

| Plot Assignments for ANOVA | PR>F-value (ANOVA) | TREATMENT MEANS Weeded | Colonized | Legumed | Enhanced |
|---|---|---|---|---|---|
| #15 & #18 Neglected | .0604 | 41.360 A | 41.105 A | 39.645 A | 33.930 B |
| #15 = Weeded #18 Neglected | .0347 | 42.127 A | 41.105 A | 39.645 A | 33.930 B |

Table 34-f. Sabal palmetto.

| Plot Assignments for ANOVA | PR>F-value (ANOVA) | TREATMENT MEANS Weeded | Colonized | Legumed | Enhanced |
|---|---|---|---|---|---|
| #15 & #18 Neglected | .0841 | 25.833 A | 18.155 A B | 10.410 B | 11.910 B |
| #15 = Weeded #18 Neglected | .0300 | 27.040 A | 18.155 A B | 10.410 B | 11.910 B |

For the case of the height data summed over species the ANOVA results were the same in both combinations of assigning plots 15 and 18 (Table 35a). In both cases the F-value was significant at the .0001 level and the mean height growth for the weeded treatment was significantly different and higher than the other three treatment means. There were no significant differences between the means for the colonized, legumed, and enhanced treatments.

Breaking down the height growth data into individual species brings out an interesting trend. Generally the Quercus results mimicked the results obtained when all species are lumped together while Carya, Celtis, Liquidambar, Sabal and Magnolia all had similar results as a group that were quite different from Quercus.

As in the case with height data summed over species the Quercus data had a highly significant F-value for both ANOVA regimes. In the two cases the weeded treatment mean was found to be significantly different from and higher than the other three treatment means which were not significantly different from each other (Table 35b).

In large part the other four species, Carya glabra, Celtis laevigata, Liquidambar styraciflua, and Sabal palmetto exhibited the same growth response to the treatments. In all cases there were no statistically significant differences between treatment means (Tables 35b through 35f).

## Seedling Plot: Height Growth

The three species were first treated separately in the analysis under the assumption that the l-m spacing between seedlings didn't allow for any interaction between species. Data were also analyzed by summing growth data for all three species together.

The ANOVA of the height growth data for each of the three species showed a significant F value ($p < .05$, Table 36) indicating that at least one of the treatment means was significantly different from the rest. Duncan's multiple range test was used to determine which treatment means were significantly different (Table 36).

For Liquidambar seedlings the mean height growth for the weeded treatment was significantly different and higher than the mean height growth of the other three treatments, and there was no significant difference between the means for the colonized, enhanced and legumed treatments.

Again as with the sweetgum, the mean height change for the live oak (Quercus virginiana) seedlings was significantly different for the weeding treatment. The live oaks seedlings in the weeded plots had a higher mean growth change over the first growing season. The analysis again showed no significant difference between the mean in the other three treatments.

The Duncan's multiple range test when run on the cabbage palm data yielded different results as compared to the sweetgum and live oak.

Weeded, legumed and colonized treatments were not significantly different from each other. Neither were the colonized and enhanced treatments signifi-

**Table 35. Comparison of mean height growth (cm) of four experimental treatments (weeded, colonized, legumed, enhanced). Means compared, following an ANOVA, using Duncan's multiple range test. Means with the same letter are not significantly different (a = .05).**

Table 35-a. All Species Summed.

| Plot Assignments for ANOVA | PR>F-value (ANOVA) | TREATMENT MEANS Weeded | Colonized | Legumed | Enhanced |
|---|---|---|---|---|---|
| #15 & #18 Neglected | .0001 | 7.78 A | 6.4 B | 6.26 B | 5.8 B |
| #15 = Weeded #18 Neglected | .0001 | 8.02 A | 6.4 B | 6.26 B | 5.8 B |

**Table 35-b. Quercus.**

| Plot Assignments for ANOVA | PR>F-value (ANOVA) | TREATMENT MEANS Weeded | Colonized | Legumed | Enhanced |
|---|---|---|---|---|---|
| #15 & #18 Neglected | .0011 | 7.81 A | 6.26 B | 6.19 B | 5.63 B |
| #15 = Weeded #18 Neglected | .0001 | 8.17 A | 6.26 B | 6.19 B | 5.63 B |

Table 35-c. Carya glabra.

| Plot Assignments for ANOVA | PR>F-value (ANOVA) | TREATMENT MEANS Weeded | Colonized | Legumed | Enhanced |
|---|---|---|---|---|---|
| #15 & #18 Neglected | N.S. | 6.25 A | 5.61 A | 5.61 A | 5.17 A |
| #15 = Weeded #18 Neglected | N.S. | 5.87 A | 5.61 A | 5.61 A | 5.17 A |

**Table 35-d.** **Celtis laevigata**

| Plot Assignments for ANOVA | PR>F-value (ANOVA) | TREATMENT MEANS Weeded | Colonized | Legumed | Enhanced |
|---|---|---|---|---|---|
| #15 & #18 Neglected | N.S. | 8.5 A | 7.0 A | 6.5 A | 8.0 A |
| #15 = Weeded #18 Neglected | N.S. | 8.3 A | 7.0 A | 6.58 A | 8.0 A |

**Table 35-e.** **Liquidambar styraciflua.**

| Plot Assignments for ANOVA | PR>F-value (ANOVA) | TREATMENT MEANS Weeded | Colonized | Legumed | Enhanced |
|---|---|---|---|---|---|
| #15 & #18 Neglected | N.S. | 6.0 A | 5.0 A | 4.0 A | 4.5 A |
| #15 = Weeded #18 Neglected | N.S. | 6.0 A | 5.0 A | 4.0 A | 4.5 A |

Table 35-f. Sabal palmetto.

| Plot Assignments for ANOVA | PR>F-value (ANOVA) | TREATMENT MEANS Weeded | Colonized | Legumed | Enhanced |
|---|---|---|---|---|---|
| #15 & #18 Neglected | N.S. | 8.14 A | 8.65 A | 7.09 A | 7.67 A |
| #15 = Weeded #18 Neglected | N.S. | 8.23 A | 8.65 A | 7.09 A | 7.67 A |

**Table 36. Comparison of treatment means using Duncan's Multiple Range test on seedling height growth data for three species (Liquidambar styraciflua, Quercus virginiana Sabal palmetto) used in Gardinier plots (a = 0.5) Means with same letter are not significantly different. Height measured in cm.**

| | Treatment Means (n) | | | | |
|---|---|---|---|---|---|
| TAXA | Weeded | Colonized | Enhanced Colonizer | Legumed | ANOVA PR>F-value |
| Liquidambar Sweetgum | 5.05(40) | 0.70(40) | 0.37(40) | 0.37(40) | .0066 |
| | B | A | A | A | |
| Quercus Live oak | 9.50(40) | 4.92(40) | 4.85(40) | 4.05(40) | .0092 |
| | B | A | A | A | |
| Sabal Cabbage palm | 6.87(40) | 6.47(40) | 3.95(40) | 2.92(40) | .0476 |
| | A | A | A | | |
| | | | B | B | |
| All species | 7.15(120) | 3.17(120) | 2.74(120) | 3.63(120) | .0001 |
| | A | | | | |
| | | B | B | B | |

cantly different from each other. The weeded and legumed treatment means were both significantly different from and higher than the enhanced treatment.

When growth data were summed over species the results were the same as for Liquidambar and Sabal. The mean for the weeded treatment was significantly different and higherthan the means for the other three treatments which were not significantly different from each other.

## Discussion

### Seed Plots

The germination results and the accompanying survival rates highlight several points. First, there are wide differences in germination rates between species under field conditions as shown by the extremes of magnolia versus oaks. The survival of individuals germinated also exhibited interesting trends. The phenology, or pattern of germination and mortality also showed differences between species.

Germination was non-existent for Magnolia, low for Carya, Celtis, Liquidambar and Sabal, and moderate for Quercus. Possible causes of the differences include: seed quality, germination requirements, seed predation and erosion. The Celtis, Liquidambar, Carya, Sabal and Magnolia seed had been collected in fall of 1982 and stored dry at 4$^{0}$C, while acorns used were collected in November 1983 and stored out-of-doors in potting soil. The storage regime, as well as the initial quality of the seed could have had an effect on germination potential. Many of the acrons were nearly germinating when planted. Also, since germination was determined only from two sampling periods in March and October, any seeds that germinated but did not survive for long would have been easily overlooked. This would have been more critical for Celtis and Liquidambar since these species produce a rather delicate germling.

Seeds respond to environmental cues in order to germinate. In some cases dormancy must be overcome. It is possible that the conditions in the field plots were more generally suitable for some species than others. Losses of seed and young seedlings would also bias the germination results. Seed and seedling predation represent obvious cases. Seed losses to bird and small mammals are always a danger to any direct seeding operation, and are one of the primary causes of low germination in natural conditions. But it should be remembered that 'for large seeded species this is also a necessary cost of dispersal. Freshly disturbed soil often attracts animals to a site. Armadillos have been observed on the study plots and racoons, field mice and feral hogs have been recorded as present in the reclamation area.

Two factors were observed to lower the germination potential of Carya qlabra. One of the factors was physical and one was biological. The hickory nut is very large as seeds go and it was easily winnowed out of the soil during heavy rain. This process may have been aided by the second factor which was the removal of hickory nuts by armadillos. Tracks and signs of soil disturbance in the plots by these animals was seen on several occasions, and the signs were usually associated with the presence of only the outer husks of the hickory nut.

No other seed predators were ever in evidence although the freshly disturbed and seeded plots could well have attracted birds and small mammals alike. Seed loss due to predation is a process occurring regularly in natural systems.

The germination and survival data (see Table 33a) brought out some differences concerning seed size and seedling growth form. The species used in the seed plots fall into three categories based on seed size. Liquidambar has a small winged seed adapted for wind dispersal. The small seed contains little food storage to nourish a young seedling after germination. Both Celtis and Sabal have intermediate sized seeds. The seeds have a fleshy exocarp to attract avian and mammalian dispersal agents, and both have modified cotelydons for food storage.

Carya glabra, Quercus laurifolia, and Quercus virginiana are large-seeded species. These heavy seeds obviously do not disperse far on their own. The species are highly dependent on animals for that function. Acorns and hickory nuts have large starchy cotelydons which provide a food source for the developing seedling and for the predator.

The relationship between the food reserve of modified cotelydon and survival rate of a germinated seed is quite striking. The large-seeded species had high rates of survival. Seventy-five percent of all germinating hickories survived and 87% of all oaks survived. In contrast, only 33% of the Liquidambar individuals survived the first growing season. In the intermediate seed size group Celtis had low survival while Sabal had the highest possible survival. The Sabal results may be a bit anomalous due to the phenology of this species and the sampling schedule. In March, during the first sampling period there were no Sabal seedlings present on any of the plots. All Sabal seedlings present in the October census had germinated between in late spring and summer. Therefore, under this scheme the cabbage palm seedlings had 100% survival. This could be misleading, but may not be, due to the kind of seedling produced by the cabbage palm. The cabbage palm seedling has a single short very leathery leaf, which even if dead is resistant to decay for some time. If cabbage palm seeds had germinated and the seedling later succumbed - in all liklihood the leaf would have remained long enough to be detected. It is assumed that there was some slight mortality, but survival rates were high.

The discussion of the seedling of cabbage palm can be carried further, especially in comparison to the other species with an intermediate-sized seed: Celtis. Paired with the tough, leathery leaf, the cabbage palm seedling possesses a thick and somewhat leathery root system. The seedling as a whole is adapted to dealing with drought stress. The aboveground and belowground tissues are strong enough not to collapse under moisture tension. The Celtis seedling on the other hand has a more delicate stem and root that are more rapidly affected by moisture stress. It is reasonable to assume from this that the mortality of Celtis would be higher than Sabal if dessication were a problem - as it most certainly is on unvegetated overburden soils.

This analysis of seedling anatomy can also be extended to the other taxa in this study. Liquidambar, like Celtis, produces a seedling with delicate shoot and root, and would be expected to be killed more easily by drought stress. Carya and Quercus both produce a woody shoot and a tough fibrous root system making them better adapted to dealing with problems of water stress.

The relatively greater performance of large seeded species has been noted in other studies. Tourney and Korstian (1942) noted that seeds containing a large amount of reserve food and germinating in early spring are better adapted for direct seeding than small seeds which are slow to germinate and which produce plants of slow juvenile growth. Tackett and Grimes (1983) seeded three large-seeded species (Quercus rubra, Q. palustris, and Q. macrocarpa) with two small-seeded species (Paulownia tomentosa and Alnus glutinosa) and found that after 5 years of study the large-seeded oaks were more successfully established than the small-seeded species.

Sorting through and analyzing the results of the seed plot survival data is complicated by the four ways each ANOVA was run. While the confusion with plots 15 and 18 at first seemed a disaster, or at best an unwanted level of noise and confusion, it turns out to have provided some added insight into the affects the weeding treatment had on seedling growth. Analyzing the data following the original plot assignments (15 to enhanced and 18 to weeded) is clearly the least correct way to deal with the data since after plot 15 was weeded it was totally inappropriate to consider it as part of the enhanced treatment. Continuing to view plot 18 as part of the weeded treatment seems less offensive and easier to defend. The simplest way around these problems is to drop plots 15 and 18 from the statistical analysis altogether. Although this results in an unbalanced design it eliminates any problems of equivocation over the treatment status of plots 15 and 18. The second ANOVA configuration in which plot 15 is assigned to the weeded treatment and plot 18 is dropped is also a fairly clean case. Plot 15 was weeded during the later part of the growing season, and so beyond the germination stage the seedlings were growing under weeded conditions.

The survival data for all species summed together and using the two most realistic plot assignments (ignore plots 15 and 18, ignore 18 and assign 15 to weeded) showed that the enhanced treatment had lower values than the other three treatments. Several factors may have had an influence on the lower values for this treatment. Competition may have been more intense in the enhanced treatment plots, thereby reducing survival and possibly germination. Alleopathic inhibition of either growth or germination of woody plants by the species added is also possible. Andropogon virginicus has been reputed to produce allelochemicals affecting other species (Rice 1978) . The enhanced plots also may have suffered more seed loss to erosion before the soils were stabilized. The erosion problem, while remaining a possible cause, does not appear to warrant any great concern. At the time the major erosion problems arose in early January 1984 the extent and degree of erosion on site was assessed and mapped. An examination of the map and field notes from this time show that eight seed plots were affected by erosion rills. Quite by chance the eight affected plots were evenly distributed among the four treatments. Several plots influenced by erosion exhibited low survival while others showed high values. Only in two cases were seeds observed to have been washed out of plots (numbers 12 and 13) and these were returned to their plots by hand. This does not preclude the possibility of some seeds being lost altogether from some plots or being washed out of one plot into a down-slope plot. One indication that there may have been little significant movement of seed from plot to plot comes from the fact that no individuals of any of the species used in the legume treatment were ever found in plots other than the ones they were placed in.

For the individual species the results were varied and the picture was certainly less clear. Carya and Liquidambar showed no differences in survival

under any of the treatments. A problem in both cases may be the generally very low survival values, since some plots had no individuals or only one.

Celtis results were also affected by generally low numbers of seeds germinating and surviving, as there was less than 6% overall. The weeded treatment had higher survival rates than the enhanced treatment and the weeded mean was not different from the legumed mean for both ANOVA configurations. In the case where plot 15 is assigned to the weeded treatment and plot 18 is neglected, the weeded treatment is also significantly different from and higher than the colonized mean.

Most interesting is that once again the weeded treatment comes out on the high end of the scale and the enhanced treatment is on the low end.

The Sabal germination results point to the same conclusion for comparisons between weeded and enhanced under the two ANOVA arrangements. In fact, it is interesting that the comparisons between the means come out the same in both instances. The weeded mean was also different from and higher than the legumed treatment mean. Differences may be indications of competition, inhibition or possibly allelopathy when the "wrong" quantity and quality of seed is added. For Sabal the weeded mean was higher than the means for the two treatments in which seeds of specific colonizers were added (legumes and old field weeds), but the mean was not different from the colonized treatment where the treatment seeds were added naturally.

In both analyses for Quercus the results were the same: the weeded, colonized and legumed means were different from the enhanced mean, but the three means were not different from each other. Once again the enhanced treatment came out on the bottom, again begging the question, Why? Are competition, allelopathy or another form of inhibition involved due to the species added in this treatment? The means for the other three treatments were not significantly different and it may be similar to the Carya results, suggesting that species with large food reserves in the seed may experience less treatment-related mortality in the first growing season.

## Seed Plot Height Data

For the data summed over species the results of the analysis with plot 15 included compared to the results when the plot is not included is particularly interesting. When plot 15 is included in the enhanced treatment then the mean for that treatment is not significantly different from the weeded treatment. But when plot 18 isn't considered the enhanced treatment is significantly different from the weeded treatment. The weeding treatment inadvertently given to plot 15 made a difference in the mean height growth attained in the plot. This result gives an even stronger foundation to the analysis done without plots 15 and 18 in which the mean height growth in the weeded plots was highest lending support to. the inhibition hypothesis.

At first this result seems clear cut and profound - seedlings in the multi-species plots grew significantly better under the weeding treatment. Under the decision criteria discussed earlier this allows the rejection of the facilitation and tolerance models in favor of the inhibition model. At this juncture it would seem appropriate to conclude that this field test demonstrated

support for the operation of the inhibition hypothesis at an embryonic stage of forest development. But following the analysis through another step begins to uncover a muddled picture in which support for more than one of the hypotheses may be found. When the growth data are summed over all species the resultant data set is heavily biased by the Quercus data. The weighting is the result of two factors. First, the two oak species were lumped together to avoid skewing results by mis-identifying individual seedlings. The second and more important factor was the higher level of germination and survival shown by the oaks as compared to the other taxa. Oak seedlings made up 74% of all seedlings found. In contrast Sabal comprised 12%, Carya made up 7%, and Celtis and Liquidambar represented 5% and 2%, respectively. It comes as no surprise then that the analyses for Quercus have exactly the same results as the analysis for all species summed. The sheer number of oak seedlings masks to a large extent the response of the other species, which if examined separately, or at least apart from the Quercus data, lead to a much different conclusion.

The results of the statistical analysis for the other four species are surprizingly uniform and at odds with the Quercus results. The two ANOVA configurations yield the same results regardless of whether the species is Carya, Celtis, Liquidambar or Sabal. For each of these four species the three ANOVA's show no significant differences between any of the treatment means. By the decision criteria of hypothesis rejection/acceptance these results provide four cases where the facilitation and inhibition models would be rejected in favor of the tolerance model. As already stated, this result is quite different from that of the oaks and points out a valuable issue to keep in mind. Individual species don't always show the same response and so in field tests such as this one it is important to incorporate into the design and analysis a method for looking at individual species as well as the mix as a whole.

In view of the data just discussed for the seed plots the analysis of growth data from the seedling plots can be considered to provide either clarity or more confusion. The seed plot data had differing interpretations depending on whether species were lumped or treated separately. Surprisingly this does not seem to have been true for the transplant plots. The results were markedly similar in the seedling data whether species were lumped or separate. For three out four cases the result were the same and even for the fourth case the results were not all that dissimilar. For Liquidambar, Quercus and all species together the growth data showed the weeded treatment to have higher growth as compared to the other three treatments. In the case of Sabal the weeded treatment was not clearly superior but it was along with the colonized treatment significantly different from and higher than the legumed treatment mean.

Comparisons between the growth results from the seed and seedling plots provide another way to look for information on how late successional species interact with colonizing plants. The three species used in the seedling transplant plots were also used in the seed plots and so provide an extremely valuable means of comparison. Live oak was used in both types of plots. For reasons already discussed the data for live oak and laurel oak were lumped for the seed plots, but this should not affect the quality of comparisons discussed here. The oak seedlings had the same growth response under experimental treatments whether they were in the seed or seedling plots. The increased height growth under weeded conditions was common to both. This was also true for the instances of lumping all species data together, providing strong support for the rejection of the facilitation and tolerance models in favor of the inhibition

model. The strength of this support though must be tempered somewhat due to the oak bias in the seed plots when all species are considered together.

Sweetgum demonstrated an interesting switch in results from the seed experiment to the seedling experiment. Growth data from the seed plots favored the rejection of the facilitation and inhibition models in favor of the tolerance' model since none of the treatment means were significantly different; In the seedling plots the growth of sweetgum was best in the weeded treatment and so favoring the acceptance of the inhibition model. The-switch indicates another variable to consider--do species change during their life history such that different stages of their life history appear to favor different hypothesis? If so then the question addressing the pattern and process of ecosystem development become inextricably more complicated. A word of caution is again needed here, The very low-levels of germination/survival of sweetgum in the seed plots warrant some hesitancy in giving strong support to those results.

Like sweetgum, cabbage palm also showed signs of switching in response to treatment effects from the seed to the seedling plots. The seed plot data favored the acceptance of the tolerance model. The seedling plot results were not clear cut in which of the other two models they favored, but it was not the tolerance model. The means for the weeded, colonized and enhanced treatments were not different from each other, but the weeded and colonized treatments were different and higher than the legumed treatment. This lends some equivocal support for the inhibition and facilitation models.

The results obtained in the seedling and seed experiments provide an even stronger case to question the validity of the classical facilitation model of ecosystem development. The two experiments mesh together very nicely to provide a view of at least some of the events occurring in the earliest stages of succession. The seed tests simulated the milieu of the propagule arriving on site and encountering an open, uncolonized landscape. The seed plots provided a look at the critical period of germination, establishment and survival during the first growing season. This is a very tenuous period in the life of a plant in which the proper cues are needed for germination, the proper microenvironment is necessary for successful establishment, and adequate resources must be available on a continued basis to assure survival.

The seedling plots provided an analog to a later stage in the life of woody plants, that beyond the stage of germination and establishment more closely approximating the condition of second growing season. This stage of seedling development while not as precarious as that of the germling is still quite rigorous and still much dependent on the quality of the microenvironment. This is especially true of the moisture and nutrient status of the rooting zone, which for young seedlings is largely confined to the upper 15-20 cm of the soil profile. Moisture status of the rooting zone has a critical bearing on seedling survival and since the moisture levels in the uppermost horizon of the soil profile is largely under control of a function of time since last rainfall, there is a strong tie to the seasonal patterns of rainfall.

Interestingly, the two experiments addressing the same issues at different points in the life history of woody seedlings had some very similar results. Using overall change in height as indicatory of growth success both seeded and planted experiments showed that when species are lumped together the highest mean seedling height growth is obtained under the weeded treatment. As already mentioned, at the outset this provides a rather strong case supporting the

rejection of the facilitation and tolerance models in favor of the inhibition model--which, to reiterate, states whomever Colonizes the site first can hold it against all comers.

Curiously, the results are strongest in their call for the rejection of the facilitation model. This is a most interesting result since it was assumed at the outset that the facilitation model would be important during the early stages of ecosystem development when the microenvironment was harshest in terms of offering seeds and seedlings little amelioration or insulation from harsh physical conditions of bare soil where cold, heat, high light, dessication, erosion etc. are sources of stress to young seedlings. The erosion issue, as already discussed, may be a problem where facilitation is important. The value of colonizing herbs in soil stabilization was demonstrated in the early part of these field tests.

Classical ecological theory, as per Clements and the facilitation model, predicts that later successional woody species only enter the developmental cycle after these harsh conditions have been ameliorated by the colonizing vegetation. The pioneer plant community is commonly assumed to play a role in the following: accumulation of soil organic matter, development of soil profiles, building of vegetation structure to provide shade and reduce the kinetic energy of rainfall thereby reducing erosion, buildup of soil nitrogen levels, development of producer-consumer recycling feedbacks, development of symbiotic relations (plant/mycorrhizae for example).

The tree treatments, legumed, colonized and enhanced, provided three levels of looking at the issue of facilitation vs. competition. The colonizer treatment looks at facilitation in the classical sense. The enhanced treatment carried this one step further under the assumption that if colonizers facilitated the establishment of climax species then "more is better." The legume treatment addressed the role of nitrogen-fixing species as colonizers - tall fast growing legumes like Sesbania could provide shade for young seedlings, attract pollinators, provide perches for birds, and cover for small mammals. It was assumed all these benefits would accelerate the rate of ecosystem development.

Apparently, none of these presumed benefits were as strong as the reduction in competition for resources. It may be that scarce resources, such as moisture or soil N, limit vegetation development and that the relatively slower growing woody species when faced with herbaceous competitors show less vigorous growth. The element of time in ecosystem development should be kept in mind in interpreting the plot experiments. Forest development is a long-term process and the experimental plots provide. a very controlled view of the earliest stages.

The importance of competition that herbs and shrubs have on the successful establishment of trees is seen by devotees of the facilitation model as quite surprising. The result is less of a surprise and closer to expectations of the population ecologists and applied ecologists alike. In the later group are foresters who have long noted competition as a factor influencing successful reforestation. Tourney and Korstian (1931) note that a low dense cover of grasses and herbs is a decided disadvantage to reforestation. It not only provides a retreat for rodents and other seed-eating animals but, as soon as germination takes place, directly competes with the young seedlings. The roots of

the herbaceous competitors draw nutrients and water from the surface layers of the soil, and the intensity of competition often proves fatal to the tree.

Competition has also been documented as a cause of decreased establishment and growth of tree seedlings in reclamation. Tackett and Graves (1983) cited competition by an herbaceous cover crop as a factor significantly reducing the height growth of tree seedlings. Vogel and Berg (1973) also found competition from herbaceous plants could be detrimental to growth of tree seedlings. Brown (1973) also cited competition from herbaceous plants as one of the factors affecting germination and initial survival of seedlings.

What insight do these two field plot experiments provide into the pattern and process of forested ecosystem development? Some degree of caution is needed in interpreting the implication of the first year results from the Gardinier seed and seedling plots. Some of the most obvious considerations are:

1. Results are from a single growing season yet conclusions are extrapolated to the long term process of ecosystem development.

2. Experimental treatments were fixed not random and therefore statistical inferences are appropriate only to the treatment levels selected not to a population of possible levels.

3. A limited number of tree species were used in the test and the response of the species differ yet results are interpreted in terms of a whole forest.

4. Results are from a single set of tests carried out on one soil type at one site so conclusions about the process of forest development must be couched in appropriate scope and scale.

5. Ecosystem development is a long term dynamic process and a single growing season provides a very narrow window on this process. Different models (facilitation, inhibition etc.) may apply at different stages during development.

6. Allelopathic effects of herbaceous species on each other and on woody plants may be important and were not investigated.

7. The weeding treatment may provide indirect benefits due to the disturbance of the soil surface which could serve to increase rate of water infiltration into soil by breaking up soil crusts commonly found on exposed overburden soils.

8. In the seeded plots it was easier to locate and measure seedlings in the weeded plots. It is possible that some small seedlings were overlooked in the other treatment plots. If this occurred then germination results would be biased in favor of the weeded treatment.

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# APPENDIX A

## Successional Plant Community Structure and Litter Data (Supplement to Chapter 1)

TABLE A1. Herbaceous Biomass data obtained from 15 individual one meter square quadrats sampled at the 3 year old study site. Importance values equal the sum of relative frequency and relative biomass for each species.

| Species | Biomass g/15m$^2$ | Relative Biomass % | Biomass g/ha (x 667) | Sample | Relative Frequency % | Importance Value | Importance Value Percentage |
|---|---|---|---|---|---|---|---|
| Ambrosia artemisifolia | 370.7 | 3.3 | 247,256 | .33 | 8.5 | 11.8 | 5.9 |
| Baccharis halimifolia | 4.3 | 0.1 | 2,868 | .13 | 3.4 | 3.5 | 1.8 |
| Chenopodium ambrosioides | 0.7 | 0.1 | 466 | .07 | 1.8 | 1.9 | 1.0 |
| Crotolaria spectabalis | 187.5 | 1.7 | 125,063 | .20 | 5.2 | 6.9 | 3.5 |
| Cynodon dactylon | 1,544.6 | 13.9 | 1,030,248 | .60 | 15.5 | 29.4 | 14.7 |
| Cyperus sp. | 7.0 | 0.1 | 4,669 | .07 | 1.8 | 1.9 | 1.0 |
| Digitaria ciliaris | 327.1 | 2.9 | 218,176 | .80 | 20.7 | 23.6 | 11.8 |
| Eupatorium capillifolium | 23.2 | 0.2 | 15,474 | .13 | 3.4 | 3.6 | 1.8 |
| Eupatorium compositifolium | 35.2 | 0.3 | 23,478 | .07 | 1.8 | 2.1 | 1.1 |
| Euphorbia maculata | 0.8 | 0.1 | 534 | .07 | 1.8 | 1.9 | 1.0 |
| Indigofera hirsuta | 8236.8 | 74.1 | 5,493,945 | .73 | 18.9 | 93.0 | 46.5 |
| Paspalum urvillei | 344.9 | 3.1 | 230,048 | .33 | 8.5 | 11.6 | 5.8 |
| Rhynchelytrum repens | 28.2 | 0.3 | 18,809 | .20 | 5.2 | 5.5 | 2.8 |
| Setaria geniculata | 7.2 | 0.1 | 4,802 | .07 | 1.8 | 1.9 | 1.0 |
| Unk grass | 3.6 | 0.1 | 2,401 | .07 | 1.8 | 1.9 | 1.0 |
| | 11,121.8 | 1.004 | 7,418,240 | 3.87 | 100.1 | 200.5 | 100.7 |

TABLE A2. Herbaceous Biomass data obtained from 15 individual one meter square quadrats sampled at the 8 year old study site. Importance values equal the sum of relative frequency and relative biomass percentages for each species.

| Species | Biomass g/15m$^2$ | Relative Biomass % | Biomass g/ha (x 667) | Frequency n/15 | Relative Frequency % | Importance Value | Importance Value Percentage |
|---|---|---|---|---|---|---|---|
| Andropogon virginicus | 475.3 | 7.5 | 317,025 | .33 | 9.9 | 17.4 | 8.7 |
| Baccharis halimifolia | 243.3 | 3.8 | 162,281 | .20 | 6.0 | 9.8 | 4.9 |
| Chenopodium ambrosioides | 37.2 | 0.6 | 24,812 | .13 | 3.9 | 4.5 | 2.3 |
| Conyza canadensis | 2.0 | 0.1 | 1,334 | .07 | 2.1 | 2.2 | 1.1 |
| Eupatorium capillifolium | 154.1 | 2.4 | 102,785 | .20 | 6.0 | 8.4 | 4.2 |
| Juncus sp. | 12.4 | 0.2 | 8,271 | .07 | 2.1 | 2.2 | 1.1 |
| Momordica charantia | 143.8 | 2.3 | 95,915 | .40 | 12.0 | 14.3 | 7.2 |
| Panicum hemitomon | 104.1 | 1.6 | 69,435 | .13 | 3.9 | 5.5 | 2.8 |
| Panicum sp. | 6.6 | 0.1 | 4,402 | .07 | 2.1 | 2.2 | 1.1 |
| Rhynchelytrum repens | 3,211.1 | 50.7 | 2,141,804 | .93 | 27.8 | 78.5 | 39.3 |
| Rubus sp. | 111.8 | 1.8 | 74,571 | .07 | 2.1 | 3.9 | 2.0 |
| Solidago fistulosa | 1,718.2 | 27.1 | 1,146,039 | .47 | 14.1 | 41.2 | 20.6 |
| Unk grass | 119.2 | 1.9 | 79,506 | .27 | 8.1 | 10.0 | 5.0 |
| | 6339.1 | 100.1 | 4,228,180 | 3.34 | 100.1 | 200.1 | 100.3 |

TABLE A3. Herbaceous biomass data obtained from 15 individual one meter square quadrats sampled at the 17-year old study site. Importance values equal the sum of relative frequency and relative biomass percentages for each species.

| Species | Biomass g/15m$^2$ | Relative Biomass % | Biomass g/ha (x 667) | Sample Frequency | Relative Frequency % | Importance Value | Importance Value Percentage |
|---|---|---|---|---|---|---|---|
| Aeschynomene americana | 1.8 | 0.1 | 1,200 | .13 | 4.5 | 4.6 | 2.3 |
| Andropogon virginicus | 86.0 | 1.0 | 57,362 | .07 | 2.4 | 3.4 | 1.7 |
| Baccharis halimifolia | 2,053.0 | 24.8 | 1,369,351 | .13 | 4.5 | 29.3 | 14.7 |
| Drymaria cordata | 236.0 | 2.9 | 157,412 | .47 | 16.3 | 19.2 | 9.6 |
| Indigofera hirsuta | 2,239.6 | 27.1 | 1,493,813 | .47 | 16.3 | 43.4 | 21.7 |
| Lantana camara | 413.9 | 5.0 | 276,071 | .27 | 9.4 | 14.4 | 7.2 |
| Momordica charantia | 2.4 | 0.1 | 1,601 | .07 | 2.4 | 2.5 | 1.3 |
| Passiflora pectinata | 4.0 | 0.1 | 2,668 | .07 | 2.4 | 2.5 | 1.3 |
| Rhynchelytrum repens | 2,185.6 | 26.4 | 1,457,795 | .60 | 21.0 | 47.4 | 23.7 |
| Rubus sp. | 284.6 | 3.4 | 189,828 | .20 | 6.9 | 10.3 | 5.2 |
| Urena lobata | 751.3 | 9.1 | 501,117 | .33 | 11.5 | 20.6 | 10.3 |
| Lamiaceae sp. | 21.0 | 0.3 | 14,007 | .07 | 2.4 | 2.7 | 1.4 |
| | 8,279.2 | 100.3 | 5,522,226 | 2.88 | 100.0 | 200.3 | 100.35 |

TABLE A4. Herbaceous biomass data obtained from 15 individual one meter square quadrats sampled at the 43 year old study site. Importance values equal to the sum of relative frequency and relative biomass percentages for each species.

| Species | Biomass $g/15m^2$ | Relative Biomass | Biomass g/ha (x 667) | Sample Frequency | Relative Frequency | Importance Value | Importance Value Percentage |
|---|---|---|---|---|---|---|---|
| Andropogon sp. | 210.0 | 7.3 | 140,070 | .07 | 3.0 | 10.3 | 5.2 |
| Asplenium hetereochorum | 2.0 | 0.1 | 1,334 | .13 | 5.6 | 5.7 | 2.9 |
| Baccharis halimifolia | 107.4 | 3.8 | 71,636 | .07 | 3.0 | 6.8 | 3.4 |
| Cassia fasciculata | 178.9 | 6.3 | 119,326 | .27 | 11.7 | 18.0 | 9.0 |
| Cyperus sp. | 18.4 | 0.6 | 12,273 | .13 | 5.6 | 18.2 | 3.1 |
| Eupatorium capillifolium | 17.6 | 0.6 | 11,739 | .07 | 3.0 | 3.6 | 1.8 |
| Eupatorium compositifolium | 12.8 | 0.4 | 8,538 | .07 | 3.0 | 3.4 | 1.7 |
| Lantana camara | 113.4 | 3.9 | 75,638 | .07 | 3.0 | 6.9 | 3.5 |
| Panicum commutatum | 1.2 | 0.1 | 800 | .07 | 3.0 | 3.1 | 1.6 |
| Passiflora pectinata | 29.2 | 1.0 | 19,476 | .13 | 5.6 | 6.6 | 3.3 |
| Psidium guajava | 347.2 | 12.1 | 231,582 | .07 | 3.0 | 15.1 | 7.6 |
| Quercus virginiana | 72.8 | 2.5 | 48,558 | .07 | 3.0 | 5.5 | 2.8 |
| Rhynchelytrum repens | 1,505.7 | 52.6 | 1,004,302 | .27 | 11.7 | 64.3 | 32.5 |
| Setaria geniculata | 6.6 | 0.2 | 4,402 | .07 | 3.0 | 3.2 | 1.6 |
| Smilax bononox | 15.9 | 0.6 | 10,605 | .07 | 3.0 | 3.6 | 1.8 |
| Solidago fistulosa | 31.0 | 1.1 | 20,677 | .13 | 5.6 | 6.7 | 3.4 |
| Trifolium sp. | 3.6 | 0.1 | 2,401 | .07 | 3.0 | 3.1 | 1.6 |
| Urena lobata | 112.0 | 3.9 | 74,704 | .07 | 3.0 | 6.9 | 3.5 |
| Vitis rotundifolia | 48.9 | 1.7 | 32,616 | .20 | 8.7 | 10.4 | 5.2 |
| Unknown grass | 1.1 | 0.1 | 734 | .07 | 3.0 | 3.1 | 1.6 |
| Unknown composite | 24.4 | 0.8 | 16,275 | .13 | 5.6 | 6.4 | 3.2 |
| | 2,860.1 | 99.8 | 1,907,687 | 2.30 | 99.1 | 199.0 | 100.3 |

TABLE A5. Herbaceous Biomass data obtained from 15 individual one meter square quadrats sampled at the 60 year old study site. Importance values equal to sum of relative frequency and relative biomass percentages for each species.

| Species | Biomass $g/15m^2$ | Relative Biomass % | Biomass g/ha (x 667) | Sample Frequency | Relative Frequency % | Importance Value | Importance Value Percentage |
|---|---|---|---|---|---|---|---|
| *Ampelopsis arborea* | 1.2 | 0.1 | 800 | .07 | 1.4 | 1.5 | 0.8 |
| *Bahia sp.* | 0.7 | 0.1 | 467 | .07 | 1.4 | 1.5 | 0.8 |
| *Bidens bipinnata* | 9.6 | 1.1 | 6,403 | .20 | 4.0 | 5.1 | 2.6 |
| *Blechnum serrulatum* | 12.2 | 1.4 | 8,137 | .07 | 1.4 | 2.8 | 1.4 |
| *Callicarpa americana* | 15.5 | 1.8 | 10,339 | .07 | 1.4 | 3.2 | 1.6 |
| *Cynodon dactylon* | 0.4 | 0.1 | 267 | .07 | 1.4 | 1.5 | 0.8 |
| *Drymaria cordata* | 0.1 | 0.1 | 67 | .07 | 1.4 | 1.5 | 0.8 |
| *Desmodium sp.* | 0.8 | 0.1 | 534 | .07 | 1.4 | 1.5 | 0.8 |
| *Gelsimium sempervirens* | 83.3 | 9.6 | 55,561 | .67 | 13.5 | 23.1 | 11.6 |
| *Liquidambar styraciflua* | 33.6 | 3.9 | 22,411 | .13 | 2.6 | 6.5 | 3.3 |
| *Oplismenus setarius* | 14.7 | 1.7 | 9,805 | .20 | 4.0 | 5.7 | 2.9 |
| *Panicum commutatum* | 8.9 | 1.0 | 5,936 | .40 | 8.1 | 9.1 | 4.6 |
| *Panicum sp.* | 83.5 | 9.6 | 55,695 | .13 | 2.6 | 12.2 | 6.1 |
| *Quercus nigra* | 177.7 | 20.5 | 118,526 | .27 | 5.4 | 25.9 | 13.0 |
| *Quercus virginiana* | 1.8 | 0.2 | 1,200 | .07 | 1.4 | 1.6 | 0.8 |
| *Rubus sp.* | 10.6 | 1.2 | 7,070 | .20 | 4.0 | 5.2 | 2.6 |
| *Smilax bononox* | 249.9 | 28.9 | 166,683 | .73 | 14.7 | 43.6 | 21.8 |
| *Tilandsia unesioides* | 61.1 | 7.1 | 40,754 | .80 | 16.1 | 23.2 | 11.6 |
| *Vitis rotundifolia* | 57.1 | 6.6 | 38,086 | .27 | 5.4 | 12.0 | 6.0 |
| *Toxicodendron radicans* | 3.0 | 0.3 | 2,001 | .13 | 2.6 | 2.9 | 1.5 |
| *Urena lobata* | 18.8 | 2.2 | 12,540 | .20 | 4.0 | 6.2 | 3.1 |
| *Unknown composite* | 20.8 | 2.4 | 13,874 | .07 | 1.4 | 3.8 | 1.9 |
| | 865.3 | 100.0 | 577,155 | 4.96 | 99.6 | 199.6 | 100.4 |

Table A6. Community structure data is given for shrub species at the 8 year old surface mine site. Density and dominance values are given for species in each sample quadrat. Importance values are calculated using mean site relative density and dominance values.

| Species | Actual Stems Measured | Density,* stems/ha: Quadrat Q1 | Q2 | Q3 | TOT | Relative Density, RD†% | Dominance (Basal Area) $m^2$/ha: Quadrat Q1 | Q2 | Q3 | TOT | Relative Dominance, RDD†% | Importance Value, RD + RDD§ | Importance Value Percentage** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Baccharis halimifolia | 78 | 480 | 3000 | 4200 | 2400 | 62.9 | 0.039 | 1.151 | 1.475 | 0.824 | 25.1 | 88.0 | 44.0 |
| Psidium guajava | 1 | -- | -- | 100 | 31 | 0.8 | -- | -- | 0.035 | 0.011 | 0.3 | 1.1 | 0.5 |
| Salix caroliniana | 43 | -- | 700 | 3600 | 1323 | 34.7 | -- | 1.724 | 6.130 | 2.410 | 73.8 | 108.5 | 54.3 |
| Schinus terebinthifolius | 2 | -- | 200 | -- | 62 | 1.6 | -- | 0.121 | -- | 0.037 | 1.0 | 2.6 | 1.3 |
| TOTAL | 124 | 480 | 3900 | 7900 | 3816 | 100.0 | 0.039 | 2.996 | 7.640 | 3.282 | 100.2 | 200.2 | 100.1 |

*Quadrat 1 Area = 125 $m^2$; Quadrat 2 Area = 100 $m^2$; Quadrat 3 Area = 100 $m^2$; Total Area = 325 $m^2$.
†Maximum = 100.
§Maximum = 200.
**Maximum = 100%.

Table A7 Community structure data is given for shrub species at the 17 year old surface mine site. Density and dominance values are given for species occurring in each sample quadrant. Importance values are calculated using mean site relative density and dominance values.

| Species | Actual Stems Measured | Density,* stems/ha: Quadrat Q1 | Q2 | Q3 | TOT | Relative Density, RD†% | Dominance (Basal Area) $m^2$/ha: Quadrat Q1 | Q2 | Q3 | TOT | Relative Dominance, RDD†% | Importance Value, RD + RDD§ | Importance Value Percentage** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Baccharis halimifolia | 139 | 5400 | 4533 | 5829 | 5295 | 19.6 | 1.366 | 0.800 | 1.218 | 1.143 | 36.4 | 56.0 | 28.0 |
| Psidium guajava | 14 | 1400 | -- | | 533 | 2.0 | 0.319 | -- | | 0.121 | 3.9 | 5.9 | 2.9 |
| Myrica cerifera | 10 | -- | -- | 1143 | 381 | 1.4 | -- | -- | 1.143 | 0.381 | 12.1 | 13.5 | 6.8 |
| Urena lobata | 379 | 9900 | 30399 | 5943 | 14436 | 53.5 | 0.335 | 1.275 | 0.299 | 0.591 | 18.8 | 72.3 | 36.2 |
| Lantana camara | 166 | 10000 | 2000 | 5829 | 6323 | 23.4 | 1.794 | 0.036 | 0.632 | 0.903 | 28.8 | 52.2 | 26.1 |
| TOTAL | 708 | 26700 | 36932 | 18744 | 26968 | 99.9 | 3.784 | 2.111 | 3.292 | 3.139 | 100.0 | 199.9 | 100.0 |

*Quadrat 1 Area = 100 $m^2$; Quadrat 2 Area = 75 $m^2$; Quadrat 3 Area = 88 $m^2$; Total Area = 263 $m^2$.
†Maximum = 100.
§Maximum = 200.
**Maximum = 100%.

Table A8 Community structure data is given for shrub species at the 43 year old surface mine site. Density and dominance values are given for species in each sample quadrat. Importance values are calculated using mean site relative density and dominance values.

| Species | Actual Stems Measured | Density,* stems/ha | | | | Relative Density, RD†% | Dominance (Basal Area) $m^2$/ha | | | | Relative Dominance, RDD†% | Importance Value, RD + RDD§ | Importance Value Percentage** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Quadrat | | | TOT | | Quadrat | | | TOT | | | |
| | | Q1 | Q2 | Q3 | | | Q1 | Q2 | Q3 | | | | |
| Baccharis halimifolia | 4 | -- | 160 | 160 | 107 | 2.0 | -- | 0.004 | 0.006 | 0.003 | 0.2 | 2.2 | 1.1 |
| Psidium guajava | 3 | -- | -- | 240 | 80 | 1.5 | -- | -- | 0.001 | 0.001 | 0.1 | 1.6 | 0.8 |
| Myrica cerifera | 67 | -- | 3200 | 2160 | 1787 | 33.8 | -- | 1.121 | 0.301 | 0.473 | 33.1 | 66.9 | 33.4 |
| Lantana camara | 78 | -- | 5200 | 1040 | 2080 | 39.4 | -- | 0.101 | 0.010 | 0.037 | 2.6 | 42.0 | 20.9 |
| Serenoa repens | 12 | 960 | -- | -- | 320 | 6.1 | 0.390 | -- | -- | 0.130 | 9.1 | 15.2 | 7.6 |
| Prunus serotina | 10 | 480 | 160 | 160 | 267 | 5.1 | 0.132 | 0.131 | 0.083 | 0.115 | 8.1 | 13.2 | 6.6 |
| Quercus virginia | 16 | 160 | 960 | 160 | 426 | 8.1 | 0.053 | 1.588 | 0.016 | 0.552 | 38.7 | 46.8 | 23.4 |
| Quercus laurifolia | 5 | -- | 160 | 240 | 133 | 2.5 | -- | 0.167 | 0.128 | 0.098 | 6.9 | 9.4 | 4.7 |
| Persea palustris | 3 | -- | 240 | -- | 80 | 1.5 | -- | 0.057 | -- | 0.019 | 1.3 | 2.8 | 1.4 |
| TOTAL | 198 | 1600 | 10080 | 4160 | 5280 | 100.0 | 0.575 | 3.169 | 0.545 | 1.428 | 100.1 | 200.1 | 99.9 |

*Quadrat 1 Area = 125 $m^2$; Quadrat 2 Area = 125 $m^2$; Quadrat 3 Area = 125 $m^2$; Total Area = 375 $m^2$.
†Maximum = 100.
§Maximum = 200.
**Maximum = 100%.

Table A9 Community structure data is given for tree species at the 43 year old surface mine site. Density and dominance values are given for species in each sample quadrat. Importance values are calculated using mean site relative density and dominance values.

| Species | Actual Stems Measured | Density,* stems/ha — Quadrat Q1 | Q2 | Q3 | TOT | Relative Density, RD†§ | Dominance (Basal Area) $m^2$/ha — Quadrat Q1 | Q2 | Q3 | TOT | Relative Dominance, RDD†§ | Importance Value, RD + RDD§ | Importance Value Percentage** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Quercus virginiana | 1 | 40 | -- | -- | 13 | 9.8 | 0.415 | -- | -- | 0.138 | 2.5 | 12.3 | 6.2 |
| Quercus laurifolia | 2 | -- | -- | 80 | 27 | 20.3 | -- | -- | 1.439 | 0.479 | 8.8 | 29.1 | 14.6 |
| Pinus elliottii | 6 | -- | 40 | 200 | 80 | 60.2 | -- | 1.548 | 9.158 | 3.567 | 65.7 | 125.9 | 62.9 |
| Pinus palustris | 1 | -- | -- | 40 | 13 | 9.8 | -- | -- | 3.739 | 1.246 | 22.9 | 32.7 | 16.4 |
| TOTAL | 10 | 40 | 40 | 320 | 133 | 100.1 | 0.415 | 1.548 | 14.336 | 5.430 | 99.9 | 200.0 | 100.1 |

*Quadrat 1 Area = 250 $m^2$; Quadrat 2 Area = 250 $m^2$; Quadrat 3 Area = 250 $m^2$; Total Area = 750 $m^2$.
†Maximum = 100.
§Maximum = 200.
**Maximum = 100%.

Table A10 Community structure data is given for tree species occurring on the 60 year old mine site. Density and dominance values are given for species occurring in each sample quadrat. Importance values are calculated using mean site relative density and dominance values.

| Species | Actual Stems Measured | Density,* # stems/ha: Quadrat Q1 | Q2 | Q3 | TOT | Relative Density, RD†% | Dominance (Basal Area) $m^2$/ha: Quadrat Q1 | Q2 | Q3 | TOT | Relative Dominance, RDD†% | Importance Value, RD + RDD§ | Importance Value Percentage** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Quercus virginiana | 11 | 360 | 80 | -- | 147 | 44.1 | 25.059 | 10.381 | | 11.810 | 46.5 | 90.6 | 45.2 |
| Pinus elliottii | 6 | 160 | 40 | 40 | 80 | 24.0 | 5.736 | 0.908 | 1.195 | 2.612 | 10.3 | 34.3 | 17.2 |
| Prunus serotina | 3 | -- | -- | 120 | 40 | 12.0 | -- | -- | 5.033 | 1.678 | 6.6 | 18.6 | 9.3 |
| Quercus nigra | 4 | 80 | -- | 80 | 53 | 15.9 | 2.332 | -- | 16.478 | 6.268 | 24.7 | 40.6 | 20.3 |
| Liquidambar styraciflua | 1 | -- | 40 | -- | 13 | 3.9 | -- | 9.161 | -- | 3.053 | 12.0 | 15.9 | 8.0 |
| TOTAL | 25 | 600 | 160 | 240 | 333 | 99.9 | 33.127 | 20.450 | 22.706 | 25.421 | 100.1 | 200.0 | 100.0 |

*Quadrat 1 Area = 250 $m^2$; Quadrat 2 Area = 250 $m^2$; Quadrat 3 Area = 250 $m^2$; Total Area = 750 $m^2$.
†Maximum = 100.
§Maximum = 200.
**Maximum = 100%.

Table A11 Community structure data is given for shrub species at the 60 year old surface mine site. Density and dominance values are given for specie in each sample quadrat. Importance values are calculated using mean site density and dominance values.

| Species | Actual Stems Measured | Density,* stems/ha: Quadrat Q1 | Q2 | Q3 | TOT | Relative Density, RDt% | Dominance (Basal Area) $m^2$/ha: Quadrat Q1 | Q2 | Q3 | TOT | Relative Dominance, RDDt% | Importance Value, RD + RDD§ | Importance Value Percentage** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Baccharis halimifolia | 1 | 80 | -- | -- | 27 | 0.3 | 0.014 | -- | -- | 0.005 | 0.3 | 0.6 | 0.1 |
| Psidium guajava | 70 | 2480 | 1680 | 1440 | 1867 | 19.4 | 0.092 | 0.087 | 0.043 | 0.074 | 5.0 | 24.4 | 12.2 |
| Myrica cerifera | 14 | 400 | -- | 720 | 373 | 3.9 | 0.039 | -- | 0.191 | 0.077 | 5.2 | 9.1 | 4.6 |
| Urena lobata | 6 | -- | 480 | -- | 160 | 1.7 | -- | 0.003 | -- | 0.001 | 0.1 | 1.8 | 0.9 |
| Prunus serotina | 23 | 400 | 480 | 960 | 613 | 6.4 | -- | 0.245 | 0.810 | 0.365 | 24.8 | 31.2 | 15.6 |
| Quercus virginiana | 6 | 80 | 320 | 80 | 160 | 1.7 | 0.002 | 0.007 | 0.001 | 0.003 | 0.2 | 1.9 | 1.0 |
| Quercus laurifolia | 4 | 80 | -- | 240 | 107 | 1.1 | 0.018 | -- | 0.052 | 0.023 | 1.6 | 2.7 | 1.4 |
| Callicarpa americana | 70 | 2240 | 3200 | 160 | 1867 | 19.4 | 0.089 | 0.195 | 0.006 | 0.097 | 6.6 | 26.0 | 13.0 |
| Smilax bononox | 33 | 640 | 1680 | 320 | 880 | 9.1 | 0.049 | 0.111 | 0.017 | 0.059 | 4.0 | 13.1 | 6.6 |
| Vitis rotundifolia | 4 | -- | 160 | 160 | 107 | 1.1 | -- | 0.034 | 0.006 | 0.014 | 0.9 | 2.0 | 1.0 |
| Quercus nigra | 125 | 2560 | 2320 | 5120 | 3334 | 34.6 | 0.489 | 0.129 | 1.138 | 0.585 | 39.7 | 74.3 | 37.2 |
| Liquidambar styraciflua | 5 | 80 | 320 | -- | 133 | 1.4 | 0.018 | 0.493 | -- | 0.170 | 11.5 | 112.9 | 6.5 |
| TOTAL | 361 | 9040 | 10640 | 9200 | 9628 | 100.1 | 0.810 | 1.304 | 2.264 | 1.473 | 99.9 | 200.0 | 100.1 |

*Quadrat 1 Area = 125 $m^2$; Quadrat 2 Area = 125 $m^2$; Quadrat 3 Area = 125 $m^2$; Total Area = 375 $m^2$.
†Maximum = 100.
§Maximum = 200.
**Maximum = 100%.

Table A12 Herbaceous and litter biomass (g/m) data obtained from the three year old mine. Litter, grass, and herbaceous biomass values have been combined to form a total above-ground biomass category.

| Quadrat Sample | Grass Species | Herbaceous Species | Shrub Species | Tree Species | Total Living Biomass | Litter | Total Biomass: (Live Biomass & Litter |
|---|---|---|---|---|---|---|---|
| 1-1 | 310 | -- | -- | -- | 310 | 66 | 376 |
| 1-2 | 13 | 29 | -- | -- | 42 | 32 | 74 |
| 1-3 | 210 | -- | -- | -- | 210 | 56 | 266 |
| 1-4 | 89 | -- | -- | -- | 89 | 111 | 200 |
| 1-5 | 10 | 237 | -- | -- | 247 | 75 | 322 |
| Total | 632 | 266 | 0 | 0 | 898 | 340 | 1238 |
| Mean | 126 | 53 | 0 | 0 | 180 | 68 | 248 |
| ********** | | | | | | | |
| 2-1 | 505 | -- | -- | -- | 505 | 238 | 743 |
| 2-2 | 321 | 323 | -- | -- | 644 | 61 | 705 |
| 2-3 | 24 | 286 | 4 | -- | 314 | 108 | 422 |
| 2-4 | 6 | 243 | -- | -- | 249 | 98 | 347 |
| 2-5 | 117 | 86 | -- | -- | 203 | 24 | 227 |
| Total | 973 | 938 | 4 | 0 | 1915 | 529 | 2444 |
| Mean | 195 | 188 | 0.8 | 0 | 303 | 106 | 489 |
| ********** | | | | | | | |
| 3-1 | -- | 5604 | -- | -- | 5604 | 895 | 6499 |
| 3-2 | 19 | 758 | 1 | -- | 778 | 99 | 877 |
| 3-3 | 334 | 313 | -- | -- | 647 | 73 | 720 |
| 3-4 | 11 | 766 | -- | -- | 777 | 1761 | 2538 |
| 3-5 | 293 | 210 | -- | -- | 503 | 161 | 664 |
| Total | 657 | 7651 | 1 | 0 | 8309 | 2989 | 11298 |
| Mean | 131 | 1530 | 0.2 | 0 | 1662 | 598 | 2260 |
| ********** | | | | | | | |
| Site Total ($g/15\ m^2$) | 2262 | 8855 | 5 | 0 | 11122 | 3858 | 14980 |
| Site | 1,508,754 | 5,906,285 | 3335 | 0 | 7,418,374 | 2,573,286 | 9,991,660 |

Table A13 Herbaceous and litter biomass ($g/m^2$) data obtained from the 8 year old mine. Litter, grass, and herbaceous biomass values have been combined to form a total above-ground biomass category.

| Quadrat Sample | Grass Species | Herbaceous Species | Shrub Species | Tree Species | Total Living Biomass | Litter | Total Biomass: (Live Biomass & Litter |
|---|---|---|---|---|---|---|---|
| 1-1 | 784 | -- | -- | -- | 784 | 651 | 1435 |
| 1-2 | 426 | 14 | -- | -- | 440 | 1454 | 1894 |
| 1-3 | 358 | -- | -- | -- | 358 | 222 | 580 |
| 1-4 | 661 | 130 | -- | -- | 791 | 729 | 1520 |
| 1-5 | 492 | 5 | -- | -- | 497 | 177 | 674 |
| Total | 2721 | 149 | 0 | 0 | 2870 | 3233 | 6103 |
| Mean | 544 | 30 | 0 | 0 | 574 | 647 | 1221 |
| 2-1 | 263 | -- | 201 | -- | 464 | 482 | 946 |
| 2-2 | 152 | 83 | 4 | -- | 239 | 454 | 693 |
| 2-3 | 10 | 46 | -- | -- | 56 | 1452 | 1508 |
| 2-4 | 83 | 942 | -- | -- | 1025 | 578 | 1603 |
| 2-5 | 312 | 428 | -- | -- | 740 | 551 | 1291 |
| Total | 820 | 1499 | 205 | 0 | 2524 | 3517 | 6041 |
| Mean | 164 | 300 | 41 | 0 | 505 | 704 | 1208 |
| 3-1 | 4 | 212 | -- | -- | 216 | 808 | 1024 |
| 3-2 | -- | 97 | -- | -- | 97 | 1492 | 1589 |
| 3-3 | 69 | 24 | 150 | -- | 243 | 2066 | 2309 |
| 3-4 | 87 | 48 | -- | -- | 135 | 975 | 1110 |
| 3-5 | 230 | 20 | -- | -- | 250 | 1249 | 1499 |
| Total | 390 | 401 | 150 | 0 | 941 | 6590 | 7531 |
| Mean | 78 | 80 | 30 | 0 | 188 | 1318 | 1506 |
| Site Total ($g/15\ m^2$) | 3931 | 2049 | 355 | 0 | 6[illegible] | 13340 | 19675 |
| Site Total (g/ha:x667) | 2,621,977 | 1,366,683 | 236,785 | 0 | 4,225,445 | 8,897,780 | 13,123,225 |

Table A14 Herbaceous and litter biomass ($g/m_2$) data obtained from the 17 year old mine. Litter, grass and herbaceous biomass values have been combined to form a total above-ground biomass category.

| Quadrat Sample | Grass Species | Herbaceous Species | Shrub Species | Tree Species | Total Living Biomass | Litter | Total Biomass: (Live Biomass & Litter |
|---|---|---|---|---|---|---|---|
| 1-1 | 133 | 58 | 94 | -- | 285 | 518 | 803 |
| 1-2 | 268 | 362 | -- | -- | 630 | 12 | 642 |
| 1-3 | 473 | 7 | 132 | -- | 612 | 3020 | 3632 |
| 1-4 | 724 | -- | 56 | -- | 780 | 446 | 1226 |
| 1-5 | 231 | 19 | 194 | -- | 444 | 371 | 815 |
| Total | 1829 | 446 | 476 | 0 | 2751 | 4367 | 7118 |
| Mean | 366 | 89 | 95 | 0 | 550 | 873 | 1424 |
| 2-1 | 19 | 310 | 2006 | -- | 2335 | 669 | 3004 |
| 2-2 | 35 | 392 | -- | -- | 427 | 45 | 472 |
| 2-3 | -- | -- | -- | -- | -- | 867 | 867 |
| 2-4 | 221 | 1152 | -- | -- | 1373 | 2 | 1375 |
| 2-5 | -- | 1 | 473 | -- | 474 | 476 | 950 |
| Total | 275 | 1855 | 2479 | 0 | 4609 | 2059 | 6668 |
| Mean | 55 | 371 | 496 | 0 | 922 | 412 | 1334 |
| 3-1 | -- | -- | 257 | -- | 257 | 1002 | 1259 |
| 3-2 | -- | 14 | 34 | -- | 48 | 1126 | 1174 |
| 3-3 | 168 | 5 | 126 | -- | 299 | 331 | 630 |
| 3-4 | -- | 69 | -- | -- | 69 | 248 | 317 |
| 3-5 | -- | 117 | 132 | -- | 249 | 660 | 909 |
| Total | 168 | 205 | 549 | 0 | 922 | 3367 | 4289 |
| Mean | 34 | 41 | 110 | 0 | 185 | 673 | 858 |
| Site Total ($g/15\ m_2$) | 2272 | 2506 | 3504 | 0 | 8282 | 9793 | 18075 |
| Site Total (g/ha:x667) | 1,515,424 | 1,671,502 | 2,337,168 | 0 | 5,524,094 | 6,531,931 | 12,056,025 |

Table A15 Herbaceous and litter biomass ($g/m^2$) data obtained from the 43 year old mine. Litter, grass, and herbaceous biomass values have been combined to form a total above-ground biomass category.

| Quadrat Sample | Grass Species | Herbaceous Species | Shrub Species | Tree Species | Total Living Biomass | Litter | Total Biomass: (Live Biomass & Litter |
|---|---|---|---|---|---|---|---|
| 1-1 | 589 | -- | -- | -- | 589 | 57 | 646 |
| 1-2 | 582 | -- | -- | -- | 582 | 0 | 582 |
| 1-3 | 215 | -- | -- | -- | 215 | 0 | 215 |
| 1-4 | 126 | 63 | -- | -- | 189 | 311 | 500 |
| 1-5 | -- | -- | -- | -- | 0 | 0 | 0 |
| Total | 1512 | 63 | 0 | 0 | 1575 | 368 | 1943 |
| Mean | 303 | 13 | 0 | 0 | 315 | 74 | 389 |
| 2-1 | 1 | -- | -- | 73 | 74 | 1085 | 1159 |
| 2-2 | -- | -- | -- | -- | -- | 290 | 290 |
| 2-3 | -- | 1 | 112 | -- | 113 | 514 | 627 |
| 2-4 | 210 | 31 | 112 | -- | 353 | 547 | 900 |
| 2-5 | -- | -- | 347 | -- | 347 | 287 | 634 |
| Total | 211 | 32 | 571 | 73 | 887 | 2723 | 3610 |
| Mean | 42 | 6 | 114 | 15 | 177 | 545 | 722 |
| 3-1 | 9 | -- | -- | -- | 9 | 982 | 991 |
| 3-2 | 1 | 85 | -- | -- | 86 | 661 | 747 |
| 3-3 | -- | 10 | 52 | -- | 62 | 1265 | 1327 |
| 3-4 | 21 | 129 | 2 | -- | 152 | 1259 | 1411 |
| 3-5 | -- | 88 | -- | -- | 88 | 1265 | 1353 |
| Total | 31 | 312 | 54 | 0 | 397 | 5432 | 5829 |
| Mean | 6 | 62 | 11 | 0 | 79 | 1086 | 1166 |
| Site Total ($g/15\ m^2$) | 1754 | 407 | 625 | 73 | 2859 | 8523 | 11382 |
| Site Total (g/ha:x667) | 1,169,918 | 271,469 | 416,875 | 48,691 | 1,906,953 | 5,684,841 | 7,591,794 |

Table A16 Herbaceous and litter biomass ($g/m^2$) data obtained from the 60 year old mine. Litter, grass, and herbaceous biomass values have been combined to form a total above-ground biomass category.

| Quadrat Sample | Grass Species | Herbaceous Species | Shrub Species | Tree Species | Total Living Biomass | Litter | Total Biomass: (Live Biomass & Litter |
|---|---|---|---|---|---|---|---|
| 1-1 | 1 | 5 | 1 | 2 | 9 | 421 | 430 |
| 1-2 | 2 | 0 | 1 | 0 | 3 | 252 | 255 |
| 1-3 | 1 | 14 | 18 | 0 | 33 | 485 | 518 |
| 1-4 | 94 | 28 | 16 | 0 | 138 | 845 | 983 |
| 1-5 | 3 | 3 | 2 | 0 | 8 | 255 | 263 |
| Total | 101 | 50 | 38 | 2 | 191 | 2258 | 2449 |
| Mean | 20 | 10 | 8 | 0.4 | 39 | 452 | 490 |
| 2-1 | 0 | 0 | 30 | 0 | 30 | 589 | 619 |
| 2-2 | 3 | 26 | 102 | 0 | 131 | 1713 | 1844 |
| 2-3 | 1 | 0 | 35 | 0 | 36 | 1497 | 1533 |
| 2-4 | 0 | 1 | 22 | 4 | 27 | 810 | 837 |
| 2-5 | 0 | 0 | 32 | 9 | 41 | 347 | 388 |
| Total | 4 | 27 | 221 | 13 | 265 | 4956 | 5221 |
| Mean | 0.8 | 5 | 44 | 3 | 53 | 991 | 1044 |
| 3-1 | 1 | 8 | 50 | 29 | 88 | 787 | 875 |
| 3-2 | 2 | 3 | 25 | 60 | 90 | 1235 | 1325 |
| 3-3 | 0 | 12 | 53 | 35 | 100 | 1908 | 2008 |
| 3-4 | 0 | 2 | 3 | -- | 5 | 1611 | 1616 |
| 3-5 | 0 | 3 | 49 | 74 | 126 | 686 | 812 |
| Total | 3 | 28 | 180 | 198 | 409 | 6227 | 6636 |
| Mean | 0.6 | 6 | 36 | 40 | 82 | 1245 | 1327 |
| Site Total ($g/15\ m^2$) | 100 | 105 | 439 | 213 | 865 | 13441 | 14306 |
| Site Total (g/ha:x667) | 72,036 | 70,035 | 292,813 | 142,071 | 576,955 | 8,965,147 | 9,542,102 |

# APPENDIX B

## Sweetgum/Mycorrhizae/Fertilizer Data
## (Supplement to Chapter 2)

TABLE B1 Analysis of variance of effects of time on herbaceous biomass. Comparisons have been made (1) among six study sites (2) among three sample quadrat locations over all sites, and (3) among quadrats within sites and among sites.

Dependent Variable: Herbaceous Biomass - Comparison Among Sites

| Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | PR>F |
|---|---|---|---|---|---|
| Model | 5 | 6433422 | 1286684 | 3.28 | 0.0094 |
| Error | 84 | 32936494 | 392101 | --- | --- |
| Total | 89 | 39369916 | --- | --- | --- |

R-Square: 0.16 Mean: 328 Std. Dev: 626 Coefficient of Variation: 191.0

Dependent Variable: Herbaceous Biomass - Comparison Among 3 Quadrat Locations

| Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | PR>F |
|---|---|---|---|---|---|
| Model | 2 | 124624 | 62312 | 0.14 | 0.87 |
| Error | 87 | 39245291 | 451095 | --- | --- |
| Total | 89 | 39369916 | --- | --- | --- |

R-Square: 0.003 Mean: 328 Std. Dev: 672 Coefficient of Variation: 204.9

Dependent Variable: Herbaceous Biomass - Comparison Among Quadrats: Within Sites: Among Sites

| Source | Degree of Freedom | Sum of Squares | Mean Square | F Value | PR>F |
|---|---|---|---|---|---|
| Model | 17 | 14824133 | 872007 | 2.56 | 0.0031 |
| Error | 73 | 24545782 | 340914 | --- | --- |
| Total | 89 | 39369916 | --- | --- | --- |

R-Square: 0.38 Mean: 328 Std. Dev.: 584 Coefficient of Variation: 178.1

TABLE B2 Analysis of variance of effects of time on litter biomass accumulation. Comparisons have been made (1) among six study sites, (2) among three sample quadrat locations over all sites, and (3) among quadrats within sites and among sites.

Dependent Variable: Litter - Comparison Among Individual Sites

| Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | PR>F |
|---|---|---|---|---|---|
| Model | 5 | 9504835 | 19000967 | 7.08 | 0.0001 |
| Error | 84 | 22541470 | 268350 | --- | --- |
| Total | 89 | 32046305 | --- | --- | --- |

R-Square: 0.29 Mean: 544 Std. Dev: 518 Coefficient of Variation: 95.2

Dependent Variable: Litter - Comparison Among 3 Quadrat Locations

| Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | PR>F |
|---|---|---|---|---|---|
| Model | 2 | 3606651 | 1803325 | 5.52 | 0.0056 |
| Error | 87 | 28439654 | 326892 | --- | --- |
| Total | 89 | 32046305 | --- | --- | --- |

R-Square: 0.11 Mean: 544 Std. Dev: 571 Coefficient of Variation: 105.1

Dependent Variable: Litter - Comparison Among Quadrats: Within Sites: Among Sites

| Source | Degree of Freedom | Sum of Squares | Mean Square | F Value | PR>F |
|---|---|---|---|---|---|
| Model | 17 | 16512800 | 971341 | 4.50 | 0.0001 |
| Error | 72 | 15533505 | 215743 | --- | --- |
| Total | 89 | 32046305 | --- | --- | --- |

R-Square: 0.52 Mean: 544 Std. Dev.: 464 Coefficient of Variation: 85.4

TABLE B3 Analysis of variance of effects of time on total aboveground biomass (litter plus herbaceous biomass) accumulation. Comparisons have been made (1) among six study sites (2) among three sample quadrat locations over all sites, and (3) among quadrats within sites and among sites.

Dependent Variable: Total Aboveground Biomass: Comparison Among Sites (Litter and Herbaceous Biomass)

| Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | PR>F |
|---|---|---|---|---|---|
| Model | 5 | 16492484 | 3298496 | 4.66 | 0.0009 |
| Error | 84 | 59404100 | 707191 | --- | --- |
| Total | 89 | 75896585 | --- | --- | --- |

R-Square: 0.22 Mean: 872 Std. Dev: 841 Coefficient of Variation: 96.5

Dependent Variable: Total Aboveground Biomass - Comparison Among 3 Quadrat Locations

| Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | PR>F |
|---|---|---|---|---|---|
| Model | 2 | 4883424 | 2441712 | 2.99 | 0.0554 |
| Error | 87 | 71013160 | 816243 | --- | --- |
| Total | 89 | 75896585 | --- | --- | --- |

R-Square: 0.06 Mean: 872 Std. Dev: 903 Coefficient of Variation: 103.6

Dependent Variable: Total Aboveground Biomass - Comparison Among Quadrats: Within Sites: Among Sites

| Source | Degree of Freedom | Sum of Squares | Mean Square | F Value | PR>F |
|---|---|---|---|---|---|
| Model | 17 | 33079972 | 1945880 | 3.27 | 0.0002 |
| Error | 72 | 42816613 | 594675 | --- | --- |
| Total | 89 | 75896585 | --- | --- | --- |

R-Square: 0.44 Mean: 872 Std. Dev.: 771 Coefficient of Variation: 88.5

TABLE B4 Analysis of variance of effects of time on soil organic matter (SOM) fractions less than 600μ in diameter. Comparisons have been made (1) among six study sites (2) among three sample quadrat locations over all sites, and (3) among quadrats within sites and among sites.

Dependent Variable: SOM - <600μ: Comparison Among Sites

| Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | PR>F |
|---|---|---|---|---|---|
| Model | 5 | 5339 | 1068 | 27.30 | 0.0001 |
| Error | 84 | 3285 | 39 | --- | --- |
| Total | 89 | 8624 | --- | --- | --- |

R-Square: 0.619 Mean: 12.059 Std. Dev: 6.254 Coefficient of Variation: 51.9

Dependent Variable: Soil Organic Matter <600μ - Comparison Among 3 Quadrat Locations

| Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | PR>F |
|---|---|---|---|---|---|
| Model | 2 | 927 | 464 | 5.24 | 0.0071 |
| Error | 87 | 7696 | 88 | --- | --- |
| Total | 89 | 8624 | --- | --- | --- |

R-Square: 0.11 Mean: 12.059 Std. Dev: 9.406 Coefficient of Variation: 77.9

Dependent Variable: SOM <600μ - Comparison Among Quadrats: Within Sites: Among Sites

| Source | Degree of Freedom | Sum of Squares | Mean Square | F Value | PR>F |
|---|---|---|---|---|---|
| Model | 17 | 6769 | 398 | 15.45 | 0.0001 |
| Error | 71 | 1855 | 26 | --- | --- |
| Total | 89 | 8623 | --- | --- | --- |

R-Square: 0.78 Mean: 12.059 Std. Dev.: 5.076 Coefficient of Variation: 42.1

TABLE 35 Analysis of variance of effects of time on soil organic matter (SOM) fractions greater than 600μ but less than and equal to 1000μ in diameter. Comparisons have been made (1) among six study sites (2) among three sample quadrat locations over all sites, and (3) among quadrats within sites and among sites.

Dependent Variable: Soil Organic Matter 600μ<SOM<1000μ; Comparison Among Sites

| Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | PR>F |
|---|---|---|---|---|---|
| Model | 5 | 59360 | 11872 | 8.02 | 0.0001 |
| Error | 84 | 124291 | 1480 | --- | --- |
| Total | 89 | 183652 | --- | --- | --- |

R-Square: 0.32 Mean: 32.32 Std. Dev: 38.47 Coefficient of Variation: 119.0

Dependent Variable: SOM: 600μ<SOM<1000μ; Comparison Among Quadrats

| Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | PR>F |
|---|---|---|---|---|---|
| Model | 2 | 26139 | 13069 | 7.22 | 0.0013 |
| Error | 87 | 157513 | 1810 | --- | --- |
| Total | 89 | 183651 | --- | --- | --- |

R-Square: 0.14 Mean: 32.32 Std. Dev: 42.55 Coefficient of Variation: 131.7

Dependent Variable: SOM: 600<SOM<1000: Comparison Among Quadrats: Within Sites and Among Sites

| Source | Degree of Freedom | Sum of Squares | Mean Square | F Value | PR>F |
|---|---|---|---|---|---|
| Model | 17 | 136120 | 8007 | 12.12 | 0.0001 |
| Error | 72 | 47532 | 660 | --- | --- |
| Total | 89 | 183652 | --- | --- | --- |

R-Square: 0.74 Mean: 32.32 Std. Dev.: 25.69 Coefficient of Variation: 79.5

TABLE 86 Analysis of variance of effects of time on soil organic matter (SOM) fractions greater than 1000 μ in diameter. Comparisons have been made (1) among six study sites, (2) among three sample quadrat locations over all sites, and (3) among quadrats within sites and among sites.

Dependent Variable: SOM>1000μ - Comparison Among Individual Sites

| Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | PR>F |
|---|---|---|---|---|---|
| Model | 5 | 1452 | 290 | 15.17 | 0.0001 |
| Error | 84 | 1608 | 19 | --- | --- |
| Total | 89 | 3060 | --- | --- | --- |

R-Square: 0.47 Mean: 4.674 Std. Dev: 4.375 Coefficient of Variation: 93.6

Dependent Variable: SOM>1000μ - Comparison Among 3 Quadrat Locations

| Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | PR>F |
|---|---|---|---|---|---|
| Model | 2 | 305 | 152 | 4.82 | 0.0103 |
| Error | 87 | 2754 | 32 | --- | --- |
| Total | 89 | 3060 | --- | --- | --- |

R-Square: 0.10 Mean: 4.674 Std. Dev: 5.627 Coefficient of Variation: 120.4

Dependent Variable: SOM>1000μ - Comparison Among Quadrats: Within Sites: Among Sites

| Source | Degree of Freedom | Sum of Squares | Mean Square | F Value | PR>F |
|---|---|---|---|---|---|
| Model | 17 | 2126 | 125 | 9.65 | 0.0001 |
| Error | 72 | 935 | 13 | --- | --- |
| Total | 89 | 3060 | --- | --- | --- |

R-Square: 0.69 Mean: 4.674 Std. Dev.: 3.601 Coefficient of Variation: 77.1

TABLE B7 Analysis of variance of effects of time on soil organic matter (SOM) accumulations. Intrasite comparisons of three different size fractions ($\leq 600\mu$, $>600\mu$ and $\leq 1000\mu$ and $>1000\mu$) are presented for 17, 43 and 60 year old study sites.

Dependent Variable: SOM Site Age: 17 Years

| Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | PR>F |
|---|---|---|---|---|---|
| Model | 2 | 2004 | 1002 | 6.17 | 0.0045 |
| Error | 42 | 6817 | 162 | --- | --- |
| Total | 44 | 8821 | --- | --- | --- |

R-Square: 0.23 Mean: 11.55 Std. Dev: 12.74 Coefficient of Variation: 110.30

Dependent Variable: SOM Site Age: 43 Years

| Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | PR>F |
|---|---|---|---|---|---|
| Model | 2 | 22193 | 11096 | 21.68 | 0.0001 |
| Error | 42 | 21493 | 512 | --- | --- |
| Total | 44 | 43685 | --- | --- | --- |

R-Square: 0.51 Mean: 28.17 Std. Dev: 22.62 Coefficient of Variation: 80.31

Dependent Variable: SOM Site Age: 60 Years

| Source | Degree of Freedom | Sum of Squares | Mean Square | F Value | PR>F |
|---|---|---|---|---|---|
| Model | 2 | 33386 | 16693 | 7.33 | 0.0019 |
| Error | 42 | 95612 | 2277 | --- | --- |
| Total | 44 | 128998 | --- | --- | --- |

R-Square: 0.26 Mean: 37.63 Std. Dev.: 47.71 Coefficient of Variation: 126.81

TABLE B8 Analysis of variance of effects of time on Root Length Accumulation and distribution. Comparisons have been made (1) among six study sites (2) among three sample quadrat locations over all sites, and (3) among quadrats within sites and among sites.

Dependent Variable: Root Length - Comparison Among Individual Sites

| Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | PR>F |
|---|---|---|---|---|---|
| Model | 5 | 727615588 | 145523117 | 19.19 | 0.0001 |
| Error | 84 | 636831313 | 7581325 | --- | --- |
| Total | 89 | 1364446901 | --- | --- | --- |

R-Square: 0.53 Mean: 3551 Std. Dev: 2753 Coefficient of Variation: 77.5

Dependent Variable: Root Length - Comparison Among 3 Quadrat Locations

| Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | PR>F |
|---|---|---|---|---|---|
| Model | 2 | 7485486 | 3742743 | 0.24 | 0.7872 |
| Error | 87 | 1356961414 | 15597257 | --- | --- |
| Total | 89 | 1364446901 | --- | --- | --- |

R-Square: 0.01 Mean 3551 Std. Dev: 3949 Coefficient of Variation: 111.2

Dependent Variable: Root Length - Comparison Among Quadrats: Within Sites: Among Sites

| Source | Degree of Freedom | Sum of Squares | Mean Square | F Value | PR>F |
|---|---|---|---|---|---|
| Model | 17 | 856535099 | 50384417 | 7.14 | 0.0001 |
| Error | 72 | 507911802 | 7054330 | --- | --- |
| Total | 89 | 1364446901 | --- | --- | --- |

R-Square: 0.63 Mean: 3551 Std. Dev.: 2656 Coefficient of Variation: 74.8

# APPENDIX C

## Results of Soil Chemical Analyses
## (Supplement to Chapter 1)

Table C1 Results of soil chemical analyses from three sample quadrats of the 0 year old site. All elemental analyses were performed on double acid soil extractions and concentrations equal g/g soil.

| Quadrat | Sample | P | Ca ++ | Mg ++ | K + | Mn ++ | Na + | %H2O | pH |
|---|---|---|---|---|---|---|---|---|---|
| | 1-1 | 1095 | 2420 | 223 | 16 | 4.4 | 39 | 4.5 | 4.4 |
| | 1-2 | 131 | 2010 | 20 | 12 | 1.1 | 24 | 5.6 | 4.2 |
| | 1-3 | 4860 | 10860 | 413 | 80 | 16.5 | 106 | 10.7 | 5.3 |
| | 1-4 | 5269 | 12180 | 427 | 122 | 22.0 | 140 | 7.4 | 5.2 |
| | 1-5 | 3877 | 10980 | 113 | 46 | 17.6 | 88 | 5.6 | 4.9 |
| | 2-1 | 348 | 670 | 5 | 8 | 2.2 | 22 | 2.7 | 4.6 |
| | 2-2 | 2147 | 4640 | 13 | 32 | 6.6 | 58 | 4.9 | 5.0 |
| | 2-3 | 344 | 280 | 3 | 18 | 2.2 | 26 | 6.3 | 4.3 |
| | 2-4 | 3771 | 11960 | 200 | 91 | 28.6 | 109 | 5.9 | 5.1 |
| | 2-5 | 4293 | 6380 | 18 | 14 | 1.1 | 39 | 7.4 | 5.0 |
| | 3-1 | 2844 | 9280 | 25 | 35 | 9.9 | 59 | 2.8 | 5.2 |
| | 3-2 | 191 | 450 | 86 | 15 | 2.2 | 24 | 2.6 | 4.7 |
| | 3-3 | 1580 | 5260 | 127 | 17 | 16.5 | 46 | 2.8 | 4.6 |
| | 3-4 | 2876 | 10480 | 243 | 35 | 30.8 | 67 | 2.5 | 5.1 |
| | 3-5 | 2332 | 9820 | 271 | 46 | 35.2 | 75 | 3.8 | 4.9 |
| MEAN | | 2397 | 6511 | 146 | 39 | 13.1 | 61 | 5.0 | - |
| STD. ERROR | | 454 | 1169 | 37 | 9 | 3.0 | 9 | 0.6 | - |
| MIN | | 131 | 450 | 3 | 3 | 1.1 | 22 | 2.5 | 4.2 |
| MAX | | 5269 | 12180 | 427 | 122 | 35.2 | 140 | 10.7 | 5.3 |

Table C2 Results of soil chemical analyses from three sample quadrats of the 3 year old site. All elemental analyses were performed on double acid soil extractions and concentrations equal g/g soil.

| Quadrat | Sample | P | Ca ++ | Mg ++ | K + | Mn ++ | Na + | %H2O | pH |
|---|---|---|---|---|---|---|---|---|---|
| | 1-1 | 138 | 404 | 55 | 9 | 0.6 | 31 | 6.2 | 4.0 |
| | 1-2 | 71 | 320 | 49 | 10 | 0.6 | 21 | 6.1 | 4.0 |
| | 1-3 | 742 | 1454 | 85 | 14 | 1.5 | 42 | 7.5 | 4.5 |
| | 1-4 | 261 | 485 | 72 | 17 | 0.9 | 19 | 6.3 | 4.1 |
| | 1-5 | 2160 | 4000 | 130 | 22 | 3.9 | 74 | 8.3 | 4.9 |
| | 2-1 | 2810 | 4800 | 178 | 27 | 7.1 | 80 | 6.5 | 5.7 |
| | 2-2 | 1340 | 2000 | 85 | 3 | 2.5 | 44 | 6.3 | 4.8 |
| | 2-3 | 820 | 1520 | 90 | 5 | 2.4 | 39 | 8.9 | 4.6 |
| | 2-4 | 1453 | 2720 | 166 | 18 | 3.2 | 51 | 6.6 | 5.3 |
| | 2-5 | 227 | 560 | 73 | 16 | 0.9 | 26 | 5.8 | 4.2 |
| | 3-1 | 770 | 1440 | 88 | 16 | 2.0 | 46 | 11.8 | 4.9 |
| | 3-2 | 300 | 640 | 65 | 2 | 0.9 | 30 | 12.8 | 4.5 |
| | 3-3 | 363 | 720 | 57 | 17 | 0.9 | 24 | 12.7 | 4.2 |
| | 3-4 | 1769 | 3760 | 135 | 45 | 4.9 | 58 | 13.9 | 5.7 |
| | 3-5 | 287 | 647 | 73 | 25 | 1.0 | 25 | 13.8 | 4.5 |
| MEAN | | 901 | 1698 | 93 | 16 | 2.2 | 41 | 8.9 | - |
| STD. ERROR | | 215 | 378 | 10 | 3 | 0.5 | 5 | 0.8 | - |
| MIN | | 71 | 320 | 2 | 2 | 0.6 | 19 | 5.8 | 4.0 |
| MAX | | 2810 | 4800 | 45 | 45 | 7.1 | 80 | 13.9 | 5.7 |

Table C3 Results of soil chemical analysis from three sample quadrats of the 8 year old site. All elemental analyses were performed on double acid soil extraction and concentrations equal g/g soil.

| Quadrat | Sample | P | Ca ++ | Mg ++ | K + | Mn ++ | Na + | %H2O | pH |
|---|---|---|---|---|---|---|---|---|---|
| | 1-1 | 3818 | 10100 | 210 | 120 | 8.0 | 88 | 14.5 | 5.1 |
| | 1-2 | 1853 | 4600 | 55 | 64 | 4.0 | 36 | 6.7 | 5.0 |
| | 1-3 | 304 | 1100 | 90 | 47 | 2.0 | 29 | 9.6 | 4.5 |
| | 1-4 | 50 | 40 | 57 | 65 | 1.0 | 21 | 14.4 | 4.2 |
| | 1-5 | 4262 | 13300 | 390 | 136 | 16.0 | 120 | 15.6 | 6.0 |
| | 2-1 | 123 | 500 | 90 | 43 | 1.4 | 23 | 13.4 | 4.3 |
| | 2-2 | 195 | 800 | 69 | 81 | 0.7 | 35 | 11.1 | 4.9 |
| | 2-3 | 200 | 700 | 46 | 53 | 2.9 | 22 | 10.9 | 4.9 |
| | 2-4 | 2650 | 5800 | 210 | 78 | 4.9 | 93 | 15.1 | 5.1 |
| | 2-5 | 2143 | 5200 | 158 | 78 | 4.0 | 66 | 13.1 | 5.0 |
| | 3-1 | 1083 | 2700 | 93 | 53 | 6.1 | 43 | 13.6 | 4.9 |
| | 3-2 | 716 | 2400 | 111 | 73 | 10.4 | 42 | 12.4 | 4.9 |
| | 3-3 | 1712 | 4900 | 123 | 64 | 10.5 | 48 | 12.1 | 5.7 |
| | 3-4 | 3810 | 12400 | 158 | 69 | 14.0 | 72 | 16.9 | 5.8 |
| | 3-5 | 662 | 2300 | 119 | 53 | 6.0 | 32 | 18.3 | 5.9 |
| MEAN | | 1572 | 4456 | 126 | 72 | 5.6 | 50 | 13.2 | - |
| STD. ERROR | | 381 | 1116 | 24 | 7 | 1.3 | 8 | 2.9 | - |
| MIN | | 50 | 40 | 46 | 43 | 0.7 | 21 | 6.7 | 4.2 |
| MAX | | 5340 | 13300 | 390 | 120 | 16.0 | 120 | 18.3 | 6.0 |

Table C4 Results of soil chemical analyses from three sample quadrats of the 17 year old site. All elemental analyses were performed on double acid soil extractions and concentrations equal g/g soil.

| Quadrat | Sample | P | Ca ++ | Mg ++ | K + | Mn ++ | Na + | %H2O | pH |
|---|---|---|---|---|---|---|---|---|---|
| | 1-1 | 2317 | 6300 | 420 | 122 | 4.6 | 68 | 21.8 | 5.0 |
| | 1-2 | 4084 | 8900 | 330 | 95 | 4.4 | 101 | 12.9 | 5.1 |
| | 1-3 | 1835 | 4600 | 220 | 123 | 3.2 | 48 | 19.9 | 4.9 |
| | 1-4 | 700 | 1900 | 220 | 100 | 1.1 | 38 | 14.9 | 5.0 |
| | 1-5 | 1978 | 5600 | 250 | 87 | 4.0 | 87 | 7.4 | 4.3 |
| | 2-1 | 3398 | 7900 | 360 | 130 | 2.9 | 106 | 18.1 | 4.9 |
| | 2-2 | 700 | 2700 | 310 | 125 | 2.4 | 41 | 23.5 | 4.8 |
| | 2-3 | 2759 | 6700 | 650 | 169 | 4.5 | 68 | 24.9 | 5.5 |
| | 2-4 | 2535 | 7200 | 380 | 124 | 2.5 | 64 | 19.1 | 4.8 |
| | 2-5 | 2786 | 7400 | 390 | 156 | 6.1 | 77 | 19.7 | 5.3 |
| | 3-1 | 1896 | 5100 | 350 | 152 | 11.2 | 41 | 19.5 | 5.4 |
| | 3-2 | 3703 | 7800 | 270 | 144 | 5.2 | 65 | 21.8 | 5.3 |
| | 3-3 | 1040 | 2500 | 270 | 122 | 1.7 | 51 | 19.5 | 4.8 |
| | 3-4 | 5340 | 11100 | 520 | 140 | 5.2 | 146 | 18.6 | 5.0 |
| | 3-5 | 3262 | 4600 | 200 | 122 | 2.0 | 46 | 17.1 | 4.8 |
| MEAN | | 2555 | 6020 | 343 | 127 | 4.1 | 71 | 18.6 | - |
| STD. ERROR | | 333 | 655 | 31 | 6 | 0.6 | 7 | 1.1 | - |
| MIN | | 700 | 1900 | 200 | 87 | 1.1 | 41 | 7.4 | 4.8 |
| MAX | | 5340 | 8900 | 650 | 169 | 11.2 | 146 | 24.9 | 5.5 |

Table C5 Results of soil chemical analyses from three sample quadrats of the 43 year old site. All elemental analyses were performed on double acid soil extractions and concentrations equal g/g soil.

| Quadrat | Sample | P | Ca ++ | Mg ++ | K + | Mn ++ | Na + | %H2O | pH |
|---|---|---|---|---|---|---|---|---|---|
| | 1-1 | 268 | 575 | 21 | 19 | 5.5 | 26 | 15.5 | 4.1 |
| | 1-2 | 109 | 277 | 28 | 25 | 1.1 | 30 | 10.6 | 4.3 |
| | 1-3 | 253 | 301 | 9 | 11 | 2.2 | 22 | 10.7 | 4.6 |
| | 1-4 | 139 | 358 | 10 | 24 | 2.2 | 22 | 8.7 | 4.2 |
| | 1-5 | 52 | 76 | 8 | 15 | 2.2 | 24 | 6.7 | 3.9 |
| | 2-1 | 257 | 660 | 21 | 12 | 4.4 | 21 | 9.5 | 4.4 |
| | 2-2 | 304 | 592 | 19 | 24 | 2.2 | 33 | 13.8 | 4.6 |
| | 2-3 | 401 | 779 | 45 | 47 | 3.3 | 35 | 17.9 | 4.6 |
| | 2-4 | 219 | 542 | 26 | 19 | 11.0 | 26 | 10.1 | 4.4 |
| | 2-5 | 161 | 384 | 14 | 28 | 2.2 | 31 | 9.6 | 4.4 |
| | 3-1 | 287 | 840 | 39 | 22 | 4.4 | 38 | 11.6 | 4.4 |
| | 3-2 | 287 | 640 | 20 | 19 | 8.8 | 25 | 8.9 | 4.3 |
| | 3-3 | 211 | 483 | 19 | 24 | 2.2 | 31 | 9.7 | 4.4 |
| | 3-4 | 413 | 1195 | 41 | 35 | 3.3 | 40 | 12.4 | 4.4 |
| | 3-5 | 80 | 402 | 20 | 11 | 4.4 | 20 | 12.2 | 4.6 |
| MEAN | | 229 | 540 | 29 | 22 | 4.0 | 28 | 10.5 | - |
| STD. ERROR | | 28 | 70 | 6 | 2 | 0.7 | 2 | 1.0 | - |
| MIN | | 52 | 76 | 8 | 11 | 1.1 | 21 | 6.7 | 3.9 |
| MAX | | 413 | 1195 | 45 | 47 | 11.0 | 40 | 17.9 | 4.6 |

Table C6 Results of soil chemical analyses from three sample quadrats of the 60 year old site. All elemental analyses were performed on double acid soil extractions and concentrations equal g/g soil.

| Quadrat | Sample | P | Ca ++ | Mg ++ | K + | Mn ++ | Na + | %H2O | pH |
|---|---|---|---|---|---|---|---|---|---|
| | 1-1 | 4227 | 9590 | 84 | 20 | 11.0 | 154 | 10.2 | 5.5 |
| | 1-2 | 4310 | 10320 | 62 | 12 | 9.0 | 138 | 9.9 | 5.1 |
| | 1-3 | 3895 | 11630 | 131 | 26 | 13.2 | 151 | 13.3 | 5.4 |
| | 1-4 | 5030 | 10890 | 130 | 25 | 13.2 | 190 | 13.3 | 5.3 |
| | 1-5 | 4836 | 10950 | 101 | 22 | 19.8 | 180 | 11.3 | 5.6 |
| | 2-1 | 4642 | 12100 | 125 | 35 | 44.0 | 175 | 9.7 | 5.0 |
| | 2-2 | 4283 | 11890 | 211 | 63 | 34.1 | 158 | 16.6 | 5.7 |
| | 2-3 | 4614 | 13400 | 229 | 39 | 20.9 | 161 | 18.4 | 5.5 |
| | 2-4 | 5528 | 14500 | 142 | 22 | 19.8 | 210 | 11.7 | 5.5 |
| | 2-5 | 5514 | 13990 | 152 | 32 | 19.8 | 195 | 15.6 | 5.1 |
| | 3-1 | 5127 | 13500 | 114 | 26 | 19.1 | 191 | 13.6 | 4.9 |
| | 3-2 | 4933 | 12260 | 128 | 28 | 15.4 | 161 | 17.7 | 5.0 |
| | 3-3 | 524 | 1930 | 105 | 46 | 7.7 | 36 | 15.5 | 4.5 |
| | 3-4 | 411 | 1140 | 47 | 24 | 7.7 | 28 | 16.3 | 4.5 |
| | 3-5 | 251 | 640 | 34 | 29 | 6.6 | 28 | 19.3 | 4.4 |
| MEAN | | 3875 | 9916 | 96 | 27 | 17.5 | 132 | 14.3 | - |
| STD. ERROR | | 480 | 1213 | 18 | 4 | 2.7 | 17 | 0.8 | - |
| MIN | | 251 | 640 | 34 | 12 | 6.6 | 28 | 9.7 | 4.4 |
| MAX | | 5528 | 14500 | 229 | 63 | 44.0 | 210 | 19.3 | 5.7 |

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# ENHANCED REESTABLISHMENT OF DISTURBED NATURAL ECOSYSTEMS

G. Ronnie Best
Center for Wetlands and
Environmental Engineering Sciences Department
University of Florida

## Introduction

Disturbance of natural ecosystems in the United States has been occurring since the movement of modern man to the continent several centuries ago. These disturbances consist of a wide range of forms such as altering natural ecosystems to agroecosystems, or altering surface ecosystems through total surface disturbance in mining (strip mining, open pit mining, etc). These disturbances still continue, the primary difference now is that the scale of disturbance is expanding concomitant with increased need of resources from these areas. A few examples are oil from oil shale, increased use of coal, and increased need for phosphate. This paper is not intended to address the need for this expanded use of resources, but is meant to address the need to develop knowledge necessary to reconstruct and/or manage disturbed ecological systems concomitant with increasing needs for utilizing resources from within these areas. Our knowledge relevant to the reconstruction of disturbed natural ecological systems is almost nonexistant. The need exists for development, synthesis, and integration of information on key biotic and abiotic factors regulating construction of natural ecosystems that may be useful in their reconstruction. In summary, research is needed to develop guidelines for practical reclamation and revegetation of disturbed lands to enhance natural recovery of terrestrial, wetland, and aquatic ecosystems.

## Planning for Balanced Design of Landscape

Although the generalities dealt with in this statement primarily address reconstruction of natural ecosystems, this is not to imply that all lands should be returned to native systems. Instead, the ideal is to maintain a functional balance between man-maintained or man-used systems and natural systems. Man-used ecosystems generally require direct or indirect subsidies in the form of dollars, energy, and stewardship; an investment of resources made to yield specific products. Natural ecosystems also provide a product, albeit somwhat more indirect, to the whole system of which man is a part. Research is needed that would address the development of guidelines balancing the specific needs of the human subsystem within the constraints and limitations of the larger "whole" system. (This concept is more fully described in sections by Drs. Brown and Klopatek.)



## Enhanced Recovery of Natural Ecosystems

The seral development of disturbed natural communities (succession) has been studied in great detail in American ecological research. Therefore one might ask, "Why stress the importance of continued research on plant community succession?" Actually, the justification for additional research on succession (at least covered in this statement) lies within the need for research more in line with the development of appropriate technology for enhancing the succession process. That is, research on plant community succession needs to more clearly address the essential or primary components that lead to successful reestablishment of natural ecosystems. Certainly, it can be agreed that all components in ecosystems are essential for them to properly function. However, I feel a strong argument can be levied in favor of keying in on specific primary components, at least for enhancing reestablishment of more mature natural ecosystems. Examples are listed below:

- large-scale seed sources for native plants (trees, shrubs, grasses, and herbaceous plants)
- seed mixture combination for enhancing recovery to specific ecosystems
- survivorship of individuals (or species) in seed mixtures
- microflora (especially endo- and ectomycorrhizal fungi) enhancement of seedling establishment and seedling survivorship
- other flora and/or fauna that may be essential in the establishment phase of natural ecosystems
- native versus introduced species. Are there gaps better filled by introduced species?
- extended subsidies—example, irrigation (even in arid or semiarid regions), is this subsidy necessary for reestablishment of native systems, or does it select for individuals more dependent on the supplyment? If native species are used, they should be more generally adapted to the dominant forcing functions of the area.

## Soil Reconstruction

Are these specific requirements for soil or soil profile reconstruction to enhance successful surface revegetation? Numerous states have enacted "topsoiling" requirements on surface mined lands; the assumption being that topsoil provides "all" the essential soil components for successful surface revegetation. How well founded (scientifically) is this practice? Is there a need for subsurface soil profile development in some systems? Also, what is the cost (energy use) effectiveness of soil reconstruction? Is topsoiling a cost (or energy use) effective method? Much new research is needed on understanding the interrelationship of reestablished natural communities concomitant with disturbed and/or reconstructed surface and subsurface soil systems.

## Summary

In summary, research is needed to address the appropriate technology for reclaiming disturbed lands. Research is needed on characteristic systems to accumulate data necessary to develop practical (i.e., cost effective and

applicable on a large scale) methodology and technology for reclaiming disturbed lands to natural ecological systems as one of many viable reclamation alternatives. The main thrust of the research should be towards whole ecosystem reconstruction—that is, reconstruction of aquatic, wetland, terrestrial, and transitional communities within watersheds or regions—through enhancing conditions more conducive of rapid reestablishment of more mature stages of succession, i.e., through enhancing ecological succession.

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# Enhancing Ecological Succession: 4. Growth, Density, and Species Richness of Forest Communities Established from Seed on Amended Overburden Soils

by

G. Ronnie Best, Peter M. Wallace, William J. Dunn,
and James A. Feiertag
Center for Wetlands and
Environmental Engineering Sciences Department
University of Florida
Gainesville, FL 32611

Abstract. Effects of VA-mycorrhiza, Pt-mycorrhiza, straw mulch, topsoil, fertilizer, and gypsum soil amendments on seedling establishment from seed of 25 tree species native to forest communities in Florida were tested in microplots. A "Community Development Index" (CDI) comprised of relative values for growth, density, and species richness of tree seedlings was developed and used to assess the effectiveness of the various soil amendments on community establishment. During initial establishment in the first growing season, mulch, topsoil, and VA-mycorrhizal inoculum treatments had a positive effect on the CDI, Pt-mycorrhizal inoculum had almost no effect, whereas gypsum and phosphate-free fertilizer had a negative effect on the CDI. By July of the second growing season, differences between mean CDI's for each treatment were reduced, partially as a result of the overpowering dominance of the pioneer successional species, Albizzia and Catalpa.

## Introduction

The primary goal of this research project is to accumulate data necessary to develop practical (e.g., cost effective and applicable on a large scale) methodology and technology for reclaiming phosphate mined lands to native forested ecological systems as one viable reclamation alternative. The main thrust of the project is towards whole ecosystem reconstruction—that is, recontruction of wetland, transitional, and terrestrial plant communities—through enhancing ecological succession (see related papers [1, 2] this symposium proceeding and 3, 4, 5). To achieve this goal research is directed towards identifying components essential for enhancing development of native forest ecosystems. Certainly, it could correctly be argued that almost all components in ecosystems are essential. However, is it possible that there may be some components more important or essential in the actual establishment phase of ecosystems with the additional components primarily functioning in long-term ecosystem maintenance? Presently, research is focusing on four "essential" components that may enhance reclamation to native forest ecosystems. They are 1) seeds (multispecies mixture), 2) mycorrhizal fungi symbionts, 3) soil nutrients, and 4) organic matter. Techniques for enhancing plant community succession being tested through this project and related research include direct seeding of late-successional and climax trees coupled with establishing the symbiotic microfloral component (mycorrhizal fungi) of the soil ecosystem necessary for more rapid growth and development of woody plants.

Direct seeding offers several distinct advantages over conventional means of tree establishment. Capital and energy invested in reclamation through direct seeding should be less than for more conventional revegetation approaches, albeit at the expense, perhaps, of a greater investment of seed resources (especially since the success of seed-to-seedling establishment will probably be less in direct seeding). In multispecies seeding several plant species have the opportunity to exploit the environmental conditions of the planting zone. In other words, the microenvironment of the planted zone coupled with the environmental tolerances of the germinating seedling of each species determines initial establishment and ultimate survivorship of plants. Conversely, when seedlings are direct planted, the reclamation planner and ultimately the person planting the seedlings, are predetermining the microenvironmental setting under which the species and individual plant are expected to survive or not survive. Disadvantages to direct seeding do exist. Seed use is less efficient since the number of propagules established is generally lower in direct seeding than in seed-to-seedling development in a nursery operation. Plus the technology presently does not exist to efficiently and economically plant seed mixtures with widely varying seed sizes, especially seed planting concomitant with mycorrhizal fungi inoculation. However, the technology is currently being developed (6). Finally, large quantities of seed necessary for large-scale reclamation are currently not availa[illegible] Will the seed producing technology develop if the technology is developed for efficiently planting and establishing multispecies forest through direct seeding?

Goals of this microplot experiment are to assess germination (propagule establishment), survivorship, growth, density, and species richness of tree seeds as a function of the following soil amendments on phosphate mined overburden: 1) vesicular-arbuscular fungi (Glomus mosseae and Glomus occultum) inoculum; 2) ectomycorrhizal fungi

(Pisolithus tinctorius) inoculum; 3) fertilizer (15-0-15); 4) soil surface organic matter (straw mulch); 5) soil amendment (phosphogypsum); and 6) topsoil.

## Literature Review

Ecosystem succession on mined land has been noted by several authors. Breedlove and Adams (7) discussed the effectiveness of the successional process in revegetation of some mined lands. Humphrey (8) and Kangas (9) describe the aesthetically pleasing ecosystems that had developed on spoil mounds that were simply abandoned after mining. Although only 50–60 years had passed, the mounds resembled a mesic oak forest. However, that was when mined tracks of land were smaller, allowing for more rapid and complete encroachment of organisms from the surrounding natural communities. Natural encroachment of the necessary components of forest communities has probably been hampered by the greater distances involved in the current large-scale mining activities of the phosphate industry in Florida such that now there are problems with simply abandoning mined lands. One of these problems is time. On less-than-ideal sites and sites where natural encroachment by forest plants is hampered by distance, compounding the time problem, initial plant establishment can be slow, resulting in a low percent cover even after several years. In some cases very persistent scrub communities, dominated by wax myrtle (Myrica cerifera) (10) or vines (9, 11) gain a foothold during an early successional stage. Often these pioneer early-successional species become so well established before seeds from the later, more mature forest species (i.e., climax forest species) are disseminated to the area that germination, growth, and establishment of these climax species are greatly reduced.

There are several approaches to enhancing establishment of "desirable" woody shrubs and trees. A common silvicultural technique used in forestry is planting seedlings (12). This technique is generally adapted to planting monospecific forests for enhancing growth and production of wood resources. One current project in reclaiming phosphate mined lands has used this technique at the multispecies level (13). Direct seeding is also an alternative revegetation method (14, 15, 16, 17, 18). A seed mixture composed of several species has been used successfully in enhancing the reestablishment of natural communities in oil shale–mined test plots in Colorado (19). Direct seeding offers distinct advantages over other revegetation methods in that it is applicable to large-scale reclamation at reasonable costs and effort levels (20). One current disadvantage, at least in the Southeast, is seed source and availability. However, as has been demonstrated in central and western United States where state and federal regulations place an emphasis on restoring diverse natural communities, considerable attention is being paid to developing technology needed for adequately supplying a seed source for native plants. Johnson (21), in his opening address to the symposium on "Trees for Reclamation" placed "increasing the production of high quality seed . . . with emphasis on native species" (p. 2) as an area where "research must be strengthened." The above mentioned revegetation methods coupled with additional methods—combinations and variations—are certainly functional and viable reclamation alternatives (22). Each revegetation alternative provides a unique set of advantages and disadvantages that can be assessed in developing the most functional reclamation plan for a specific area.

"Direct seeding, an attractive alternative to planting, is not a simple method of reforestation" (16). Early reports of reclamation to forest indicate that direct seeding has been attempted in many regions as an alternative to tree planting. However, most of the earlier attempts were direct seeding of a single species. Schavilji (23) reported good first-year germination and survival of black walnut (Juglans nigra) with an average seedling height of 12 inches. However, others (16, 20, 24) have reported only moderate to poor success in direct seeding of single species. Other recent experiments include direct seeding of loblolly (Pinus taeda), shortleaf (P. echinata), Virginia (P. virginiana), and white (P. strobus) pine (25). First-year results were moderately successful with some species performing better than others. Plass (18), in a test using multispecies seed mixtures, was able to select a combination of several compatible species of herbs and trees for direct seeding. Identification of the promising species resulted from a species evaluation trial in which 34 species of trees, shrubs, and herbaceous plants were hydroseeded on five sites. Others have developed seed handling methods (26), collection techniques (27), and planting strategies for specific environmental conditions (28).

The concept of enhanced ecological succession of devasted lands implies man's manipulation of natural processes. Ashby et al. (29) noted that some formerly mined lands in Illinois, though planted successfully with several species of trees 30 years earlier, had a greater number of pioneer trees than planted trees suggesting that total reclamation involves more than simply revegetating the surface (a problem being addressed within the goals of this research project). In order to develop a "holistic approach to natural ecosystem reconstruction" an understanding of both macrocomponents and microcomponents is mandatory (30). Most recent studies of revegetation have examined only aboveground components of the plant community while completely ignoring belowground microbial participation. One very important microbial entity warranting special attention is mycorrhizal fungi. Termed by Hacskaylo (31) as "indispensable invasions by fungi," mycorrhizae have been proven to be essential for survival of most plants. Studies of mycorrhizae importance in reclamation of phosphate mined lands in Florida are relatively nonexistent although the significance of mycorrhizae in revegetation of disturbed lands is well documented in other regions (32, 33, 34). Reeves et al. (35) stated that "the reestablishment and maintenance of the mycorrhizal fungus component is vital in producing stable plant ecosystems on disturbed areas." It is imperative that successful reclamation studies include examinations of the mycorrhizal fungi that could possibly be used for inoculation of native forest species. (For a more complete discussion on the role of mycorrhizae in reclamation see Wallace and Best [2], this symposium proceedings.)

## Methods

The statistical design for the microplot experiment is a $2^6$ factoral design, resulting in 64 different treatment cells. This design is excellent

Table 1. Essential components, possible techniques for providing "essential" components, and experimental treatments and application rates for field microplot experiments.

| Essential Components | Possible Technique | Method Used and Application Rate | Materials |
|---|---|---|---|
| Seed Bank | Multiple species seeding | Direct seeding | 25 species of trees (see Table 2). |
| Soil Microflora | Inoculation with mycorrhizae | VA-mycorrhizae (endomycorrhizae) Glomus mosseae & occultum | Greenhouse inoculum cultures consisting of soil, plant roots, fungal hyphae and spores. |
| | | Pt-mycorrhizae (ectomycorrhizae) Pisolithus tinctorius | Vegetative hyphae mycorrhizal inoculum in vermiculite carrier (purchased from Abbott Labs). |
| Organic Matter | Mulching (straw, woodchips, etc.) | Straw (6500 kg/ha; 2 tons/acre) | Commercially available material. |
| Nutrients | Fertilizer, sludge amendments | 15-0-15 (320 kg/ha; 285 lb/ac) | Commercially available Marico brand. |
| Others | Soil structure amendments | Gypsum (1750 kg/ha; 1500 lb/ac) | Phosphogypsum from a chemical plant's gypsum stack. |
| All of above | Topsoiling....? | Premined surface soil (6-8 cm; 2-3 in) | Surface soil from upland forested area that was being site prepped for mining |

when the goal is to screen direct and possible interactive effects of a large number of parameters. The six treatments were 1) inoculation with an endomycorrhizal fungi; 2) inoculation with an ectomycorrhizal fungi; 3) application of straw mulch; 4) fertilizing; 5) soil amendments with gypsum; and 6) topsoiling. Application rates for the treatments are presented in Table 1. The "topsoil treatment" serves a dual purpose. First, topsoil is a viable reclamation alternative worth testing in its own right. Second, the topsoil treatments can serve as a control to which the other soil amendment parameters can be compared.

The microplots are 50 x 50-cm cells 75 cm deep constructed from plywood. Prior to planting microplots were filled with strip mined overburden soil supplied by IMC and AMAX. The two supplies of overburden soil were thoroughly homogenized in a 50/50 mixture using a large cement mixer. Overburden-filled microplots were sterilized with methyl bromide and covered with plastic for 48 hours in order to standardize all microplots with regard to soil microflora and fauna. The plastic was removed to allow the microplots to "air out" for 21 days to insure complete dissipation of the methyl bromide gas.

A week prior to experiment set up, surface soil (for topsoiling) was collected from several upland forest areas in north-central Florida that were being site prepped for mining by Occidental. The area was intermixed with predominantly young pine and a few hardwoods. Saw palmetto (Serenoa repens) was a significant component of the ground cover. Nonsterilized surface soil (topsoil) was added to appropriate cells to simulate the practice of topsoiling the top 2-3 inches of a reclaimed area.

Inoculation material of the vesicular-arbuscular (VA) mycorrhizal fungi (endomycorrhizal fungi) Glomus mosseae and Glomus occultum was cultured in a soil matrix planted to bahia grass (Paspalum notatum) and soybean (Glycine max) as host plants. The cultures were maintained in a greenhouse until prepared for use. The fresh inoculum actually used consisted of ground up soil matrix that included mineral soil, plant roots, fungal hyphae, and spores. Microplots receiving the endomycorrhizal treatment were treated with 500 grams of the VA-mycorrhizal inoculum. All other microplots (those not receiving VA-mycorrhiza) were treated with an equivalent amount of the same material that had first been sterilized through autoclaving (in other words, a nonmycorrhizal equivalent). The material was worked into each seed planting row to simulate actual field planting conditions.

The ectomycorrhizal fungi (Pisolithus tinctorius [Pt]) inoculum material was purchased from Abbott Labs.* The material consists of mycorrhizal vegetative hyphae inoculum in a vermiculite carrier. Microplots receiving the ectomycorrhizal inoculum were treated with 75 grams of Pt-inoculum. A "non-mycorrhizal" equivalent of the Pt-inoculum was prepared using sterilized vermiculite and nutrient broth matrix minus the Pt-fungi. The nutrient broth was subsequently leached from the inoculum to simulate actual preparation conditions.

Nutrient rich soils, especially soils high in available phosphate, generally reduce mycorrhizal fungi colonization on host plants. In addition, high levels of fertilizer application tend to favor growth and aggressive weedy plants. Therefore, the fertilizer treatment was limited to a one-time, low-level application of a nonphosphate fertilizer, hopefully at a level sufficient to stimulate growth of the planted species without encouraging overcompetition of weedy plants. The application rate was 8 grams/microplot (equivalent to 320 kg/ha or 285 lbs/acre) of 15-0-15 (N, P, K).

*The Pt-inoculum is being developed as part of an experiment to produce commercially available ectomycorrhizal fungi for nursery operations. For further information contact: Dr. Donald Marx, Institute of Mycorrhizal Research and Development, U.S. Forest Service, Carlton Street, Athens, Georgia 30602 (404-546-2435).

Phosphogypsum, a by-product of chemical processing of phosphate ore, is occasionally used as an agricultural soil amendment (36). The phosphogypsum for the experiment was obtained from Occidental's Suwannee River chemical plant. Phosphogypsum, added at a rate of 1750 kg/ha (1500 lbs/acre), was worked into the surface soil of the respective microplot.

Straw mulch was applied to appropriate treatment plots at a rate equivalent to 4500 kg/ha (2 tons/acre). To prevent introduction of extraneous microbes in the experiment the straw mulch was autoclaved prior to use.

The field microplot experiments were planted on 17 July 1982. All microplots were planted with a seed mix of 25 tree species (Table 2) using 5 seeds of each species (125 seeds/microplot). The species chosen represent a wide variety of trees typically found in natural systems in Florida, and/or are either early-, mid-, or late-successional woody plants. Seeds were collected fall 1981 and spring 1982 and were stored in a large walk-in cooler at 4°C until 2 weeks prior to use.

Table 2. Tree species used in the microplot experiments. A seeding rate of five individuals of each species was used in the microplot experiments.

| | |
|---|---|
| Acer rubrum | Nyssa ogeche |
| Albizzia julibrissins | Persea palustris |
| Catalpa bignoniodes | Pinus elliottii |
| Celtis laevigata | Platanus occidentalis |
| Cercis canadensis | Prunus angustifolia |
| Chamaecyparis thyoides | Prunus serotina |
| Cornus florida | Quercus laurifolia |
| Diospyros virginiana | Quercus virginiana |
| Fraxinus americana | Sabal palmetto |
| Fraxinus caroliniana | Sambucus canadensis |
| Gordonia lasianthus | Taxodium distichum |
| Juniperus silicicola | Taxodium ascendens |
| Liquidambar styraciflua | |

The microplots were sampled at the end of 3 months (end of first growing season) and 12 months for number and height of seedlings by species. The 3-month sampling was done in October 1982 just prior to the onset of winter dormancy. The 12-month sampling was done in July 1983.

Data were analyzed using analysis of variance to test significant main and interactive effects, Student-Newman-Kuels (SNK) test for differences between means of treatments, and the Student's t-test for comparison of "with treatment" and "without treatment" means. Differences were tested at $\alpha = 0.05$ level.

## Results

Growth. During the first growing season, the presence of mulch, topsoil, and VA-mycorrhiza significantly increased growth of tree seedlings over plots without these treatments, whereas Pt-mycorrhiza, gypsum, and fertilizer significantly decreased growth. When treatment effects alone are considered, mulch and topsoil had a significantly higher mean growth than the other treatments (Table 3a). However, during the second growing season differences between treatments were significantly reduced (Table 3a). In fact, only the VA-mycorrhiza and fertilizer treatments differed significantly.

Density. Initially, surviving propagules ranged from a low of 7% for microplots without mulch to a high of 9% for cells with mulch. Considering that seeds of most tree species generally have better germination after overwintering, the 7-to-9% range in propagule survivorship represented a reasonable level of germination. After overwintering the number of surviving propagules increased to 14 to 15%.

During the first 3 months, mulch, topsoil, and VA- and Pt-mycorrhizae had a significant positive effect on tree seedling density whereas fertilizer and gypsum significantly reduced tree seedling density (Table 3b). There were no significant differences between the effects of VA- and Pt-mycorrhizae on tree density. The second growing season yielded different results. There were no significant differences between treatments on density.

Species Richness. Species richness, a measure of the number of species having seedlings established, ranged from a mean low of 15% for gypsum-treated plots to a mean high of 20% for mulch-treated plots at the end of the first growing season. The grand mean for all treatments was 17%; that is, out of 25 tree species planted an average of 4.24 species established seedlings. Eleven species (44%) of the 25 tree species planted had at least one individual seedling established after only 3 months. This trend changed after overwintering. During the second growing season species richness almost doubled, ranging from a mean of 30 to 33%. In other words of the 25 species planted at least 7 to 8 species were established per treatment.

Table 3. Average values for (a) height (in cm), (b) number of individuals (density) and (c) species richness for treatments. Similar means are connected by the bar. The vertical "*" presented for the first growing season separates those treatment means where the "without treatment" mean was significantly ($\alpha = 0.05$) higher than the "with treatment" means. "With treatment" and "without treatment" means were not significantly different during the second growing season.

a. Mean height (cm) of tree species

| Growing Season | Mulch | Topsoil | VA-mycorrhizae | | Pt-mycorrhizae | Gypsum | Fertilizer |
|---|---|---|---|---|---|---|---|
| First | 13.71 | 12.86 | 11.08 | * | 10.86 | 10.53 | 10.21 |
| Second | 23.03 | 22.91 | 22.14 | | 22.51 | 22.49 | 23.29 |

b. Mean density of tree species

| Growing Season | Mulch | Topsoil | VA-mycorrhizae | Pt-mycorrhizae | | Fertilizer | Gypsum |
|---|---|---|---|---|---|---|---|
| First | 11.16 | 10.38 | 10.30 | 9.93 | * | 9.63 | 9.40 |
| Second | 18.91 | 19.09 | 17.41 | 18.19 | | 17.69 | 18.36 |

c. Mean tree species richness (mean number of tree species)

| Growing Season | Mulch | Topsoil | VA-mycorrhizae | | Pt-mycorrhizae | Fertilizer | Gypsum |
|---|---|---|---|---|---|---|---|
| First | 4.79 | 4.62 | 4.28 | * | 4.21 | 3.97 | 3.91 |
| Second | 8.25 | 8.12 | 7.62 | | 7.69 | 7.47 | 7.69 |

Initially, mulch, topsoil, and VA-mycorrhiza had a significant positive influence on species richness, whereas Pt-mycorrhiza, fertilizer, and gypsum significantly lower species richness (Table 3c). These differences were not realized in the second growing season.

Community Development Index. Numerous factors contribute to community structure of an ecosystem. Total biomass production (growth), whether by a few or many individuals; total number of individuals (density), whether of one or many species; and the number of species (species richness) are all significant components of community structure. The "community development index" (CDI) was developed as a cumulative measure of the relative contribution of growth, density, and species richness to community structure. Mean growth for all individuals in each microplot was normalized to the maximum growth. Density was normalized by dividing the number of individuals per microplot by 125 (total number of seeds planted per microplot). Species richness was normalized by dividing the number of species per microplot by 25 (number of species planted). Normalized growth, density, and species richness were summed to yield the CDI (Table 4). Hopefully, the CDI or some variant of the CDI will have application to experimental field revegetation programs.

During the first growing season, mulch, topsoil, and VA-mycorrhiza soil amendments had significantly higher CDI's than did Pt-mycorrhiza, fertilizer, and gypsum. In fact, the ranked order of means for treatments in Tables 3 and 4 are almost identical. The only anomaly occurs in the reversed ranking of treatment means for gypsum and fertilizer treatments on plant growth (Table 3a). This trend shifted during the second growing season where there were no treatment effects on the CDI.

## Discussion

During initial establishment in the first growing season, the straw mulch treatment had the most significant positive effect on all aspects of community development in the microplots. There are several positive aspects of mulched surface soils such as reducing eroding impact of raindrops, binding soil against wind erosion, etc. (37). But perhaps the most important function of mulching is through its effect on decreasing both surface soil moisture loss and soil crusting. The importance of preventing surface soil crusting was realized about 2 weeks after the microplots were planted. During the 2-week period after planting several afternoon thundershowers had occurred. The observation had been made that overburden-only soils had crusted to a very hard surface, whereas straw mulched or topsoil treated microplots had not crusted as hard. About 2 weeks after planting, the microplots were deluged with an intense afternoon thundershower. All microplots containing only overburden soil as the surface soil (i.e., no mulch or topsoil) were overflowing the walls of the microplot (> 5–7 cm of standing water). All microplots treated with topsoil but without mulch had 2–3 cm of standing water but were not overflowing. However, none of the microplots treated with straw mulch, whether directly over overburden or topsoil, had standing water. The straw mulch, by preventing surface crusting, had apparently increased the rate of infiltration. Therefore, the straw mulch, by increasing water infiltration and decreasing soil moisture loss, increased water availability and, in turn, propagule survivorship and growth. However, as plants became established during the first growing season, the significance of mulch, albeit still important, was reduced. Perhaps by the second growing season the "role" mulch originally played was replaced by standing plant biomass and leaf litter produced during the first growing season.

Initially, topsoil also had a positive effect on community development. Although this trend carried over into the second growing season, topsoiling as a treatment was not significantly different from any of the other treatments. In fact, when one considers the overall negative economic, energy and environmental effects of topsoiling (indirectly resulting from increased fuel/energy use), the potential benefits that may be realized from topsoiling must be closely weighed with the associated negative impacts of topsoiling.

During the first growing season, inoculating the microplots with VA-mycorrhiza had a slightly positive, significant effect on the community development parameters of growth, density, and species richness. Conversely, the Pt-mycorrhizal inoculum appeared to have little or no influence on community development. This result was not unexpected since the Pt-mycorrhizal inoculum is known to stimulate growth of only one of the species planted—pine. Although oaks are also ectomycorrhizal it is not know if they are colonized by Pt. The importance of mycorrhizae did not carry over into the second growing season. Careful examination of the species list (Table 2) reveals that several of the species are "pioneer invaders" (e.g., Albizzia, Catalpa, etc.). True to their descriptive namesake, these "pioneer invaders" became dominant features of the microplots during the second growing season, masking, both in means as well as through actual competition, the establishment of the more climax species. Often these pioneer species are facultative-mycorrhizal; therefore, the reduced significance of the mycorrhizal treatment is not unexpected since pioneer species were sown in equal proportion to other climax species.

Table 4. Mean community development index (CDI). The CDI is the sum of normalized values of growth, density, and species richness. The maximum possible CDI value is 3 and would represent maximum means and equal contributions of all three parameters. Similar means are connected by the bar. The vertical "*" presented for the first growing season separates those treatment means where the "without treatment" mean was significantly ($\alpha = 0.05$) higher than the "with treatment" means. "With treatment" and "without treatment" means were not significantly different during the second growing season.

| Growing Season | Mulch | Topsoil | VA-mycorrhizae | * | Pt-mycorrhizae | Fertilizer | Gypsum |
|---|---|---|---|---|---|---|---|
| First | 1.98 | 1.87 | 1.73 | * | 1.70 | 1.62 | 1.61 |
| Second | 2.12 | 2.11 | 1.99 | | 2.03 | 2.02 | 2.04 |

## Summary

1. Tree seedlings establishment ranged from 7 to 9% during the first growing season and increased to about 15% during the second growing season. Mulch, topsoil, VA-mycorrhiza, and Pt-mycorrhiza had a significant positive effect on seedling density during initial establishment.

2. Although 44% of the tree species planted established seedlings, the average for each treatment ranged from 15 to 20% during the first growing season and from 30 to 33% during the second growing season.

3. During the initial establishment phase of the first growing season, the community development index (CDI) was highest for the mulch, topsoil, and VA-mycorrhiza treatments. A net decrease in the mean CDI resulted from treatment of Pt-mycorrhiza, fertilizer, and gypsum. Mulch, topsoil, and VA-mycorrhizal (endomycorrhizal) fungi significantly increased community development components such as growth, density, and species richness of tree seedlings. Differences between CDI's for each treatment were reduced during the second growing season partially as a result of the overriding dominance of the "pioneer species," Albizzia and Catalpa.

4. Pt-mycorrhizal fungi slightly decreased average growth and species richness and slightly increased density of tree seedlings. However, this may partially be due to the fact that only a few of the tree species planted may be potential ectomycorrhizal hosts.

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# Enhancing Ecological Succession: 5. Seed Bank Survey of Some Florida Marshes and Role of Seed Banks in Marsh Reclamation

by

William J. Dunn and G. R. Best
Environmental Engineering Sciences Department and
Center for Wetlands
University of Florida
Gainesville, Florida 32611

Abstract. Wetland soils typically contain large reservoirs of long-lived, viable seeds and/or propagules known as seed banks. Seed bank composition of the upper soil profile represents a primary mechanism by which wetland ecosystems recover from natural perturbations (fire, water level fluctuation, etc.). Seed banks buffer ecosystem perturbation by providing a diverse array of species. Wetland seed bank establishment in appropriately landscaped postmined lands may provide a promising avenue for wetland ecosystem restoration. Presently, little is known of the seed bank composition of wetland ecosystems in Florida. Data presented compare seed bank composition of selected natural marshes and marshes in postmining landscapes (both unreclaimed and reclaimed wetlands). Seed bank samples from all but the youngest reclamation sites fall within or just below the range of densities, species diversity (H'), and species richness found in natural wetlands in Florida, Iowa, New Jersey, and Ontario. The seed bank samples from central Florida are dominated by four species, Juncus effusus, Polygonum punctatum, Ludwigia virgata, and Cyperus rotundus. To date, long-lived perennial marsh species are conspicuous by their absence from seed bank samples.

## Introduction

Reclamation of "high quality" wetland ecosystems is a desirable if not defineable goal of land reclamation efforts in the phosphate mining industry. It is a problem companies must often wrestle with under the varied and conflicting input from regulatory agencies, consultants, and university researchers. All involved agree with the goal, though few agree on what the best method of reclamation is. Reclamation is a type of ecosystem engineering and should be done using ecological principles. One goal of reclamation should be to design self-maintaining ecosystems in concert with nature, especially native ecosystems. Ecosystems have evolved mechanisms that allow them to survive naturally occurring disturbances such as fire, flood, and drought. Reclamation goals will be best met if through a better and clearer understanding of recovery and maintenance processes in ecosystems we can enhance reestablishment of nature's self-maintaining systems.

The establishment of vegetation is one of the first stages in primary succession on an abiotic substrate whether it results from geologic uplift, glacial retreat, volcanic lava flow, landslide, or strip mining. The question arises, How can humans best use their energies to enhance the recovery of vegetation? For wetland systems lessons may be learned from the disturbance recovery processes observed in established communities. In several cases, to be discussed below, stored viable seed in wetland soils plays a critical role in the recovery process. This storage of dormant, viable seed in the soil is known as the seed bank.

The functional significance of seed banks lies in providing the plant community with an in situ means of regenerating from naturally occurring disturbances (1). Van der Valk (2) and van der Valk and Davis (3, 4) have aptly documented and demonstrated the role seed banks play in the vegetation dynamics of prairie glacial marshes that undergo cyclic patterns of flooding-drawdown-drought. In prairie glacial marshes and other marsh systems (5, 6), seeds remain dormant, yet viable, in the seed bank during periods in which environmental conditions are unfavorable for germination, growth, and development of the population. Seed banks provide a mechanism for rapid recovery from catastrophic mortality due to fire (7), clear-cutting (8), and drought (9). Marks (8) has shown that the rapid response of pin cherry to clear-cutting in the Hubbard Brook ecosystem helped minimize the effect of canopy removal on nutrient losses from the ecosystem.

As an initial step in understanding and using ecological principles to design self-maintaining ecosystems, this study was undertaken to examine seed bank dynamics in marsh ecosystems. The objectives of the study were 1. to assess the size and species composition of seed banks in selected marsh ecosystems from natural and postmining landscapes, 2. to elucidate ecological role and significance of seed banks in marsh community dynamics, and 3. to evaluate the feasibility of establishing marsh ecosystems by mimicking natural recovery processes such as those known to occur with seed banks.

## Materials and Methods

Seven wetland sites were sampled in the present study (Table 1, Figure 1). At each site one to several major vegetation zones were sampled if the water was not more than 1 m deep, assuring that samples came from the shallow littoral zone—the area of greatest water level fluctuation. Samples were taken with a 5-cm-diameter hand core sampler which was pushed into the substrate to the mineral soil layer. The depth of any overlying organic layer was noted, and only the upper 10 cm of the core was retained. Four individual cores were combined to yield a sample with three samples taken in each vegetation zone selected. Sampling took place over a 3-week period from October 28 to November 18. Samples were stored in sealed plastic bags at 4°C until they were processed in late November.

All live plant material was removed from the samples to prevent any vegetative regeneration from confusing the seed germination results. Once the plant material was removed, the samples were placed in wooden flats (25 cm x 25 cm) containing approximately 4 cm of sterilized gravel mixed with tailings sand; the samples were approximately 2 cm deep when spread out evenly in the flats. The flats were then placed outdoors in large plastic tubs containing sufficient water to maintain a saturated soil condition.

Seedling emergence by species was monitored through time. Unidentified seedlings were counted, transplanted to flower pots, and allowed to mature until they could be identified.

Table 1. Sample Locations and Site Characteristics.

| Site | County | Vegetation Zone | Substrate |
|---|---|---|---|
| *Unreclaimed Mine Area* | | | |
| Sanlan (Clay Settling Area) (10 yr old) | Polk | Juncus-Polygonum | Clay |
| | | Eichhornia | Clay |
| *Reclaimed Mined Areas* | | | |
| Clear Springs (4 yr old) | Polk | Polygonum-Ludwigia | Clay-overburden |
| Fort Green (2 yr old) | Polk | Pontederia (Mulched) | Organic muck-overburden |
| | | Open water (Mulched) | Organic muck-overburden |
| | | Open water (Unmulched) | Overburden |
| Four Corners (3 yr old) | Manatee | Pontederia (Mulched) | Organic muck-sand |
| | | Pontederia (Planted) | Some organic matter-sand |
| | | Eleocharis (Control) | Sand |
| | | Polygonum-Ludwigia (Swamp) | Some organic matter-sand |
| *Natural Wetland* | | | |
| Pasture marsh | Polk | Juncus-Pontederia | Muck-sand |
| Peace River bayhead | Polk | Taxiurus | Muck-peat-sand |
| Lake Kanapaha* | Alachua | Amaranthus | Muck-sand |
| | | Echinochloa | Muck-sand |
| | | Open water | Muck-sand |
| Four Corners marsh | Manatee | Pontederia-Juncus | Muck-sand |

*Lake Kanapaha not sampled in this study, but results of previous sampling was graciously provided by Dr. Ken Myers.

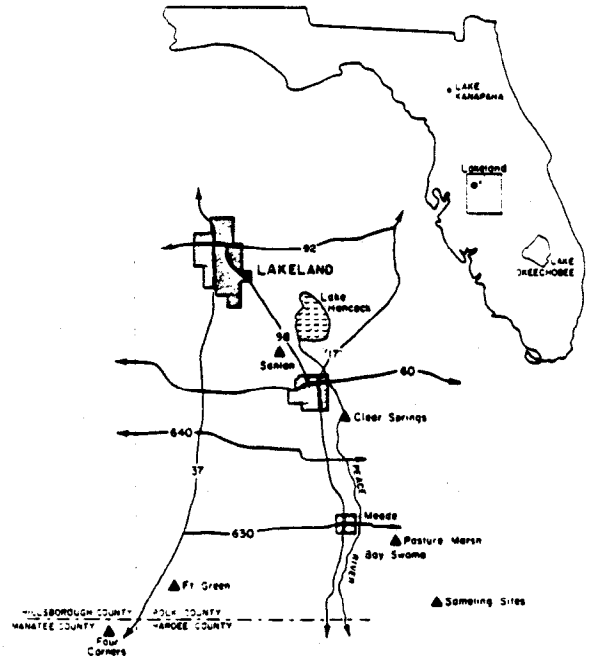


Figure 1. Site location map.

## Results

Information gathered from a survey of native and postmining wetlands in central Florida is prepented in Tables 2a and 2b. The information contained in these tables is further summarized in Tables 3 and 4. The results are presented in four general areas: seed bank density, species importance values, floristic similarity between samples, and species diversity of samples.

### Seed Bank Densities

Table 3 summarizes the results of seed bank samples and includes values from other seed bank studies from Florida, Iowa, New Jersey, and Ontario. The overall range of seed bank size (density) covers three orders of magnitude with the lowest density of 1877/$m^2$ in the topsoiled (peat mulched), unvegetated sample from Fort Green project and the high value of 156,000/$m^2$ from the Sacciolepis striata zone at Lake Kanapaha, Florida.

The range for natural wetlands samples is 4,000–156,000 seeds/$m^2$ with the lowest value coming from bay swamp (incidentally the only forested wetland sample) and the high value again for the Sacciolepis zone at Lake Kanapaha. A trend evident in the results from studies at Lake Kanapaha is that the species richness and size of the seed bank appears to decrease as the water depth increases in the Sacciolepis zone-Amaranthus zone-Echinochloa zone-Pond zone. For the wetland samples cited from outside Florida the densities range from 6,000 to 40,000, and for the three natural systems sampled in this study the range of densities is 8,000–72,000. The two marsh samples (Four Corners natural marsh and pasture marsh) had densities of 41,000/$m^2$ and 72,500/$m^2$, respectively.

The unreclaimed wetland sampled—the Sanlan sample—had densities of 12,000/$m^2$ and 62,000/$m^2$ from the Eichhornia and Juncus marshes, respectively. Interestingly, here as with the Kanapaha samples seed bank size apparently decreases with depth, the water depth being over a meter in the Eichhornia marsh.

The Sanlan samples fall in the range of the natural wetlands already discussed, especially the 62,000/$m^2$, which represents one of the higher densities encountered and indicates that sizeable seed banks can develop in absence of any reclamation efforts in postmining wetlands.

Wetland samples from reclaimed mine lands had a range of 1,800 to 33,000/$m^2$, which is low to moderate by comparison to natural wetland systems. Samples from the three basins at Clear Springs ranged from 7,000 to 11,000/$m^2$, while at Four Cor-

ners project the range was 2,200–33,000. More specifically the treated plots had densities well within the range of the natural systems already discussed; topsoiled (peat) marsh plot (33,000), planted marsh plot (31,000), and swamp planted plot (11,300). The lowest density found at the Four Corners project came from the control plot (2,200), indicating that seed bank establishment is facilitated by reclamation efforts.

Finally, samples from the Fort Green project had the lowest and narrowest range of densities 1,800–3,900/$m^2$, but it should be remembered that this project is only in it's second growing season. Surprisingly the lowest density value from Fort Green and for all samples, came from an unvegetated topsoiled (peat) area with open water. It may be due to the vagaries of sampling, or it may be that the seed bank in the peat at this spot is either dominated by 1) short-lived seeds or species that only germinate under flooded conditions (which were not duplicated in this study), or 2) that the topsoil material (peat) had been stockpiled (as is known to have occurred with some peat material at this site).

## Species Importance Values

As an estimate of the overall influence or importance of the species in the seed bank survey, a modified importance value was calculated using the density and frequency totals for each species (Table 2b). The importance value is calculated by adding relative density and relative frequency for each species, where relative density is defined as the density of the species divided by the sum of all densities and where relative frequency is defined as the frequency of occurrence of the species divided by the sum of all species frequencies. Both relative density and frequency were connected. When calculated this way both relative frequency and relative density are constrained to values between 0 and 100% and therefore the importance value of each specie takes on a value in the range 0 to 200. The most striking aspect of all these calculations is the numerical dominance of Juncus effusus, which accounted for 84% of germinating seeds in this study, and in addition to its numerical dominance it was the only species found in all samples yielding an absolute frequency of 1.0.

The 20 species of highest importance value are listed in Table 4; the 20 acccount for 185 out of the total importance value of 200. In fact, the four species of highest importance value (Juncus effusus, Polygonum punctatum, Cyperus rotundus, and Ludwigia virgata) account for 94% of the relative density and 132 of the total importance value. These four species can be considered the dominant species so far in this study and can serve in general to characterize the seed banks sampled from central Florida.

## Floristic Similarity

A measure of floristic similarity of seed bank samples was calculated using the similarity index of Czekanowski for binary data. The index is defined as follows:

$$\text{Czekanowski's index} = 2a/(2a + b + c)$$

Table 2a. Mean Number of Germinating Seeds per $m^2$* by Species for Natural Wetlands and Reclaimed and Unreclaimed Marshes in the Central Florida Phosphate District.

| | | Four Corners | | Sanlon | | Four Corners | | | | Clear Springs | | | Fort Green | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Bay Swamp | Natural Marsh | Pasture Marsh | Juncus-Polygonum | Eichhornia | Topsoiled Marsh | Planted Marsh | Control Marsh | Planted Swamp | South Basin #1 | South Basin #2 | North Basin | Topsoiled, Vegetated | Topsoiled, Unvegetated | Unmulched |
| Aster subulata | --- | --- | --- | --- | --- | --- | --- | --- | --- | 416 | 500 | 210 | --- | --- | --- |
| Boehmeria hellmiifolia | --- | --- | --- | --- | --- | --- | --- | --- | --- | 125 | 42 | 292 | --- | --- | --- |
| Carex sp. | --- | --- | --- | --- | 125 | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Cyperus brevifolius | --- | --- | --- | --- | 416 | --- | --- | --- | --- | 84 | --- | --- | --- | --- | --- |
| Cyperus sp. | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | 915 | 334 | 960 |
| Cyperus rotundus | 84 | --- | --- | 416 | 3,750 | 166 | --- | --- | 500 | 1,666 | 4,125 | 1,500 | --- | --- | --- |
| Cyperaceae ? | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | 84 | --- | --- | --- |
| Echinochloa walteri | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | 125 |
| Eclipta alba | --- | --- | --- | 125 | --- | --- | --- | --- | --- | 250 | 541 | 42 | --- | --- | --- |
| Eupatorium compositifolium | 84 | --- | --- | 541 | --- | --- | --- | 125 | 46 | 125 | 250 | 375 | --- | 42 | 84 |
| Gnaphalium obtusifolium | --- | --- | --- | --- | --- | --- | --- | --- | --- | 84 | --- | --- | --- | --- | --- |
| Grasses, unknown #1 | --- | --- | 42 | --- | --- | --- | --- | --- | 125 | --- | --- | --- | --- | --- | --- |
| #2 | --- | --- | --- | --- | --- | --- | --- | --- | 42 | --- | --- | --- | --- | --- | --- |
| #3 | --- | --- | --- | --- | --- | --- | 84 | --- | --- | --- | --- | --- | --- | --- | --- |
| #4 | 42 | --- | --- | --- | --- | --- | --- | --- | --- | --- | 250 | 125 | --- | --- | --- |
| #5 | --- | --- | --- | --- | --- | --- | --- | --- | --- | 1,625 | --- | --- | --- | --- | --- |
| #6 | --- | 42 | 84 | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Hydrocotyle verticillata | 42 | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Hypericum mutilum | 42 | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | 166 | --- | --- | --- |
| Juncus effusus | 292 | 67,210 | 40,832 | 58,625 | 7,625 | 32,750 | 31,416 | 1,500 | 10,625 | 1,875 | 1,916 | 1,460 | 209 | 42 | 1,210 |
| Juncus bufonius | 125 | --- | --- | 42 | --- | 42 | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Ludwigia virgata | 292 | --- | --- | --- | --- | --- | --- | --- | --- | 500 | 416 | 2,666 | 1,834 | 1,375 | 1,416 |
| Ludwigia palustris | 334 | --- | --- | --- | --- | --- | --- | --- | --- | 42 | --- | --- | --- | --- | --- |
| Ludwigia leptocarpa | --- | --- | --- | --- | --- | --- | --- | --- | --- | 84 | 210 | --- | --- | --- | --- |
| Polygonum punctatum | --- | 5,250 | 292 | 2,460 | 125 | 42 | 126 | --- | 42 | 42 | 125 | 84 | --- | --- | --- |
| Ptilimnium capillaceum | --- | --- | --- | --- | --- | --- | --- | --- | --- | 42 | 1,042 | 84 | --- | --- | --- |
| Rumex verticillatus | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | 42 | --- | --- | --- | --- |
| Sumolus parviflorus | 250 | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | 42 | --- | --- | --- |
| Scrophulariaceae ? | --- | --- | --- | --- | --- | --- | --- | --- | --- | 42 | --- | --- | --- | --- | --- |
| Stellaria media | --- | --- | --- | --- | --- | --- | --- | 334 | --- | --- | --- | --- | --- | --- | --- |
| Unknown species #1 | --- | --- | --- | --- | --- | --- | --- | --- | --- | 375 | 1,834 | 2,750 | 375 | --- | --- |
| #2 | --- | --- | --- | --- | --- | --- | 84 | 250 | 42 | --- | --- | --- | --- | --- | --- |
| #3 | 2,541 | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | 84 | 125 |
| Mean # seeds/m2 | 4,125 | 72,502 | 41,250 | 62,250 | 12,040 | 33,000 | 31,710 | 2,210 | 11,460 | 7,375 | 11,300 | 9,880 | 3,334 | 1,877 | 3,920 |
| # species | 11 | 3 | 4 | 6 | 5 | 4 | 4 | 4 | 7 | 16 | 13 | 14 | 4 | 5 | 6 |

*Results from core samples 10 cm deep with actual area sampled corrected to standard reference area of 1 $m^2$.

where a = species common to sites 1 and 2, b = species found at site 1 but absent at site 2, and c = species found at site 2 but absent at site 1. The index has a range of 0—1.0, where 0 represents no similarity and 1.0 represents complete similarity.

There were few cases of high floristic similarity (Figure 2). One was a comparison between the two natural marshes sampled, another was the comparison between the Sanlan-Juncus marsh and the Four Corners mulched plot. These two cases are both comparisons of samples with low species richness. The other cases of high floristic similarity are within site sample comparisons, one from Clear Springs and one from Fort Green.

The Clear Springs samples had the largest number of species and had moderately high to high within-site floristic similarity. The species assemblage at Clear Springs had several unique or less frequently encountered species including: Aster subulata, Baccharis halimifolia, Eclipta alba, and Ptilimnium capillaceum. The samples from Fort Green also exhibited moderately high to high within-site floristic similarity, largely due to three species (Juncus effusus, Ludwigia virgata, and a species of Cyperus).

Figure 2 also shows many comparisons of low to moderate similarity largely due to the near ubiquity of Juncus effusus and Polygonum punctatum in all samples.

Table 2b. Seed Bank Density Data from Table 2a Summarized Across Sites for Species Totals of Density, Relative Density, Frequency, Relative Frequency, and Importance Value.

| | Density Total (mean #/m²) | % Relative Density | Sampling Site Frequency | % Relative Frequency | Importance Value |
|---|---|---|---|---|---|
| Aster subulata | 1,226 | 0.36 | 0.20 | 2.60 | 3.16 |
| Baccharis halimifolia | 450 | 0.13 | 0.20 | 2.80 | 2.95 |
| Carex sp. | 125 | 0.04 | 0.07 | 0.95 | 0.99 |
| Cyperus brevifolius | 500 | 0.16 | 0.13 | 1.90 | 2.06 |
| Cyperus sp. | 2,209 | 0.70 | 0.20 | 2.80 | 3.50 |
| Cyperus sp. | 12,207 | 4.00 | 0.53 | 7.50 | 11.50 |
| Eleocharis rotundata | 84 | 0.03 | 0.07 | 0.95 | 0.98 |
| Gramineae | 125 | 0.04 | 0.07 | 0.95 | 0.99 |
| Echinochloa walteri | 958 | 0.30 | 0.27 | 3.80 | 4.10 |
| Eclipta alba | 1,672 | 0.50 | 0.60 | 8.50 | 9.00 |
| Eupatorium compositifolium | 84 | 0.03 | 0.07 | 0.95 | 0.98 |
| Eupatorium oblusifolium | 167 | 0.05 | 0.13 | 1.90 | 1.95 |
| Grasses, unknown #1 | 42 | 0.02 | 0.07 | 0.95 | 0.97 |
| Grasses, unknown #2 | 84 | 0.03 | 0.07 | 0.95 | 0.98 |
| Grasses, unknown #3 | 417 | 0.13 | 0.20 | 2.80 | 2.93 |
| Grasses, unknown #4 | 1,625 | 0.50 | 0.07 | 0.95 | 1.45 |
| Grasses, unknown #5 | 126 | 0.04 | 0.13 | 1.90 | 1.94 |
| Grasses, unknown #6 | 42 | 0.02 | 0.07 | 0.95 | 0.96 |
| Hydrocotyle verticillata | 206 | 0.07 | 0.13 | 1.90 | 1.97 |
| Hypericum mutilum | 257,587 | 84.00 | 1.00 | 14.00 | 98.00 |
| Juncus effusus | 209 | 0.07 | 0.20 | 2.80 | 2.87 |
| Juncus gutonius | 8,499 | 3.00 | 0.67 | 9.50 | 9.60 |
| Ludwigia virgata | 376 | 0.12 | 0.13 | 1.90 | 2.00 |
| Ludwigia palustris | 294 | 0.09 | 0.13 | 1.90 | 2.00 |
| Ludwigia leptocarpa | 5,588 | 3.00 | 0.67 | 9.50 | 12.50 |
| Polygonum punctatum | 1,168 | 0.40 | 0.20 | 2.80 | 3.20 |
| Ptilimnium capillaceum | 42 | 0.02 | 0.07 | 0.95 | 0.97 |
| Rumex verticillatus | 292 | 0.09 | 0.13 | 1.90 | 1.99 |
| Samolus parviflorus | 42 | 0.02 | 0.07 | 0.95 | 0.95 |
| Scrophulariaceae | 334 | 0.10 | 0.07 | 0.95 | 1.30 |
| Stellaria media | 5,336 | 1.70 | 0.27 | 4.00 | 5.50 |
| Unknown species #1 | 378 | 0.10 | 0.20 | 2.80 | 2.90 |
| #2 | 2,750 | 0.90 | 0.20 | 2.80 | 3.70 |
| #3 | | | | | |
| Column total | 308,000 | 100.00 | 7.07 | 100.00 | 200.00 |

## Species Diversity

Species richness and species diversity were compiled for data from this study and Lake Kanapaha (Table 3). Diversity was calculated using the Shannon-Weaver diversity index, given as H' and defined as

$$H' = \sum_{i=1}^{n} - P_i \ln P_i$$

where $P_i$ is the ratio of number of individuals of the ith species divided by the total number of individuals in the sample. The value of H' is influenced by two factors: the number of species, known as species richness, and the equitability with which

Table 3. Seed Bank Densities, Species Richness, and Shannon-Weaver Diversity Index from Florida Wetlands and Selected Marsh Studies from Temperate North America.

| | Mean # seeds.m² | Number of Species | Shannon-Weaver Diversity, H' | Source |
|---|---|---|---|---|
| Natural Systems, Florida | | | | |
| Bay Swamp | 4,125 | 12 | 1.45 | This study |
| Lake Kanapaha | | | | |
| Sacciolepis zone | 156,000 | 38 | 2.64 | (11) |
| Amaranthus zone | 28,000 | 17 | 1.72 | (11) |
| Echinochloa zone | 30,000 | 13 | 0.98 | (11) |
| Pond zone | 9,000 | 8 | 1.17 | (11) |
| Four Corners Marsh | | | | |
| Juncus-Panicgrass zone | 72,502 | 3 | 0.06 | This study |
| Pasture Marsh | | | | |
| Juncus-Panicgrass zone | 41,250 | 4 | 0.26 | This study |
| Unreclaimed Systems | | | | |
| Sanlan | | | | |
| Juncus-Polygonum Marsh | 62,250 | 6 | 0.30 | This study |
| Eleocharis Marsh | 12,040 | 5 | 0.86 | This study |
| Reclaimed Systems | | | | |
| Four Corners Reclamation Project | | | | |
| Mulched plot | 33,000 | 4 | 0.05 | This study |
| Planted plot | 31,710 | 4 | 0.05 | This study |
| Control plot | 2,210 | 4 | 0.95 | This study |
| Planted swamp plot | 11,460 | 4 | 0.30 | This study |
| Clear Springs Reclamation Project | | | | |
| South Basin #1 | 7,375 | 16 | 2.33 | This study |
| South Basin #2 | 11,100 | 13 | 1.92 | This study |
| North Basin | 9,880 | 14 | 1.89 | This study |
| Fort Green Reclamation Project | | | | |
| Mulched, vegetated | 3,334 | 4 | 1.11 | This study |
| Mulched, unvegetated | 1,877 | 5 | 0.84 | This study |
| Unmulched | 3,920 | 6 | 1.38 | This study |
| Other Natural Systems | | | | |
| Iowa, Prairie glacial marsh | 20—40,000 | 7—16 | Not calculated | (3, 5) |
| Ontario, Lakeshore marsh | 5—20,000 | 31 | Not calculated | (2) |
| New Jersey, Freshwater tidal marsh | 5—32,300 | 17—20 | Not calculated | (8) |

the individuals of the population are apportioned among the species, the greater the species richness and/or the equitability the greater the value of H'.

The overall range of H' values was 0.05—2.64, the lower value is from the mulched, vegetated plot at Fort Green while the highest value was from the Sacciolepis-zone at Lake Kanapaha. This Kanapaha sample also had the highest seed density (156,000/$m^2$) and the highest species richness (38). As can be seen in Table 3, the samples with the greater number of species typically had H' values in the upper range. The most diverse samples from natural wetlands came from Lake Kanapaha and the bay swamp, while the highest diversity in the mined wetlands group was in the Clear Springs samples. In several cases (Four Corners natural marsh, pasture marsh, Four Corners mulched plot, Four Corners planted plot, and Sanlan-Juncus-zone) the seed bank had relatively few species and dominated numerically by one species in particular—Juncus effusus. This situation more or less defined the low end of the H' range.

## Discussion

Seed bank samples from all but the youngest sites in the postmining landscape fall within or just below the range of densities and species diversity found in natural wetlands of Florida, Iowa, New Jersey, and Ontario. The indications from the results in this study are that it is possible for nature to reestablish a seed bank of approximately the same size and diversity as that occurring in some natural marshes such as with the Juncus-Polygonum marsh at Sanlan (30 yr old). The time it takes for the seed bank to reach the point of being a "reasonable facsimile" to that of a natural marsh is an open question. If Clear Springs is any indication then modest sized seed banks with higher diversity can develop in 4 yr with little actual marsh

Table 4. Twenty Species With Highest Importance Values Along With the Two Components Used to Calculate the IV. All Data Taken From Tables 2a and 2b.

| Species | % Relative Density | % Relative Frequency | Importance Value |
|---|---|---|---|
| Juncus effusus | 84.00 | 14.00 | 98.00 |
| Polygonum punctatum | 3.00 | 9.50 | 12.50 |
| Cyperus rotundus | 4.00 | 7.50 | 11.50 |
| Ludwigia virgata | 3.00 | 6.60 | 9.60 |
| Eupatorium compositifolium | 0.50 | 8.50 | 9.00 |
| Unknown #1 | 1.70 | 3.80 | 5.50 |
| Eclipta alba | 0.30 | 3.80 | 4.10 |
| Unknown #3 | 0.90 | 2.80 | 3.70 |
| Cyperus sp. | 0.70 | 2.80 | 3.50 |
| Ptilimnium capillaceum | 0.40 | 2.80 | 3.20 |
| Aster subulata | 0.36 | 2.80 | 3.16 |
| Baccharis halimifolia | 0.15 | 2.80 | 2.95 |
| Grass #4 | 0.13 | 2.80 | 2.93 |
| Unknown #2 | 0.10 | 2.80 | 2.90 |
| Juncus bufonius | 0.07 | 2.80 | 2.87 |
| Cyperus brevifolius | 0.16 | 1.90 | 2.06 |
| Ludwigia palustris | 0.12 | 1.90 | 2.02 |
| Ludwigia leptocarpa | 0.09 | 1.90 | 1.99 |
| Samolus parviflorus | 0.09 | 1.90 | 1.99 |
| Hypericum mutilum | 0.07 | 1.90 | 1.97 |
| | | | 185.00 |

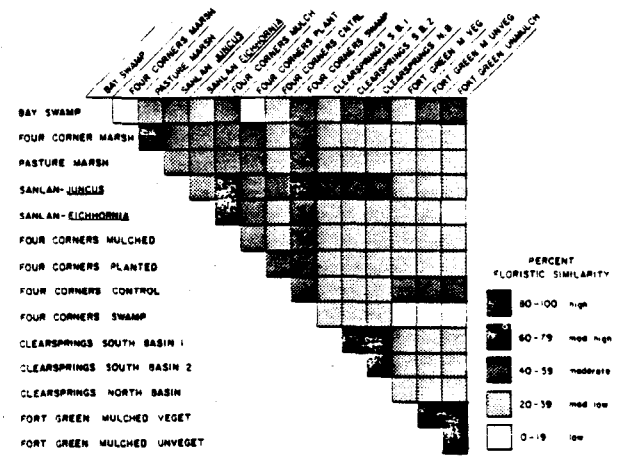


Figure 2. Summary matrix of floristic similarity.

reclamation, and with some reclamation efforts seed banks that compare very favorably in size with natural marshes can develop in 5 yr as demonstrated at Four Corners.

It appears that in some cases the seed banks in some of the postmining wetlands are not all that different in size and species composition from the natural marshes sampled in this study, but the actual vegetation present is not always as diverse, dense, or well developed except in cases where "mulch" (topsoil) from a donor wetland was applied. As an example, in line intercept transects run by the authors in mulched and unmulched areas of the marsh at Fort Green it was found that the mulched areas had almost 100% cover, while the unmulched areas had less than 30% cover. Scanning the list of species in Table 2 it is striking to see the kinds of species represented in these seed bank samples. They are a mixture of annuals and perennials that are for the most part found on exposed mudflats, wetland transition zones, and shallow littoral zones. The list is notably bereft of emergent macrophytes, submergent macrophytes, and free-floating aquatic species. This may in part be due to the germination conditions, which were similar to that of an exposed mudflat. The species present are largely the wetland ruderal species characteristic of environments subject to disturbance such as water level fluctuations. They are the annuals and short-lived perennials of mudflats and gravel bars (10).

This begs the question that if Pontederia cordata, Sagittaria lancifolia, Nuphar luteum, and Nymphaea odorata were present in at least a few of the wetlands sampled, why were none of their seeds detected in this assay? These species are the long-lived perennials of freshwater marshes—the late successional marsh species. It may be beneficial to examine the species present versus those conspicuous by their absence in terms of successional status and life history characteristics of the adult established phase and juvenile regenerative phase. Persistent seed banks represent only one of three regenerative strategies used by wetland plants the other two are vegetative expansion and production of numerous wind-dispersed seeds. It may be that regenerative strategies are related to successional status of the individual species. Generally speaking vegetative expansion is successful in situations where the adult plant is already established especially in the case of long-lived perennials where vegetative expansion allows for rapid colonization of open space under conditions unconducive to seed germination. In habitats of chronic but unpredictable disturbance (fire, flood, drought) where most of the vegetative structure is destroyed, the persistent seed bank is the primary regenerative mechanism. The third regenerative strategy employed by wetland plants is the production of numerous wind-dispersed seeds (Typha, Salix, and Baccharis). Ultimately the key is seed source, even for species whose primary regenerative strategy is vegetative, the production of viable seed represents a critically essential component of the total regenerative strategy.

The pertinence of considering life history characteristics and regenerative strategies is supported by a qualitative model of freshwater marsh succession proposed by van der Valk in which the plant community response to climatic cycles of flood and drought is analyzed through the life history characteristics of the species found to reside in the seed bank. The model depicts the plant community as the result of prevailing environmental conditions "screening" species from the seed bank. The life history characteristics considered in the model include: propagule longevity (short-lived dispersing or long-lived seed bank forming), life span of the established plant (annual, perennial, etc.), propagule establishment requirements (germination under drawdown or flooded conditions). It is the contention of van der Valk that once the composition of the seed bank is known the course of successional response to environmental changes, such as water level fluctuations, can be predicted by a knowledge of the species-specific life history characteristics. A wetland whose seed bank contains species representative of all regenerative strategies and life history characteristics is well buffered against all disturbance regimes. It is this type of wetland we should try to establish in the postmining landscape through the use of seeding, mulching, and planting.

What are the implications of this consideration of successional status and regenerative strategy for vegetation management and wetlands restoration? We are trying to make the case for a closer melding of wetlands ecology and reclamation with the goal of restoring stable, self-maintaining marsh systems in the postmining landscape. Studies of wetlands on unreclaimed lands have documented the paucity of the more "desirable climax" species in both marshes (11) and swamps (11, 12). These studies describe arrested succession in which the initial floristic composition, sensu Egler (13), of primary invading species is perpetuated. The question remains to be answered whether the succession is arrested due to the inability of the later successional species to 1) arrive on abandoned sites or 2) to arrive and find adequate resources available for germination, growth, and establishment. Initial colonizing species, once established, may be able to resist invasion by other species, but as suggested by Egler (13) the ability of established plants to resist invasion may be independent of the position of species in the normal sequence of community development. Consequently, it may be feasible to establish self-maintaining, stable marsh communities dominated by desirable late successional species and able to resist invasion by aggressive weedy species such as Typha. The key will be in creating wetlands well buffered against the disturbance regime typically encountered in marshes—fire, flood, drought. As already mentioned, a well-buffered system has representatives of all successional stages, regenerative strategies, and life histories. For reclamation the emphasis should be on two goals: establishing those components that have been found to be lacking in unreclaimed systems—the late successional long-lived perennials—and control of aggressive weed species capable of arresting succession such as Typha. (To date topsoiling (peat mulching) experiments using soil/sediment from donor marshes has been successful in both of these areas). Topsoiling, may not be feasible in all cases due to quality of donor material or budgetary constraints of transporting the material. In such situations a combination of seed bank establishment through direct seeding coupled with transplant of an array of long-lived perennials might accomplish the two goals.

In final analysis, seed banks are not a panacea for restoration of native marsh systems, but are critical components of stable, self-maintaining ecosystems. The persistent seed bank is one of the regenerative strategies by which wetlands respond to disturbance. This capacity needs to be established either through seeding or application of topsoil (peat mulch).

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# Enhancing Ecological Succession: 6. Succession of Vegetation, Soils and Mycorrhizal Fungi Following Strip Mining for Phosphate

by

Peter M. Wallace and G. Ronnie Best
Center for Wetlands and
Environmental Engineering Sciences Department
University of Florida
Gainesville, FL 32611

Abstract. Several nonreclaimed mined sites in central Florida were selected to study belowground succession and endomycorrhizal dynamics following strip mining for phosphate. Site ages range from recently mined to 60 years postmining. The study was divided into the major tasks of 1) assessing aboveground vegetative composition changes; 2) analyzing organic matter dynamics and soil nutrient parameters; 3) analyzing changes in root structure; and 4) assessing mycorrhizal population density changes through time and identifying indigenous mycorrhizae. Results indicate invasion of some mycorrhizal fungi species to mined areas is relatively rapid with most plants examined exhibiting mycorrhizal colonization. Root length and root biomass data indicate a continuing increase in belowground structure with time. Soil nutrient analyses revealed phosphorus concentrations ranged from 50 to 5528 mg/L (ppm). The importance of endomycorrhizal fungi to succession of native forest ecosystems on mined areas will be related to the potential use of mycorrhizal fungi inoculum as a reclamation practice.

## Introduction

The concept of enhanced ecological succession of devastated lands implies man's manipulation of natural processes. Designing reclamation techniques requires an understanding of both aboveground and belowground processes of ecosystem succession. In order to develop a holistic approach to natural ecosystem reconstruction, it must be emphasized that total reclamation involves more than simply revegetating the surface of the land. Reclamation of drastically disturbed lands (e.g., coal mining, oil spills, phosphate mining) in the United States is currently in the experimental stage. Critical parameters in natural ecosystem reconstruction need to be identified and analyzed to insure success of current reclamation efforts. One such parameter warranting special attention in reclamation studies is the potential role of mycorrhizal fungi. The function and importance of mycorrhizal infection in plant growth and survival are well documented (1, 2); however, mycorrhizal population dynamics and function following disturbance have only received limited attention. It has been observed in several instances in the American Midwest that a majority of the invading species in disturbed areas are typically nonmycorrhizal (3, 4, 5) Reeves et al. (5) found that 99% of the plant species adjacent to a disturbed area (old roadbed) were mycorrhizal compared to only 1% in the disturbed area. Similar results were observed in strip mined areas of the Red Desert in Wyoming (4). Land disturbance is accredited with reducing the availability of viable mycorrhizal propagules thus inhibiting the invasion of mycorrhizal plants. Although mycorrhizal infection may be mediated through spores, hyphal fragments, or infected plant roots, the most probable form of endomycorrhizal inoculum is via infected plant roots and not spores (5). Therefore a reduction in host availability by drastic land alteration (i.e., mining) could subsequently limit invasion of disturbed areas by mycorrhiza-dependent species. This is very important when considering that a majority of naturally occurring late successional plants typically form mycorrhizal associations (see 1, 6).

In contrast to these findings, investigations of coal spoils in Pennsylvania and Scotland showed a majority of colonizing plants to exhibit typical vesicular-arbuscular mycorrhizal infections (7, 8). It was suggested that mycorrhizae are necessary for plant survival on these harsh anthracite spoils. A similar study of bituminous coal mining wastes in New South Wales also found that colonizing species were generally endomycorrhizal (9). It appears that in these areas plant colonization and subsequent survival may in fact be limited and controlled by mycorrhizal presence. Invasion by nonmycorrhizal species is limited, which is in contrast to the previously cited examples.

To date no information exists concerning vesicular-arbuscular mycorrhizae dynamics following phosphate mining in Florida. During mining, nonweathered overburden is removed from various depths and deposited on the surface. This "abiotic" soil must be invaded and naturally colonized by native plant species. The degree or rate at which mycorrhizal reinvasion occurs could possibly determine rates of plant community establishment (4, 10).

In central Florida numerous nonreclaimed areas exist that were mined at different dates. These areas offer an excellent opportunity to study community succession following phosphate mining. In

response to a need to develop reclamation techniques to establish natural ecosystems on mined lands, a study was initiated to investigate both aboveground and belowground changes during succession following phosphate mining disturbance. Emphasis is placed upon mycorrhizal dynamics during succession; however, numerous ecosystem parameters were examined to investigate total community changes influencing the rate and direction of succession in these disturbed areas.

## Materials and Methods

### Site Selection

Six different aged nonreclaimed phosphate mined areas in southwest Polk County in central Florida were selected for study. Sites included an area presently being mined (0 years) and areas mined 3, 8, 17, 43, and 60 years ago. Criteria used for site selection were 1) that sites had not been subjected to any reclamation procedures following mining and 2) that sites were sufficiently isolated so that natural vegetation was undisturbed and not subjected to any interfering activities (e.g., cultivation, pasturing, construction, etc.). Study sites were originally selected using aerial photographs to eliminate any bias towards community composition. All sites selected represent typical areas strip mined for phosphate and characterized by a mosaic of overburden mounds having extremely steep slopes and generally surrounded by deep water-filled channels (for site locations see 11).

### Sampling Technique

Three 5 x 50-m quadrats were established on all sites. Quadrat locations were positioned as follows: quadrat 1 was located on the crest (ridge) of the overburden mounds; quadrat 2 corresponded to the midslope region of the overburden mounds; and quadrat 3 was located at the base of overburden piles adjacent to the water's edge. Quadrats were placed in this stratified-random fashion because during initial observations of these sites it was determined that vegetation differences were prominent between the more hydric area at the base and the somewhat xeric situation that exists at the crest of the overburden piles.

### Aboveground Biomass

Within each quadrat, aboveground biomass estimates were made for all tree, shrub, and herbaceous species. Total tree basal area estimates were made for all species having a diameter at breast height (dbh) measurement greater than 10 cm. Shrub biomass estimates were determined for all species where dbh was less than 10 cm and height was at least 1.3 m. All individuals that had not attained a height of 1.3 m, regardless of species, were harvested as herbaceous vegetation. Herbaceous plots were 1 $m^2$ and were located at 10-m intervals within the quadrat. For each quadrat five herbaceous plots were harvested, totaling 15 plots per site (this particular sample group will be referred to as herbaceous biomass). Herbaceous biomass was separated by species, oven dried at 70°C for 48 hours, and dry weight was determined. Following harvest of all live herbaceous material within a given plot, all litter was completely removed, exposing the mineral soil. Litter was oven dried at 70°C for 48 hours, and total dry weights were determined.

### Belowground Components

Several categories of belowground parameters were examined. All belowground data were collected directly below the areas in which herbaceous plants were harvested. Sample categories, techniques, and processes are explained below.

Soil Organic Matter (SOM). SOM consists of total root biomass and detrital components located within the soil column. Three size fractions were analyzed to best determine organic matter dynamics as affected by time.

SOM >1 mm. Soil cores averaging 25 x 25 x 15 cm (9375 $cm^3$) were excised from within the herbaceous sample areas. Samples were weighed and subsequently sieved through a 1-mm mesh screen using flowing water. All organic material retained on the screen was oven dried at 70°C for 48 hours and weighed.

SOM <0.60 mm and SOM 0.60 < X < 1.0 mm. To separate and analyze the small and intermediate SOM size fractions, cores (6.5 cm dia. x 15 cm deep [500 $cm^3$]) were removed using a soil auger. Samples were air dried and passed through consecutive 1-mm and 0.6-mm sieves. Material that passed through the 1-mm sieve but was retained on the 0.6-mm sieve was collected and processed as an independent size fraction. Material passing through the 0.6-mm sieve was also collected and analyzed. Organic matter content within these size fractions was determined by the Walkley-Black wet combustion technique (12).

Soil Chemical Analysis. Three soil cores measuring 6.5 cm dia. x 15 cm deep (500 $cm^3$) from each herbaceous plot were composited and an aliquot (1000 g) removed for chemical analysis. Samples were dried at 70°C for 48 hours and subsequently ground to pass through a 0.6-mm sieve. Phosphorus determinations were performed on these samples using double acid, ammonium fluoride, and sodium bicarbonate extracts (13).

Root Length and Mycorrhizal Colonization. Single cores (6.5 cm dia. x 15 cm deep) were removed from herbaceous plots to determine total root length. Sample weights and volumes were determined prior to sieving through a 0.6-mm sieve. All material retained by the sieve was collected, and total root length measurements were determined using a grid-line intercept method (14, 15). Roots were then cleared in potassium hydroxide and stained with trypan blue or acid fuchsin (16), and percent mycorrhizal colonization was determined using a modified grid-line intercept procedure (14).

Mycorrhizal Infectivity. A multiple dilution series technique was employed to determine the "most probable number" (MPN) of mycorrhizal propagules in a given volume of soil (17, 18, 19). Soil samples used in these analyses were composites of five samples collected from the herbaceous plots in each quadrat. Therefore an infectivity value was obtained for each independent quadrat. A fourfold dilution series was prepared using mixtures of test soil and autoclaved test soil. Fifty grams of soil was placed in five replicate vials of each dilution and planted with seeds of Cassia obtusifolia, a naturally occurring legume common in central Florida. Plants were grown for 45 days, and roots were removed, cleared and stained, and examined for mycor-

rhizal infection. Any form of colonization, regardless of intensity, was denoted as positive. The MPN of mycorrhizal propagules was determined from tables presented in Fisher and Yates (20; see table $VIII_2$).

Mycorrhizal Colonization of Plant Species. The mycorrhizal status of several plant species was examined at each site. Roots of commonly occurring herbaceous, shrub, and tree species were carefully excavated, sealed in plastic containers, and refrigerated. Percent root colonization was quantified using a modified grid-line intercept method (14).

## Results

### Plant Community Structure

Aboveground Biomass. Aboveground biomass data are presented in several general categories as follows. Occurrence of herbaceous, shrub, and tree species populations is discussed for each site and associated dynamics through time. Litter dynamics will be treated as an aboveground component, and, in addition, litter and herbaceous data have been combined into a total aboveground biomass category for analysis. Vegetation data are tabulated so that total importance values (IV) are presented for herbaceous vegetation (Table 1), and density, basal area, and IV's are provided for tree and shrub species (Table 2). IV's have been calculated for all species that were sampled to simplify review and discussion of data. IV's serve as a measure of the significance of each species as a structural component of the plant community. Herbaceous IV's were calculated using relative frequency and relative biomass data and are presented in Table 1. Shrub and tree IV's are a combination of relative density and relative dominance (basal area) determinations (Table 2). Although standard methods for calculating density, dominance, frequency, and IV were used (21), IV's for each species were converted to a percentage of the total importance for all species.

Zero-year-old mine (recently mined site). Eupatorium compositifolium, Digitaria sanguinalis, and Sesbania macrocarpa were actively colonizing this area. Salix caroliniana was also present but individuals occurred as resprouts from pre-existing rhizomes redistributed at various levels during mining. S. caroliniana seedlings were not observed to be colonizing the site at the time sampling was performed. Representative members of the species encountered were not found to occur within the herbaceous sample area; therefore, zero values exist for all herbaceous harvest data at this site. The site can generally be characterized as having barren mineral soil with no litter deposition or accumulation and extremely limited herbaceous colonization.

Three-year-old mine. Herbaceous vegetation is very abundant within this area, and substantial plant cover has been established since mining was discontinued. Indigofera hirsuta is the most important plant species present (IV = 46.0) with Cynodon dactylon (IV = 14.7) and Digitaria ciliaris (IV = 11.8) being very abundant also. It is necessary to emphasize that even though I. hirsuta is very common within the sample area, dominance by this species does not seem to be prevalent on all sites within this age classification. Observation of overburden mounds within the general locality adjacent to the sample site revealed that I. hirsuta populations may vary from very dense to very sparse. Dominance of herbaceous species on any one site is probably a result of the mining period being incidental with species seed dispersal.

Table 1. Importance Values of Herbaceous Plants. Importance value is the sum (normalized to 100%) of relative biomass and relative frequency of herbaceous plants at each site.

| | Site Age, Years | | | | | |
|---|---|---|---|---|---|---|
| Plant Species | 0 | 3 | 8 | 17 | 43 | 90 |
| GRASSES | | | | | | |
| Cynodon dactylon | — | 14.7 | — | — | — | 0.8 |
| Cyperus sp. | — | 1.0 | — | — | — | — |
| Digitaria ciliaris | — | 11.8 | — | — | — | — |
| Paspalum urvillei | — | 5.8 | — | — | — | — |
| Rhynchelytrum repens | — | 2.8 | 39.3 | 23.5 | 32.4 | — |
| Setaria geniculata | — | 1.0 | — | — | 1.6 | — |
| Unknown Grass | — | 1.0 | 5.0 | — | 1.6 | — |
| Andropogon virginicus | — | — | 8.7 | 1.7 | — | — |
| Juncus sp. | — | — | 1.1 | — | — | — |
| Panicum hematomon | — | — | 2.8 | — | — | — |
| Panicum sp. | — | — | 1.1 | — | — | 6.0 |
| Andropogon sp. | — | — | — | — | 5.1 | — |
| Cyperus sp. | — | — | — | — | 3.1 | — |
| Panicum commutatum | — | — | — | — | 1.6 | 4.6 |
| Paspalum sp. | — | — | — | — | — | 2.8 |
| Oplismenus setarius | — | — | — | — | — | 2.9 |
| HERBACEOUS | | | | | | |
| Ambrosia artemisiifolia | — | 5.9 | — | — | — | — |
| Chenopodium ambrosioides | — | 1.0 | 2.3 | — | — | — |
| Crotolaria spectabalis | — | 3.5 | — | — | — | — |
| Eupatorium capillifolium | — | 1.8 | 4.1 | — | 1.7 | — |
| Eupatorium compositifolium | — | 1.1 | — | — | 1.7 | — |
| Euphorbia maculata | — | 1.0 | — | — | — | — |
| Indigofera hirsuta | — | 46.0 | — | 21.5 | — | |
| Conyza canadensis | — | — | 1.1 | — | — | — |
| Momordica charantia | — | — | 7.2 | 1.3 | — | — |
| Solidago fistulosa | — | — | 20.5 | — | 3.4 | — |
| Aeschynomene americana | — | — | — | 2.3 | — | — |
| Drymaria cordata | — | — | — | 9.6 | — | 0.8 |
| Passiflora pectinata | — | — | — | 1.3 | 3.3 | — |
| Lamiaceae sp. | — | — | — | 1.6 | — | — |
| Asplenium heterochorum | — | — | — | — | 2.9 | — |
| Cassia fasciculata | — | — | — | — | 9.0 | — |
| Trifolium sp. | — | — | — | — | 1.6 | — |
| Unknown composites | — | — | — | — | 3.2 | 1.9 |
| Ampelopsis arborea | — | — | — | — | — | 0.8 |
| Bidens bipinnata | — | — | — | — | — | 2.6 |
| Blechnum serrulatum | — | — | — | — | — | 1.4 |
| Desmodium sp. | — | — | — | — | — | 0.8 |
| Tillandsia unesioides | — | — | — | — | — | 11.6 |
| Toxicodendron radicans | — | — | — | — | — | 1.5 |
| SHRUBS | | | | | | |
| Baccharis halimifolia | — | 1.6 | 4.8 | 14.7 | 3.4 | — |
| Lantana camara | — | — | — | 7.2 | 3.5 | — |
| Rubus sp. | — | — | 2.0 | 5.2 | — | 1.5 |
| Urena lobata | — | — | — | 10.3 | 3.5 | 3.1 |
| Psidium guajava | — | — | — | — | 7.6 | — |
| Smilax bononox | — | — | — | — | 1.5 | 11.5 |
| Vitis rotundifolia | — | — | — | — | 5.2 | 9.0 |
| Callicarpa americana | — | — | — | — | — | 1.5 |
| Gelsimium sempervirens | — | — | — | — | — | 11.5 |
| TREES | | | | | | |
| Quercus virginiana | — | — | — | — | 2.8 | 0.8 |
| Liquidamber styraciflua | — | — | — | — | — | 3.3 |
| Quercus nigra | — | — | — | — | — | 13.2 |
| TOTAL | | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |

Eight-year-old site. In addition to numerous herbaceous and grass species, several shrub species were sampled at this site. S. caroliniana (IV = 54.3) and Baccharis halimifolia (IV = 44.0) were the most important shrub species encountered. These species, although not attaining tree size (>10 cm dbh), were very large shrubs forming a low, closed canopy in the most-basal areas of quadrat 3. Rhynchelytrum repens (IV = 39.3) and Solidago fistulosa (IV = 20.5) were the most important herbaceous species, however, occurrence was restricted to different areas within quadrats. R. repens was dominate in areas of quadrats 1 and 2 in which full intensity sunlight occurred.

Seventeen-year-old site. The 17-year-old mine represented a stage of transition from a herbaceous-dominated community to a herbaceous-shrub community. Unlike the 8-year-old mine, shrubs were generally not restricted to slope bases but were also distributed throughout the ridge and midslope regions of

Table 2. Shrub (<10 cm dbh) and tree (>10 cm dbh) biomass data obtained from succession study sites: Density (stems/ha), basal area ($m^2$/ha), and importance values (%) are reported for all species having a dbh at 1.3 m (dbh = diameter at breast height). Note: the younger aged sites (0 and 3 years) did not have shrub or tree size class individuals.

| | SITE AGE, YEARS | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 8 | | | 17 | | | 43 | | | 60 | | |
| Species | Density | Basal Area | I.V. | Density | Basal Area | I.V. | Density | Basal Area | I.V. | Density | Basal Area | I.V. |
| SHRUB | | | | | | | | | | | | |
| Baccharis halimifolia | 2400 | 0.824 | 44.0 | 5295 | 0.913 | 25.5 | 147 | 0.003 | 1.5 | 27 | 0.005 | 0.3 |
| Psidium guajava | 31 | 0.011 | 0.5 | 533 | 0.121 | 3.1 | 80 | 0.001 | 0.8 | 1867 | 0.074 | 12.2 |
| Salix caroliniana | 1323 | 2.410 | 54.3 | — | — | — | — | — | — | — | — | — |
| Schinus terebinthifolius | 62 | 0.037 | 1.3 | — | — | — | — | — | — | — | — | — |
| Myrica cerifera | — | — | — | 381 | 0.381 | 7.3 | 1787 | 0.473 | 33.4 | 373 | 0.077 | 4.6 |
| Urena lobata | — | — | — | 14436 | 0.591 | 36.9 | — | — | — | 160 | 0.001 | 0.9 |
| Lantana camara | — | — | — | 6323 | 0.903 | 27.2 | 2080 | 0.037 | 20.9 | — | — | — |
| Serenoa repens | — | — | — | — | — | — | 320 | 0.130 | 7.6 | — | — | — |
| Prunus serotina | — | — | — | — | — | — | 267 | 0.115 | 6.6 | 613 | 0.365 | 15.6 |
| Quercus virginiana | — | — | — | — | — | — | 426 | 0.552 | 23.4 | 160 | 0.003 | 1.0 |
| Quercus laurifolia | — | — | — | — | — | — | 133 | 0.098 | 4.7 | 107 | 0.023 | 1.4 |
| Persea palustris | — | — | — | — | — | — | 80 | 0.019 | 1.4 | — | — | — |
| Callicarpa americana | — | — | — | — | — | — | — | — | — | 1867 | 0.097 | 13.0 |
| Smilax bononox | — | — | — | — | — | — | — | — | — | 880 | 0.059 | 6.6 |
| Vitis rotundifolia | — | — | — | — | — | — | — | — | — | 107 | 0.014 | 1.1 |
| Quercus nigra | — | — | — | — | — | — | — | — | — | 3334 | 0.585 | 37.2 |
| Liquidambar styraciflua | — | — | — | — | — | — | — | — | — | 133 | 0.170 | 6.5 |
| TOTAL | 3816 | 3.282 | 100.1 | 26968 | 2.909 | 100.0 | 5320 | 1.428 | 100.3 | 9628 | 1.473 | 100.4 |
| TREE | | | | | | | | | | | | |
| Quercus virginiana | — | — | — | — | — | — | 13 | 0.138 | 6.2 | 147 | 11.810 | 45.2 |
| Quercus laurifolia | — | — | — | — | — | — | 27 | 0.479 | 14.6 | — | — | — |
| Pinus elliottii | — | — | — | — | — | — | 80 | 3.567 | 63.0 | 80 | 2.612 | 17.2 |
| Pinus palustris | — | — | — | — | — | — | 13 | 1.246 | 16.4 | — | — | — |
| Prunus serotina | — | — | — | — | — | — | — | — | — | 40 | 1.678 | 9.3 |
| Quercus nigra | — | — | — | — | — | — | — | — | — | 53 | 6.268 | 20.3 |
| Liquidambar styraciflua | — | — | — | — | — | — | — | — | — | 13 | 3.053 | 8.0 |
| TOTAL | | | | | | | 133 | 5.430 | 100.2 | 333 | 25.421 | 100.0 |

the site. Baccharis halimifolia (IV = 14.7) and Urena lobata (IV = 10.3) were very important components within the herbaceous strata. R. repens was the most important herbaceous species, comprising 23.5% of the total IV. In addition, in the shrub size class B. halimifolia (IV = 25.5) and U. lobata (IV = 36.9) along with Lantana camara (IV = 27.2) attained densities of approximately 26,000 stems/ha (note: dbh <10 cm; height ≥1.3 m).

Forty-three-year-old mine. R. repens was the most important herbaceous species sampled. Although numerous tree and shrub species occur on this site, occurrence is generally restricted to areas located within quadrats 2 and 3. Quadrat 1, which is located on ridge areas of the overburden mounds, is generally restricted to colonization by grasses and forbs. R. repens is common in this area where intense sunlight and lower moisture levels prevail. Ridge areas occurring at this mine are very similar in appearance to ridge areas of younger mines with the exception of the presence of occasionally occurring oaks. Pinus elliottii (IV = 63.0) and Pinus palustris (IV = 16.4) are the most important species encountered and are the largest individuals occurring on the site. Quercus virginiana and Q. laurifolia are important components of both tree and shrub size class categories; however, tree size does not approach that attained by pines within this area.

Sixty-year-old mine. The 60-year-old site differs somewhat from the younger mines previously described. This area was mined before flotation methods were used to remove small particulate phosphate. In this location, large debris piles surround somewhat rectangular-shaped dredge pits as contrasted to the ridge and furrows characteristic of present day mining. Quercus virginiana (IV = 45.2) and Q. nigra (IV = 20.3) were the dominant tree species observed on the site. Q. virginiana attained very large dimensions with many exhibiting dbh measurements greater than 1 m (not located in sample area). Q. nigra individuals were by far the most dominant individuals in the shrub size class and represented 13% of the IV comprising the herbaceous harvest. At this site, the greatest total number of individuals (species number) was encountered in all vegetation categories. Several species were encountered that had not occurred at any previous sites. Gelsimium sempervire... Oplismenus setarius, and Callicarpa americana were prevalent and are generally considered late successional species and indicators of mature climatic conditions (22).

Herbaceous and litter. Mean values for herbaceous and litter biomass harvests for individual quadrats are illustrated in Figure 1. The highest mean herbaceous biomass (742 g/$m^2$) was obtained from the 3-year-old site with values decreasing to 58 g/$m^2$ at the 60-year-old mine. The largest

amount of litter accumulation occurs at the 60-year-old site with the least values obtained at the 3-year-old site. As might be expected, zero values for litter and herbaceous biomass were obtained at the most recently mined site.

Trends are that litter biomass generally increases with time with a corresponding decrease in herbaceous biomass as communities approach mature stages. It is obvious that these communities rapidly establish biomass in the form of litter and herbaceous material. Means for total aboveground biomass (sum of litter and herbaceous biomass) are given in Figure 1. These means of cumulative aboveground biomass are not significantly different (Duncan's, $\alpha = 0.10$ [see 11]). Statistical analyses (Duncan's, $\alpha = 0.10$ [see 11]) indicate that no significant differences exist between any sites regardless of age (excluding site 0).

Belowground Components

Organic matter values and results of statistical analyses of three size fractions are presented in Figure 2. SOM values in the >1-mm size fraction range from 0.400 g/kg at site 0 to 11.7 g/kg soil at the 60-year-old mine. Significant differences were obtained between the two oldest sites and the remaining four younger sites, indicating that there is definite litter accumulation with time. SOM values for the intermediate size fraction range from 5.89 g/kg soil at the most recent mine to 75.07 g/kg soil at the 60-year-old mine. SOM values for the <0.6-mm size fraction range from 4.28 g/kg soil at the 3-year-old mine to 26.74 g/kg soil at the 60-year-old mine. SOM <6-mm values at all sites are considerably higher than for the SOM >1-mm fraction. SOM values for the intermediate size fractions are significantly higher than the <0.6 mm and >1.0 mm size fractions at all sites, indicating that substantial organic matter storage within the soil probably occurs as relatively small refractory particulate fractions.

Root length and mycorrhizal colonization percentage. Average values for root length determinations and mycorrhizal colonization percents are given in Figure 3. Root length mean values range

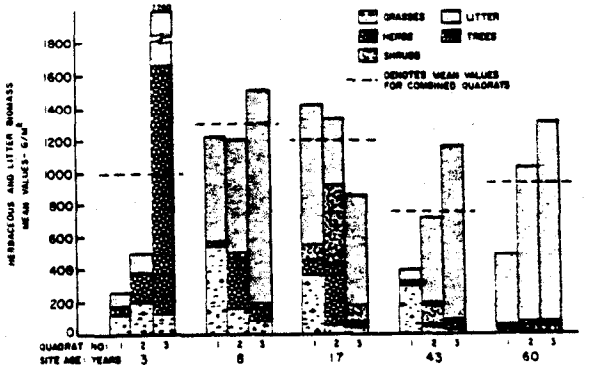


Figure 1. Cumulative mean values for herbaceous and litter biomass (total aboveground biomass). Data are combined and given for each quadrat. Herbaceous data have been divided into grasses, herbs, shrubs, and tree categories to allow for intra- and inter-site comparisons.

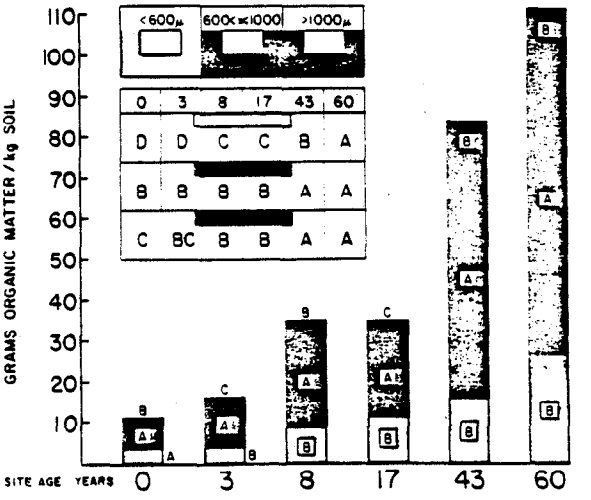


Figure 2. Mean values for soil organic matter concentrations (g/kg soil) are given for each particulate size fraction. Results of statistical analyses (Duncan's, $\alpha = 0.10$) are given for both inter- (table values) and intra-site (bar values) comparisons. Means with same letter were not significantly different.

from 53 cm/kg soil at the recent mine to 8,310 cm/kg soil at the 60-year-old mine. Values as high as 20,520 cm/kg soil were obtained from samples at the oldest site. Data indicate that significant differences (Duncan's, $\alpha = 0.1$ [see 11]) between means occur at 60 years, 43 years, 17 and 8 years, and 3 and 0 years. Root length continually increases with ecosystem development. It cannot be determined from the present results if the trend of increasing root length with time will continue toward some maximum value beyond 60 years, or if this is the highest average value maintained. Root length as a measure of accumulated belowground structure may prove to be an important community development indicator.

Mean values for sample root lengths and mean mycorrhizal colonization percentages (Figure 3) have been used to calculate mean colonized root length.

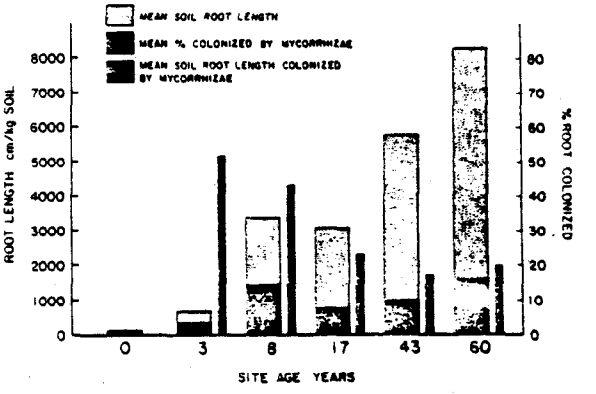


Figure 3. Mean values for total root length (cm/kg soil) and percent mycorrhizal colonization of root length are given. Mean root length colonization by mycorrhiza is presented as the product of mean root length x percent colonization.

Mean percent values for root colonization are shown to substantially decrease with time from a maximum value of 51.5% at 3 years to a minimum value of 16.8% and 19.5% obtained at the 43- and 60-year-old sites, respectively. Percent colonization values, however, are misleading when interpreted independently of total root length measurements obtained within the sample. Total estimated colonized root length (e.g., root length mean x percent colonization mean) exhibits a somewhat different distribution. Actual total mycorrhizal occurrence within a given soil core indicates minimum values at the 3-year-old site (339 cm/kg soil colonized) with maximum values obtained at the 60-year-old site (1620 cm/kg soil colonized). Although colonization percentage of any random root length within a sample may be minimal, when total root length is considered fungal biomass may be quite high.

Mycorrhizal colonization of plant species. Table 3 gives results obtained from observing mycorrhizal colonization of commonly occurring plant species at each mine site. The data indicate that mycorrhizal invasion is rapid into the areas following termination of mining activity. Of the plant species sampled on the most recent site, Eupatorium compositifolium and Salix caroliniana exhibited colonization albeit at very low levels. Species sampling at the 3-year-old site revealed a majority of species exhibiting mycorrhizal colonization with percent root colonization ranging from 2% in Salix caroliniana to 90% in Paspalum notatum.

Results obtained from the 8-year-old mine were generally the same as those from the 3-year-old site. A majority of plants exhibited typical endomycorrhizal infection. Colonization percentages were very high in a number of species (e.g., Heterotheca subaxillaris, Salix caroliniana). Several species gave highly variable results with individuals exhibiting both very high and very low root colonization (e.g., R. repens, A. artemisiifolia). Chenopodium ambrosioides was the only species encountered not to exhibit colonization. Typically

Table 3. Mycorrhizal Colonization of Commonly Occurring Plant Species for the Different Aged Study Sites. Values represent the percentage of root examined that was colonized by mycorrhizal fungi.

| | SITE AGE, YEARS | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | | | 3 | | | 8 | | | 17 | | | 43 | | | 60 | | |
| Plant Species | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Digitaria sanguinalis | N* | N | N | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Eupatorium compositifolium | 2 | - | - | - | - | - | 82 | 64 | 96 | - | - | - | - | - | - | - | - | - |
| Salix caroliniana | 5 | - | - | 17 | 87 | 2 | 76 | 61 | 72 | - | - | - | - | - | - | - | - | - |
| Ambrosia artemisiifolia | - | - | - | 19 | 28 | 26 | 4 | 98 | 13 | - | - | - | 2 | 25 | 16 | - | - | - |
| Baccharis halimifolia | - | - | - | 32 | 16 | 52 | 91 | 47 | 75 | 34 | 4 | 41 | - | - | - | - | - | - |
| Crotolaria spectabilis | - | - | - | 49 | P† | P | - | - | - | - | - | - | - | - | - | - | - | - |
| Cynodon dactylon | - | - | - | 13 | 15 | 4 | - | - | - | - | - | - | - | - | - | - | - | - |
| Cyperus sp. | - | - | - | 3 | 7 | 20 | - | - | - | - | - | - | - | - | - | - | - | - |
| Eupatorium capillifolium | - | - | - | 25 | 29 | 25 | 39 | 85 | N | - | - | - | 100 | 79 | 51 | - | - | - |
| Heterotheca subaxillaris | - | - | - | 41 | 74 | 75 | 86 | 79 | 81 | - | - | - | | | | | | |
| Indigofera hirsuta | - | - | - | 11 | P | 83 | - | - | - | - | - | - | N | 7 | 47 | - | - | - |
| Lactuca floridana | - | - | - | 39 | 49 | 45 | - | - | - | - | - | - | - | - | - | - | - | - |
| Lepidium virginicum | - | - | - | 27 | N | N | - | - | - | - | - | - | - | - | - | - | - | - |
| Ludwigia leptocarpa | - | - | - | 19 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Ludwigia peruviana | - | - | - | 19 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Paspalum urvillei | - | - | - | 49 | 79 | 74 | 53 | 50 | 74 | - | - | - | - | - | - | - | - | - |
| Paspalum notatum | - | - | - | 32 | 90 | 78 | - | - | - | - | - | - | - | - | - | - | - | - |
| Paspalum repens | - | - | - | N | N | N | - | - | - | - | - | - | - | - | - | - | - | - |
| Polygonum punctatum | - | - | - | N | N | N | N | 4 | 5 | - | - | - | - | - | - | - | - | - |
| Rhyncelytrum repens | - | - | - | N | 10 | P | 5 | 48 | 44 | 20 | 48 | 20 | 48 | 25 | 18 | - | - | - |
| Setaria geniculata | - | - | - | 82 | 75 | 45 | - | - | - | - | - | - | - | - | - | - | - | - |
| Chenopodium ambrosioides | - | - | - | - | - | - | N | N | N | - | - | - | - | - | - | - | - | - |
| Gnaphalium obtusifolia | - | - | - | - | - | - | 69 | 83 | 91 | - | - | - | - | - | - | - | - | - |
| Imperata cylindrica | - | - | - | - | - | - | 8 | 24 | 50 | 62 | - | - | - | - | - | - | - | - |
| Lactuca floridana | - | - | - | - | - | - | 30 | 26 | 70 | - | - | - | - | - | - | - | - | - |
| Psidium guajava | - | - | - | - | - | - | P | P | P | 69 | 50 | 71 | 63 | 40 | 50 | N | 14 | 22 |
| Solidago fistulosa | - | - | - | - | - | - | 18 | 68 | - | - | - | - | N | 56 | 1 | - | - | - |
| Thelypteris normalis | - | - | - | - | - | - | P | P | P | N | 20 | 8 | - | - | - | - | - | - |
| Drymaria cordata | - | - | - | - | - | - | - | - | - | 13 | P | N | - | - | - | - | - | - |
| Lantana camara | - | - | - | - | - | - | - | - | - | 67 | 45 | 92 | 65 | 68 | 26 | - | - | - |
| Myrica cerifera | - | - | - | - | - | - | - | - | - | N | 1 | - | - | - | - | N | N | N |
| Rubus sp. | - | - | - | - | - | - | - | - | - | 67 | 52 | - | - | - | - | - | - | - |
| Schinus terebinthifolius | - | - | - | - | - | - | - | - | - | 86 | - | - | - | - | - | - | - | - |
| Urena lobata | - | - | - | - | - | - | - | - | - | 84 | 95 | 71 | 86 | 44 | 81 | 35 | 60 | 39 |
| Ampelopis arborea | - | - | - | - | - | - | - | - | - | - | - | - | 44 | 81 | 81 | - | - | - |
| Andropogon sp. | - | - | - | - | - | - | - | - | - | - | - | - | N | 3 | 17 | - | - | - |
| Asplenium heterochorum | - | - | - | - | - | - | - | - | - | - | - | - | N | N | N | - | - | - |
| Bidens bipinnata | - | - | - | - | - | - | - | - | - | - | - | - | 10 | 58 | 22 | 44 | 11 | 29 |
| Cassia fasiculata | - | - | - | - | - | - | - | - | - | - | - | - | 60 | 60 | 33 | - | - | - |
| Chrysopsis graminifolia | - | - | - | - | - | - | - | - | - | - | - | - | 50 | 43 | 75 | - | - | - |
| Euchamia minor | - | - | - | - | - | - | - | - | - | - | - | - | 34 | 33 | 52 | - | - | - |
| Liatris sp. | - | - | - | - | - | - | - | - | - | - | - | - | 36 | 8 | 11 | - | - | - |
| Phlebodium aureum | - | - | - | - | - | - | - | - | - | - | - | - | N | N | N | - | - | - |
| Vitis rotundifolia | - | - | - | - | - | - | - | - | - | - | - | - | 85 | 98 | - | N | N | 1 |
| Callicarpa americana | - | - | - | | - | - | - | - | - | - | - | - | - | - | - | P | 72 | 23 |
| Gelsimium sempervirens | - | - | - | | - | - | - | - | - | - | - | - | - | - | - | P | 1 | N |
| Liquidambar styraciflua | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | P | - | - |
| Opliamenus setarius | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | N | P | N |
| Panicum commutatum | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 14 | 12 | 15 |
| Prunus serotina | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | N | N | N |
| Psychotria nervosa | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 24 | 2 | 23 |
| Quercus laurifolia | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ECTO | ECTO | ECTO |
| Quercus nigra | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ECTO | ECTO | ECTO |
| Quercus virginiana | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ECTO | ECTO | ECTO |
| Smilax bononox | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | P | 43 | 13 |
| Solanum sp. | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 60 | 28 | 35 |
| Tillandsia utriculata | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | N | N | N |

*Negative
†Positive

members of the family Chenopodiaceae do not form mycorrhizal associations (1).

A greater proportion of shrub size class individuals were sampled at the 17-year-old site than at the younger mines. All species sampled exhibited endomycorrhizal colonization; however, Myrica cerifera infection levels were extremely low. Urena lobata and Lantana camara, which were the most common shrub size class plants on the site, were extensively colonized by mycorrhizal endophytes. Extremely dense stands of these two species occurred within the site. Such high colonization percentages may indicate these species are maintaining a large mycorrhizal propagule reservoir within the soil.

Plant species sampled on the 43-year-old mine also exhibited generally high colonization percentages. Endophytes were typically found in all plant growth forms examined, i.e., vines, grasses, herbs, and shrubs. Vitis rotundifolia and Ampelopsis arborea, which are two common vine species, exhibited extremely high levels of colonization. Lantana camara and Urena lobata were extensively infected as was also the case noted on the 17-year-old mine. Although ectomycorrhizal species compose a significant component of the vegetation on this site, reductions in occurrence in endomycorrhizal colonization were not noted.

The 60-year-old mine yielded the most highly variable results of mycorrhizal occurrence within plant roots. Tillandsia utriculata, Prunus serotina, Vitis rotundifolia, and Myrica cerifera were all found to be typically nonmycorrhizal in this area. Similar results for M. cerifera were obtained at the 18-year-old site; however, it is surprising that V. rotundifolia was not colonized since extremely high levels of infection were noted at the 43-year-old site. Endomycorrhizal infection of species at these sites were substantially reduced compared to all younger sites (except site 0). Ectomycorrhizal species comprise a significant proportion of the aboveground biomass, which may tend to decrease endomycorrhizal occurrence due to a reduction in potential host availability. Also the typically endomycorrhizal herbaceous component is a very sparse component of the aboveground vegetation. All individuals of Q. nigra, Q. virginiana, and Q. laurifolia that were sampled were exhibiting ectomycorrhizal infection characterized by fungal mantle formation on simple, unforked feeder roots.

*Dilution series: MPN determinations of mycorrhizal propagules.* MPN determinations of mycorrhizal propagules in the different aged soils were obtained for each individual quadrat within all sample sites (Table 4). Mean MPN values were calculated for each site to facilitate comparisons of inoculation potential between the different areas. The 3-year-old site had the highest mean value estimate of 0.52 propagules per gram. Results indicate a trend of general reduction of mycorrhizal infectivity with time. The lowest mean value obtained was 0.11 propagules per gram, which occurred at the 60-year-old mine. It is quite surprising that the highest values were obtained at a very young site. Several possible explanations exist for this observation. Mycorrhizal invasion is extremely rapid and a high level of inoculum source is maintained to increase inoculum potential for invading plant species. Also, it is possible that the fungal species present had a greater affinity for the test plant used than species occurring in the later aged sites. In the MPN analyses, difference in host plant infection responses should be minimized, since colonization is simply based on positive or negative results and not colonization intensity analyses (17).

Table 4. Estimates of the dilution series results of the "Most probable number" (MPN) of mycorrhizal fungi propagules at the different aged mine spoils. Values represent propagule number per gram of soil.

| Site Age Years | Quadrat Location | Mycorrhizal Propagule Density (#/g soil) | |
|---|---|---|---|
| 3 | 1 | 0.83 | |
| | 2 | 0.31 | $\bar{x}$ = 0.52 |
| | 3 | 0.42 | |
| 8 | 1 | 0.23 | |
| | 2 | 0.17 | $\bar{x}$ = 0.17 |
| | 3 | 0.12 | |
| 17 | 1 | 0.42 | |
| | 2 | 0.17 | $\bar{x}$ = 0.27 |
| | 3 | 0.23 | |
| 43 | 1 | 0.23 | |
| | 2 | 0.12 | $\bar{x}$ = 0.16 |
| | 3 | 0.12 | |
| 60 | 1 | 0.07 | |
| | 2 | 0.17 | $\bar{x}$ = 0.11 |
| | 3 | 0.09 | |

*Comparisons between percent mycorrhizal root colonization and MPN determinations.* Comparisons between the different methods for assessing mycorrhizal populations previously described are difficult. In review, the dilution series indicated highest infection potential at the 3-year site with the minimum value obtained at the 60-year site. This general trend corresponds well when compared to mean percent infection of root lengths; however, comparisons with total colonized root length (Figure 3) indicate negative correlations. The differences obtained cannot readily be explained. Cassia obtusifolia, which was used in the dilution series test procedure is an opportunistic colonizer of disturbed habitats, roadsides, ditchbanks, etc. occurring in Florida. The plant may be more readily colonized by mycorrhizal species occurring in the herbaceous dominated recent mines as opposed to the mature ecosystems. This, however, infers that host specificity may be a factor, although this is not typical of endomycorrhizal associations. Another explanation may be offered when considering root infection intensity. It was noted that the roots sampled from the younger sites were more intensively colonized than roots from the more mature sites (site ages 43 and 60). Root cortical areas were massively infected in both random root samples and samples from selected species obtained from the earlier sites. In contrast infection noted in later sites was often restricted to single hyphal lengths with sporadic vesicle production. Similar observations were also noted in roots observed from the dilution series experiments. The most intense infections on Cassia obtusifolia were noted in samples from sites 3 and 8 years old. Extensive infection was noted extending through the cortex from the initial penetration

area. In contrast, the 60-year-old site plants were characterized by very little proliferation of cortical infection extending from the initial penetration point. The grid-line intercept method (14) does not distinguish between intensities of infection (e.g., 5 hyphal filaments/cm or 1 hyphal filament/cm) only that colonization occurs within a given root section. Intensity of root infection may be the factor determining inoculum potential and not total root length infected. These data should not be interpreted without considering quantitative estimates of total spore counts at these sites.

### Soil Chemical Analysis

Phosphorus concentrations obtained from all extraction procedures (Table 5) indicate phosphorus values generally exceed critical soil phosphorus levels at which plants would not respond to phosphorus fertilization (23). The high concentrations of phosphorus are very important when considering mycorrhizal effects on plant growth. Phosphorus is believed to be the most important nutrient involved in the plant-mycorrhizal relationship. It is hypothesized that mycorrhizae are able to explore soil phosphate resources that nonmycorrhizal roots are unable to use (24, 25). However, high plant or soil phosphate levels have been shown to inhibit mycorrhizal colonization and activity (2, 26).

## Discussion

### Community Succession

Aboveground Structure. The present study indicates that well-developed ecosystems have been established in a 60-year period following mining. Kangas (27) in a similar study stated that "within a period of approximately 50 years barren, undifferentiated mounds develop into a forest ecosystem with fertile soil." Results obtained from these studies may tend to be misleading. Although 60-year-old sites resemble somewhat nondisturbed climatic communities (27), these areas were mined at a time when the scale of disturbance was small. Perhaps a better indicator of ecosystem direction and development can be ascertained by examining the 17-year-old site.

Table 5. Results of Double Acid Extract Phosphorous Concentrations (ppm; mg/L) from Each Study Site. For comparison purposes, various extraction solutions were used on six samples from the 3-year-old site.

| | SITE AGE, YEARS | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 3 | 8 | 17 | 43 | 60 |
| Mean | 2397 | 901 | 1572 | 2555 | 229 | 3875 |
| S.E. | 456 | 215 | 381 | 333 | 28 | 480 |
| Minimum | 131 | 71 | 50 | 700 | 52 | 251 |
| Maximum | 5269 | 2810 | 4262 | 5340 | 413 | 5528 |

| Sample Number | Extracting Solution | | | | |
|---|---|---|---|---|---|
| | Double Acid* | Bray 1† | Bray 2§ | Olsen** | Olsen†† |
| 1-1 | 138 | 47 | 218 | 9.1 | 15.2 |
| 1-2 | 71 | 34 | 97 | 7.5 | 11.4 |
| 2-1 | 2810 | 106 | 2272 | 14.2 | 22.2 |
| 2-2 | 1340 | 101 | 1086 | 15.3 | 21.9 |
| 3-1 | 770 | 59 | 671 | 12.0 | 16.7 |
| 3-2 | 300 | 67 | 360 | 13.1 | 14.7 |

*(0.025 N $H_2SO_4$ + 0.050 N HCl).
†(0.03 N $NH_4F$ in 0.025 HCl).
§(0.03 N $NH_4F$ in 0.1 N HCl).
**(0.5 M $NaHCO_3$ [5 min extraction]).
††(0.5 M $NaHCO_3$ [30 min extraction]).

These areas are principally colonized by bird dispersed (e.g., Myrica cerifera, Psidium guajava, Prunus serotina, Rubus sp.) or windblown species (Baccharis halimifolia) that are capable of long distance dispersal. Seedlings of Quercus virginiana, Q. nigra, Liquidambar styraciflua, or Pinus species were not observed to be colonizing this site or similar aged sites within the adjacent localities. If succession on these sites is to lead to a mature oak-dominated ecosystem (60-year-old site), then invasion of oaks must occur early in site development. Dominance of a plant species can only occur after initial invasion into a site and successful reproduction with time. Due to the vast areas of disturbed land currently present in these areas, seed sources for late successional species have essentially been restricted to floodplain forests. Removal of late successional seed sources would tend to insure the possibility of an arrested succession situation (for discussion see 28), i.e., sites will remain at some stage of ecosystem development, which may possibly be similar to conditions observed at the 17-year-old site. It is only speculation, however, and further research must provide information on succession in these areas. This may prove to be a very difficult task, since succession analysis requires static analysis of dynamic processes. It implies that information can be obtained from areas disturbed at different times, which have developed under conditions that are constantly changing with time. The example previously described—seed source availability changes that occur from 60 years ago to the present—adequatetly illustrates this point.

Belowground Structure. Past research of belowground processes during succession of phosphate mined lands is limited; therefore, comparison of the present data with previous findings is difficult. The extent to which vegetation modifies the soil environment and how these modifications affect successive plant communities is generally unknown. Accumulation of soil organic matter is a very important factor in succession of phoshate mined areas. During mining, the original surface soil is removed and large quantities of unweathered mineral material are exposed. Production of organic matter by plants conserves nutrients released by weathering, prevents leaching from the profile, and establishes a readily available nutrient pool (29). Whereas nutrients are continually cycled within a community and conservation is mediated by organic matter, organic matter itself represents net additions to the system, hence, development of ecosystem structure. Data obtained from the study indicate that a continual increase in both root biomass and soil organic matter occurs through time. It appears that accumulation is a constant process that has not attained maximum limits within the 60-year time span studied (i.e., values in Figures 2 and 3 have not yet begun to plateau). An intriguing question occurs concerning the degree to which belowground succession is a time controlled or community controlled phenomena. In the case of arrested succession, will belowground parameters of a shrub community (17-year-old site) approach those of a mature hammock community (60-year-old site) especially if the site retains the same aboveground characteristics for the same time period?

### Mycorrhizal Succession

Mycorrhizal invasion into phosphate mined areas is extemely rapid. Within 3 years, the majority of invading plant species exhibit extensive mycorrhizal

colonization. Infection levels in early sites have been shown to be comparatively higher than levels obtained in the most mature ecosystem studied. Allen and Allen (3) demonstrated that mycorrhizal occurrence in reclaimed coal strip mined areas in Wyoming attained levels of 50% of undisturbed values within 2–3 years. Comparisons of mycorrhizal infectivity and occurrence between mined and undisturbed areas in Florida are difficult to perform. Disturbed lands in Florida may have origially been dominated by sandhill, oak shrub, pine flatwood, mesic hammocks, marsh, bayhead, or cypress dome type communities. Phosphate mining produces areas, characterized by soil conditions, that are totally different than areas not subjected to disturbance activities. Therefore, it is difficult to determine levels of infection to which these mined areas may approach with time. Mycorrhizal species occurring on phosphate mined lands would also have to be adapted for survival in extremely high phosphorus soils. Phosphorus has repeatedly been shown to inhibit mycorrhizal colonization and performance. It is indeed an interesting question concerning the survival strategy that these fungi have adapted for these soils. Also, questions arise as to the extent to which mycorrhizae will promote a growth enhancement response in plants growing in phosphate mined soils. Data regarding such growth responses are not available; however, other research within the scope of this project is presently in progress to determine such effects.

The question of whether mycorrhizal inoculation can affect survival of plant species used in reclamation attempts has recently prompted considerable attention. Numerous questions exist regarding the possibility that field inoculation may enhance survival of naturally colonizing or planted individuals and whether or not large-scale inoculation can feasibly be performed. Data presented in this report may seem to question whether inoculation should be performed, since mycorrhizal invasion has been shown to be very rapid and colonization occurs in the majority of species present. However, it is possible that mycorrhizal species occurring in early successional communities may, in fact, be adapted to biotrophic relationships with early colonizing grass and shrub species. Endomycorrhizae have generally been shown to be nonhost specific. Yet, different growth responses and inoculation potential may be mediated by different species or different ecotypes (30, 31). Hence, mycorrhizae that occur in early aged successional systems might not be as efficient in promoting growth in L. styraciflua—a later successional plant. Schenck and Kinloch (32) reported that yearly changes did occur in incidence of root colonization and spore occurrence in six monoculture crops in a recently cleared woodland area. Species type and spore numbers were noticeably affected by the plant host species. They concluded that mycorrhizae occurrence changes were the result of host species interaction and not site edaphic factors. Crush (33) demonstrated differences in the abilities of endomycorrhizal populations, originating from different stages of pasture development, to enhance growth of white clover. Endophytes from improved pasture more efficiently elicited a growth response than those obtained from systems characteristic of earlier stages of pasture development. Crush (33) indicated that these results may have been attributed to cultivar origin, which had been selected from a high fertility pasture. It is apparent from these studies that host-endophyte interactions are very important in determining mycorrhiza occurrence in the soil. Crush (33) discusses the possibility that through host selection, from an initially heterogeneous mycorrhizal soil population, certain fungal species may eventually dominate. Possibly during succession, mycorrhizal population changes (densities and species) occur in response to selection by changing host populations (or vice-versa?). This may account for the initially high infectivity potential and root colonization occurring very rapidly in phosphate mine succession.

Another problem warranting investigation pertains to the use of indigenous versus introduced species for inoculation purposes. Variable results have been obtained from using indigenous versus introduced strains as mycorrhizal inoculum (30, 34, 35). The survival of an introduced fungus will be controlled by the ability to adapt to fertility, moisture, and temperature regimes present within the soil. Lambert et al. (30) suggests that generally introduced species would not improve plant yield in soils with indigenous fungi. They indicate these conditions may change if vegetation, pH, and fertility factors to which the indigenous fungi have adapted are altered. This view is also expressed by Crush (33) pertaining to inoculation of New Zealand pasture soils. Introduced mycorrhizal fungi species must be adapted to environmental and soil conditions to effect an advantage for plant growth enhancement and survival. Investigations in this area are urgently needed to determine if the indigenous mycorrhizal fungi species from phosphate mined systems can be increased in culture and used to enhance growth in reclamation attempts.

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# APPLICATION OF MYCORRHIZAL FUNGI IN RECLAMATION OF PHOSPHATE-MINED LANDS

G. Ronnie Best and Peter M. Wallace

## INTRODUCTION

The role of mycorrhizal fungi in plant establishment on drastically disturbed lands is receiving increasing research attention. Much of the research focuses primarily on natural mycorrhizal fungi recolonization or recolonization through replacement of topsoil (especially on lands disturbed through surface mining activities)(see discussion below for references). This paper (1) presents data on the occurrence of mycorrhizal fungi on different age surface-mine spoils in the central phosphate mining region of Florida, (2) assesses the potential role of mycorrhizal fungi in soils with moderate-to-high residual extractable phosphates, and (3) reviews current technology for large-scale inoculation of reclaimed mine spoils with select mycorrhizal fungi.

## SIGNIFICANCE OF MYCORRHIZAL FUNGI ON SURFACE MINED LANDS

The function and importance of mycorrhizal infection in plant growth and survival are well documented (10, 21); however, mycorrhizal population dynamics and function following disturbance such as surface mining have only received limited attention. It has been observed in several instances in the American midwest that a majority of the invading species in disturbed areas are typically non-mycorrhizal (1, 19, 24). Reeves et al. (24) found that 99% of the plant species adjacent to a disturbed area were mycorrhizal compared to only 1% in the disturbed area. Similar results were observed in surface-mined areas of the Red Desert in Wyoming (19). Land disturbance is accredited with reducing the availability of viable mycorrhizal propagules, thus inhibiting the invasion of mycorrhizal plants. Although mycorrhizal infection may be mediated through spores, hyphal fragments, or infected plant roots, the most probable form of endomycorrhizal inoculum is via infected plant roots not spores (24). Therefore, a reduction in host availablity by drastic land alteration (i.e. surface mining) could subsequently limit invasion of disturbed areas by mycorrhizal dependent species. This is very important when considering that a majority of naturally occurring late successional plants typically form mycorrhizal associations (see 10, 18).

In contrast to these findings, investigations of coal spoils in Pennsylvania and Scotland showed a majority of colonizing plants to exhibit typical vesicular-arbuscular mycorrhizal infections (5, 6). It was suggested that mycorrhizae are necessary for plant survival on these harsh anthracite spoils. A similar study of bituminous mining wastes in New South Wales also found that colonizing species were generally endomycorrhizal (15). It appears that in these areas plant colonization and subsequent survival may in fact be limited and controlled by mycorrhizal presence. Invasion by nonmycorrhizal species is limited, which is in contrast to the previously cited examples.

## SIGNIFICANCE OF MYCORRHIZAL FUNGI IN RECLAMATION OF PHOSPHATE SURFACE-MINED LANDS

Reclamation of mined lands in Florida presents several unique and interesting problems with regard to community development. During mining, nonweathered overburden material is removed from varying depths and deposited on the surface, creating a mosaic of steep sided hills surrounded by deep water filled canals. Reclamation of mined lands has been mandatory since the mid-1970's, requiring a recontouring of the mined areas and restoration to useful systems. Generally

reclamation has been to pasture ecosystems, or pasture and lake systems. Since Florida law now requires that a portion of the disturbed area must be reclaimed to natural ecosystems problems have developed which were not encountered in traditional pastureland restoration situations. Two avenues exist by which these areas may be returned to natural systems. The method of natural system reclamation generally utilized requires that extensive labor be used in replanting. An alternative to this approach is natural ecosystem reconstruction via succession (2). Can nature restore a mature self maintaining system in a time frame which is acceptable? Although these approaches are vastly different, success of either may be affected in part and limited by common components such as soil, organic matter, nitrogen, mycorrhizae and/or others. Premining ecosystems in Florida may range from xeric communities dominated by oaks (Quercus spp.) to less well drained pine flatwood to hydric bottomland hardwood or cypress dominated communities. Although many systems of varying hydrologic regimes are mined, generally restoration efforts produce mesic to xeric upland habitats. In addition, post mining soils characteristically possess very different physical and chemical properties than their undisturbed counterparts (see Hawkins (13) for general description). Typically, mined soils have higher concentrations of major nutrients, e.g. Ca, P, K, Mg, than premined soils, and contain considerably greater proportions of clay to sand. Mined soils, however, contain extremely low concentration of organic matter and nitrogen. The creation of these new habitats proposes the question as to whether locally occurring native plant species which have evolved in generally acid, low clay, low nutrient sandy soils can be expected to invade, survive, and successfully regenerate on these newly generated soil systems.

Success of reclamation, whether through the intervention of man or natural ecosystems regeneration, involves an understanding of both macrocomponents and microcomponents of the developing ecosystems. Whether seedlings are planted or occurrence is through natural invasion, success will be determined through interaction with abiotic and biotic environmental parameters. One such parameter warranting special attention is the potential role of mycorrhizal fungi. During the process of mining, overburden soil is removed from depths ranging from 15-80 feet. This soil which can generally be assumed to be abiotic, thus becomes the site of not only aboveground biomass invasion but also of microbial recolonization, i.e. mycorrhizal fungi. Successful inhabitance by planted or invaded species may in fact be governed by the degree and rate at which mycorrhizal populations become reestablished within the disturbed area.

Presently, little information exists on mycorrhizal occurrence in surface mined land in Florida (26). The goals of this paper are to present data regarding occurrence of mycorrhizae on phosphate surface-mined spoils and reclaimed lands, plus discuss information on potential for use of mycorrhizal fungi inoculum in reforestation of native Florida communities.

## Natural Occurrence of Mycorrhizal Fungi on Phosphate Surface-Mined Spoils

How commonplace are vesicular-arbuscular mycorrhizal fungi on surface-mined lands in Florida? Six different aged (0, 3, 8, 17, 43 and 60 years post-mining) unreclaimed areas in Polk County, Florida were studied to determine occurrence of mycorrhizal fungi on different age mine spoils.

To determine mycorrhizae populations in these areas two methods were employed. Most probable number determinations (MPN) were utilized employing a four fold dilution series with propagules per gram of soil determined from tables presented in Fisher and Yates (9). Cassia obtusifolia, a commonly occurring Florida legume, was used as the test plant. Mean (3 determinations each site) MPN values were calculated for each site (Table 1) to facilitate comparisons of inoculum potential between the different areas. The 3-year-old site had the highest mean value

Table 1. Post-mining age and most probable number (MPN) of mycorrhizal propagules.

| Site Age Years | Mycorrhizal Propagule Density* (#/g soil) |
|---|---|
| 0 | ** |
| 3 | 0.52 |
| 8 | 0.17 |
| 17 | 0.27 |
| 43 | 0.16 |
| 60 | 0.11 |

* Values represent means of three separate determinations from different locations within each site.

** Initial test for the "0-year" age class was damaged during a freeze in December, 1983, retesting currently underway.

estimate of 0.52 propagules per gram. The results indicate a general trend of reduction of vesicular-arbuscular mycorrhizal infectivity with time. The lowest mean value obtained was 0.11 propagules/gram, which occurred at the 60-year-old mine. It is quite surprising that the highest values were obtained at a very young site. Several possible explanations exist for this observation. Mycorrhizal invasion may be extremely rapid or a high level of residual inoculum may remain at or near the surface even though the surface mining technique generally results in significant soil inversion. Also, it is possible that the fungal species present had a greater affinity for the plant used in the MPN test than species occurring in the later aged sites. C. obtusifolia is typically a ruderal strategist which is not normally found in older mature systems. However, in the MPN analysis, difference in host plant infection responses should be minimized, since colonization is simply based on positive or negative results and not colonization intensity analyses (8).

The mycorrhizal status of several plant species was also examined. Roots of commonly occurring plant species were collected from the different age sites and percent root colonization was quantified using a grid-line intercept method (11).

Data (Table 2) indicate that mycorrhizal invasion is rapid into areas following termination of mining activity. Of the plant species sampled on the most recent site, Eupatorium compositifolium and Salix caroliniana exhibited colonization albeit at very low levels. Plants sampled at the 3-year-old site revealed a majority of species exhibiting mycorrhizal colonization with percent root colonization ranging from 2% in Salix caroliniana to 90% in Paspalum notatum.

Results obtained form the 8-year-old mine were generally the same as those from the 3-year-old site. Most plants exhibited typical endomycorrhizal infection. Colonization percentages were very high in a number of species (e.g., Heterotheca subaxillaris, Salix caroliniana). Several species gave highly variable results with individuals exhibiting both very high and very low root colonization (e.g., Rhynchelytrum repens, Ambrosia artemisiifolia). Chenopodium ambrosioides was the only species encountered not to exhibit colonization. Typically members of the family Chenopodiaceae do not form mycorrhizal associations (10).

A greater proportion of shrub size class individuals were sampled at the 17-year-old site than at the younger mines. All species sampled exhibited endomycorrhizal colonization; however, Myrica cerifera infection levels were extremely low. Urena lobata and Lantana camara, which were the most common shrub size class plants on the site, were extensively colonized by mycorrhizal endophytes. Extremely dense stands of these two species occurred within the site. Such high colonization percentages may indicate these species are maintaining a large mycorrhizal propagule reservoir within the soil.

Table 2. Mycorrhizal Colonization of Commonly Occurring Plant Species for the Different Aged Study Sites. Values represent the percentage of root examined that was colonized by mycorrhizal fungi.

| | SITE AGE, YEARS | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | | | 3 | | | 8 | | | 17 | | | 43 | | | 60 | | |
| Plant Species | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Digitaria sanguinalis | N* | N | N | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Eupatorium compositifolium | 2 | - | - | - | - | - | 82 | 64 | 96 | - | - | - | - | - | - | - | - | - |
| Salix caroliniana | 5 | - | - | 17 | 87 | 2 | 76 | 61 | 72 | - | - | - | - | - | - | - | - | - |
| Ambrosia artemisiifolia | - | - | - | 19 | 28 | 26 | 4 | 98 | 13 | - | - | - | 2 | 25 | 16 | - | - | - |
| Baccharis halimifolia | - | - | - | 32 | 16 | 52 | 91 | 47 | 75 | 34 | 4 | 41 | - | - | - | - | - | - |
| Crotolaria spectabilis | - | - | - | 49 | P† | P | - | - | - | - | - | - | - | - | - | - | - | - |
| Cynodon dactylon | - | - | - | 13 | 15 | 4 | - | - | - | - | - | - | - | - | - | - | - | - |
| Cyperus sp. | - | - | - | 3 | 7 | 20 | - | - | - | - | - | - | - | - | - | - | - | - |
| Eupatorium capillifolium | - | - | - | 25 | 29 | 25 | 39 | 85 | N | - | - | - | 100 | 79 | 51 | - | - | - |
| Hetereotheca subaxillaris | - | - | - | 41 | 74 | 75 | 86 | 79 | 81 | - | - | - | | | | | | |
| Indigofera hirsuta | - | - | - | 11 | P | 83 | - | - | - | - | - | - | N | 7 | 47 | - | - | - |
| Lactuca floridana | - | - | - | 39 | 49 | 45 | - | - | - | - | - | - | - | - | - | - | - | - |
| Lepidium virginicum | - | - | - | 27 | N | N | - | - | - | - | - | - | - | - | - | - | - | - |
| Ludwigia leptocarpa | - | - | - | 19 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Ludwigia peruviana | - | - | - | 19 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Paspalum urvillei | - | - | - | 49 | 79 | 74 | 53 | 50 | 74 | - | - | - | - | - | - | - | - | - |
| Paspalum notatum | - | - | - | 32 | 90 | 78 | - | - | - | - | - | - | - | - | - | - | - | - |
| Paspalum repens | - | - | - | N | N | N | - | - | - | - | - | - | - | - | - | - | - | - |
| Polygonum punctatum | - | - | - | N | N | N | N | 4 | 5 | - | - | - | - | - | - | - | - | - |
| Rhyncelytrum repens | - | - | - | N | 10 | P | 5 | 48 | 44 | 20 | 48 | 20 | 48 | 25 | 18 | - | - | - |
| Setaria geniculata | - | - | - | 82 | 75 | 45 | - | - | - | - | - | - | - | - | - | - | - | - |
| Chenopodium ambrosioides | - | - | - | - | - | - | N | N | N | - | - | - | - | - | - | - | - | - |
| Gnaphalium obtusifolia | - | - | - | - | - | - | 69 | 83 | 91 | - | - | - | - | - | - | - | - | - |
| Imperata cylindrica | - | - | - | - | - | - | 8 | 24 | 50 | 62 | - | - | - | - | - | - | - | - |
| Lactuca floridana | - | - | - | - | - | - | 30 | 26 | 70 | - | - | - | - | - | - | - | - | - |
| Psidium guajava | - | - | - | - | - | - | P | P | P | 69 | 50 | 71 | 63 | 40 | 50 | N | 14 | 22 |
| Solidago fistulosa | - | - | - | - | - | - | 18 | 68 | - | - | - | - | N | 56 | 1 | - | - | - |
| Thelypteris normalis | - | - | - | - | - | - | P | P | P | N | 20 | 8 | - | - | - | - | - | - |
| Drymaria cordata | - | - | - | - | - | - | - | - | - | 13 | P | N | - | - | - | - | - | - |
| Lantana camara | - | - | - | - | - | - | - | - | - | 67 | 45 | 92 | 65 | 68 | 26 | - | - | - |
| Myrica cerifera | - | - | - | - | - | - | - | - | - | N | 1 | - | - | - | - | N | N | N |
| Rubus sp. | - | - | - | - | - | - | - | - | - | 67 | 52 | - | - | - | - | - | - | - |
| Schinus terebinthifolius | - | - | - | - | - | - | - | - | - | 86 | - | - | - | - | - | - | - | - |
| Urena lobata | - | - | - | - | - | - | - | - | - | 84 | 95 | 71 | 86 | 44 | 81 | 35 | 60 | 39 |
| Ampelopis arborea | - | - | - | - | - | - | - | - | - | - | - | - | 44 | 81 | 81 | - | - | - |
| Andropogon sp. | - | - | - | - | - | - | - | - | - | - | - | - | N | 3 | 17 | - | - | - |
| Asplenium heterochorum | - | - | - | - | - | - | - | - | - | - | - | - | N | N | N | - | - | - |
| Bidens bipinnata | - | - | - | - | - | - | - | - | - | - | - | - | 10 | 58 | 22 | 44 | 11 | 29 |
| Cassia fasiculata | - | - | - | - | - | - | - | - | - | - | - | - | 60 | 60 | 33 | - | - | - |
| Chrysopsis graminifolia | - | - | - | - | - | - | - | - | - | - | - | - | 50 | 43 | 75 | - | - | - |
| Euthamnia minor | - | - | - | - | - | - | - | - | - | - | - | - | 34 | 33 | 52 | - | - | - |
| Liatris sp. | - | - | - | - | - | - | - | - | - | - | - | - | 36 | 8 | 11 | - | - | - |
| Phlebodium aureum | - | - | - | - | - | - | - | - | - | - | - | - | N | N | N | - | - | - |
| Vitis rotundifolia | - | - | - | - | - | - | - | - | - | - | - | - | 85 | 98 | - | N | N | 1 |
| Callicarpa americana | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | P | 72 | 23 |
| Gelsimium sempervirens | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | P | 1 | N |
| Liquidambar styraciflua | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | P | - | - |
| Oplismenus setarius | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | N | P | N |
| Panicum commutatum | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 14 | 12 | 15 |
| Prunus serotina | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | N | N | N |
| Psychotria nervosa | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 24 | 2 | 23 |
| Quercus laurifolia | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ECTO | ECTO | ECTO |
| Quercus nigra | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ECTO | ECTO | ECTO |
| Quercus virginiana | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ECTO | ECTO | ECTO |
| Smilax bononox | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | P | 43 | 13 |
| Solanum sp. | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 60 | 28 | 35 |
| Tillandsia utriculata | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | N | N | N |

*Negative
†Positive

Plant species sampled on the 43-year-old mine also exhibited generally high colonization percentages. Endophytes were typically found in all plant growth forms examined, i.e., vines, grasses, herbs, and shrubs. Vitis rotundifolia and Ampelopsis arborea, which are two common vine species, exhibited extremely high levels of colonization. Lantana camara and Urena lobata were extensively infected as was also noted on the 17-year-old mine. Although ectomycorrhizal species compose a significant component of the vegetation on this site, reductions in occurrence in endomycorrhizal colonization were not noted.

The 60-year-old mine yielded the most highly variable results of mycorrhizal occurrence within plant roots. Tillandsia utriculata, Prunus serotina, Vitis rotundifolia, and Myrica cerifera were all found to be typically nonmycorrhizal in this area. Similar results for M. cerifera were obtained at the 18-year-old site; however, it is surprising that V. rotundifolia was not colonized since extremely high levels of infection were noted at the 43-year-old site. Endomycorrhizal infection of species at these sites were substantially reduced compared to all younger sites except the most recently mined site. However, ectomycorrhizal host species do comprise a significant proportion of the aboveground biomass and may

tend to decrease endomycorrhizal occurrence due to a reduction in potential host availability. Also, the typically endomycorrhizal herbaceous component is a very sparse component of the aboveground vegetation. All individuals of Quercus nigra, Q. virginiana, and Q. laurifolia sampled exhibited ectomycorrhizal infection characterized by fungal mantle formation on simple, unforked feeder roots.

## Role of Mycorrhizal Fungi in Soils with Elevated Residual Extractable Phosphate

Mycorrhiza occurrence in phosphate overburden presents various interesting questions. Primarily, what is the role or function of mycorrhizae in high phosphorus soils? Do mycorrhizae enhance growth in soils with relatively high extractable phosphorus levels? Phosphorus analyses (Table 3) were performed on fifteen soil cores obtained from each site. Double acid extractions were performed

Table 3. Comparison of extractable phosphorus concentrations (ppm: mg/l) obtained using various common extracting solutions. Six soil samples taken from the 3-year-old site were used for analysis.

| Sample Number | Extracting Solution | | | | |
|---|---|---|---|---|---|
| | Double Acid | Bray 1 | Bray 2 | Olsen A | Olsen B |
| 1-1 | 138 | 47 | 218 | 9.1 | 15.2 |
| 1-2 | 71 | 34 | 97 | 7.5 | 11.4 |
| 2-1 | 2810 | 106 | 2272 | 14.2 | 22.2 |
| 2-2 | 1340 | 101 | 1086 | 15.3 | 21.9 |
| 3-1 | 770 | 69 | 671 | 12.0 | 16.7 |
| 3-2 | 300 | 67 | 360 | 13.1 | 16.7 |

Double Acid - (0.025 N $H_2SO_4$ + 0.050 N HCl).

Bray 1 - (0.03 N $NH_4F$ in 0.025 HCl). Olsen A - (0.5 M $NaHCO_3$ [5 min extraction]).

Bray 2 - (0.03 N $NH_4F$ in 0.1 N NCl). Olsen B - (0.5 M $NaHCO_3$ [30 min extraction]).

on all soils and P determined as described by Watanabe and Olson (27). Double-acid was originally employed because it is the primary procedure used for Florida and southeastern sandy soils. Mean values obtained from the study sites revealed double-acid P ranges from 229 ppm-P to 3875 ppm-P. In order to facilitate comparisons with other research three additional extraction techniques were performed. Six samples obtained from the 3-year-old site were utilized. It is difficult to assess which values indicate the actual plant available phosphorus. It is evident that P concentrations are related to the normality of the acid within the extractant. The Olsen extraction is generally performed on alkaline and calcareous soils. Although calcium concentrations are quite high in these soils, calcium apatites are generally the major form of occurrence. Soil pH values are generally below 6; for example sample 1-2 in Table 3 has a pH of 4.9 in distilled water and 3.9 in .01 M $CaCl_2$. Although the P values obtained from the different extractants are highly variable, each generally exceeds critical levels at which a plant would probably not respond to phosphorus fertilization (16).

Growth enhancement studies were performed to investigate factors affecting survival and growth of sweetgum (Liquidambar styraciflua) on phosphate mined overburden soils. Sweetgum was chosen because of its often poor performance when planted in overburden soils, although it is a common species in adjacent unmined systems. A factorial design experiment was used in which four levels of phosphorus (0, 25, 75, and 150 ppm-P), three levels of nitrogen (0, 50, 150 ppm-N), and endomycorrhiza

additions were evaluated. Phosphorus was incorporated into the soil as 20% superphosphate at the experiment's initiation, while nitrogen as ammonium nitrate was added biweekly in a liquid form. Endomycorrhizal treatments consisted of control, Glomus macrocarpum and a composite sample (CS8) of several unidentified species isolated from a phosphatic clay settling area. Glomus macrocarpum was obtained from Dr. N.C. Schenck, University of Florida, and is a commonly occurring Florida species. The composite mycorrhizal species (CS8) was obtained by trapping Salix caroliniana roots in large pot cultures. Although the experiment was monitored for 120 days, data presented in Figure 1 is for change in height of sweetgum seedlings at 90 days. Special notice should be given to the fact that the CS8 mycorrhiza

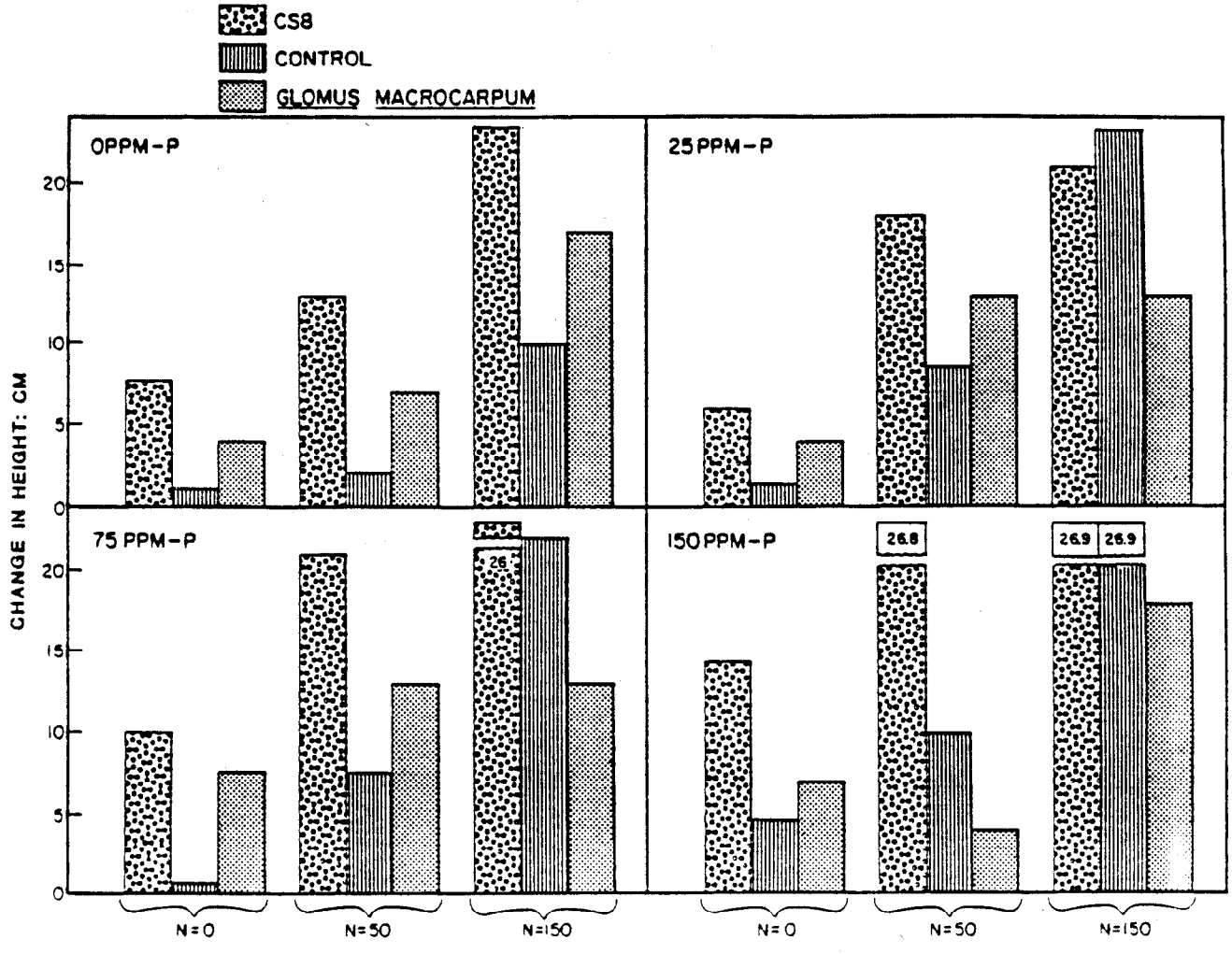


Figure 1. Net change in height (at 90 days) of sweetgum (Liquidambar styraciflua) seedlings as a function of mycorrhizal and nutrient (nitrogen and phosphorus) treatments.

promoted greater growth than Glomus macrocarpum at all nutrient levels. Also, treatments containing 150 ppm-N and in which additional P was added (25, 75, 150 ppm-P) control plants had greater growth than did plants inoculated with Glomus macrocarpum. Size of control plants generally only approach mycorrhizal plants in treatments with 150 ppm-N added. Control plants without added nitrogen (N=0) at all levels exhibited P deficiency systems. Mycorrhizal plants at all nutrient levels appeared healthy with no nutrient deficiency symptoms. Increasing amount of fertilizer simply increased the rate of growth as indicated by growth curves. Leaf area data (determined at 120 days) indicated a significant growth response of

CS8 plants over control plants at nutrient levels of 150 ppm-P and 150 ppm-N, indicating that these mycorrhizal plants were responding to both high P and N fertilization. Leaf area values also showed that with treatments in which macro and micronutrients were added with no additional P or N, CS8 increased growth over Glomus macrocarpum 1.8 times and 30 times greater than control plants. Glomus macrocarpum provided 17 times greater leaf area than control treatments.

## Techniques for Field Inoculation with Mycorrhizal Fungi - Status of Pilot Studies

If mycorrhizal fungi are important, as has been discussed, in establishment of late successional plants on surface-mined reclaimed lands, then what technologies are available for inoculation of "desirable" plants? Presently, the most feasible approach is to inoculate nursery grown seedlings for transplanting to field sites. Even this approach is subject to several limitations. Inoculation of seedlings in nursery operations is not presently a standard practice. Also, although some "broad spectrum" mycorrhizal fungi are currently being tested on seedlings of several tree species planted on reclaimed land, little data presently exist relating the more common trees, shrubs or herbaceous plants in natural Florida communities to either specific or groups of "beneficial" mycorrhizal fungi. This is especially true for vegetation commonly found in native plant communities in the phosphate mining region in south-central Florida.

Another approach, the direct application of a standard mycorrhizal inoculum in a soil matrix, has been tested in a pilot scale project and is currently being further evaluated. The approach used consisted of mixing seeds in a matrix of inoculated (Glomus occultum and G. mosse) soil and directly drilling the seed and soil matrix with a standard John Deere "Flexi Planter." Both seed and inoculum-matrix were planted through a "corn" plate. This procedure resulted in approximately 90 kg/ha (80 lbs/ac) of inoculum being sown directly with the seed. Using the seed/inoculum-matrix mixing technique did not allow for achieving a desired application rate for inoculum (desired rate for pilot experiment was 360 kg/ha) and seed. Therefore, additional tests are currently being conducted using unit seed drills coupled with granular fertilizer-pesticide applicators to allow for gauging rate of mycorrhizae inoculum application.

Topsoiling, that is the reapplication of surface soil material to a reclaimed mine site, is one approach currently being used on a limited scale in phosphate mined land reclamation. Although topsoiling appears to offer some potential for inoculating reclaimed soils, there are several problems in achieving desired results from topsoiling. Under the current surface mining scheme used by the phosphate industry, for topsoiling to be implemented on a large scale would require long-term stockpiling (6 months to 2 years) of topsoil, thereby reducing viability of the microflora. Even when immediately reused, topsoil may not provide the ecotypes of mycorrhizae best suited for the system being reclaimed. In addition, topsoil often contains numerous seeds of weedy plants. Best et al. (2) showed that after two growing seasons, there were no significant differences between growth, density and species richness of woody plants grown in microplots treated with topsoil and several other treatments (mulch, VA-mycorrhizae, Pt-mycorrhizae, fertilizer and gypsum). In fact, when one considers the overall negative economic, energy and environmental effects (if topsoil is "borrowed" from an unmined donor site and/or indirect results of an increase in fuel/energy use), the potential benefits that may be realized from topsoiling must be closely weighed with the associated negative impacts of topsoiling. This is not to say that topsoiling is not a viable approach for amending and preparing reclaimed sites for vegetation. Topsoiling and/or planting inoculated nursery grown seedlings are presently perhaps the most efficient means of inoculating reclaimed surface-mined lands. Further research on all mycorrhizae inoculation methods is needed.

## DISCUSSION

Data are abundant indicating the beneficial effects obtained from mycorrhizal inoculation of agricultural crops and important timber species. However, research regarding the inoculation of a multispecies component for reclamation practices has been minimal. Numerous questions exist regarding the possibility that field inoculation may enhance survival of naturally colonizing or planted individuals and whether or not large-scale inoculation can feasibly be performed. Data presented in this paper may seem to question whether inoculation should be performed, since mycorrhizal invasion has been shown to be very rapid and colonization occurs in the majority of species present. However, it is possible that mycorrhizal species occurring in early successional communities may, in fact, be adapted to biotrophic relationships with early colonizing grass and shrub species. Endomycorrhizae have generally been shown to be nonhost specific. Yet, different growth responses and inoculation potential may be mediated by different species or different ecotypes (17, 23). Hence, mycorrhizae that occur in early aged successional systems might not be as efficient in promoting growth, e.g. in L. styraciflua, a later successional plant. Mycorrhizal inoculation would select for a host-mycorrhiza association that would have an efficient relationship between the transfer of photosynthate from the host to fungus in exchange for fungal absorbed nutrients (12). Schenck and Kinloch (25) reported that yearly changes did occur in incidence of root colonization and spore occurrence in six monoculture crops in a recently cleared woodland area. Species type and spore numbers were noticeably affected by the plant host species. They concluded that mycorrhizae occurrence changes were the result of host species interaction and not site edaphic factors. Crush (3) demonstrated differences in the abilities of endomycorrhizal populations, originating from different stages of pasture development, to enhance growth of white clover. Endophytes from improved pasture more efficiently elicited a growth response than those obtained from systems characteristic of earlier stages of pasture development. He indicated that these results may have been attributed to cultivar origin, which had been selected from a high fertility pasture. It is apparent from these studies that host-endophyte interactions are very important in determining mycorrhiza occurrence in the soil. Crush (3) discusses the possibility that through host selection, from an initially heterogeneous mycorrhizal soil population, certain fungal species may eventually dominate. Possibly during succession, changes in mycorrhizal populations (densities and species) occur in response to selection by changing host populations (or vice-versa?). This may account for the initially high infectivity potential and root colonization occurring very rapidly in phosphate mine succession.

Another problem warranting investigation pertains to the use of multispecies, single species, and indigenous versus introduced species for inoculation purposes. Multispecies inoculation may offer several advantages over use of single species inoculum. First, multiple species being present allows the plants to select for the symbiont or quite probably allow for multiple symbiosis to occur. Numerous studies have shown that a plant may form symbiotic associations with many mycorrhizal species at the same time (20). The presence of several different endophytes may be advantageous because each may have variable importance at different life cycles or times during the year (4, 7). Daft (4) recently offered several criteria describing the properties an ideal endophyte would have to possess to ensure successful field inoculation. These are abilities to (1) infect plants early in the growth period, (2) efficiently exploit the soil, (3) transfer nutrients readily to the host, (4) spread and multiply, (5) compete effectively and (6) infect a wide range of plants under variable environment conditions. He suggests all these characteristics could possibly not be found in a single endophyte, however a multispecies inoculum may be the answer. Utilizing a single species inoculum requires that the researcher, rather than "mother nature," make the selection. Possible drawbacks for this approach are numerous, especially if

an indigenous source is desired. Selecting endophytes from field samples may result in a bias selection of the more common sporulating and/or large-spored species because of ease of isolating. Non sporulating species may not be seen even though they may possibly be the most abundant and efficient at enhancing plant growth.

Variable results have been obtained from using indigenous versus introduced strains as mycorrhizal inoculum (15, 17, 22). The survival of an introduced fungus will be controlled by the ability to adapt to fertility, moisture, and temperature regimes present within the soil. Lambert et al. (17) suggest that generally introduced species would not improve plant yield in soils with indigenous fungi. They indicate these conditions may change if vegetation, pH, and fertility factors to which the indigenous fungi have adapted are altered. This view is also expressed by Crush (3) pertaining to inoculation of New Zealand pasture soils. Introduced mycorrhizal fungi species must be adapted to environmental and soil conditions to effect an advantage for plant growth enhancement and survival.

Additional investigations in this area are urgently needed to determine if indigenous mycorrhizal fungi species from phosphate mined systems can be propagated in culture and used to enhance growth in reclamation attempts.

## ACKNOWLEDGEMENTS

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Center for Wetlands Contribution #125.

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# Applications of Mycorrhizal Fungi in Crop Production

February 22-23, 1984
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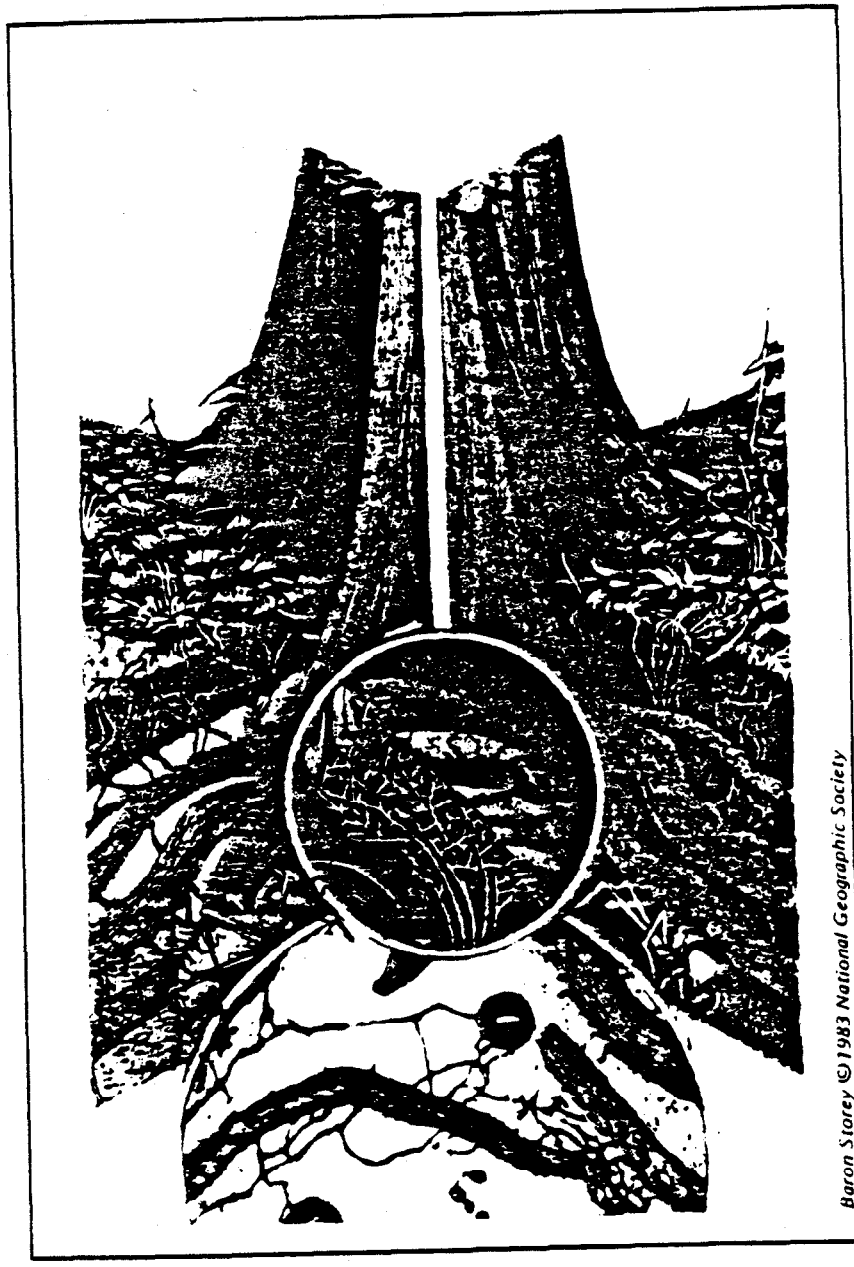
University of Florida, Gainesville

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Applications of VA Mycorrhizal Fungi in Fruit Crops
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"Applications of Mycorrhizal Fungi in Crop Production"

1984 Symposium on Surface Mining, Hydrology, Sedimentology, and Reclamation
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# Mycorrhizae Enhanced Growth of Sweetgum (Liquidambar Styraciflua) in Phosphate Mined Overburden Soils

Peter M. Wallace, G. Ronnie Best, James A. Feiertag and Karen M. Kervin
Center for Wetlands and Environmental
Engineering Sciences Department
University of Florida
Gainesville FL 32611

Abstract. Growth enhancement studies were performed to investigate factors affecting growth of sweetgum (Liquidambar styraciflua) on phosphate mined overburden soils. A factorial design experiment was used in which five levels of phosphorus (0, 12.5, 25, 75, and 150 ppm-P), three levels of nitrogen (0, 50, 150 ppm-N), and endomycorrhiza additions were evaluated.

Endomycorrhizal treatments consisted of control, Glomus macrocarpum and a composite sample of several species isolated from a phosphate mine clay settling area. Results indicate that the addition of the composite mycorrhizae promoted greater growth than either the control or G. macrocarpum treatments at all nutrient levels. Leaf area measurements indicated that the composite endomycorrhizal treatment resulted in 30 times greater area than control and 1.7 times greater area than Glomus macrocarpum. Although soil P levels equal 2592 ppm (double-acid), in each treatment block significant growth responses were noted with addition of mycorrhizal fungi.

## INTRODUCTION

Reclamation of mined lands in Florida presents several unique and interesting problems with regard to community development. During mining, non-weathered overburden material is removed from varying depths and deposited on the surface, creating a mosaic of steep sided hills surrounded by deep water filled canals. Reclamation of mined lands has been mandatory since the mid-1970's, requiring a recontouring of the mined areas and restoration to useful systems. Generally reclamation has been to pastured ecosystems, or pasture and lake systems. Since Florida law now requires that a portion of the disturbed area must be reclaimed to natural ecosystems problems have developed which were not encountered in traditional pastureland restoration situations. Two avenues exist by which these areas may be returned to natural systems. The method of natural system reclamation generally utilized requires that extensive labor be used in replanting. An alternative to this approach is natural ecosystem reconstruction via succession (1). Can nature restore a mature self maintaining system in a time frame which is acceptable? Although these approaches are vastly different, success of either may be affected in part and limited by common components such as soil organic matter, nitrogen, mycorrhizae and/or others. Premining ecosystems in Florida may range from xeric communities dominated by oaks (Quercus spp.) to less well drained pine flatwood to hydric bottomland hardwood or cypress dominated communities. Although many systems of varying hydrologic regimes are mined generally restoration efforts produce mesic to xeric upland habitats. In addition, post mining soils characteristically possess very different physical and chemical properties compared with their undisturbed counterparts (see Hawkins (2) for general description). Typically, mined soils have higher concentrations of major nutrients e.g. Ca, P, K, Mg, than premined soils, and contain considerably greater proportions of clay to sand. Mined soils, however, contain extremely low concentrations of organic matter and nitrogen. The creation of these new habitats proposes the question as to whether locally occurring native plant species which have evolved in generally acid, low clay, low nutrient sandy soils can be expected to invade, survive, and successfully regenerate on these newly created soil systems.

Success of reclamation, whether through the intervention of man or natural ecosystem regeneration, involves an understanding of both macrocomponents and microcomponents of the developing ecosystems. Whether seedlings are planted or occurrence is through natural invasion, success will be determined through interaction with abiotic and biotic environmental parameters. One such parameter warranting special attention is the potential role of mycorrhizal fungi. During the process of mining, overburden soil is removed from depths ranging from 15-80 feet. This soil which can generally be assumed to be abiotic, thus becomes the site of not only aboveground

biomass invasion but also of microbial recolonization i.e. mycorrhizal fungi. Successful inhabitance by planted or invaded species may in fact be governed by the degree and rate at which mycorrhizal populations become reestablished within the disturbed area.

Presently little information exists regarding mycorrhiza occurrence in surface mined lands in Florida or the potential role and function of mycorrhizae in these high phosphorus overburden soils. The consideration of mycorrhizal inoculation for enhanced plant growth for reclamation activities presents several difficult but intriguing questions. The source of endophyte inoculum should be an important consideration. High soil phosphate levels have generally been shown to substantially reduce mycorrhizal colonization (3, 4) thus decreasing or alleviating plant dependence on the symbiosis. Presently mycorrhizae that can be used for inoculum purposes does not originate from phosphate mined areas and these ecotypes may not be adapted to soil conditions such as high phosphate which would be encountered in this area. Indigenous mycorrhizae from previously mined areas may infact be adapted to these conditions which have been shown to be antagonistic to plant mycorrhiza interactions, hence, offering the most viable alternative. Although plant mycorrhizal interactions involving phosphate absorption are well known, information regarding interactions with other nutrients is generally inadequate. However, it is reasonable to assume that in situations of high phosphorus availability, mycorrhizae may enhance uptake of nutrients which exist at suboptimal concentrations. On phosphate overburden soil nitrogen generally occurs in very low concentrations and is thus considered the major nutrient limiting growth of invading plant species. A growth experiment was designed to investigate factors which affect growth of sweetgum (Liquidambar styraciflua) in overburden soils. Sweetgum was used because of its often poor performance when planted in overburden soils, although it is a common species in adjacent unmined systems. Mycorrhizal additions were evaluated and growth responses determined in relation to both increasing nitrogen and phosphorus additions.

## MATERIALS AND METHODS

### Experimental Design

A 5x3x3 factorial design experiment was utilized in which five levels of phosphorus (0, 12.5, 25, 75, 150 ppm - P), three levels of nitrogen (0, 50, 150 ppm - N) and endomycorrhiza additions were evaluated. This design allowed for forty-five separate treatments to be analyzed (Table 1). Five replications were employed for each individual treatment. Data obtained were analyzed using analysis of variance techniques contained within the Statistical Analysis System (SAS).

### Endomycorrhiza Inoculum Preparation

Endomycorrhizal treatments consisted of control, Glomus macrocarpum and a composite sample (CS8) of unidentified species isolated from a phosphatic clay settling area. G. macrocarpum (a commonly occurring Florida species) was obtained from Dr. N. C. Schenck, University of Florida, in the form of soil and infected plant roots. This inoculum was increased by placing in large 1x2 ft. flats containing sterilized native Florida soil and planted with bahia grass (Paspalum notatum) and soybean (Glycine max).

These pot cultures were allowed to grow for four months, at which time roots and soil were separated by sieving and stored in plastic containers at approximately 5% soil moisture until initiation of the experiment. The CS8 mycorrhiza was obtained from Salix caroliniana roots collected from a partially inundated eight month old clay settling area in southwest Polk County (International Minerals and Chemical Corporation Clear Springs Mine clay settling area #8). Roots were placed in large 1x2 ft. flats containing phosphate overburden soil and inoculum prepared as previously described.

### Test Plant Inoculation

Seeds of sweetgum (Liquidambar styraciflua) were collected from Polk County in central Florida. Surface sterilzed seeds were placed in germination flats containing autoclaved vermiculite. Two weeks following germination seedlings were removed and transferred to pots containing 1100 grams of 1:1 phosphate overburden - sand tailings mix. Mycorrhizal inoculum consisting of 0.5 grams sieved roots and 5 grams soil was added directly below the seedling prior to planting. The inoculum used consisted of infected root fragments, mineral soil, fungal hyphae, mycorrhizal spores, and rhizosphere microflora.

In order to standardize microflora for all plants a rhizosphere microflora inoculum was prepared from both inoculum types by washing roots and soil in deionized water. The soil and root wash was subsequently sieved through 45 and 42 u soil sieves followed by filtration through Whatman No. 4 filter paper (20-25u). The microflora inoculum was added to all plants including controls. Control and CS8 plants in addition received 5 grams of sterilized G. macrocarpum inoculum soil because it was chemically

Table 1. GROWTH EXPERIMENT DESIGN

| | FACTOR = CS 8 PHOSPHORUS (ppm) | | | | | FACTOR = G. MACROCARPUM PHOSPHORUS (ppm) | | | | | FACTOR = CONTROL PHOSPHORUS (ppm) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NITROGEN (ppm) | 0 | 12.5 | 25 | 75 | 150 | 0 | 12.5 | 25 | 75 | 150 | 0 | 12.5 | 25 | 75 | 150 |
| 0 | 1 | 2 | 3 | 4 | 5 | 16 | 17 | 18 | 19 | 20 | 31 | 32 | 33 | 34 | 35 |
| 50 | 6 | 7 | 8 | 9 | 10 | 21 | 22 | 23 | 24 | 25 | 36 | 37 | 38 | 39 | 40 |
| 150 | 11 | 12 | 13 | 14 | 15 | 26 | 27 | 28 | 29 | 30 | 41 | 42 | 43 | 44 | 45 |

different than the phosphate overburden matrix. Control plants also received 0.5 grams of sterilized root fragments.

Fertilizer Addition

Phosphorus treatments were performed by incorporating 20% superphosphate fertilizer into the entire contents of each pot at the designated concentration. All phosphorus treatments were based upon concentrations (parts per million: ppm) of elemental phosphorus. Nitrogen fertilization was performed biweekly for 16 weeks by adding 6.25 mg-l and 18.25 mg-l as ammonium nitrate in 20 ml deionized water. This resulted in the total addition of 50 ppm-N (100 lbs./acre) and 150 ppm-N (300 lbs./acre) respectively.

All treatments received an additional standard nutrient solution (from (5)), described, as follows: (mg/l) $CaCl_2 \cdot 2H_2O$, 294; $K_2SO_4$, 174; $MgSO_4 \cdot 7H_2O$, 184. $MnSO_4 \cdot 4H_2O$ 0.23 $ZnSO_4 \cdot 7H_2O$, 0.03; $CuSO_4 \cdot 5H_2O$, 0.024; $FeSO_4 \cdot 7H_2O$, 1.10; $(NH_4)_6 Mo_7O_{24} \cdot 4H_2O$, 0.0035. All plants received 40 ml of nutrient solution biweekly for 16 weeks. After the final nutrient addition plants were allowed to grow four weeks before harvesting was performed.

Data Collection and Harvest

Seedling height was recorded biweekly for the first eight weeks, then monthly until harvest to allow for construction of growth curves. To best determine grow response for individual treatments both leaf area and root length were determined for each plant. At harvest stems were clipped at the soil surface and leaves pressed in paper bags and stored at 4°C for no more than 48 hrs. Leaf area was determined with a Hayashi Denko Automatic Area Meter model AAM-5. Roots were removed from the soil by washing and sieving and stored in a 90:5:5 Alcohol-Acetic Acid - Formalin (FAA) mixture until further processing. Total root length was determined using a grid-line intercept method (6). Following total root length analysis smaller aliquots were removed for determination of the percent of root length colonized by mycorrhizal fungi. Roots were cleared in 10% potassium hydroxide for 1 hour, bleached 15 minutes in alkaline $H_2O_2$ solution and subsequently stained in 0.05% Trypan Blue staining solution. Percent mycorrhizal colonization was obtained by using the grid line intercept method. Having two measurements of root length, i.e. total root length and percent colonized of the aliquot, allows for a conversion of total plant root length colonized by mycorrhizae. Root length was considered a better indicator of plant response than root biomass because it gives a better estimate of root absortive surface. Large diameter root segments can contain a proportiately large biomass although possess only very little nutrient exploiting capacity and significantly bias results.

Soil Nutrient Analysis

Soil phosphorus determinations were performed on both the 1:1 overburden-sand tailing mix (test soil) and each of the individual components (Table 2). Although double acid extracts are commonly employed in southeast sandy soils, two additional extracts were analyzed to facilitate comparisons with other research. Double acid extractions were performed on both sterilized and unsterilized test soil. P analyses were performed by the Environmental Science and Engineering Laboratory, Gainesville, Florida.

Table 2. Comparisons of Extractable Phosphorus Concentration (ppm; mg/l) obtained using various common extracting solutions.

| 1:1 Overburden/sandtailings Mix Sterilized | | | 1:1 OB/ST Mix Nonsterilized |
|---|---|---|---|
| Double Acid* | Bray 1** | Bray 2*** | Double Acid |
| 2592 | 400 | 2313 | 2404 |

| Overburden Nonsterilized | Sandtailings Nonsterilized |
|---|---|
| 2408 | 2068 |

*Double Acid - (0.025 N $H_2SO_4$ + 0.050 N HCl)
**Bray 1 - (0.030 N $NH_4F$ H 0.025 N HCl)
***Bray 2 - (0.030 N $NH_4F$ H 0.100 N HCl)

Results

Results of analysis of variance (ANOVA) for both root length and leaf area growth responses are given in Table 3. Comparisons of model R-square (0.87/0.67) values indicate that leaf area measurements produced the least amount of variability among samples within each individual treatment than did root length. Leaf area as a measure of photosynthetic area and root length as an indicator of nutrient absortive surface are both significant indicators of plant vigor. However, interpretations of leaf area data may offer the most simplistic and precise interpretation of growth response to the various treatments. Due to the difficulty associated with determination of root length, variability may in fact be largely a reflection of error in sample processing rather than actual response differences. The leaf area F statistic indicates that all main effects of P, N, and Myc (Mycorrhiza) are

Table 3. Analysis of Variance of Effects of Phosphorus (P) Nitrogen (N) and Mycorrhiza Type (Myc) on Root Length and Leaf Area Growth Responses.

Growth Response Variable: Root Length: cm

ANOVA

| SOURCE | DF | SS | F | PR>F | $R^2$ |
|---|---|---|---|---|---|
| Model | 44 | 79329167 | 8.24 | .0001 | 0.67 |
| Error | 180 | 39392395 | | | |
| TOTAL | 224 | 118721562 | | | |

| SOURCE | DF | F VALUE | PR>F |
|---|---|---|---|
| P | 4 | 6.15 | 0.0001 |
| N | 2 | 113.09 | 0.0001 |
| P x N | 8 | 1.55 | 0.1431 |
| MYC | 2 | 17.15 | 0.0001 |
| P x MYC | 8 | 4.38 | 0.0001 |
| N x MYC | 4 | 2.06 | 0.0879 |
| P x N x MYC | 16 | 1.36 | 0.1667 |

Growth Response Variable: Leaf Area: $mm^2$

ANOVA

| SOURCE | DF | SS | F | PR>F | $R^2$ |
|---|---|---|---|---|---|
| Model | 44 | 27477331081 | 21.06 | 0.0001 | 0.87 |
| Error | 135 | 4003226878 | | | |
| Total | 179 | 31480557959 | | | |

| SOURCE | DF | F VALUE | PR>F |
|---|---|---|---|
| P | 4 | 7.24 | 0.0001 |
| N | 2 | 250.40 | 0.0001 |
| P x N | 8 | 1.83 | 0.0774 |
| MYC | 2 | 116.35 | 0.0001 |
| P x MYC | 8 | 9.74 | 0.0001 |
| N x MYC | 4 | 5.05 | 0.0008 |
| P x N x MYC | 16 | 3.22 | 0.0001 |

highly significant. However, responses to N and mycorrhiza additions were far greater than those associated with P. To facilitate interpretation of these results response surface curves (Figure 1) have been constructed so that differential responses of each mycorrhizal treatment to P and N fertilization may be compared and optimum levels of nutrients and mycorrhizal type may be seen.

Generally, responses of CS8 mycorrhiza and control plants followed the same general trend. Addition of increasing amounts of N at each P level resulted in substantial growth enhancement. Responses to increased P concentration at each N level also generally resulted in greater growth however not of the magnitude that was noted in the previous situation. In both cases it is evident from the response surfaces that maximum production was obtained at levels of 150 ppm - N and 150 ppm - P indicating that both control and mycorrhizal plants were positively responding to fertilizer additions. Comparisons between CS8 and control plants indicate that at all nutrient levels leaf area of CS8 plants were higher than those of corresponding control plants. At levels of 150 ppm - P and 150 ppm - N, CS8 plants possessed significantly greater leaf area (446.7 $cm^2$/352.2 $cm^2$: $\alpha$ = .05) than control plants, indicating mycorrhizal enhanced growth. In the treatment in which no additional P or N was added CS8 resulted in a 30 times (113.78 $cm^2$/3.79 $cm^2$) increase in leaf area over control.

Response of G. macrocarpum plants to the various treatments offers somewhat of a variable and difficult pattern to analyze. Response curves indicate that maximum mean values for both root length and leaf area were obtained when nutrient levels equalled 150 ppm - N and 75 ppm - P. Variable results were obtained when P levels were elevated to 150 ppm. Intratreatment variability of growth responses was greater for G. macrocarpum plants than either control or CS8 plants making interpretation of response exceedingly difficult. However, generally at nutrient levels in which no concurrent additions of P or N were made, G. macrocarpum resulted in greater growth than control treatments, yet generally significantly less than CS8 inoculated individuals. Combinations of added P and N levels resulted in highly variable results. G. macrocarpum plants with no additional P or N possessed 17 times (65.02/3.79 $cm^2$) more leaf area than control plants but produced only 0.57 (113.78/65.02 $cm^2$) the area produced by CS8.

The significant second order PxNxMyc interaction makes overall experimental analysis difficult to interpret. The insignicant ($\alpha$ = 0.05) P X N interactions indicates that possibly responses to P and N additions are dependent upon the particular mycorrhizal component, i.e. CS8, G. macrocarpum, and control however effects of P and N additions within each mycorrhizal group are simply additive.

The most simplistic method in which to analyze these data may be to momentarily ignore interactive effects and just examine the main effects of the three factors within the experiment i.e. nitrogen, phosphorus and mycorrhizae. Table 4 gives main effect results of the various levels of each factor over all levels of the remaining two factors. For example, mean values are given for 150 ppm - P obtained for all levels of mycorrhizae and nitrogen.

Table 4. Analysis of Variance of Main Effects of Phosphorus (P), Nitrogen (N) and Mycorrhiza Type on Sweetgum Leaf Area.

MAIN EFFECTS

Dependent Variable: Leaf Area: $cm^2$

| MEAN | N | P (ppm) | DUNCANS | $\alpha$=.05 |
|---|---|---|---|---|
| 200.42 | 36 | 150 | A | |
| 191 48 | 36 | 75 | A | |
| 156.07 | 36 | 25 | B | |
| 151.70 | 36 | 0 | B | |
| 148.31 | 36 | 12.5 | B | |

| MEAN | N | N (ppm) | DUNCANS | $\alpha$=.05 |
|---|---|---|---|---|
| 287.26 | 60 | 150 | A | |
| 155.40 | 60 | 50 | B | |
| 66.13 | 60 | 0 | C | |

| MEAN | N | MYCORRHIZAE | DUNCANS | $\alpha$=.05 |
|---|---|---|---|---|
| 256.72 | 60 | CS8 | A | |
| 133 60 | 60 | G. macrocarpum | B | |
| 118.47 | 60 | CONTROL | B | |

Briefly, results indicate that a significant growth response was obtained when P concentrations were elevated to 75 ppm or more. In addition, increasing the amount of nitrogen resulted in doubling the leaf area of that obtained at the next lower level. CS8 mycorrhizae exhibited significantly greater growth than that obtained by both control and G. macrocarpum when means were averaged over all nutrient treatments whereas no significant differences appeared between average response of G. macrocarpum and controls.

Response of CS8 root infection to various treatments is presented in Figure 2. The response surface indicates that very little change in the percent of root length colonized occurrred with respect to treatment and no trends were associated with increasing either nitorogen or phosphorus supply. Infection percentage remained high for all treatments with values ranging from 66.8% where P = 0 and N = 0 to 75.2% where P = 75 and N = 150. Infections recorded in G. macrocarpum roots were significantly lower with generally all values being less than one percent. Although spores were seen in soil and attached to roots, large areas of infected

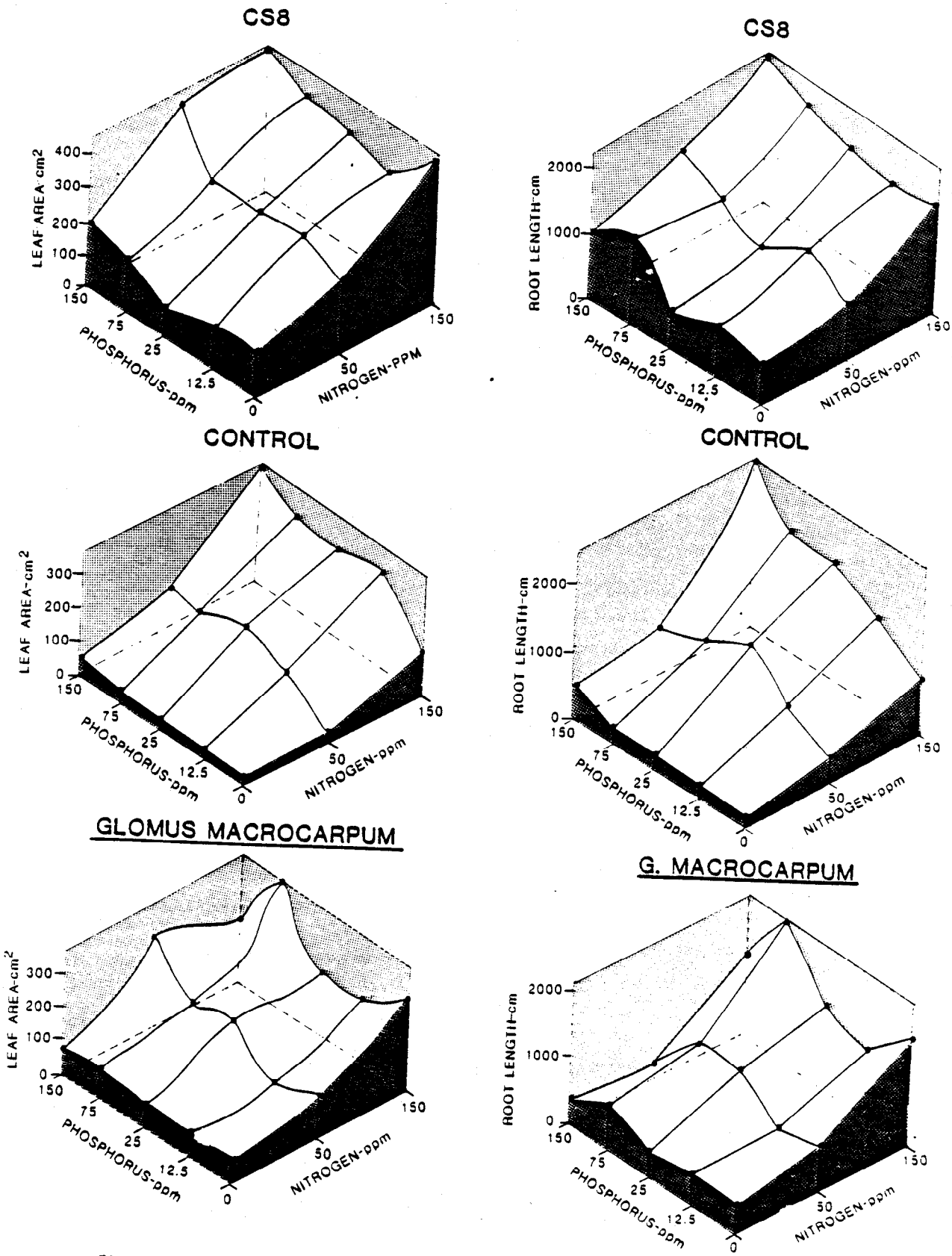


Figure 1: Response Surface Curves for the Root Length and Leaf Area Obtained From the Various Nutrient Treatments Within Each Mycorrhizal Group.

root length were generally not detected.

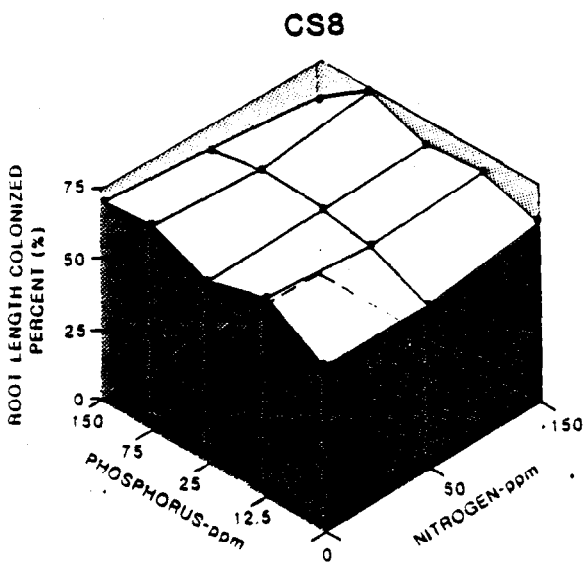


Figure 2: Response of CS8 Mycorrhizae Percent Root Infection to Increasing Nitrogen and Phosphorus Concentrations.

## DISCUSSION

Data are abundant indicating the beneficial effects obtained from mycorrhizal inoculation of agricultural crops and important timber species. However, to date only limited information exists concerning plant response to mycorrhiza and fertilizer additions in overburden soils. The results presented here clearly indicate the dramatic effect which mycorrhizal fungi have on growth of sweetgum. Control plant biomass approached that of mycorrhizal plants only when relatively high concentrations of nitrogen and phosphorus were added. Although both mycorrhizal types resulted in greater growth of sweetgum, it is interesting to note the differences in the degree of response which was obtained. G. macrocarpum was orginally selected because it is a readily obtainable native Florida species. Inoculum is also maintained by local mycorrhizal research labs and thus is a species which may be available for field inoculation in Florida. CS8 was utilized because it an indigenous group occurring in the phosphate district and may possess adaptations to high soil phosphorus concentrations. Single species were not isolated from the composite simply because it was felt that if they existed together within the roots of field plants, then there may be some functional significance to the association. Inoculation with individual species components could have dramatically affected experimental outcome. The overall greater enchancement effect of CS8 over G. macrocarpum may be attributed to many factors. However without further investigations any attempt at explanation would merely be speculation. CS8 may in fact be adapted to conditions of high soil phosphorus and function to supply other valuable nutrients needed in plant growth. G. macrocarpum may have been affected by soil edaphic factors such as pH. G. macrocarpum has evolved in soil with comparatively lower phosphorus levels where its function is to supply phosphorus to the host plant. At high P soils the ability of G. macrocarpum to supply the plant with other essential nutrients e.g. nitrogen may not be as efficient as that of CS8.

Multispecies inoculation may offer several advantages over use of single species inoculum. First, multiple species being present allows the plants to select for the symbiont or quite probably allow for multiple symbiosis to occur. Numerous studies have shown that a plant may form symbiotic associations with many mycorrhizal species at the same time (7). The presence of several different endophytes may be advantageous because each may have variable importance at different life cycles or times during the year (8, 9). Daft (8) recently offered several criteria describing the properties an ideal endophyte would have to possess to ensure successful field inoculation. These are abilities to (1) infect plants early in the growth period (2) efficiently exploit the soil, (3) transfer nutrients readily to the host, (4) spread and multiply, (5) compete effectively and (6) infect a wide range of plants under variable environment conditions. He suggests all these characteristics could possibly not be found in a single endophyte, however a multispecies inoculum may be the answer. Utilizing a single species inoculum requires that the researcher, rather than "mother nature," make the selection. Possible drawbacks for this approach are numerous, especially if an indigenous source is desired. Selecting endophytes from field samples may result in a bias selection of the more common sporulating and/or large-spored species because of ease of isolating. Non sporulating species may not be seen even though they may possibly be the most abundant and efficient at enhancing plant growth.

Variable results have been obtained from using indigenous versus introduced strains as mycorrhizal inoculum (10, 11, 12). The survival of an introduced fungus will be controlled by the ability to adapt to fertility moisture, and temperature regimes present within the soil. Lambert et al. (11) suggest that generally introduced species would not improve plant yield in soils with indigenous fungi. They indicate these conditions may change if vegetation, pH, and fertility factors to which the indigenous fungi have adapted are altered. This view is also expressed by Crush (13) pertaining to inoculation of New Zealand pasture soils. Introduced mycorrhizal fungi species must be adapted to environmental and soil conditions to effect an advantage for plant growth enhancement and survival.

Another result which warrants specific consideration is the effect of nutrients on mycorrhizal root infection. It is not clear why such low (<1%) root infection levels were obtained for G. macrocarpum. Although a growth enhancement over control plants (at low nutrient levels) was recognized, very little colonization was maintained by the plant at any of the various nutrient levels tested. CS8 root colonization followed an extremely different pattern. High levels of colonization were maintained at all nutrient levels and no variation was associated with either increasing or decreasing levels of N or P. Generally it has been shown that increasing P concentrations results in subsequent reduction in mycorrhizal colonization (3, 4, 14). Effects of nitrogen on colonization have been less well documented however both decreases (15) and increases (16) in mycorrhizal colonization levels have been noted with increasing nitrogen concentrations. Hepper (5) showed that the extent of the depression in mycorrhizal colonization at high phosphate levels was dependent upon the ratio of nitrogen to phosphorus concentrations. Increasing amounts of nitrogen tended to increase root colonization at a given phosphate level. Results obtained in this study us-

ing the CS8 composite, significantly differ from those found in the above studies.

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Center for Wetlands Contribution #127

# MYCORRHIZAE ENHANCE GROWTH OF SWEETGUM IN PHOSPHATE MINED OVERBURDEN SOILS

Peter M. Wallace, G. Ronnie Best, and James A. Feiertag
Center for Wetlands and Environmental
Engineering Sciences Department
University of Florida
Gainesville, FL 32611

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Abstract. Growth enhancement studies were performed to investigate factors affecting growth of sweetgum (Liquidambar styraciflua) on phosphate mined overburden soils. A factorial design experiment was used in which five levels of phosphorus (0, 12.5, 25, 75, and 150 ppm-P), three levels of nitrogen (0, 50, 150 ppm-N), and endomycorrhiza additions were evaluated.

Endomycorrhizal treatments consisted of control, Glomus macrocarpum, and a composite sample of several species isolated from a phosphate mine clay settling area. Results indicate that the addition of the composite mycorrhizae promoted greater growth than either the control or G. macrocarpum treatments at all nutrient levels. Leaf area measurements indicated that the composite endomycorrhizal treatment resulted in 30 times greater area than control and 1.7 times greater area than Glomus macrocarpum. Although soil P levels equal 2592 ppm (double-acid), in each treatment block significant growth responses were noted with addition of mycorrhizal fungi.

## INTRODUCTION

Reclamation of mined lands in Florida presents several unique and interesting problems with regard to community development. During mining, nonweathered overburden material is removed from varying depths and deposited on the surface, creating a mosaic of steep sided hills surrounded by deep water filled canals. Reclamation of mined lands has been mandatory since the mid-1970's, requiring a recontouring of the mined areas and restoration to useful systems. Generally reclamation has been to pastured ecosystems, or pasture and lake systems. Since Florida law now requires that a portion of the disturbed area must be reclaimed to natural ecosystems problems have developed which were not encountered in traditional pastureland restoration situations. Two avenues exist by which these areas may be returned to natural systems. The method of natural system reclamation used requires that extensive labor be used in replanting. An alternative to this approach is natural ecosystem reconstruction via succession (1). Can nature restore a mature self maintaining system in a time frame which is acceptable? Although these approaches are vastly different, success of either may be affected in part and limited by common components such as soil, organic matter, nitrogen, mycorrhizae and/or others. Premining ecosystems in Florida may range from xeric communities dominated by oaks (Quercus spp.) to less well drained pine flatwood to hydric bottomland hardwood or cypress dominated communities. Although many systems of varying hydrologic

regimes are mined, restoration efforts more commonly produce mesic to xeric upland habitats. In addition, post mining soils characteristically possess very different physical and chemical properties compared with their undisturbed counterparts (see Hawkins (2) for general description). Typically, mined soils have higher concentrations of major nutrients, e.g. Ca, P, K, Mg, than premined soils, and contain considerably greater proportions of clay to sand. Mined soils, however, contain extremely low concentrations of organic matter and nitrogen. The creation of these new habitats proposes the question as to whether locally occurring native plant species which have evolved in generally acid, low clay, low nutrient sandy soils can be expected to invade, survive, and successfully regenerate on these newly created soil systems.

Success of reclamation, whether through the intervention of man or natural ecosystem regeneration, involves an understanding of both macrocomponents and microcomponents of the developing ecosystems. Whether seedlings are planted or occurrence is through natural invasion, success will be determined through interaction with abiotic and biotic environmental parameters. One such parameter warranting special attention is the potential role of mycorrhizal fungi. During the process of mining, overburden soil is removed from depths ranging from 15 to 80 feet. This soil which can generally be assumed to be abiotic, thus becomes the site of not only aboveground biomass invasion but also of microbial recolonization, i.e. mycorrhizal fungi. Successful inhabitance by planted or invaded species may in fact be governed by the degree and rate at which mycorrhizal populations become reestablished within the disturbed area.

Presently little information exists regarding mycorrhiza occurrence in surface mined lands in Florida or the potential role and function of mycorrhizae in these high phosphorus overburden soils. The consideration of mycorrhizal inoculation for enhanced plant growth for reclamation activities presents several difficult but intriguing questions. The source of endophyte inoculum should be an important consideration. High soil phosphate levels have generally been shown to substantially reduce mycorrhizal colonization (3, 4) thus decreasing or alleviating plant dependence on the symbiosis. Presently mycorrhizae that can be used for inoculum purposes does not originate from phosphate mined areas and these ecotypes may not be adapted to soil conditions such as high phosphate which would be encountered in this area. Indigenous mycorrhizae from previously mined areas may infact be adapted to these conditions which have been shown to be antagonistic to plant mycorrhiza interactions, hence, offering the most viable alternative. Although plant mycorrhizal interactions involving phosphate absorption are well known, information regarding interactions with other nutrients is generally inadequate. However, it is reasonable to assume that in situations of high phosphorus availability, mycorrhizae may enhance uptake of nutrients which exist at suboptimal concentrations. On phosphate overburden soil nitrogen occurs in very low concentrations and is thus considered the major nutrient limiting growth of invading plant species. A growth experiment was designed to investigate factors which affect growth of sweetgum (Liquidambar styraciflua) in overburden soils. Sweetgum was used because of its often poor performance when planted in overburden soils, although it is a common species in adjacent unmined systems. Mycorrhizal additions were evaluated and growth responses determined in relation to both increasing nitrogen and phosphorus additions.

## MATERIALS AND METHODS

### Experimental Design

A 5x3x3 factorial design experiment was utilized in which five levels of phosphorus (0, 12.5, 25, 75, 150 ppm - P), three levels of nitrogen (0, 50, 150 ppm - N) and endomycorrhiza additions were evaluated. This design allowed for forty-five separate treatments to be analyzed (Table 1). Five replications were employed for each individual treatment. Data obtained were analyzed using analysis of variance techniques contained within the Statistical Analysis System (SAS).

Table 1. Growth experiment design.

| | FACTOR = CS 8 PHOSPHORUS (ppm) | | | | | FACTOR = G. MACROCARPUM PHOSPHORUS (ppm) | | | | | FACTOR = CONTROL PHOSPHORUS (ppm) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NITROGEN (ppm) | 0 | 12.5 | 25 | 75 | 150 | 0 | 12.5 | 15 | 75 | 150 | 0 | 12.5 | 25 | 75 | 150 |
| 0 | 1 | 2 | 3 | 4 | 5 | 16 | 17 | 18 | 19 | 20 | 31 | 32 | 33 | 34 | 35 |
| 50 | 6 | 7 | 8 | 9 | 10 | 21 | 22 | 23 | 24 | 25 | 36 | 37 | 38 | 39 | 40 |
| 150 | 11 | 12 | 13 | 14 | 15 | 26 | 27 | 28 | 29 | 30 | 41 | 42 | 43 | 44 | 45 |

### Endomycorrhiza Inoculum Preparation

Endomycorrhizal treatments consisted of control, Glomus macrocarpum and a composite sample (CS8) of unidentified species isolated from a phosphatic clay settling area. G. macrocarpum (a commonly occurring Florida species) was obtained from Dr. N. C. Schenck, University of Florida, in the form of soil and infected plant roots. This inoculum was increased by placing in large 1x2 ft. flats containing sterilized native Florida soil and planted with bahia grass (Paspalum notatum) and soybean (Glycine max).

These pot cultures were allowed to grow for four months, at which time roots and soil were separated by sieving and stored in plastic containers at approximately 5% soil moisture until initiation of the experiment. The CS8 mycorrhiza was obtained from Salix caroliniana roots collected from a partially inundated eight month old clay settling area in southwest Polk County (International Minerals and Chemical Corporation; Clear Springs Mine clay settling area Δ8). Roots were placed in large 1x2 ft. flats containing phosphate overburden soil and inoculum prepared as previously described.

### Test Plant Inoculation

Seeds of sweetgum (Liquidambar styraciflua) were collected from Polk County in central Florida. Surface sterilzed seeds were placed in germination flats containing autoclaved vermiculite. Two weeks following germination seedlings were removed and transferred to pots containing 1100 grams of 1:1 phosphate overburden - sand tailings mix. Mycorrhizal inoculum consisting of 0.5 grams sieved roots and 5 grams soil was added directly below the seedling prior to planting. The inoculum used consisted of infected root fragments, mineral soil, fungal hyphae, mycorrhizal spores, and rhizosphere microflora.

In order to standardize microflora for all plants a rhizosphere microflora inoculum was prepared from both inoculum types by washing roots and soil in deionized water. The soil and root wash was subsequently sieved through 45 and 42 μ soil sieves followed by filtration through Whatman No. 4 filter paper (20-25μ). The microflora inoculum was added to all plants including controls. Control and CS8 plants in addition received 5 grams of sterilized G. macrocarpum inoculum soil because it was chemically different than the phosphate overburden matrix. Control plants also received 0.5 grams of sterilized root fragments.

Fertilizer Addition

Phosphorus treatments were performed by incorporating 20% superphosphate fertilizer into the entire contents of each pot at the designated concentration. All phosphorus treatments were based upon concentrations (parts per million: ppm) of elemental phosphorus. Nitrogen fertilization was performed biweekly for 16 weeks by adding 6.25 mg-l and 18.75 mg-l as ammonium nitrate in 20 ml deionized water. This resulted in the total addition of 50 ppm-N (100 lbs./acre) and 150 ppm-N (300 lbs./acre) respectively.

All treatments received an additional standard nutrient solution (from (5)), described, as follows: (mg/l) $CaCl_2 \cdot 2H_2O$, 294; $K_2SO_4$, 174; $MgSO_4 \cdot 7H_2O$, 184; $MnSO_4 \cdot 4H_2O$, 0.23; $ZnSO_4 \cdot 7H_2O$, 0.03; $CuSO_4 \cdot 5H_2O$, 0.024; $FeSO_4 \cdot 7H_2O$, 1.10; $(NH_4)_6 Mo_7O_{24} \cdot 4H_2O$, 0.0035. All plants received 40 ml of nutrient solution biweekly for 16 weeks. After the final nutrient addition plants were allowed to grow four weeks before harvesting was performed.

Data Collection and Harvest

Seedling height was recorded biweekly for the first eight weeks, then monthly until harvest to allow for construction of growth curves. To best determine grow response for individual treatments both leaf area and root length were determined for each plant. At harvest stems were clipped at the soil surface and leaves pressed in paper bags and stored at 4°C for no more than 48 hrs. Leaf area was determined with a Hayashi Denko Automatic Area Meter, model AAM-5. Roots were removed from the soil by washing and sieving and stored in a 90:5:5 Alcohol-Acetic Acid - Formalin (FAA) mixture until further processing. Total root length was determined using a grid-line intercept method (6). Roots were cleared in 10% potassium hydroxide for 1 hour, bleached 15 minutes in alkaline $H_2O_2$ solution and subsequently stained in 0.05% Trypan Blue staining solution. Percent mycorrhizal colonization was obtained by using the grid line intercept method. Having two measurements of root length, i.e. total root length and percent colonized of the aliquot, allows for a conversion of total plant root length colonized by mycorrhizae. Root length was considered a better indicator of plant response than root biomass because it gives a better estimate of root absortive surface. Large diameter root segments can contain a proportiately large biomass although possess only very little nutrient exploiting capacity and significantly bias results.

Soil Nutrient Analysis

Soil phosphorus determinations were performed on both the 1:1 overburden-sand tailing mix (test soil) and each of the individual components (Table 2).

Although double acid extracts are commonly employed in southeast sandy soils, two additional extracts were analyzed to facilitate comparisons with other research. Double acid extractions were performed on both sterilized and unsterilized test soil. P analyses were performed by the Environmental Science and Engineering Laboratory, Gainesville, Florida.

Table 2. Comparisons of Extractable Phosphorus Concentration (ppm: mg/l) obtained using various common extracting solutions.

| 1:1 Overburden/sandtailings Mix - Sterilized | | | 1:1 OB/ST Mix Nonsterilized |
|---|---|---|---|
| Double Acid* | Bray 1** | Bray 2*** | Double Acid |
| 2592 | 400 | 2313 | 2404 |

| Overburden Nonsterilized | Sandtailings Nonsterilized |
|---|---|
| 2408 | 2068 |

*Double Acid - (0.025 N $H_2SO_4$ + 0.050 N HCl)
**Bray 1 - (0.030 N $NH_4F$ H 0.025 N HCl)
***Bray 2 - (0.030 N $NH_4F$ H 0.100 N HCl)

Results

Results of analysis of variance (ANOVA) for both root length and leaf area growth responses are given in Table 3. Comparisons of model R-square (0.87/0.67) values indicate that leaf area measurements produced the least amount of variability among samples within each individual treatment than did root length. Leaf area as a measure of photosynthetic area and root length as an indicator of nutrient absortive surface are both significant indicators of plant vigor. However, interpretations of leaf area data may offer the most simplistic and precise interpretation of growth response to the various treatments. Due to the difficulty associated with determination of root length, variability may in fact be largely a reflection of error in sample processing rather than actual response differences. The leaf area F statistic indicates that all main effects of P, N, and Myc (Mycorrhiza) are highly significant. However, responses to N and mycorrhiza additions were far greater than those associated with P. To facilitate interpretation of these results response surface curves (Figure 1) have been constructed so that differential responses of each mycorrhizal treatment to P and N fertilization may be compared and optimum levels of nutrients and mycorrhizal type may be seen.

Generally, responses of CS8 mycorrhiza and control plants followed the same trend. Addition of increasing amounts of N at each P level resulted in substantial growth enhancement. Responses to increased P concentration at each N level also resulted in greater growth however not of the magnitude that was noted in the previous situation. In both cases it is evident from the response surfaces that maximum production was obtained at levels of 150 ppm - N and 150 ppm - P indicating that both control and mycorrhizal plants were positively responding to fertilizer additions. Comparisons between CS8 and control plants

Table 3. Analysis of Variance of Effects of Phosphorus (P), Nitrogen (N), and Mycorrhiza Type (Myc) on Root Length and Leaf Area Growth Responses.

Growth Response Variable: Root Length: cm

ANOVA

| SOURCE | DF | SS | F | PR>F | $R^2$ |
|---|---|---|---|---|---|
| Model | 44 | 79329167 | 8.24 | .0001 | 0.67 |
| Error | 180 | 39392395 | | | |
| TOTAL | 224 | 118721562 | | | |

| SOURCE | DF | F VALUE | PR>F |
|---|---|---|---|
| P | 4 | 6.15 | 0.0001 |
| N | 2 | 113.09 | 0.0001 |
| P x N | 8 | 1.55 | 0.1431 |
| MYC | 2 | 17.15 | 0.0001 |
| P x MYC | 8 | 4.38 | 0.0001 |
| N x MYC | 4 | 2.06 | 0.0879 |
| P x N x MYC | 16 | 1.36 | 0.1667 |

Growth Response Variable: Leaf Area: $mm^2$

ANOVA

| SOURCE | DF | SS | F | PR>F | $R^2$ |
|---|---|---|---|---|---|
| Model | 44 | 27477331081 | 21.06 | 0.0001 | 0.87 |
| Error | 135 | 4003226878 | | | |
| Total | 179 | 31480557959 | | | |

| SOURCE | DF | F VALUE | PR>F |
|---|---|---|---|
| P | 4 | 7.24 | 0.0001 |
| N | 2 | 250.40 | 0.0001 |
| P x N | 8 | 1.83 | 0.0774 |
| MYC | 2 | 116.35 | 0.0001 |
| P x MYC | 8 | 9.74 | 0.0001 |
| N x MYC | 4 | 5.05 | 0.0008 |
| P x N x MYC | 16 | 3.22 | 0.0001 |

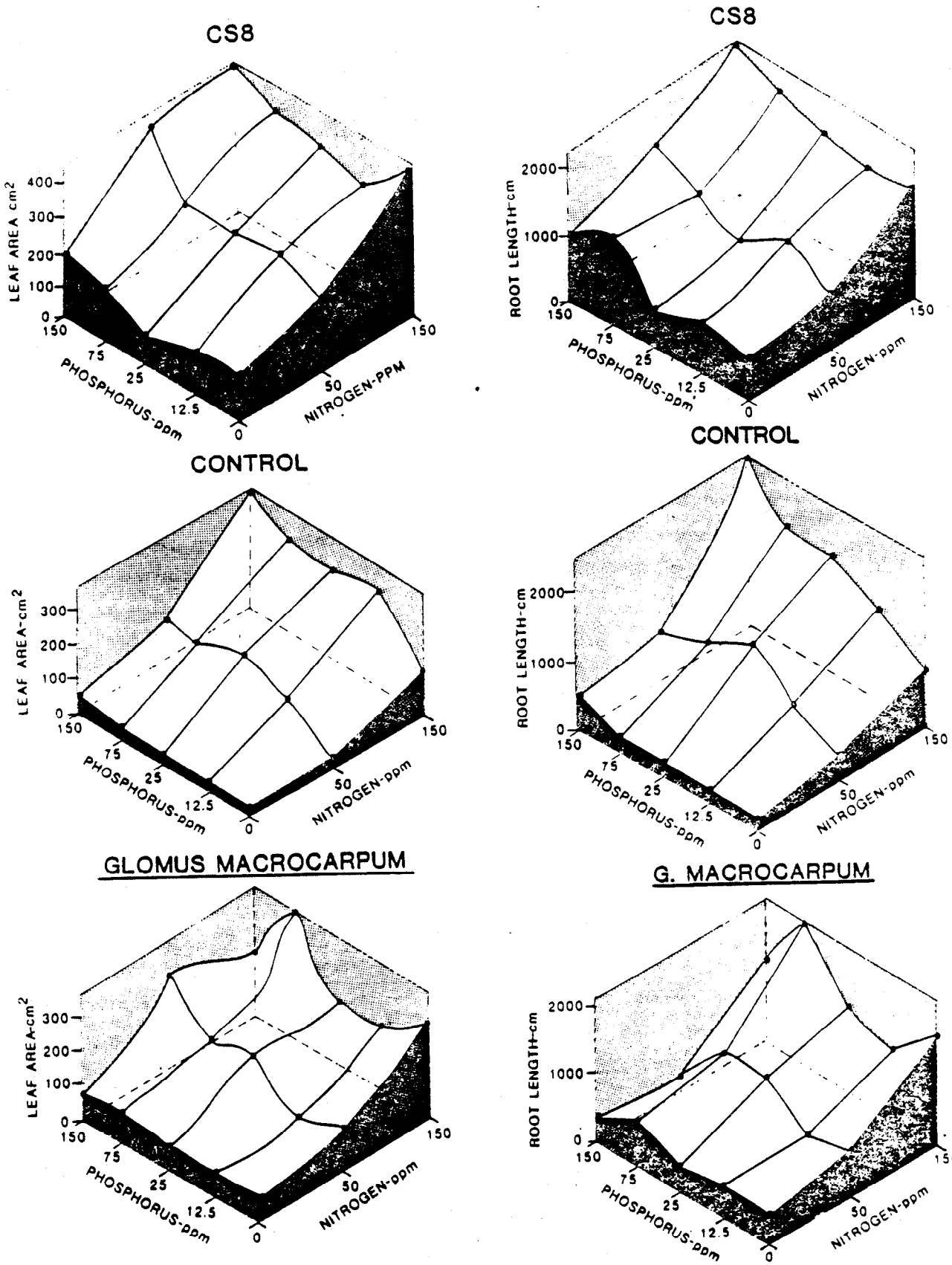


Figure 1. Response surface curves for the root length and leaf area obtained from the various nutrient treatments within each mycorrhizal group.

indicate that at all nutrient levels leaf area of CS8 plants were higher than those of corresponding control plants. At levels of 150 ppm - P and 150 ppm - N, CS8 plants possessed significantly greater leaf area (446.7 $cm^2$/352.2 $cm^2$: $\alpha = .05$) than control plants, indicating mycorrhizal enhanced growth. In the treatment in which no additional P or N was added CS8 resulted in a 30 times (113.78 $cm^2$/3.79 $cm^2$) increase in leaf area over control.

Response of G. macrocarpum plants to the various treatments offers somewhat of a variable and difficult pattern to analyze. Response curves indicate that maximum mean values for both root length and leaf area were obtained when nutrient levels equalled 150 ppm - N and 75 ppm - P. Variable results were obtained when P levels were elevated to 150 ppm. Intratreatment variability of growth responses was greater for G. macrocarpum plants than either control or CS8 plants making interpretation of response exceedingly difficult. However, at nutrient levels in which no concurrent additions of P or N were made, G. macrocarpum resulted in greater growth than control treatments, yet generally significantly less than CS8 inoculated individuals. Combinations of added P and N levels resulted in highly variable results. G. macrocarpum plants with no additional P or N possessed 17 times (65.02/3.79 $cm^2$) more leaf area than control plants but produced only 0.57 (113.78/65.02 $cm^2$) the area produced by CS8.

The significant second order PxNxMyc interaction makes overall experimental analysis difficult to interpret. The insignicant ($\alpha = 0.05$) P X N interactions indicates that possibly responses to P and N additions are dependent upon the particular mycorrhizal component, i.e. CS8, G. macrocarpum, and control, however effects of P and N additions within each mycorrhizal group are simply additive.

The most simplistic method in which to analyze these data may be to momentarily ignore interactive effects and just examine the main effects of the three factors within the experiment, i.e. nitrogen, phosphorus and mycorrhizae. Table 4 gives main effect results of the various levels of each factor over all levels of the remaining two factors. For example, mean values are given for 150 ppm - P obtained for all levels of mycorrhizae and nitrogen. Briefly, results indicate that a significant growth response was obtained when P concentrations were elevated to 75 ppm or more. In addition, increasing the amount of nitrogen resulted in doubling the leaf area of that obtained at the next lower level. CS8 mycorrhizae exhibited significantly greater growth than that obtained by both control and G. macrocarpum when means were averaged over all nutrient treatments whereas no significant differences appeared between average response of G. macrocarpum and controls.

Response of CS8 root infection to various treatments is presented in Figure 2. The response surface indicates that very little change in the percent of root length colonized occurrred with respect to treatment and no trends were associated with increasing either nitorogen or phosphorus supply. Infection percentage remained high for all treatments with values ranging from 56.8% where P = 0 and N = 0 to 75. [illegible] where P = 75 and N = 150. Infections recorded in G. macrocarpum roots were significantly lower with all values being less than one percent. Although spores were seen in soil and attached to roots, large areas of infected root length were not detected.

Table 4. Analysis of Variance of Main Effects of Phosphorus (P), Nitrogen (N) and Mycorrhiza Type on Sweetgum Leaf Area.

MAIN EFFECTS

Dependent Variable: Leaf Area: $cm^2$

| MEAN | N | P (ppm) | DUNCANS | $\alpha$=.05 |
|---|---|---|---|---|
| 200.42 | 36 | 150 | A | |
| 191.48 | 36 | 75 | A | |
| 156.07 | 36 | 25 | B | |
| 151.70 | 36 | 0 | B | |
| 148.31 | 36 | 12.5 | B | |

| MEAN | N | N (ppm) | DUNCANS | $\alpha$=.05 |
|---|---|---|---|---|
| 287.26 | 60 | 150 | A | |
| 155.40 | 60 | 50 | B | |
| 66.13 | 60 | 0 | C | |

| MEAN | N | MYCORRHIZAE | DUNCANS | $\alpha$=.05 |
|---|---|---|---|---|
| 256.72 | 60 | CS8 | A | |
| 133.60 | 60 | G. macrocarpum | B | |
| 118.47 | 60 | CONTROL | B | |

## DISCUSSION

Data are abundant indicating the beneficial effects obtained from mycorrhizal inoculation of agricultural crops and important timber species. However, to date only limited information exists concerning plant response to mycorrhiza and fertilizer additions in overburden soils. The results presented here clearly indicate the dramatic effect which mycorrhizal fungi have on growth of sweetgum. Control plant biomass approached that of mycorrhizal plants only when relatively high concentrations of nitrogen and phosphorus were added. Although both mycorrhizal types resulted in greater growth of sweetgum, it is interesting to note the differences in the degree of response which was obtained. G. macrocarpum was orginally selected because it is a readily obtainable native Florida species. Inoculum is also maintained by local mycorrhizal research labs and thus is a species which may be available for field inoculation in Florida. CS8 was utilized because it an indigenous group occurring in the phosphate district and may possess adaptations to high soil phosphorus concentrations. Single species were not isolated from the composite simply because it was felt that if they existed together within the roots of field plants, then there may be some functional significance to the association. Inoculation with individual species components could have dramatically affected experimental outcome. The overall greater enchancement effect of CS8 over G. macrocarpum may be attributed to many factors. However without further investigations any attempt at explanation would merely be speculation. CS8 may in fact be adapted to conditions of high soil phosphorus and function to supply other valuable nutrients needed in plant growth. G. macrocarpum may have been

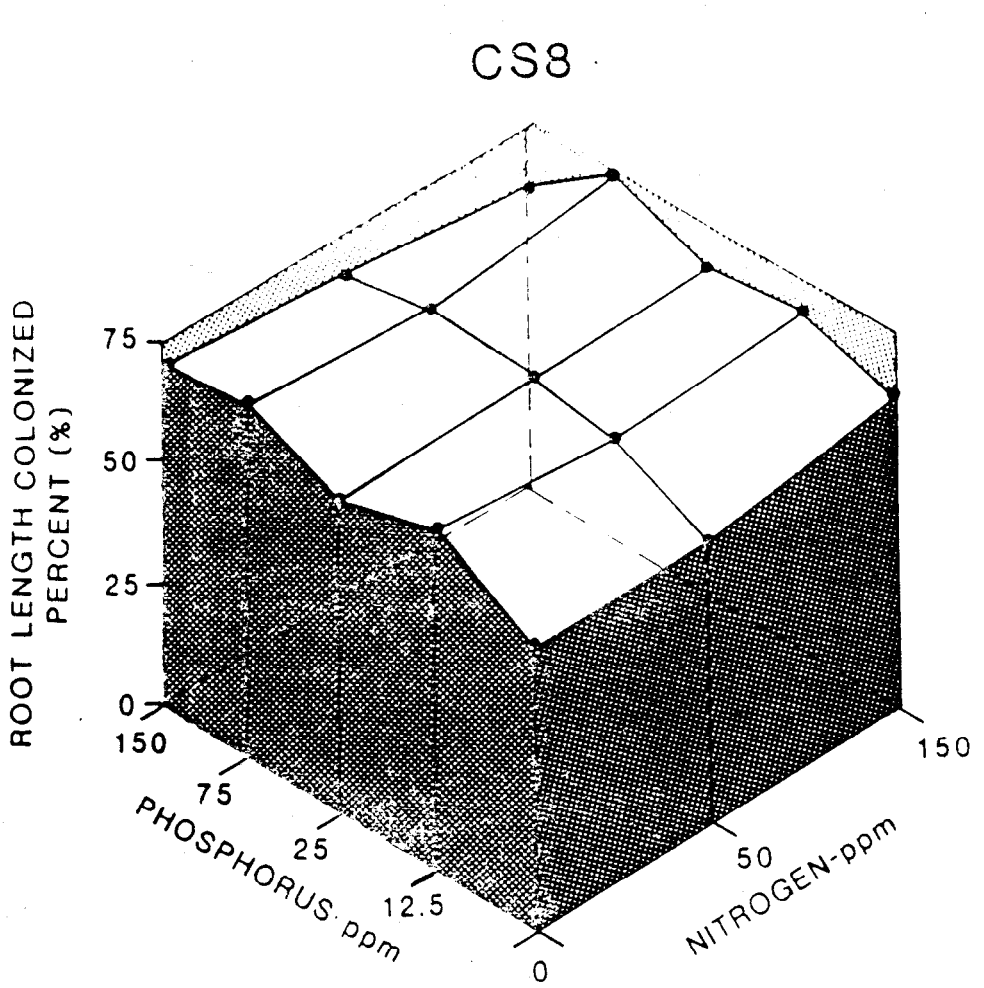


Figure 2. Responses of CS8 mycorrhizae percent root infection to increasing nitrogen and phosphorus concentrations.

affected by soil edaphic factors such as pH. G. macrocarpum has evolved in soil with comparatively lower phosphorus levels where its function is to supply phosphorus to the host plant. At high P soils the ability of G. macrocarpum to supply the plant with other essential nutrients e.g. nitrogen may not be as efficient as that of CS8.

Multispecies inoculation may offer several advantages over use of single species inoculum. First, multiple species being present allows the plants to select for the symbiont or quite probably allow for multiple symbiosis to occur. Numerous studies have shown that a plant may form symbiotic associatic with many mycorrhizal species at the same time (7). The presence of several different endophytes may be advantageous because each may have variable importance at different life cycles or times during the year (8, 9). Daft (8) recently offered several criteria describing the properties an ideal endophyte would have to possess to ensure successful field inoculation. These are abilities to (1) infect plants early in the growth period (2) efficiently exploit the soil, (3) transfer nutrients readily to the host, (4) spread and multiply, (5) compete effectively and (6) infect a wide range of plants under variable environment conditions. He suggests all these characteristics could possibly

not be found in a single endophyte, however a multispecies inoculum may be the answer. Utilizing a single species inoculum requires that the researcher, rather than "mother nature," make the selection. Possible drawbacks for this approach are numerous, especially if an indigenous source is desired. Selecting endophytes from field samples may result in a bias selection of the more common sporulating and/or large-spored species because of ease of isolating. Non sporulating species may not be seen even though they may possibly be the most abundant and efficient at enhancing plant growth.

Variable results have been obtained from using indigenous versus introduced strains as mycorrhizal inoculum (10, 11, 12). The survival of an introduced fungus will be controlled by the ability to adapt to fertility, moisture, and temperature regimes present within the soil. Lambert et al. (11) suggest that generally introduced species would not improve plant yield in soils with indigenous fungi. They indicate these conditions may change if vegetation, pH, and fertility factors to which the indigenous fungi have adapted are altered. This view is also expressed by Crush (13) pertaining to inoculation of New Zealand pasture soils. Introduced mycorrhizal fungi species must be adapted to environmental and soil conditions to effect an advantage for plant growth enhancement and survival.

Another result which warrants specific consideration is the effect of nutrients on mycorrhizal root infection. It is not clear why such low (<1%) root infection levels were obtained for G. macrocarpum. Although a growth enhancement over control plants (at low nutrient levels) was recognized, very little colonization was maintained by the plant at any of the various nutrient levels tested. CS8 root colonization followed an extremely different pattern. High levels of colonization were maintained at all nutrient levels and no variation was associated with either increasing or decreasing levels of N or P. Generally it has been shown that increasing P concentrations results in subsequent reduction in mycorrhizal colonization (3, 4, 14). Effects of nitrogen on colonization have been less well documented however both decreases (15) and increases (16) in mycorrhizal colonization levels have been noted with increasing nitrogen concentrations. Hepper (5) showed that the extent of the depression in mycorrhizal colonization at high phosphate levels was dependent upon the ratio of nitrogen to phosphorus concentrations. Increasing amounts of nitrogen tended to increase root colonization at a given phosphate level. Results obtained in this study using the CS8 composite, significantly differ from those found in the above studies.

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