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NATIVE REVEGETATION

with Pitch Pine, *Pinus rigida* Mill.

for

Cape Cod National Seashore

Fire Island National Seashore

Gateway National Recreation Area

Final Report

NATIVE REVEGETATION

Containerized Production of Seedlings of Native Provenances

of Pitch Pine, *Pinus rigida* Mill.

for

Cape Cod National Seashore

Fire Island National Seashore

Gateway National Recreation Area

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EXECUTIVE SUMMARY

Native Revegetation: Containerized Production

of Seedlings of Native Provenances

of Pitch Pine, *Pinus rigida* Mill.,

and related studies

A method was developed to produce containerized pitch pine seedlings specifically adapted to each of the Parks (Cape Cod, Fire Island, and Gateway). Seed may be collected from about the second week of September to mid-November. The average germination rate of 54 families was 50.2% in the nursery situation. The mean number of seeds/cone was 95.2 for 46 families and the number of expected germinants per cone was 45.5 for these same 46 families. In an experiment to determine optimum potting medium pH seedlings growing in a mix of peat/vermiculite/sand (1/1/1) and amendments, with a pH of approximately 4.8, were significantly larger in volume ($p < 0.05$) than those growing at either lower or higher pH. Seedlings growing in more acid media also suffered less mortality, with 79% of total mortality occurring in media with pH greater than 6.0. The probable pathogen, a species of Fusarium , was found to grow significantly faster ($p < 0.05$) at pH values of 6.5 and 7.0 than at 4.5 and 5.0. Progeny testing was used to select parent trees producing the most vigorous seedlings for use at both Cape Cod and Fire Island. Analysis of surviving seedlings from the Cape Cod data set suggests that seedlings should be grown to above a minimum stem diameter of 2.8 mm before outplanting. Other general information is contained within this report that should enable Park Service personnel to successfully grow and outplant pitch pine seedlings.

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INTRODUCTION

Unlike National Parks in the western United States, the three parks (Fig. 14) involved with this project have had a long history of intensive usage by man since the time of European settlement along the East Coast. This, in conjunction with the National Park Service's policy of managing resources in pristine condition, immediately raises two questions: 1) what was pristine condition at Cape Cod National Seashore, Fire Island National Seashore, and Gateway National Recreation Area, and 2) to what degree is it feasible or desirable to restore these areas to such condition? Our project was undertaken to demonstrate how one resource, pitch pine (Pinus rigida Mill.), could be managed to maintain it in pristine condition where it now exists and establish it in pristine-like condition in areas where it is lacking. Because the three parks differ in regard to presence/absence of pitch pine, they are discussed separately.

Cape Cod National Seashore has extensive natural stands of pitch pine. These vary from open forests of 10m-15m tall trees on sheltered sites near Provincelands, to windswept scrub forests as short as 5m at many places in the park. Because management of the pitch pine resource will probably involve collecting seed from pitch pines and growing seedlings to plant in areas where it is desirable to restore pristine condition, we set these objectives: 1) determine whether there is genecological variation among the various pitch pine populations (i.e., can one seed source serve the whole park, or must seed be collected from each stand to produce seedlings best adapted to that stand or to similar sites?), 2) quantify differences among seedlings grown from different parent trees, and identify the best parents with respect to cone crops, number of viable seedlings per cone, and seedling survival and growth, and 3) develop a system for container-growing seedlings, with particular attention to type of planting medium, greenhouse environment, and timing of seed collection and planting.

Fire Island National Seashore has two areas where pitch pine is a significant ecosystem component, Sunken Forest and Wilderness Area. An objective of the Fire Island National Seashore Wilderness Management Plan (NPS 1983) is to "---manage so that the character of the area will be molded primarily by forces of nature and so that human influence will be minimal ------ management actions will be directed at restoring the natural character of the area and preserving and maintaining natural systems. Evidence of man's previous actions will be removed ----". Fire Island has extensive areas of summer resort development where pitch pine is rare or absent and its place is taken by the introduced Japanese black pine (P. thunbergii Parl.). If native revegetation were undertaken to replace the latter with pitch pine, a more pristine-like condition would result. Assuming that the

Park Service might wish to undertake such a project, we set the same research objectives with pitch pine for Fire Island as for Cape Cod.

Gateway National Recreation Area has no native pitch pine populations at either Floyd Bennett Field or Sandy Hook, although the New Jersey mainland adjacent to Sandy Hook has extensive stands of both pitch pine and shortleaf pine (P. echinata Mill.) . Floyd Bennett Field is a manmade island in Jamaica Bay, with some developed areas, large expanses of Phragmites, a few planted stands of Japanese black pine (including some natural regeneration), and scattered specimens of pioneer tree species such as tree-of-heaven (Ailanthus altissima (Mill.) Swingle) and black cherry (Prunus serotina Ehrh.). Floyd Bennett Field's soils are probably suitable for pitch pine, although mycorrhizal inoculation may be necessary for the best growth.

Sandy Hook has been extensively impacted by man since before the Revolutionary War, and most recently during its administration by the U.S. Army from 1874 through 1974. Here and there on the peninsula are small planted stands and scattered individual specimens of Austrian pine (P. nigra Arnold), Japanese black pine, Scots pine (P. sylvestris L.), Virginia pine (P. virginiana Mill.), and pitch pine. Both Scots pine and Virginia pine are naturalizing (the latter aggressively), but pitch pine is not. The apparent reason for this is that the whole Sandy Hook pitch pine population is serotinous. Very likely of Pine Plains (NJ) origin, Sandy Hook's pitch pines were probably planted by the Army during the mid-1950's to trap blowing sand and prevent it from drifting around a new radar installation. Since there have been no fires to open the cones and release seed since then, naturalization has not occurred.

Our objectives for Gateway were: 1) to determine whether Fire Island or New Jersey provenances of pitch pine are best adapted for planting at Floyd Bennett Field; 2) identify the best parent trees from either source; 3) determine whether mycorrhizae enhance survival or growth at Floyd Bennett, and; 4) develop a containerized-seedling production system which makes advantageous use of the Park Service greenhouse at Floyd Bennett to provide vigorous pitch pine seedlings for Gateway and possibly the two other parks concerned.

Chapter 1. Progeny testing

INTRODUCTION

Evidence that local provenances of pitch pine are best adapted for planting in their native areas (Kuser and Knezick 1985) indicated that seedlings for each park unit should come from local seed sources. Following provenance selection, individual parent tree selection is the next important step for producing hardy, well-adapted seedlings. Parent trees should exhibit and transmit the traits that are desired by the planter. In this case the trees are to be used primarily for revegetating areas that are barren, windy and exposed to a very harsh environment. It is appropriate given these circumstances, to select trees in good health that exhibit tree form best adapted to the site. This may vary with site characteristics with exposed sites demanding a low spreading form, and sheltered sites a more treelike form. Once putative parent trees have been selected, the next step is to collect the seed and grow their progeny under uniform conditions. In this manner the offspring from each parent may be compared against all of the others. Measuring growth rates and counting survival will determine the parent trees that provide the most vigorous offspring. The objective of this experiment is to identify superior parent trees within local provenances so that park personnel can return to them and collect seed for future planting. In conjunction with the progeny test, three nursery soils were tested to identify the most appropriate for growing our seedlings in the future.

METHODS AND MATERIALS

Parent trees for Cape Cod National Seashore and Fire Island National Seashore were selected and permanently marked with numbered aluminum tags during visits in June 1986. Parent trees for Gateway National Recreation Area were selected from three sources, Fire Island, Colt's Neck, New Jersey, and farther south along the New Jersey

coastal plain. Trees were selected on the basis of form and coning characteristics, that is, we needed a minimum of at least 10-15 cones/tree. Their locations were noted for future reference.

Cape Cod initially provided 25 parent trees from 3 locations. These locations varied in exposure from a sheltered area along Airport Road near the Provincelands Visitors Center to a moderately exposed area at the Marconi site, and a windswept site at Highhead, off of Highhead Road. Nine trees were tagged at Provincelands (designated PS1-PS9) and Highhead (HH1-HH9) sites. Seven trees were tagged at the Marconi site (M1-M7).

Fire Island initially provided 19 trees from 2 sites. Ten trees were selected from the Sunken Forest (SF1-SF10) area and nine trees were chosen at the Wilderness Area (WA1-WA9). These two sites were similar in exposure.

Parent trees for planting at Gateway National Recreation Area, which has no native population of pitch pine, were selected from nearby sources in New Jersey and Fire Island. New Jersey provided 16 parent trees, one-half from the Naval Weapons Station at Colt's Neck (nearest mainland pitch pines to Sandy Hook), and the others from farther south, along the Coastal Plain (selections from the New Jersey Pitch Pine Improvement Project). The Fire Island selections came from among the parents selected for Fire Island.

Ripe cones were collected from the Cape Cod, Fire Island, and Colt's Neck sites in early October 1986. Some difficulty was encountered at both Cape Cod and Fire Island as the local squirrel population also likes to pick ripe seed. Many trees had only a few cones taken, while a few trees had lost the majority of their seed. Trees # M2, M3, M6, M7, PS2, HH2, HH4, HH5, SF2, SF3, and SF7 had lost enough seed so that replacements were needed. All cones were placed in separate paper sacks and marked accordingly. There was no evidence of cone predation at the New Jersey sites.

The cones were allowed to air dry for two months. In December 1986 they were placed in an oven at 48° C for c. 15 mins. to melt the resin seals on the cone scales and initiate opening. The cones were then placed in a sunny, dry space to complete the opening process.

In late January of 1987, over the course of several days, seed representing each parent was sown into one rack of 98 163.9 cc (10 cu.in.) Leach super-cells. A total of 53 racks were seeded. In order to test three separate nursery potting mixes, (1) standard greenhouse peat/vermiculite mix, (2) same with 10% native soil added, and (3) a commercial blend, Fafards pine tree mix, the racks were divided into three separate sections of 32 cells/each. All parents, except for three, provided enough seed so that 3 seeds could be placed in each cell, for a total of 296 seeds per rack.

Seedlings were maintained in the greenhouse until April, when they were moved outside. The racks were placed on black plastic sheeting to prevent seedlings from rooting into the ground. Areas adjacent to the racks were herbicided with glyphosate, at recommended rates, as needed. A timed sprinkler was set up to provide watering. The trees were treated with a solution of Peters 20-20-20 soluble plant food at the recommended rate of 350 grams per 75 gallons of water every other week. The fungicide Benlate was applied to all racks on three separate occasions March 17, March 27, and April 10 to try to alleviate damping -off. The seedlings in the center of each rack, growing in Fafard's Pine Tree blend, started to become noticeably chlorotic as of March 9, after c. 6 weeks of growth. On April 28 measurements of soil pH were made from each of the three soil types. The two peat/vermiculite blends had values between 6.0-6.5, while the Fafards mix was especially high between 6.8-7.0. These trees were selectively watered with ferrous sulphate at a rate of 1.5 tablespoons per gallon of water. Each rack received about 0.5 gallons of this solution on four separate occasions: May 2, May 15, May 27, and June 10. This treatment resulted in a noticeable

improvement. These trees regained their green color and soon outgrew the trees in each of the other two nursery soils.

The trees were allowed to grow in their cells until the fall of 1987, when they were outplanted at their respective locations. In late August 1987, 1558 trees grown from Cape Cod parents were planted at Cape Cod, in a recently roto-tilled plot adjacent to the NPS biological research facilities in Truro, Mass. They were lined out in whole rows by nursery soil mix/parent at 12 inch spacing.

In September 1987, 480 trees were delivered to the facilities at Floyd Bennett Field. They represented five parents from Fire Island sources and five parents from New Jersey sources. They were lined out in rows and were planted under four separate treatments. This is discussed in detail in the outplanting experiment.

In October 1987, 523 Fire Island trees were lined out, by parent, in a recently tilled plot near the NPS maintenance facilities in Patchogue, N.Y.. Another 300 trees were given to park personnel to plant on the Island as they saw fit.

Progeny at all locations were measured in the spring of 1988 for height and diameter. Volumes were determined by the simple equation diameter^2 x height. This measure has been used by others for comparing progeny (Franklin 1983). Survival was tallied and used to determine minimum size requirements to grow seedlings to prior to outplanting. Duncan's Multiple Range Test was used to separate the individual treatment means when the Anova indicated a significant treatment effect ($p \leq 0.05$).

RESULTS

Progeny testing

Analysis of the progeny of Cape Cod trees, grown in the Fafards nursery soil, found significant differences ($p < 0.05$) among the volume growth of the seedlings. The trees ranged in size from a maximum mean volume of 2.7 cc. to a minimum mean volume

of 0.9 cc (Fig. 1). The largest progeny were provided by tree PS2, but they were not significantly different from the next four largest families of progeny. The volume differences among the seedlings in the middle ranges are rather closely grouped and so there are few significant differences among them. There was no significant difference among the eight smallest families of seedlings.

An interesting and unexpected result was that the Provincelands sheltered area provided 5 of the 6 most vigorous offspring. If the families are grouped by collection site (PS, HH, M) the PS progeny are significantly larger ($p < 0.05$) in volume (2.0 cc) than either the HH progeny (1.7 cc) or M progeny (1.5 cc), which do not differ significantly.

The mean volumes among the offspring of the Fire Island trees, grown in all three nursery soils ranged from 4.10 cc. to 2.07 cc. (Fig. 2). These trees have larger mean volumes than the Cape Cod trees partly because they were measured later in the spring. They showed significant differences ($p < 0.05$), and were more closely grouped than the Cape Cod seedlings. The top five families of seedlings were not significantly different, while the fifth largest family was not considered significantly different from the fourth smallest. This covers 10 families of seedlings that are not considered significantly different. Progeny from Fire Island did not show size differences between seedlings from the two sites.

Seedlings planted at Floyd Bennett Field had mean volumes ranging from 3.03 cc to 1.24 cc. The two largest came from selections from the New Jersey Pitch Pine Improvement Project, Nos. 108 and 100. It is too early to tell how provenances from New Jersey or Fire Island will perform on this landfill where no native population of pitch pine exists.

Mortality

The data used to analyze outplanted seedling mortality came from the Cape Cod data set. This was the largest data set and had the greatest number of seedlings that had died over the winter, 63 out of 1558. The majority of seedlings that died at Floyd Bennett Field were eaten by rabbits and only 11 of 523 seedlings succumbed at Fire Island. A significant difference was found between stem diameters of alive versus dead seedlings ($p < 0.05$). Mean stem diameter for live trees was 3.25 mm while mean stem diameter for dead trees was 2.6 mm. No significant difference was found between heights ($p > 0.05$) of alive versus dead trees; each had a mean near 12 cm. Figures 3a and 3b illustrate the results of the spring measurements of the stem diameters of live versus dead trees from the Cape Cod data set. In Figure 3a stem diameters are broken into ten quantiles with the height of each bar representing the percent mortality within that quantile. The greatest percentages of mortality occurred among the smaller diameter seedlings. Figure 3b illustrates that approximately 70 % of the mortality occurred among seedlings with stem diameter less than .280 cm.

Nursery soils

Comparing the three nursery soils, using the Cape Cod data set, showed that the Fafards mix grew seedlings with both significantly ($p < 0.05$) greater height (means of 14.4 cm versus 11.5 cm) and stem diameter (means of 3.38 mm versus 3.16 mm) than either the peat/vermiculite (p/v) or the peat/vermiculite with 10% native soil (p/vns) added. These last two were not significantly different for any of the values. The trees grown in Fafards mix for Floyd Bennett field proved to be significantly different only for height. There are no data relating tree measurements to the three nursery soils for the Fire Island trees.

The seedlings growing in the Fafards mix were also subject to the greatest degree of damping-off, 41% as compared to 30.5% for the peat/vermiculite with native soil, and 27.4% for the peat/vermiculite potting mixes.

DISCUSSION

Progeny testing is a simple method of selecting trees that produce seedlings with better survival and juvenile growth rates by comparing the survival and growth of their half-sib open- pollinated offspring. One of the problems that may arise from this type of selection method, requiring us to return to the parent tree for seed, once it has been selected, is the infrequency of good crops of cones. Pitch pine may bear poor crops for two successive years, with an excellent crop every 4-9 years (Little 1959). So, obtaining seed is dependent on the vagaries of trees' cone crops. It should be noted that 1986, the year that parent trees were selected, was a particularly poor seed production year.

Pitch pine is subject to a great degree of genotype x environment interaction, also termed phenotypic plasticity (Ledig and Fryer 1974). In other words, its general appearance can be greatly modified by its environment. Anyone who has observed pitch pine in the wild has witnessed this high degree of variability. The sites in both Cape Cod and Fire Island are extremely harsh, characterized by virtually sterile soils and strong prevailing winds laden with salt. Under these conditions, trees are scrubby and misshapen, rarely attaining heights above 5 m. The trees are so homogeneous that selecting for any of the characteristics of tree form (stem straightness, branching habits, etc.) becomes virtually impossible. As a result, our selections were essentially random. Progeny testing will give us an indication of the range of survival and growth characteristics transmitted by the parents. We will undoubtedly be able to select parents that are superior to the others, but by how much we can't tell. It would require

a much larger sample population to establish baselines. Of all the trees collected, there were three that did exhibit the characteristics we were selecting for on the exposed sites. Selection was based on adult tree form. These trees appeared exceptionally healthy and had a very low, broad crown with many branches partially buried by the windswept sand. Notable specimens were Cape Cod HH7 and Fire Island SF9 and SF10. Fortunately, these three trees were prolific "coners" during this otherwise poor cone production year.

One possible benefit of our selection method may be a result of the fact that 1986 was a poor cone production year. We were essentially limited to selecting any tree that bore cones, so we may have inadvertently selected for trees that are better seed producers. This would be of benefit in the long run because these trees, if they are more prolific seeders, will have the effect of revegetating the areas where they are planted more efficiently than a less prolific seeder. If this is truly a quality of our selections, it will be difficult to measure. Cones are not usually borne on open grown trees until they are 8 to 12 years old (Little 1959), plus, all of our selections were "good-coners", and so we will have nothing to measure them against. They should all be relatively equal.

Little is known about juvenile/mature height correlation in pitch pine. Two closely related species, shortleaf and loblolly pine (Ledig and Fryer 1974) have both had early stage growth measurements correlated with measurements taken later in the trees' life. Dorman (1976) suggests that in loblolly pine, growth rates seem to be established in nursery beds a year or so following planting, which indicates the performance to be expected in following years, but predictions for a wide range of years may be less accurate. He also states that in a racial variation study of shortleaf pine, the taller seedlings after one year of growth continued to be significantly taller 5 to 7 years later. On the other hand Goddard et al. (1983) claim that measurements at one year are seldom correlated with later measurements. Most of the available evidence seems to support this opinion. Another important aspect that will bear on growth over the course

of years is the fertility of the planting site. A good site will tend to bring out genetic differences, while a poor site will tend to mask them (Goddard et al. 1983). In our case, all three planting sites are good approximations of the natural environment in which pitch pine occurs, that is, they are very poor. Given this, and the fact that there are no studies correlating early and later growth of pitch pine, it would seem prudent to assume that our first year measurements may have little relation to eventual growth rates.

The only information that our measurements really give us is a measure of the vigor of the seedlings. This may prove to be important as time passes, as the larger, more vigorous seedlings are better able to survive. We have found that there are significant differences in survival, related to stem diameter, of the seedlings that were fall planted. The smaller seedlings had a higher rate of mortality, succumbing to desiccation during their first winter. Seedlings should not be planted until they have reached a certain size. Another important and well studied factor related to survival is stress. Stress plays a major role in the growth and survival of individual plants. Plants that are under stress from factors such as drought, winter desiccation, nutrient deficiencies, etc, are much more likely to perish. These factors may also predispose seedlings to additional damage by diseases and insects.

The diameters of the alive versus dead seedlings from the Cape Cod data set showed a significant ($p < 0.05$) relation to seedling mortality. The dead trees had a mean diameter of 2.6 mm, while the living trees averaged 3.25 mm mean stem diameter. The dead trees, though dry, did not appear shrunken. When these trees were planted they all had developed well-lignified stems, which tend to retain their diameter upon drying. The data were then broken into ten quantiles, each representing one-tenth of the total measured range in diameters (Fig. 3a). Mortality decreases as stem diameter increases. The graph of cumulative mortality (Fig. 3b) shows us that at the point at which mean stem diameter reaches 2.8 mm, approximately 70% of the total mortality has occurred.

This may be a good minimum size specification for seedlings in order to decrease mortality following fall planting. A minimum stem caliper of 2.5 mm is recommended for plantable container grown western conifers, but this is a judgment value that has not been tested (Owston 1974). Stem diameters from 2.5 to 3.0 are the recommended target sizes for outplanting conifers in British Columbia (Van Eerden 1974). All southern pines, excepting shortleaf, should attain a diameter of 3.2 mm (1/8 inch) before outplanting (Balmer and Williston 1974). Our estimate of 2.8 mm or greater is within the target range for many species of container grown, one year old seedlings, prior to outplanting. Pitch pine suffers from "inbreeding depression", and a certain amount of self-pollination may occur which may result in a small proportion of aberrant phenotypes (Ledig and Fryer 1974). Some of these were probably the seedlings that were particularly small and undoubtedly contributed to the mortality over the winter. Such small trees should be screened out prior to outplanting.

The overall value of the results of the analysis of variance among the three nursery soils tested is minimal because the trees grown in the Fafard's mix, though larger, were subject to the greatest degree of damping-off. Over 41% of the seedlings died before they reached the transplanting stage. The peat/vermiculite with 10% native soil (p/vns) added and the peat/vermiculite (p/v) potting mixes lost 30.5% and 27.4% respectively. The trees grown in the Fafards mix were also selectively watered with ferrous sulphate in an attempt to restore their health. After c. 6 weeks of growth they had become noticeably more chlorotic and stunted than either the p/v or p/vns trees. The ferrous sulphate lowered the soil pH which resulted in the trees' regaining their normal dark green color and boosting their growth. They soon outgrew the trees in either of the other two soils. Although the selective addition of the ferrous sulphate confounded our comparison of the three nursery soils, it provided useful information for later studies (see pH experiment).

In the data analysis of the progeny we found that there were significant differences among parents and among treatments, and that there was also a significant ($p < 0.05$) parent and treatment interaction. This means that the effect of parent on seedling growth was different at each level of treatment (ff, p/v, p/vns). Therefore, it would be inappropriate to isolate either of the two factors (parent or treatment) and do an analysis of means. In the future, if we want to perform a progeny test, we should grow the seedlings under uniform conditions in order to avoid interactions. We should grow the trees in different nursery soils only if we want to test nursery soils. The information that we do have enables us to make good generalizations about the relative sizes of the seedlings. The Cape Cod data set is large enough that we are able to use only one of the three nursery soils to analyze the mean volumes. The Fafard nursery soil provided the largest seedlings, so we should use it for the analysis, because we are interested in the seedlings that perform the best under the best conditions. This eliminates the interaction, but is a much smaller data set, so it doesn't provide as accurate an analysis as a larger data set would. This is the data set used for the analysis of the Cape Cod progeny.

M6, the tree ranked number two (figure 1), was the one tree that showed a number of very branchy offspring. Nine of the seedlings had developed from 3 to 6 branches that were often as large as the main stem itself. No other parents provided more than one offspring with any degree of branchiness. This "branchiness" is obviously an inherited trait, and would make an excellent tree for revegetating. Progeny of HH7, that was the best looking tree in the wild, were ranked first of all the trees selected from the Highhead site (figure 1), but were not significantly different in volume from trees HH6, HH3 or HH1. One interesting result was that the Provincelands sheltered site provided 5 of the 6 seedlots of offspring which grew largest (as expressed by D^2*H). The other sites, Marconi and Highhead are both exposed to the onslaught of

salt laden winds, and no discernible growth pattern among the seedlings occurred for these two sites. These results may be due to the powerful effect that selection has on creating a population best suited to an environment. The seedlings provided by the parents from the sheltered site have adapted to their particular environment where selection is probably concerned with competition and the advantages that rapid juvenile growth confers with respect to this. The slower growing seedlings from the exposed sites may be the result of different selection pressures. They may not be programmed for rapid growth, rather, their best strategy may be to stay closer to the ground and develop branches, in order to stabilize themselves and avoid the assaults of their windy habitat. There would seem to be little selective advantage in growing excessively tall. We may characterize these two types of seedlings, if they exist, as ecotypes. An ecotype is defined as the product arising as a result of the genotypical response of a population to a "definite habitat" or "particular habitat"(Spurr and Barnes 1980). The development of genetic variation between nearby stands of pitch pine is not unusual. For example, the distance between the Pine Plains, covered with a growth of dwarf pitch pine (<4 m tall), is only 35 km from Egg Harbor where some of the largest (30 m tall) specimens of this species grow. That this difference in growth is genetically controlled has been convincingly demonstrated by Good and Good (1975). In light of this, it does not seem unlikely that ecotypes may occur over the short distances (20 km) between our stands. We know that site and climate play significant roles in selection and that seedlings from a particular site are likely to be genetically the best adapted to that site. Depending on where revegetating is to be done, future sources of seed should be picked from the "best" individual trees nearest to each site to be revegetated . For revegetating on or near the Provincelands sheltered site, seed should come from trees PS2, PS4 or PS9. For High Head, select seed from trees HH1, HH3, HH6, and HH7. For Marconi, collect seed from tree M6.

The offspring of the 17 parents representing Fire Island have volume means that are grouped a little more closely than those of the Cape Cod trees' offspring. There is no predominant site among the five top trees; two come from the Sunken Forest and three from the Wilderness Area. There does not seem to be any ecotypic difference as expressed by growth rates. The five half-sib parents that produced the five top families of seedlings should be used for future seed sources, since there was a relatively large decrease in volume after the fifth ranked tree.

Seedlings planted at Floyd Bennett Field had mean volumes ranging from 3.0 cc to 1.2 cc. The two best families came from selections from the New Jersey pitch pine improvement project, Nos. 108 and 100. These two parent trees were originally selected on the basis of size and form, so it is not surprising that they provided the largest seedlings. It is too early to tell how provenances from New Jersey or Fire Island will perform on this landfill where no native population of pitch pine exists.

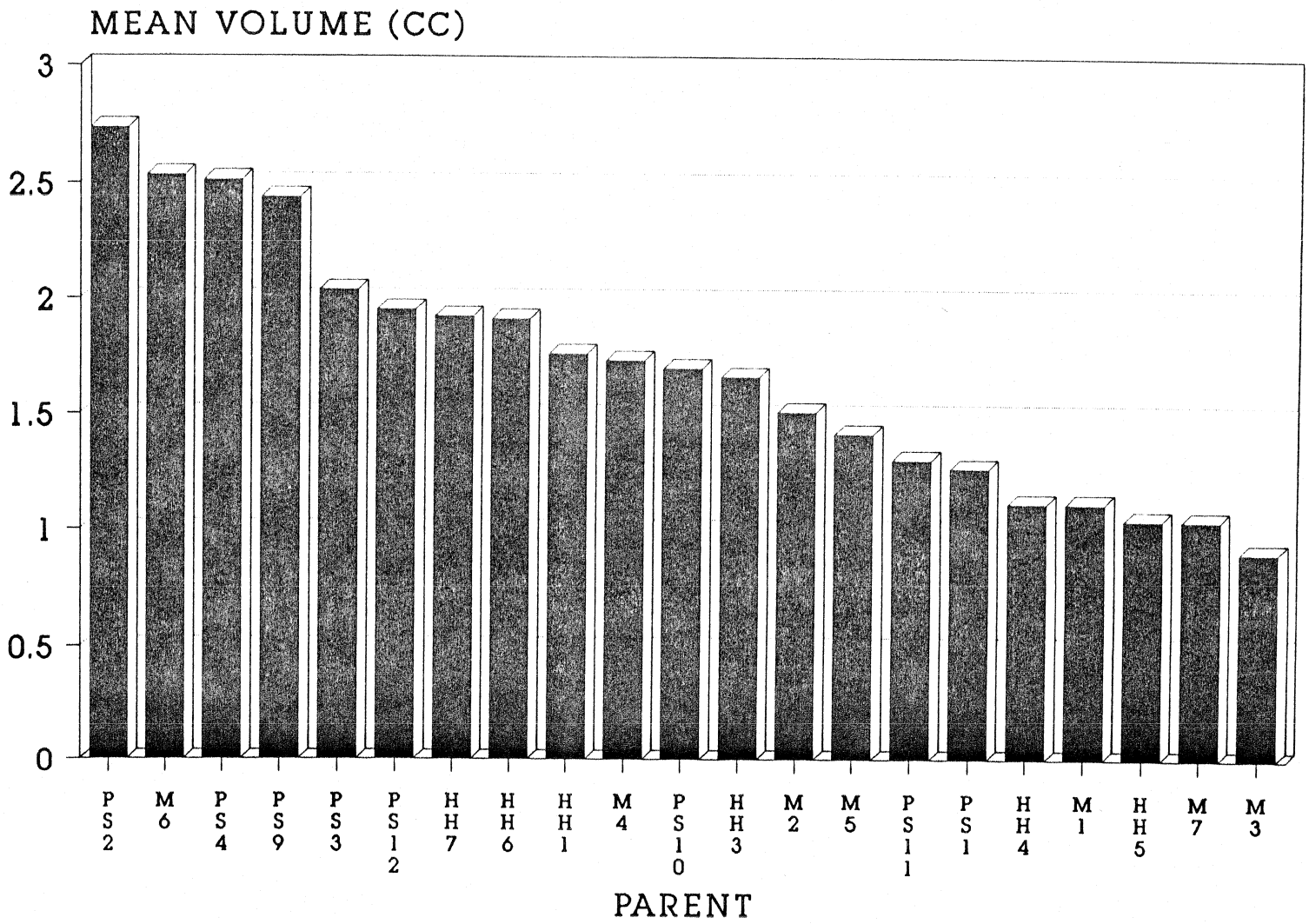


Fig. 1. Mean Volumes for the progeny of 21 parent trees from Cape Cod.

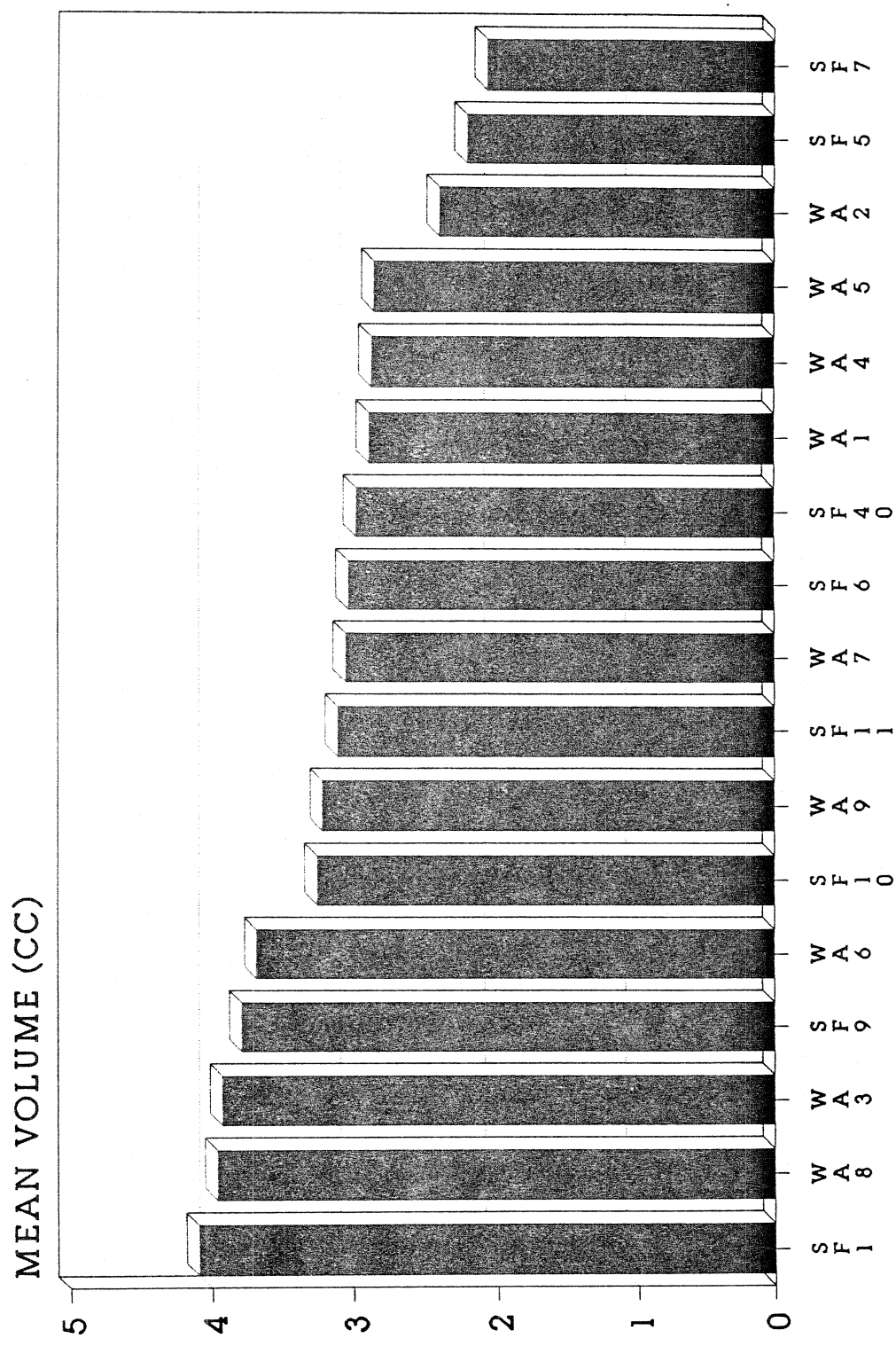


Fig. 2. Mean Volumes from the progeny of 17 parent trees from Fire Island.

Results obtained from Spring Measurements of seedlings at Cape Cod

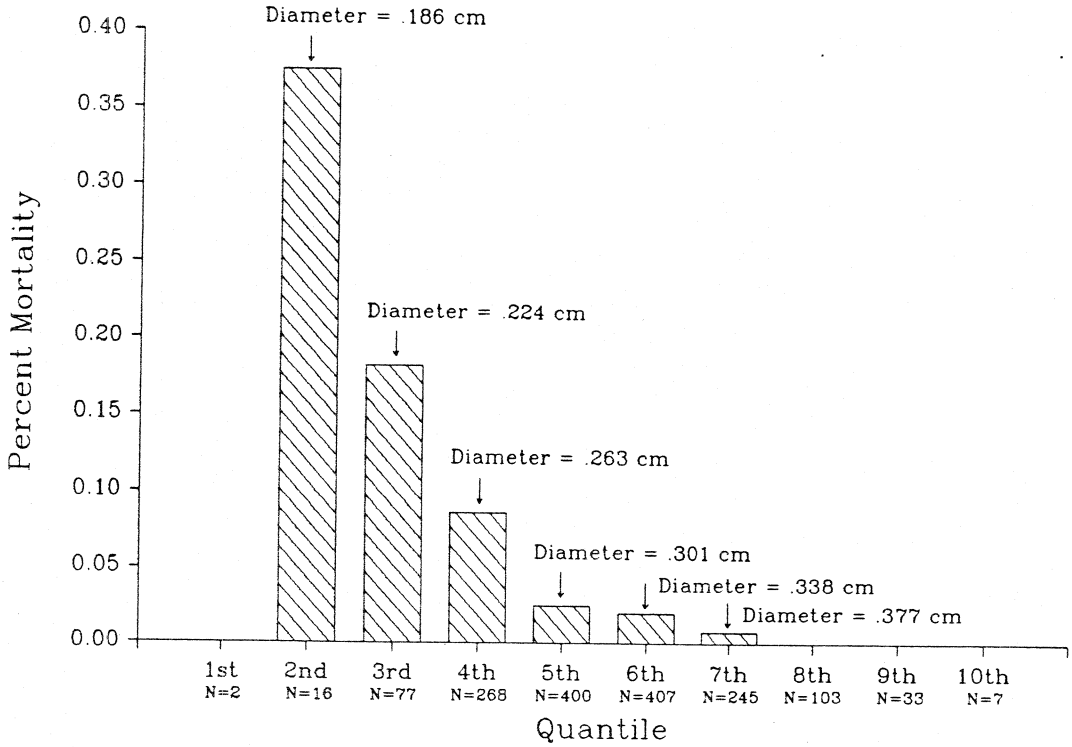


Fig. 3a. Percent mortality broken into 10 diameter quantiles.

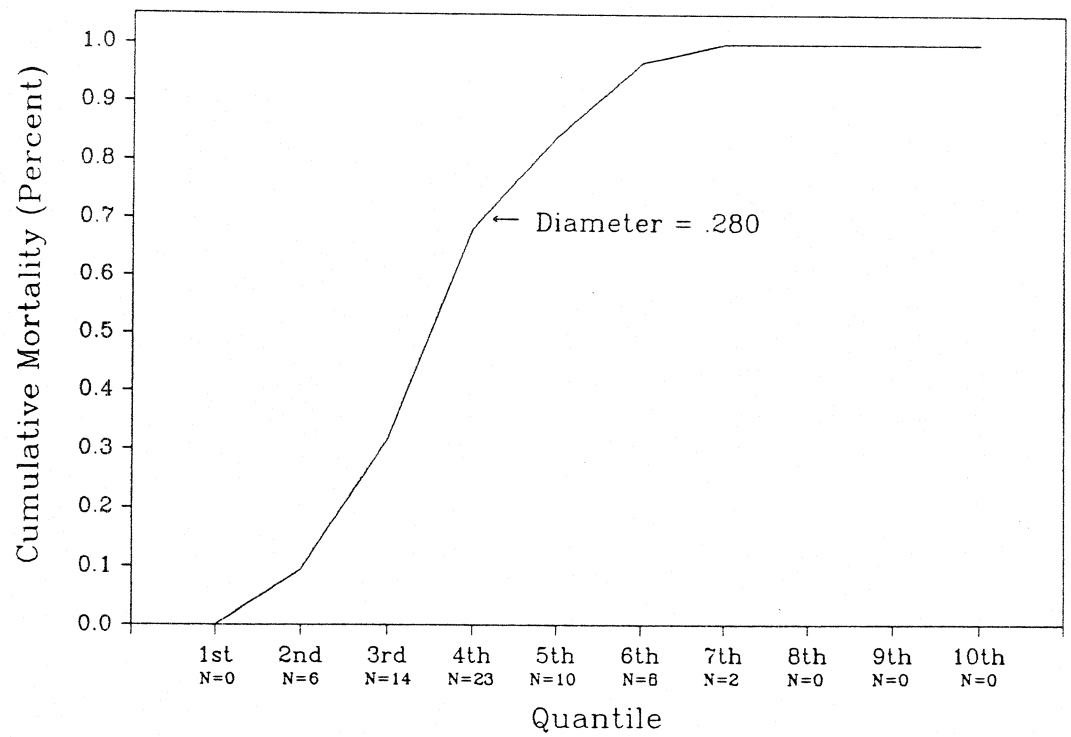


Fig. 3b. Cumulative percent mortality as diameter increases.

Chapter 2. Germination rates and number of seeds/cone

INTRODUCTION

In any nursery operation germination rates of seeds are important to know prior to seeding, and can be especially useful when working with containerized systems. The rates give an indication of the number of seed needed to sow in order to obtain a desired number of seedlings. Additionally, knowing the number of seeds/cone will permit determination of the number of cones required to obtain a known quantity of seed. Combining this with the germination rate gives an indication of the number of cones required to obtain a certain number of germinated seedlings. A knowledge of this information will facilitate future nursery operations and enable the nursery operator to better plan cone picking requirements and seeding rates.

METHODS AND MATERIALS

To determine germination rates a known number of seed, 296 in all but three cases, was sown directly into flats of 98 164 cc (10 cu. in.) Leach super-cells. Seeding individual racks can be facilitated by spreading the seed on a clear piece of glass or plexiglass, which rests on top of the rack of tubes. Seeds can be easily slid off of the edge of the glass and into the appropriate tube. As each row of tubes is finished, the glass is simply moved to the next row, and the process continued. When the far edge of the glass needs to be supported, it can be easily done with another rack of tubes.

This method can be used to sow 3 seeds/container, at a rate of about 500 cells/hour. The seed then received a 1 cm layer of potting soil and a small quantity of fine gravel to hold everything in place. The racks were then watered regularly, every other day. After three weeks germination was counted. Any seeds that germinated after this point were considered non-germinants. Such seedlings were a month or more behind schedule by this time, and if allowed to continue, would produce a non-uniform

rack containing many small seedlings by outplanting time. To determine the number of seeds/cone, 4 non-insect damaged cones were set aside from each parent tree. These four cones were placed together in a paper sack and allowed to dry. Some cones opened naturally; serotinous cones were heated in an oven at 48° C for 5 to 10 minutes, until the resin seals holding the cone scales melted allowing the scales to open and free the seed. When the cones had opened, all of the seed was extracted from the four cones, counted, and divided by 4 to obtain an average. An alternate method of opening cones required drilling the center out, 3 cm deep with a 1 cm (3/8") drill bit. This destroys the integrity of the cone so that it may then be easily pried apart. The seed were then separated from the cone debris and counted.

Germination rates were multiplied by the number of seeds per cone to enable us to determine the number of germinants per cone.

RESULTS

The overall germination rate for all 54 parents was 50.2% with a standard deviation of 22% (Table 1). The highest rate was 93% while the lowest was 2%. My average value was somewhat lower than the results listed by USDA (1974) for pitch pine of 31 samples at a rate of 69%. This is not unexpected as these were from laboratory tests that take place under the most favorable of conditions of moisture and temperature. Consequently they are often higher than what would occur under greenhouse conditions, often by 20 to 50% (USDA 1948).

The overall mean number of seeds/cone for all 46 parent trees is 95.2 with a standard deviation of 26.7 (Table 2). The lowest area mean came from cones collected at the Wilderness Area, 81.3 seeds/cone, while the highest area mean came from cones from Highhead, 115.0 seeds/cone. The tree with most seeds per cone (151) was from tree M6, and the one with the fewest (22) was tree PS9.

The overall average of germinants per cone is 45.5 for all 46 parents, with a standard deviation of 25.8 (Table 3). The lowest area mean of 39.1 came from cones collected at the Wilderness Area, while the highest rate of 69.2 germinants per cone came from the Provincelands sheltered area. A low value of 2.2 germinants/cone came from tree HH2, which had the lowest germination rate (2.1%), while a high value of 106.3 germinants/cone was obtained from PS2.

DISCUSSION

The germination rates from this experiment give a practical answer to the question of how many healthy pitch pine seedlings will sprout from a given quantity of seed sown in containers.

The sowing rate of 3 seeds per container worked quite well across the range of germination. This means that there is some thinning that will need to be done in most cases, but also that there are very few empty tubes even with the seed with poor germination. With 3 seeds per container the germination rate can drop as low as 33 % and a reasonably full flat of trees will be obtained. Tinus and McDonald (1979) suggest that seed with viabilities below 75% should not be used. If low viability seed is used it requires heavier sowing with thinning later. This recommendation does not apply to small scale operations such as those appropriate for the National Park Service, only to a large mechanized operation where these factors can create a lot of extra labor.

Frasco and Good (1976) studied pitch pine cone characteristics and found means of 102, 94, 87, and 77 seeds/cone from different populations in the Pine Barrens. Ledig and Fryer (1974) came up with an average of 74 seeds/cone with a range from 3 to 151. A study by McIntyre (1930) of the cones of a single tree found an average of 38.12 seeds/cone. We can see that the number of seeds/cone for any given tree or stand of trees is variable. The values obtained in this experiment seem to fall within the range of what others have found. These results allow us to estimate the number of cones to pick

in order to produce a given amount of seed. Once the seeds have been extracted from the cones they should be briefly examined, and any off color seeds should be discarded. A healthy pitch pine seed is dark brown to black in color with an echinulate or rough surface. Any seed that have reddish-brown streaks or light tan spots are invariably sterile. McIntyre (1932) found that the greatest number of viable seeds are found in the cones located in the middle portions of the trees' crowns, and come from the middle portions of the individual cones. Once the seed has been cleaned, a brief "cutting test" can be performed that will give a rough estimation of seed viability. Seed should be cut open and those with a fully developed, firm, healthy looking embryo can be considered viable. From this knowledge it can be decided whether 2 or 3 seeds should be seeded into each container. If viability over 50% is estimated then 2 seeds/container should be used. If it is less than 50%, then 3 seeds should be used. It can also be useful to seed an extra flat of cells. You can then replace blank ones with the extras.

The fertility of any particular individual is a function of genetic make-up, its proximity to neighboring compatible trees, vagaries of the weather, and cone predatory insect populations. Neither of the first two factors should change appreciably from year to year, while weather is unpredictable and may affect pollination. Conifers release pollen during dry weather (Eis and Craigdallie 1981), so extended wet periods during the peak of pollen shedding may affect the cones that will be produced 16-18 months later. Wind direction may also affect pollination, with wind shifts away from the prevailing direction responsible for changes from the usual. Populations of insects predatory on cones will also fluctuate from year to year. All of these factors will affect the number of viable seeds produced by an individual tree's cones from year to year.

Assuming that the values do not change appreciably from year to year for a given individual, then the results of this experiment can be used to forecast the number of viable seedlings to be obtained from the cones of a selected parent.

TABLE 1. Germination rate in Leach Super-cells

Parent	Percent germination	Parent	Percent Germination
WA1	39.2	M1	22.9
WA2	36.7	M2	92.7
WA3	69.1	M3	67.0
WA4	57.6	M4	47.2
WA5	14.2	M5	19.8
WA6	35.4	M6	55.5
WA7	55.5	M7	28.5
WA8	56.6	**mean**	**47.7**
WA9	65.6		
mean	**45.5**	CN1	52.4
		CN2	46.9
SF1	23.9	CN3	25.2
SF4	23.2	CN4	33.3
SF5	35.8	CN5	62.1
SF6	44.4	CN6	68.4
SF7	39.7	CN7	15.3
SF9	55.2	**mean**	**43.4**
SF10	56.6		
SF11	32.9	DK86	54.9
mean	**39.8**	DK89	61.8
		DK98	75.3
PS1	67.4	DK99	74.6
PS2	83.7	DK100	77.4
PS3	69.4	DK102	19.1
PS4	82.3	DK107	65.6
PS9	66.3	DK108	67.4
PS10	36.1	**mean**	**62.0**
PS11	86.8		
PS12	83.7		
mean	**70.3**		
HH1	59.4		
HH2	2.1		
HH3	52.1		
HH4	21.9		
HH5	19.8		
HH6	62.8		
HH7	22.6		
mean	**34.4**		

TABLE 2. Average number of seeds/cone for all parents

Parent Tree:	Avg. No. Seeds	Parent Tree:	Avg. No. Seeds
Marconi 1*	103	Sunken Forest 1*	90
Marconi 2	92	Sunken Forest 4*	82
Marconi 3*	103	Sunken Forest 5*	134
Marconi 4**	48	Sunken Forest 6*	103
Marconi 5	56	Sunken Forest 7*	100
Marconi 6*	151	Sunken Forest 9	94
Marconi 7	110	Sunken Forest 10	115
Total mean	**94.7**	Sunken Forest 11	87
Std. Dev.	32.1	**Total mean**	**100.6**
		Std. Dev.	15.9
Provinceland Shelt. 1	108		
Provinceland Shelt. 2	127	Colt's Neck 1	58
Provinceland Shelt. 3	117	Colt's Neck 2*	84
Provinceland Shelt. 4	119	Colt's Neck 3*	64
Provinceland Shelt. 9	22	Colt`s Neck 4	78
Provinceland Shelt. 10	112	Colt's Neck 5	92
Provinceland Shelt. 11	96	Colt's Neck 6	93
Provinceland Shelt. 12	68	Colt's Neck 7	132
Total mean	**96.1**	**Total mean**	**85.9**
Std. Dev.	32.8	Std. Dev.	22.5
High Head 1	99	Wilderness Area 1**	89
High Head 2	103	Wilderness Area 2	26
High Head 3	126	Wilderness Area 3*	89
High Head 4	115	Wilderness Area 4	71
High Head 5	119	Wilderness Area 5	110
High Head 6	138	Wilderness Area 6*	65
High Head 7	108	Wilderness Area 7*	94
Total mean	115.0	Wilderness Area 8*	98
Std. Dev.	12.6	Wilderness Area 9	90
		Total mean	**81.3**
		Std. Dev.	23.3

Trees with an * were serotinous

Trees with an ** exhibited both serotinous and open cones.

TABLE 3. Germinants per cone

Parent	Germinants/Cone	Parent	Germinants/ Cone
WA1	34.9	M1	23.6
WA2	9.5	M2	85.3
WA3	61.5	M3	69.0
WA4	40.9	M4	22.6
WA5	15.6	M5	11.1
WA6	23.0	M6	83.8
WA7	52.2	M7	31.4
WA8	55.5	**mean**	**46.7**
WA9	59.0		
mean	**39.1**	CN1	30.4
		CN2	39.4
SF1	21.5	CN3	16.1
SF4	19.0	CN4	26.0
SF5	47.9	CN5	57.1
SF6	45.7	CN6	63.6
SF7	39.7	CN7	20.2
SF9	51.9	**mean**	**36.1**
SF10	65.1		
SF11	28.6	HH1	58.8
mean	**39.9**	HH2	2.2
		HH3	65.6
PS1	72.8	HH4	2.2
PS2	106.3	HH5	23.6
PS3	81.2	HH6	86.7
PS4	97.9	HH7	24.4
PS9	14.6	**mean**	**40.9**
PS10	40.4		
PS11	83.3		
PS12	56.9		
mean	**69.2**		

Chapter 3. Cone picking experiment

INTRODUCTION

Because of the problems encountered in picking cones for progeny testing (Chapter 1) it would be advantageous to determine the earliest feasible date to pick pitch pine cones. Many of the tagged trees on Cape Cod and Fire Island were robbed by squirrels of their most recent cone crop by the first week in October. In many cases the branchlets that had borne the cones were lying beneath the trees and appeared to have been removed no more than one to two weeks earlier. This would indicate that it would be wise to pick cones in the first or second week of September, if seeds are viable. At this time of year the cones are still very green with only a hint of brown on the tips of the cone scales. It is generally thought that cones are "ripe" when they lose this green color and begin to dry and turn brown. One might also assume that squirrels know when the seeds are ripe and wait to cut cones at this time.

The literature regarding picking dates is somewhat conflicting. Sudworth (1900) states that ripe pitch pine cones are light russet brown in color and should be collected in October-November, but he also says,"cones and cone-like fruits are exceptional in not always giving external signs of maturity of their concealed seeds. Thus, the cones of the pines may have mature seeds under their scales from one to several weeks before the cones appear to be ripe, in fact cones with ripe seed may appear quite green externally". In Seeds of Woody Plants in the United States (USDA 1974) it is stated that the pre-ripe color of cones is green, whereas the ripe color is a yellow brown and that the cones should be collected in September. However, in September the cones will normally still appear quite green. To confuse matters more, Pettis (1909) states that no specific rule can be given nor definite time stated because of variation from year to year. Drought in August will delay the maturity of some species, while a warm wet August will hasten ripening. Slope and exposure will also

species, while a warm wet August will hasten ripening. Slope and exposure will also affect ripening dates.

METHODS AND MATERIALS

Cones used for this study were collected from an open grown pitch pine tree located at the intersection of Route 539 and McMahon Rd. in Ocean County. The tree was approximately 12 meters tall with a 30 cm dbh and was chosen because it had a large number of cones distributed throughout its crown. Cones were picked on each of nine dates: 8/28, 9/4, 9/11, 9/20, 9/25, 10/3, 10/9, 10/16, and 11/16 of 1987. On each picking date six to ten cones were selected from throughout the tree's crown. This was done in order to minimize the effect crown position has on germination (McIntyre 1932). Cones were stored in brown paper bags to allow drying and prevent excess moisture accumulation. After the cones were air dry they were measured for length, width, weight, and volume displacement. The data were analyzed for significant differences by Duncan`s multiple range test. In January 1988 the serotinous cones were opened by dipping them briefly (2 to 4 seconds) into boiling water. They were then placed in a box with a 100 watt incandescent bulb to provide warmth and complete the opening process.

The recommendations for the number of seeds to use for a germination test are not less than 400, and preferably 800-1000 seeds per test (USDA 1974). For my test 500 seeds were used. Seeds were collected from the cones from each picking date and the wings were removed. Each lot of 500 seeds was weighed. The seeds were then placed in petri dishes lined with filter paper. Each picking date was represented by five dishes containing 100 seeds apiece. The filter paper was moistened with distilled water and the dishes were placed in the seed germinator at 30° C for 16 hours alternating with 8 hours at 20° C. This is the recommended germination test condition for pitch pine

(USDA 1974). Germination counts were made at 7 and 14 days. At 7 days a seed was counted germinated if it had a radicle 1 cm or more long. At 14 days germination was broken into three categories; **fully germinated** (radicle and cotyledons fully developed), **incomplete germination** (radicle emerged and cotyledons partially developed), or **ungerminated** (no evidence of any germination. The data were analyzed for significance with a simple regression analysis.

In order to determine if the seeds from the earlier picking dates were more prone to disorders over the course of several months of growth, 21 healthy, germinated seeds from each picking date were transplanted into Leach super-cells on February 24 in a soil mixture of peat:sand:vermiculite (1:1:1) with no amendments. At planting time the seedlings were one week old, the radicle was 2-3 cm long, and the cotyledons were still contained within the seed coat. Eight days later, on March 3, all the seedlings had lost their seed coats and the cotyledons were fully expanded. All seedlings were healthy and looked alike. The seedlings were fertilized with Peters soluble 14-15-16 on two occasions, March 16 and April 14, at a rate of 2 grams/liter of water. All seedlings were measured for height on May 17. Duncan's Multiple Range Test was used to separate the individual treatment means when the Anova indicated a significant treatment effect ($p \leq 0.05$).

RESULTS

At 7 days the average germination was 86.7%, with a low of 72.8% for cones picked 8/28, and a high of 97.8% for cones picked 10/3. At 14 days, if the first two categories (**fully germinated** and **incomplete germination**) are combined and consider germinated, then average germination rate is 91%, with a standard deviation of 3.75%. However, if I consider only the **fully germinated** seed as germinated then the average drops to 80%. Most of this drop is attributable to the greater number of

incomplete germination seed from the earlier picking dates (Fig. 4). There is an obvious trend toward increasing amounts of **fully germinated** seed on the later picking dates ($t = 2.58$, $p = 0.036$) with a concomitant decrease in the number of **incomplete germination** seed ($t = 3.95$, $p = 0.006$). There is no apparent pattern to the line depicting **ungerminated** seed ($t = .29$, $p = 0.78$) with the highest amounts on early (8/28), late (11/16), and middle (9/25) picking dates.

An analysis of the heights of the 21 planted seedlings found that there was no significant difference between the means of seedlings from the first (8/28) and the last (11/16) picking dates. Some significant differences ($p < 0.05$) were detected among the treatments but this appeared to be due to cultural practices. In order to minimize this, the seedlings should have been randomized within the rack. It appears that the pre-germinated seed from the earliest picking dates grew normally, and as rapidly as seed from the later dates.

The analysis of the cone measurements shows that the cones picked 11/16 had the greatest mean in all four measurements (length, width, weight, and volume) and there seems to be a general trend for the cones picked on the later dates to be longer, wider, heavier, and with a greater volume (Table 4). However, these differences are in almost all cases not large enough to be considered significant.

From the weights of the 500 seed there appears to be no obvious pattern. The average weight was 3.75 grams for 500 seeds, with a standard deviation of .151 grams. The seed lot having the heaviest weight of 4.055 was picked 10/3, while the lightest seed lot, 3.544 grams, came from the preceding week, 9/25. The average weight of 500 seeds being 3.75 grams gives us a figure of 60,400 seeds per pound. This figure is quite close to the number of 61,700 seeds/pound listed by the Seeds of Woody Plants in the United States (USDA 1974).

DISCUSSION

Seeds which exhibit epigeal germination, (most conifers, including pitch pine) may be considered normally germinated when the radicle is four times the length of the seed, provided all structures appear normal. Seedlings infected or damaged by bacteria or fungi may be counted normal if they appear otherwise normal and infection has not originated from within (USDA 1974). After one week in the chamber most of the seeds had produced a radicle about 1 cm long, which is about four times the length of the seed itself. This is when the first count of germination was made. The average of 86.7 % is very good germinative capacity, better than the average of 67% for pitch pine that is the result of 31 seed lots tested by various authors for the germination test results in the Seeds of Woody Plants in the United States (USDA 1974). This is also considerably better than my rate of 50.2% as an average for the 54 families germinated for the progeny test. This rate was determined in the greenhouse situation, not in a germination chamber, so it is probably somewhat lower than it would otherwise be. At 14 days the final count of germination was made. This had to be the final count as the seeds in the plates from 8/28 and 9/4 had become partially overgrown by fungi and were starting to become digested. The seeds from the later dates, 9/25 and on, were **fully germinated** in almost all instances, while the dates preceding this had more and more seed that exhibited **incomplete germination**. More rapid and complete germination occurred in seedlots collected on 9/25 or later. This can be an important factor as seeds and seedlings are very vulnerable during this period, and the sooner they are past this stage, the less likely they are to perish.

The high incidence of contamination in the first two seed lots, 8/28 and 9/4, suggests that these seedlots contained substances that enhanced fungal contamination. However, it is important to bear in mind that the moisture and temperature conditions

within the germination chamber are not only optimal for seed germination, but also enhance the growth of fungi. This does not explain the reason that the seed from later dates suffered little or no contamination, restricted to individual seeds at most. The seed picked on these first two dates, though it germinated well, may not have matured enough to have elaborated its easily digestible sugars into the more complex storage starches and fats of the endosperm, before it was picked from the tree. It seems likely that a mature seed will develop its reserves (endosperm) into forms that are not easily utilizable by pathogens. A seed must often endure long periods before it has a chance to germinate. If it is to survive then it should make itself as "unattractive" as possible. Obviously, the seed coat is the first line of defense, but during germination, after the seed imbibes water and the seed coat has split open, there is a period before the endosperm has been absorbed by the growing seedling. It is during this time that the endosperm can be easily attacked by any of the ubiquitous fungi and bacteria that are present on and around it.

Germination in the laboratory takes place under the most favorable conditions of moisture and temperature. Consequently it is generally higher that what would occur in nature, often by 50 to 20 percent (USDA 1948). The information that we have tells us that under the most optimum conditions, we had more rapid and complete germination from seed picked 9/25 and after. It tells us little about what would occur under the more natural circumstances of nursery conditions. It would prove interesting to run this experiment again with a nursery performance check that would tell us if the seed from the early picking dates are capable of surviving the vulnerable period following seed coat opening in a normal potting soil.

Another important facet of an experiment of this type that should not be overlooked is the specific gravity of the cones at picking time. Specific gravity is the fresh weight of the cone divided by the volume. This is a measure that is accepted for predicting ripeness for a number of pine species, but no figure is given for pitch pine.

For most species the value is between .80 and 1.0. Perry (1922) has picked very green pitch pine cones on Sept.9 (spec. grav. > 1.0), which opened normally and the seed germinated as rapidly and as completely as that from "ripe" cones picked one month later. So, specific gravity of the cones should be obtained at each picking date. It would also prove useful to germinate seed from each picking date directly into a potting medium along with the regular germination test. We would then have a measure of how well the seed would perform under less optimal circumstances.

Germination rates were exceptionally good for all weeks that this seed was picked, though it was more rapid from seed picked 9/25 and after. For future picking I would recommend waiting to pick seed until the 2nd or 3rd week of September. The cones will still appear green, or unripe, but the seed contained should be viable. Pitch pine is apparently one of those species that Sudworth (1900) considers exceptional in that the condition of the cone is not indicative of the ripeness of the seed therein. At this relatively early picking date we should also be free of squirrel predation.

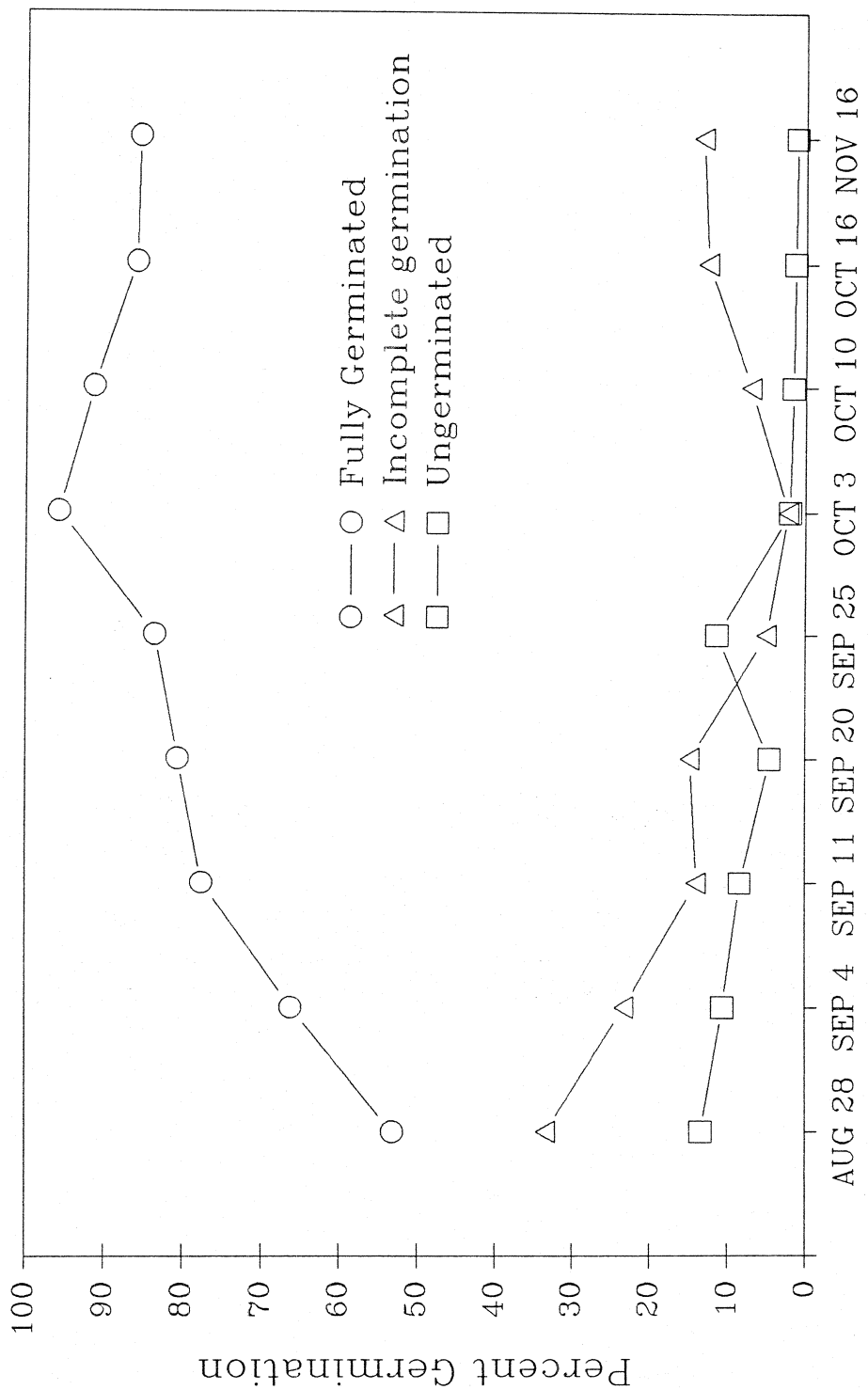


Fig. 4. Germination at two weeks

TABLE 4. Means of measurements of cones picked on nine successive dates in 1987

DATES	LENGTH cm	DIAMETER cm	VOLUME cc	WEIGHT gm
8/28	5.91 a	2.95 b	24.42 abc	13.35 ab
9/4	5.76 abc	3.10 ab	26.38 abc	14.16 ab
9/11	5.66 abc	3.00 ab	23.75 bc	13.12 ab
9/20	5.50 c	3.04 ab	24.20 bc	13.70 ab
9/25	5.57 bc	3.01 ab	23.10 c	12.38 b
10/3	5.96 a	3.10 ab	26.35 abc	14.72 a
10/9	5.87 ab	3.10 ab	27.20 ab	14.42 a
10/16	5.93 a	3.11 ab	27.35 ab	14.85 a
11/16	6.00 a	3.15 a	27.95 a	14.97 a

Means with the same letter are not significantly different

Chapter 4. Time of year to plant

INTRODUCTION

During the initial phase of nursery production, seeding in January was not early enough to produce seedlings for spring planting, but did produce seedlings large enough for fall planting. Because spring planting is desired, a flat of seedlings was seeded in each of the months June through December 1987, for spring planting in 1988. In this way it can be determined how early it is necessary to plant the seeds in order to get a seedling that is the appropriate size for spring outplanting.

METHODS AND MATERIALS

Each mid-month, from June through December, a rack of 98 Leach 163.9 cc (10 cu.in.) super-cells was filled with potting soil, and seeded at a rate of 2 seeds per seedling tube. Mixed seed collected from Colt's Neck, N.J. in September 1986 was used as a seed source. The basic soil for all 7 racks was peat/vermiculite/sand (1/1/1) with the following nutrients:

56 grams triple super phosphate (46% P_2O_5)

28 grams sulfate of potash (60% K_2O)

336 grams 14-14-14 Osmocote

216 grams dolomitic limestone

8 grams fritted trace elements

28 grams Aqua-Gro (a wetting agent)

These nutrients were added to each .076 cubic meters (2 bushels) of potting medium. Seedlings were thinned to 1 per tube 2-3 weeks following seeding. The fungicide Benlate was applied as a drench at this time to prevent damping-off. Racks of trees were transported up to the greenhouse at Floyd Bennett Field on three occasions where they overwintered. The seedlings were maintained under the normal winter

greenhouse conditions at Floyd Bennett Field. The low temperatures averaged 11.4° C and the highs averaged 23.7° C for 29 readings taken Dec. 15 through March 11. They received no additional illumination beyond ambient winter daylight. Height and diameter of the seedlings were measured in April 1987.

RESULTS

The seeds planted in June 1987, the earliest planting date, produced the largest seedlings, with an average height of 14.6 cm and average diameter of 0.44 cm. The July-started seedlings were the next largest, followed by the September seedlings. It is interesting that the August-grown seedlings were very small, similar in size to the November-sown seedlings. The October, November, and December started racks were successively smaller than the September seedlings and each other (Table 5).

DISCUSSION

The June-planted seedlings, though the largest, were deemed unsuitable for outplanting. The roots of these seedlings were twined into an extremely thick fibrous mass that had completely filled the tubes, and was emerging in a knot at the base. This is one of the drawbacks of growing containerized seedlings, where the root configuration is determined by the shape and size of the container. Circling (girdling) roots and a reduction in lateral roots are the two major problems associated with containers (Tinus and McDonald 1979). There seems to be little reference to the appropriate size that a root system should grow to before outplanting. This is because of the large variety of shapes, sizes, and materials from which tree container systems are constructed of, each one of which will have an optimum size for root growth. In one instance, a dry weight of 0.3-0.5 grams of root mass for British Columbia conifers is recommended for Styro-plug 2 containers (Van Eerden and Arnott 1974). They also state, from their

observations, that planted seedlings free of any container restraint results in relatively symmetrical root form, and that an excessive and overly compacted root mass may result from undue extension of the growing period. Generally, most of the recommendations for containerized seedling outplanting sizes are for heights and diameters, and it is assumed that this includes a proportional root system that is not overdeveloped. The root systems of the June planted seedlings were overdeveloped and had very poor form. There was no system of laterals, and there were many intertwined roots. The container had probably restricted growth for some time, and the nature of the present root system will possibly create future problems. Scarratt (1974) found that by increasing the size of a container he could significantly increase the size of the seedling, in a given growing period. The optimum size for a root system in any of the myriad of containers is rather subjective. For pitch pine in Leach super-cells the roots may be judged optimum when; 1)several roots have emerged from the base of the container and air-pruning of these roots is occurring; 2)the root mass will hold the soil in place when the seedling is pulled from the tube; 3)the seedling has formed a firm but unbound root plug. This should provide a seedling that has grown to capacity in this container, but is not yet restricted by it. All of the other seedlings had root development that was subjectively rated as good to excellent, even among the youngest seedlings.

Another interesting result was that the August-started seedlings were very small, smaller than either of the "younger" September or October-started seedlings. A possible explanation for this is that the August seedlings germinated, grew a little bit, and then responded to the shortening day lengths and became dormant. September is normally the time of year that pitch pine sets its next year's buds. The September and October-started seedlings germinated and began growing after this signal had passed, and so may not have become dormant.

Overall, it was surprising to find how small the seedlings from the later months were by spring planting time. Under the conditions at the Floriculture Greenhouse, Cook College, seed sown in Dec.-Jan. will produce seedlings of a suitable size for spring planting, much larger than the Oct.-Nov.-Dec. started seedlings that overwintered at Floyd Bennett Field. In a study of pitch pine grown under three temperature regimes, cool, moderate, and warm it was concluded that pitch pine is tolerant to a wide range of temperatures (Good and Good 1976). Ledig(1977) states that optimum photosynthesis in seedlings occurs at 25° C. The average greenhouse temperature at the Floriculture Greenhouse, Cook College fluctuated from an average low of 12.1° C to a high of 34° C for 17 readings taken November 20 through February 10. These are considerably higher averages than the winter temperatures taken at the Floyd Bennett Field greenhouse (see METHODS AND MATERIALS). However, the temperatures at Floyd Bennett Field, though below optimum for pitch pine, should be sufficient to provide for adequate photosynthesis, and are probably not completely responsible for the stunted growth of the seedlings at Floyd Bennett Field. Alternately it may be the lack of an artificially extended photoperiod, that slowed growth during the shortening winter daylengths. There does not seem to be any information available concerning photoperiod requirements for pitch pine. However, all of the controlled growth studies on pitch pine that have been reviewed have used a standard 16 hour light/8 hour dark photoperiod to simulate growing conditions (Good and Good 1976, Ledig et al. 1977, Schier 1985). As insurance when producing a fall crop of pitch pine this is the recommended lighting regime.

Trees started in Jan.-Feb. will be subject to increasing day lengths along with increasing temperatures in the greenhouse. Trees started in Sept.-Dec. will receive mixed messages that may slow growth and might even require the trees to be subjected to a cold requirement period before they will resume normal growth. This may explain the slow growth rates of the fall sown seed at Floyd Bennett Field. A photoperiod of 16 hour

light/ 8 hour dark was used for the pH experiment in which seedlings were started in late October. These seedlings grew normally and were suitable for spring planting.

This experiment was originally set up because of the faulty cultural conditions under which a January 1987 (see Chapter 1. Progeny experiment) crop of pitch pine seed was sown in. It has now become clear that by improving the potting soil a satisfactory crop of spring plantable pitch pine seedlings can be produced from January-sown seed in the Floriculture Greenhouse, Cook College. The results of this experiment have become academic in light of the improved potting soils that will allow the seedlings to grow faster. However, this experiment still leaves an unanswered question. How well will January-sown seedlings perform under the relatively cooler temperature regimes of greenhouse at Floyd Bennett Field? This could be easily tested by sowing seed in January at both the Floyd Bennett Field Greenhouse and the Floriculture Greenhouse, Cook College, monitoring the daily highs and lows, and measuring the seedlings after several months growth. Everything else being relatively equal, we may then find whether the lower average temperatures at Floyd Bennett Field are responsible for a difference in growth rates.

TABLE 5. Average heights, diameters, and volumes of seedlings planted over 7 consecutive months

	Avg. Ht.	Avg. Dia.	Avg. vol.
June	14.6cm	.44cm	2.83cc
July	10.0cm	.30cm	.90cc
August	6.3cm	.22cm	.30cc
September	9.6cm	.27cm	.70cc
October	10.9cm	.24cm	.62cc
November	8.9cm	.18cm	.28cc
December	6.2cm	.14cm	.12cc

Chapter 5. pH experiment

INTRODUCTION

Recent interest in growing pitch pine expressed by the National Park Service and the State of New Jersey has prompted an effort to produce containerized seedlings for outplanting. Initial studies, testing three nursery soils, produced slow growing seedlings with a high incidence of damping-off. "Damping-off" is a catch-all term for any of a variety of diseases that are pathogenic to seedlings. It was suggested that a common denominator for our troubles might be soil pH's in the range of 6.0-7.0, deemed too high for pitch pine. In a study of pitch pine growing naturally in southern New Jersey, soil pH ranged from 3.5 to 5.1, and in northern New Jersey it varied between 4.0 and 4.5 (Ledig and Little 1979). Illick and Aughanbaugh (1930) state that pitch pine grows naturally on acid soils and that for the best results it should have acid soil in the nursery.

Soil pH is probably the most commonly measured soil property because of its relationship to many aspects of soil fertility and plant growth (Adams 1984). Brix and van den Driessche (1974) suggest that the pH of the rooting medium for conifers should be adjusted to a value between 4.5 and 5.0. Tinus and Macdonald (1979) recommend target pH's of 5.0-6.0 for container grown conifers, and Benzian (1966) suggests that optimum growth of many conifers occurs over a very limited pH range. These figures represent a broad range of pH values, with the optimum being quite specific for any given species. From preliminary observations we know that a potting soil of pH 6.0 and above produces chlorotic and stunted pitch pine seedlings. At pH values below 4.5 in organic soils, many plant nutrients become limited in availability (Lucas and Davis 1961). The optimum pH for growing containerized pitch pine likely falls somewhere between these values. In a related study, Leyton (1952) examined the effect of pH and the form of nitrogen on the growth of Sitka spruce in culture solutions. He found that

regardless of the form of nitrogen, the trees amassed the greatest dry weight within a very specific range of pH, between 4.0 and 5.0.

The second problem that we addressed was the high incidence of damping-off that occurred in our preliminary experiments, and the role that soil pH might play with regards to this. Out of 4637 trees growing at the start, 1527, or 33% of them had died before they left the nursery 8 months later. This is an unacceptably high rate.

Because of the ubiquitous nature of the fungal spores that cause damping-off, it is difficult to exclude them from the nursery environment. Once pathogens are present there are several factors that help exacerbate the problems they may pose. Manion (1981) states that cool, wet, highly organic, and neutral to basic soils all benefit damping-off organisms, and that losses of 15% or more are not uncommon. Additionally, the high rates of fertilization required by containerized tree seedlings produce an environment unfavorable for mycorrhizae, which confer protection to unsuberized roots and can play a major role in preventing root infection and subsequent disease (Zak 1964). Thus, the greenhouse can provide an ideal environment for the buildup of pathogens that do not ordinarily represent a threat under natural circumstances.

Illick and Aughanbaugh (1930) state that if there is an excess of lime in the soil, nursery grown pitch pine seedlings turn yellow and are more susceptible to mortality. Damping-off may kill practically the entire stand of seedlings. In an experiment to control damping-off in Pennsylvania nurseries, Retan (1918) experimented with "a naturally acidic leachate from leaves". This treatment reduced damping-off of pitch pine seedlings by 30%. Perry (1930) has observed, that in 20 years of forest nursery experience, wood ashes, raw bone meal, or lime in any form applied to seed beds before germination will multiply damping-off losses many fold, especially for such species as pitch pine, shortleaf pine, and red pine. From the above statements it is apparent that

soil pH is an important variable related to (or influencing) incidence of disease. This study was undertaken to determine if pitch pine seedling growth and damping-off rates are related to soil pH in containerized tree seedling production.

METHODS AND MATERIALS

A simple method was used to test growth rates and disease incidence of pitch pine seedlings growing in different pH potting mediums. This involved starting with as low a pH soil as possible, and modifying it with increments of lime to raise the pH. This was deemed easier and more accurate than the alternative method of acidifying a neutral soil with increasing concentrations of sulfur (personal communication H. Motto). The initial soil mix used was a peat/vermiculite/sand (1/1/1) mixture. These materials are commonly used as a basis for any nursery growing medium, and provided a highly acidic starting point (pH 3.8). One drawback of potting soils containing peatmoss and vermiculite is their high cation exchange capacity (C.E.C), or resistance to a shift in pH (buffering). Other factors being equal, an organic soil such as this will require more lime (or sulfur) to effect an equivalent change in pH than will a mineral soil (Brady 1960). Twelve racks of soils of varying pH were constructed. The basic soil for all racks was peat/vermiculite/sand (1/1/1) with the nutrients:

56 grams triple super phosphate (46% P_2O_5)

28 grams sulfate of potash (60% K_2O)

336 grams 14-14-14 Osmocote

8 grams fritted trace elements

28 grams Aqua-Gro (a wetting agent)

This quantity of nutrients was added to each 0.076 cubic meters (2 bushels) of potting medium. This quantity of potting mix is sufficient to fill 4 racks of 98 164 cc (10 cu.in.) Leach super-cells. The potting soil was thoroughly mixed, spread 6-10 cm

deep on clean plastic film, and allowed to dry for 3 days. It was re-mixed several times during this period to insure uniform drying. The amount of dry-weight potting soil required to fill one rack of tubes weighs approximately 9.3 kg. To this amount of soil, increasing increments of finely ground dolomitic limestone (Baker's Ag Dolomite, J.E. Baker Co. York, PA) were added. The chemical composition of the limestone is CaO 30%, MgO 20%, $CaCo_3$ 53.5%, $MgCo_3$ 42%, with a $CaCo_3$ equivalent of 103.4%, and a CaO equivalent of 58%. For each treatment dolomitic limestone was added as shown in Table 6.

The potting soil for each rack (9.3 kg) was weighed on the beam balance and the requisite amounts of lime were thoroughly incorporated, by hand mixing, for each individual treatment. After filling, the racks were watered several times to re-wet the very dry peat moss fraction of the medium. The soils were prepared on 15 October 1987 and seeded one week later to allow the lime time to react. Upon incorporation into acid soils, limestone particles immediately begin to dissolve, and raise the soil pH (Barber 1984). Seed for the experiment was purchased from the Croshaw Nursery in Columbus, New Jersey. It was reported to have been collected from the Pine Plains, an area of pygmy forest in New Jersey's Outer Coastal Plain. Growth rates of Plains seedlings and those from (normal) barrens populations, under ordinary greenhouse conditions, are statistically identical for the first two years (Good and Good 1975). This experiment ran for 5 months so there should be no growth differences between these seedlings and average pitch pine seedlings. Three seeds were placed in each tube, for a total of 294 seeds/rack. The seeds were top dressed with a small amount of the same soil as in the rack, and then topped with a layer of fine gravel to hold them in place while they germinated. Germination was counted, on December 15 1987, 55 days after seeding. After germinants had been counted, the seedlings were thinned to one per tube. Any additional germinants which appeared during the experiment were weeded out.

Soil pH's were taken at 7-10 day intervals for the duration of the experiment. A standard method was used to measure soil pH (J. Walworth, personal communication). On each sampling date 15 to 20 grams of soil was removed from the center of one randomly selected tube from each treatment. The samples were placed in individually marked 50 ml beakers and an equivalent weight of distilled water was added. The mixture was stirred, and allowed to sit for 30 minutes. The electrode of the pH meter was submerged in the supernatant liquid, and pH was read after it equilibrated. The electrode was rinsed with distilled water after each reading. Soil pH's were measured on 15 separate dates.

Damping-off of seedlings was monitored on a regular basis, at approximately weekly intervals, beginning on 7 November 1987, about one week after germination. At this point, 2 weeks following seeding, seeds had germinated and seedlings were 2-4 cm tall. When a seedling died, it was removed and replaced by a toothpick. The number of seedlings that damped-off during the interval between observations were recorded. On three separate occasions tissue from dying seedlings was plated onto culture media. Tissue samples were taken from the area just above and below the soil surface. Sections approximately 3-4mm long were surface sterilized in 10% (by volume) bleach solution for 2 minutes, briefly blotted to remove excess liquid, and plated onto Difco potato dextrose agar (PDA). These were incubated at room temperature. After several days of growth the plates were observed. Of 14 seedlings tested for the presence of pathogens, all produced mycelium that was pigmented the characteristic pinks, reds, and purples of Fusarium spp. In most cases the Fusarium emerged from the cut ends of all four tissue samples from each diseased seedling. There was very little evidence of any other fungi, only one zygomycete, and little bacterial contamination. Representative colonies of the various shades of mycelium were examined for microscopic characteristics. Small ovoid microconidia, and fusiform macroconidia with a foot cell

were observed. These, along with the highly pigmented mycelium, are identifying features which separate Fusarium from the closely related genus Cylindrocarpon. Fusarium spp. consistently grew from all of the tissue samples.

In order to compare the growth rate of one of the strains of Fusarium, at various pH's, a single hyphal tip was taken from one of the representative purple colonies (the most common). The hyphal tip provides us with a single strain of the fungus, so that we aren't comparing the growth rates of dissimilar (unrelated) colonies. The hyphal tip was plated onto PDA and grown for several days until the colony was 4-5 cm. in diameter. In order to make plates of PDA of varying pH we started with 750 ml of distilled water with 29.75 grams of PDA added to it. This solution had an initial pH of 5.75 and was adjusted to pH 7.0 with 1M NaOH. A 125 aliquot ml was set aside in a 250 ml flask. The pH of the remainder was lowered to pH 6.5 with the addition of lactic acid, and another 125 ml aliquot was collected. We continued adding increments of lactic acid so that we lowered the pH to the values of 6.0, 5.5, 5.0, and 4.5, each time removing 125 ml aliquots to separate flasks. These flasks were autoclaved and the media was poured into 9 cm Petri dishes such that there were 6 plates of PDA at each of 6 pH levels. Plugs of actively growing Fusarium were cut with a #3 cork borer, from the margins of an actively growing colony, and one was placed in the center of each of the 36 plates of various pH plates of PDA. The growth of the colonies were measured at 2 day intervals, starting 2 days after the plates were inoculated, until the fastest growing colonies had completely filled the 9 cm Petri dishes at which time the experiment was terminated. An analysis of variance was performed on the data and the means were separated using Tukey's Studentized Range Test.

The seedlings were grown in the greenhouse for just over 5 months. Incandescent lights were set up on 30 November 1987 to increase the photoperiod to 16 hours. Temperatures ranged from a low of 8° C to a high of 41° C. The seedlings were watered

(water pH 7.5) on a regular basis, about every other day, for the duration of the experiment. The racks of trees were shifted to different positions on the bench on several occasions to minimize greenhouse effects. No added fertilizer or fungicide was applied. At the end of the experiment 30 seedlings were randomly selected from each treatment. Shoot height, shoot diameter, shoot weight, and root weight were measured; and presence of fascicled needles, and the number of primary branches were tabulated. Duncan's Multiple Range Test was used to separate the individual treatment means when the Anova indicated a significant treatment effect ($p \leq 0.05$). Additionally, the foliage of the 30 randomly selected seedlings growing in each treatment was combined and each lot was analyzed for 10 nutrients (N, P, K, Ca, Mg, Fe, Mn, Cu, Al, and Zn). To identify the linear relations between nutrients and pH, Pearson Correlation Coefficients were computed. The statistically significant values ($p \leq 0.05$) are reported in the text.

RESULTS

The germination rate for all racks was a mean of 101 seeds germinated, for a rate of just over 34%, with a standard deviation of 11.94. The minimum was 78 seeds germinated in rack 4 (4g lime/1 kg soil) and a maximum of 121 seeds germinated in rack 15 (15g lime/1 kg soil). There was no apparent correlation between soil pH and germination rate.

An analysis of the growth measurements of the seedlings found significant differences among treatments ($p < 0.05$) for all measurements except root weight. Potting medium #2, with 2g lime/1 kg soil provided the seedlings with the greatest height, diameter, volume, and root weight; however, they were significantly different from the next largest set of seedlings only in diameter and volume (Table 7). The pH of the soil that these seedlings grew in averaged 4.8 over the course of the experiment. The seedlings growing in the soils with 1 (#1) and 4 (#4) g lime/1 kg soil were largest or

next to largest in a dimension if the seedlings in potting medium #2 were not. That is, seedlings in #1 were greatest in shoot weight and seedling total weight and next to greatest in shoot height and volume, while trees #4 were next to greatest in shoot diameter and root weight. The seedlings grown with no lime added /1 kg soil (#0), with an average pH of 4.3, came in third greatest in height, diameter, volume, and root weight (Figs. 5 - 9, Table 7).

The trees growing in the soils with the least amounts of lime also appeared to be more mature than their counterparts in the more alkaline soils. The more acidic treatments exhibited fewer seedlings without fascicled needles as well as more seedlings having secondary branches. In treatments #0 and #1, 14 and 13 seedlings, respectively, had developed secondary branches, while only 2 seedlings, in each treatment, were fully juvenile leaved. Seedlings in treatments #30 and #40 had 10 and 13 seedlings that were still completely juvenile leaved, and only 3 and 0 seedlings, respectively, that had developed secondary branches.

A significant correlation was found between foliar nutrient content and rising pH values for the nutrients N, P, Ca, Al, Mn, and near significant for Mg. The elements K, Fe, and Zn displayed no apparent trends as pH changed (Table 8, Figs. 10 - 11). These results are treated individually in more detail in the discussion section.

The relationship between rising soil pH and seedling mortality is illustrated by Fig. 12. A total of 216 seedlings died during the course of the experiment, with 79% of this mortality occurring in soils with pH's of 6.0 and above. The remaining 21%, or 46 dead seedlings were distributed among the 7 racks of 98 trees with pH's below 6.0. This is mean damping-off rate of about 7%.

From the study of the growth rate of an isolate of Fusarium, from one diseased seedling, on varying pH's of PDA, we found that there was a significant increase in the growth of this isolate at the highest pH ranges. In an analysis of variance of the growth

rates, the fungus grew significantly faster($p < 0.05$) on PDA with the pH's 7.0 and 6.5, than on PDA with pH's of 4.5 and 5.0 (Fig. 13).

DISCUSSION

This experiment was designed to help us become better able to grow containerized pitch pine tree seedlings. It served the dual purpose of developing a medium that provides an optimum pH for pitch pine, as well as alleviating pressures from damping-off organisms. Because the seedlings were grown under uniform conditions, the only variable being increasing increments of dolomitic limestone, we can be sure that the modification of the potting medium by this product is responsible for the differences in growth that we had. We found that potting medium #2 provided the seedlings with the greatest height, diameter, volume, and root weight, and that the other mediums with small increments of dolomitic limestone added (#1 and #4) also grew healthy, large seedlings. The seedlings grown with no lime (#0), also grew quite well despite the fact that no dolomitic limestone was added to this treatment. Peatmoss, which was 1/3 of the potting soil, generally contains small quantities of Ca and Mg (Tinus and McDonald 1979). An analysis of the the foliage of the seedlings grown in the #0 soil shows that they were not deficient in these elements. It seems that pitch pine seedlings benefitted from the addition of trace amounts of dolomitic limestone, but also grew quite well at the lowest pH, with no dolomitic limestone added. Whether the slight rise in pH caused some of the nutrients to become more available, or the presence of additional calcium and magnesium cations in itself was responsible for the improved growth, we can not be sure. We do know that calcium and magnesium are both essential elements, required in satisfactory amounts, for the growth of higher plants (Epstein 1972), but it has been recognized that the effects of lime applications are indirect in most cases, as far as higher plants are concerned, and that though calcium is required, forest trees exhibit a

higher plants are concerned, and that though calcium is required, forest trees exhibit a limited demand for it (Tamm 1964).

In order to analyze the foliage for nutrients, the leaves of 30 seedlings growing in each treatment were combined. When sampling plant tissue for foliar nutrient analysis, the usual recommendation is to bulk together samples from about 20 trees from each treatment to eliminate between-tree variation (Mead 1984). The rationale behind the analysis of the foliage of plants is that the concentration of the nutrients within the tissue is an indication of the nutritional status of the plant, its growth potential, and overall health. It is also widely accepted as an indication of the nutritional content of the soil in which the plants are growing (Fowells and Krauss 1959). There is evidence that the nutritional requirements of plants varies widely among species, and to a lesser degree within species (Goddard and Hollis 1984). Concentration of nutrients in forest tree tissue can be affected by crown position, foliage age, between-tree differences, and seasonal or annual variation (Mead 1984). Mead (1984) suggests that current foliage selected from the upper crown has the highest concentration of nutrients and the lowest between tree variability, and that it should be used for sampling. Because of the marked seasonal changes in nutrient levels, the more mobile elements N, P, K, have their greatest concentrations in the summer, while the reverse is true for the non-mobile elements (Leaf 1973). There is not agreement on a standardized sampling period for conifers. Generally one should be aware of the phenology of the species one is sampling and collection should be made when the foliage is fully expanded, or actively growing in the case of conifers (Mead 1984). From the above discussion it is apparent that there may be wide variation in the results obtained depending on numerous variables when analyzing plant tissue. This limitation allows us to use foliar analysis values by others only as very general guidelines from which we can extrapolate to interpret our results. Epstein (1972) lists values of concentrations of nutrient

elements in plant materials at levels considered adequate. These values are for crop plants, but he adds that analyses of forest trees have given similar values. Leaf (1973) has published values for forest trees compiled from numerous authors; they are consistently lower than Epstein's, and he emphasizes that they are general guidelines at best. Motto (personal communication) states that he would expect pitch pine to have a lower requirement for elements N, P, K, Ca, and Mg, while it may be able to tolerate (or require) higher concentrations of Al, Fe, Mn, Cu, and Zn than would crop plants.

Pitch pine may exhibit little difference among provenances in nutrient requirements. In a review of studies on the nutritional requirements of 2 species with broad distributions (loblolly and Scots pine), Goddard and Hollis (1984) suggest that due to their heterozygous nature, or genetic diversity within provenances, they are well buffered for variations in edaphic factors. They state that all groups of provenances seemed to grow better in more favorable environments, and there were limited differences among provenances. This means that because of their genetic make-up, they are able to readily adapt to different soil conditions. Pitch pine is also a widely distributed tree species that has adapted to a diverse variety of sites. It is described by Ledig (1974) as an extremely variable species. With regards to its distribution and variability it may respond to edaphic factors in a fashion similar to loblolly pine, i.e., the genetic diversity within local populations may allow successful growth under a variety of soil conditions.

The foliar nutrient analysis found significant correlation between foliar nutrient content and rising pH values for the nutrients N, P, Ca, Al, Mn, and near significant for Mg. The elements K, Fe, and Zn displayed no apparent trends as pH changed (Table 8, Figs. 10 - 11). These elements will now be individually discussed in more detail.

There was a significant correlation between declining levels of foliar phosphorus and rising soil pH ($p < 0.05$). This is in accord with Lucas and Davis's report (1961),

suggesting that as pH rises, phosphorus forms the less soluble calcium phosphate, which is less available to the growing trees. We can not be sure whether this reduction in foliar phosphorus, as pH rose, is due to less phosphorus available, or a reduced ability of the tree to obtain phosphorus due to the interaction of another element. However, the lowest level of phosphorus, 0.18 percent, found in the treatment (#30) 30 g lime/1 kg soil seedlings, is well above Fowells and Krause's (1959) level of 0.10 percent as a critical level for either loblolly or Virginia pine, and even above 0.14-0.16 percent, the level at which their seedlings grew best. Though phosphorus was present in lower percentages in the trees grown in higher pH soils, it was undoubtedly supplied by the fertilizer at a high enough rate that it could not become unavailable. Phosphorus was present in all treatments at levels well above the minimum, and even in amounts that might be considered "luxury consumption" (amounts greater than required for normal growth).

Decreases in foliar nitrogen content were also correlated with increases in pH ($p < 0.05$). Acidic organic soils, such as this potting medium, require substantial amounts of nitrogen for satisfactory plant growth (Lucas and Davis 1961). This is due to low soil nitrogen content, between 0.6 and 1.4% N in peat mosses (Nelson 1985), and poor microbial activity in this acidic environment. The Osmocote fertilizer supplied enough nitrogen so that under the most acid treatments the trees were able to absorb sufficient quantities of the element. As pH rose to near neutral, the trees were absorbing less and less of this element, possibly to the point at which they may have approached a critical level of 1.2-1.5% (H. Motto, personal communication; Ellis 1972; Leaf 1973). The range of foliar nitrogen in seedlings ranged from 2.2% in the #0 treatment, to a low of 1.3% in the #30 treatment. The #6 seedlings were reported to contain 1.1% foliar nitrogen but this figure is an outlier, probably due to experimental error. In a study of loblolly and Virginia pines, the seedlings making the

best growth had foliar nitrogen concentrations of 1.7 - 2.3%, while at amounts below 1.2% the seedlings exhibited nitrogen deficiency symptoms (Fowells and Krause 1959). Schier (1985) grew pitch pine seedlings and treated them with simulated acid rain of varying pH's. The trees subject to the most acidic treatment had the best growth and contained foliar nitrogen of 0.89%, in a range that most would consider deficient. Several of the concentrations of elements in the foliage of his pitch pine seedlings are 2 to 5 times less than what are generally considered critical amounts for macronutrients, so his nitrogen content may also be low. In a study (Roth and Copeland 1957) comparing healthy and diseased shortleaf pines, foliar nitrogen of healthy trees had a mean of 1.3%, while littleleaf diseased trees had a mean of 0.9% foliar nitrogen. Additions of nitrogen fertilizer to the soil increased foliar nitrogen content of diseased trees and aided in their recovery. This suggests that a foliar nitrogen content of less than approximately 1.0% can lead to diseased, unthrifty trees. Despite the excess of nitrogen available throughout the course of this experiment some of the seedlings (in the higher pH ranges) were undoubtedly absorbing less than an optimal amount, possibly close to the point where it may have become limiting. This is probably due to the slow growth and photosynthesis of these seedlings as imposed by another element.

Potassium did not show any significant correlation ($p > 0.05$) between changing pH and foliar content. The mean value of 1.21% was well above Leaf's value of 0.4-0.5% that he considers low, and is within the range that others (H. Motto, personal communication; Ellis 1972) consider adequate. Sucoff (1961) lists foliar values of 0.26% and 0.28% as being deficient for loblolly and Virginia pines, respectively, and a value of 0.75% as being sufficient for Virginia pine. In this study values for potassium are all well above this range.

Magnesium showed near significant correlation ($p = 0.07$) between foliar nutrient content and changing pH. The trend was to increase as pH rose. This is in

accord with the added inputs of magnesium, as dolomitic limestone, to create the higher pH's. Sucoff (1961) lists a value of .08 as an amount of foliar Mg below which both loblolly and Virginia pines will exhibit deficiency symptoms. Values of from 0.08% to 1.0% were obtained in this experiment, from seedlings in treatments #0, #1, #2, and #4. These seedlings, that were growing in the lowest pH soils, are the ones that grew the largest and healthiest, yet their foliar Mg content was within the range where they might be considered low or deficient for this element. This tolerance of especially low levels of magnesium may be partially explained by the soils of the Pine Barrens, the natural "home" of pitch pine. These soils are a product of the parent material, weathering processes, and the acidic foliage of the native species that are naturally low in calcium. The development of these soils, termed podzolization, provides an acidic soil that is excessively leached and has had the soluble minerals K, Ca, and Mg largely removed from the solum. The less soluble elements Al and Fe have been leached from the upper layers and have accumulated in the subsoil (Tedrow 1952). This edaphic environment is one that pitch pine has adapted to and has helped maintain (through the deposition of its acidic foliage) over the years. Where little else will survive, pitch pine thrives. The fact that it will grow as well as it does under these impoverished conditions suggests that this is a species that requires less of the essential elements than others might, and can probably tolerate excessive quantities of Al and Fe. This may explain why the low to deficient quantities of magnesium present in the foliage of the healthiest trees does not interfere with their growth. Pitch pine seems to require little of this element, or is very efficient in using what little it is able to obtain from its naturally depauperate soil environment.

Foliar calcium was significantly correlated ($p < 0.05$) with the rising soil pH. The trend was for the trees growing in the higher pH soils to contain more calcium. A similar trend was observed by Roth and Copeland (1957) who added gypsum to the soil

beneath shortleaf pines and found this to be responsible for increased Ca content in the foliage. In this experiment foliar calcium rose from a low of 0.64% in the #0 treatment seedlings, to a high of 0.87% in the #40 treatment seedlings. The lowest pH soil, that had no calcium added as dolomitic limestone still provided enough calcium so that the trees growing in this soil contained a foliar level above what is generally considered inadequate. Levels below 0.5% are considered inadequate (H. Motto, personal communication; Epstein 1972), and with pitch pine's natural habitat occupying Ca-deficient soils, it may be capable of tolerating much lower levels than this.

Another element analyzed for was aluminum. Aluminum is not listed as an essential element for plant metabolism or growth (Epstein 1972), despite the fact that it is often present in plant tissue. Aluminum is a common constituent of the soil, and aluminum toxicity can be an important growth limiting factor on many acidic soils (McCormick and Steiner 1978). This is caused by excess aluminum, which becomes soluble in acidic soils, and produces symptoms resembling phosphate deficiency. The presence of aluminum ions causes precipitation of phosphate as insoluble aluminum phosphate in the soil and root tissue, thereby directly interfering with phosphate uptake under acidic conditions. The aluminum content of the foliage of the seedlings was significantly correlated ($p < 0.05$) to changing pH. The trend was an increase in Al as the pH of the soil dropped. It was especially high at the most acidic value; with concentrations almost twice (292 ppm) what the next most acidic value (158 ppm) contained. Though this value (292 ppm) is considerably higher, these trees were not significantly different in total weight than the trees (158 ppm) in the next most acidic soil. They were also not significantly different in height, diameter, or volume than seedlings with less than 1/6 (48 ppm) the concentration of aluminum in their tissue. It appears that the highest value for foliar aluminum in this experiment is not sufficient to be growth limiting. In a study of the mineral composition of foliage of shortleaf pine and

loblolly pine, Motto (1964) found Al concentrations of 281.2 and 146.3 ppm respectively, as an average for five seedlings. Both of these values approximate the range of the highest values encountered by the seedlings in this experiment in the lowest pH soils. Steiner and McCormick (1978) studied the aluminum tolerance among 11 species of trees, including pitch pine. Pitch pine was characterized as being moderately tolerant to soils with high concentrations of dissolved aluminum. Some plants are termed "aluminum-accumulators" and are capable of tolerating in excess of 40,000 ppm (Hydrangea macrophyllum) of aluminum in their tissue (Sutcliffe and Baker 1974). We can see that different species are inherently able to tolerate varying amounts of aluminum in the rhizosphere before toxic effects are noticed. Pitch pine has adapted over the course of time to an environment that commonly has excess aluminum in the subsoil, and so has likely developed some mechanism to deal with this factor.

The remaining elements analyzed were Fe, Cu, Zn, and Mn. These are are all elements that may become deficient in heavily limed acidic soils (Khana and Ulrich 1984). Neither iron ($p > 0.05$) nor zinc ($p > 0.05$) showed any significant correlation between rising pH and a decrease in nutrient concentration, though in both cases the lowest values (ppm conc.) of these elements did occur in the seedlings grown in the highest pH soils. The mechanism whereby iron becomes less available at higher pH is oxidation of the readily available ferrous form (Fe^2) to the relatively unavailable ferric form (Fe^3) under alkaline conditions (Sutcliffe and Baker 1974). The concentrations in ppm. for Fe in this experiment were from 151 to 48 ppm. The values near the low end are approaching the point where they may be limiting. Motto (personal communication) states that 30 ppm may be low, while Leaf (1973) says that 40-50 ppm is in the low range. In a study of foliar iron content of loblolly and shortleaf pine seedlings, Motto (1964) found amounts of 121 and 223 ppm respectively, in normal, healthy plants. Again, it is difficult to say whether the low values in some of the treatments are due to a

lack of available Fe or a limited ability of the plants to absorb it due to another interacting element, and also whether or not the lower amounts are even limiting.

Zinc is another element that becomes deficient under more alkaline conditions. In a study by Wilson (1953) it was shown that shortleaf and loblolly pines had marked differences in growth response to varying concentrations of the nutrient zinc. Loblolly pine was able to carry on normal growth at such low concentrations that shortleaf would die. There were no data on zinc foliar content. We see from this that there are drastic differences in zinc requirements between these two sympatric species. Motto (1964) in her analysis of the foliage of seedlings of loblolly and shortleaf pines found average values of 54.7 and 51.4 ppm Zn respectively, in normal, healthy seedlings. From Wilson (1953) it is known that under normal circumstances, when zinc is plentiful, loblolly and shortleaf absorb it at similar concentrations; however, when it is in short supply loblolly appears to be better able to utilize it. Where pitch pine falls with respect to these two species regarding its zinc requirement is unknown. The foliage of the pitch pine seedlings grown in varying pH soils had foliar Zn at concentrations between 101 and 50 ppm. These values are all above amounts generally considered deficient for pines by various authors.

The micronutrients manganese and copper both showed significant correlation between decreasing foliar content and rising soil pH (manganese $p < 0.05$, copper $p < 0.05$). Lucas and Davis (1961) state that manganese is influenced more by soil reaction (pH) than any other plant nutrient, and that serious deficiencies may occur at pHs above 6.5. In an experiment reviewed by these authors it was found that manganese in onion tops, grown in peat soils limed to raise the pH from 4.1 to 5.6, dropped from 1125 ppm. at the low pH (4.1) to 25 ppm. at the high pH (5.6), a drastic change. The liming of acid organic soils (which the potting soil for the experiment may be considered), and high pH levels associated with organic soils both favor the development of manganese deficiency

(Follett et al. 1981). At high pH levels, the readily absorbed Mn^2 ion is oxidized to the tri (Mn^3) or tetra-valent (Mn^4) forms of this element which are not absorbed by plants in calcareous soils (Sutcliffe and Baker 1974).

Motto (1964) found foliar manganese levels for loblolly and shortleaf pines to be 239 and 180 ppm. respectively, as an average value for five healthy seedlings. Leaf (1973) gives a value of 100 ppm. as a minimum for pines. The levels of foliar Mn for pitch pine seedlings growing in varying pH soils fell below 100 ppm as the soil pH approached 6.0 (treatment #10). The first signs of chlorosis in this experiment appeared on 10 January 1988, among the seedlings growing in the racks with 10 or more grams lime/1 kg potting soil and persisted for the duration of the experiment. According to Tamm (1964) the most rapid and convenient method of determining a nutrient deficiency is by observation of the visual symptoms, if they are specific enough. Unfortunately the symptoms for manganese deficiency are considered more diverse than for any other mineral element. Symptoms may appear on young or old leaves and comprise a variety of necrotic and chlorotic patterns (Sutcliffe and Baker 1974). The pitch pine seedlings that became chlorotic developed a yellowing, usually beginning with the youngest tissue and eventually involving the entire plant. These seedlings were smaller in both height and diameter and generally had an unthrifty, stunted appearance. Occasionally a slight bronzing or necrosis would develop at the tips of the leaves. These are very general deficiency symptoms and may be consistent with deficiencies caused by a variety of elements, including nitrogen, potassium, copper, iron, manganese, molybdenum, sulfur, and zinc, as described by Roberts and Boothroyd (1975).

Copper is another element that showed a significant correlation between rising soil pH and decreasing levels of copper in the seedling foliage ($p < 0.05$). This is consistent with findings that copper availability decreases with rising pH. The Cu^2 ions are precipitated as insoluble copper phosphates (Sutcliffe and Baker 1974). The lowest

level of copper, 4.4 ppm found in the seedlings grown in treatment #30, is a concentration that is approaching deficiency levels (H. Motto, personal communication, Epstein 1972, Leaf 1973). Motto (1964) found concentrations of copper in loblolly and shortleaf pines of 5.88 ppm and 5.42 ppm respectively, as an average for five healthy seedlings. The pitch pine seedlings that grew the largest in different pH potting soils had foliar copper concentrations of twice what the smallest seedlings had, yet seedlings that also grew poorly (#20 and #25) had foliar levels above what is considered inadequate. The values for these two, 7.4 ppm and 6.4 ppm, respectively, are very close to the value of 7.5 ppm foliar copper in the one best rack of trees (#2). From the literature it is apparent that there are small differences between adequacy and deficiency for copper. This factor combined with the lack of data on pitch pine and its requirements for Cu make it difficult to analyze the data from this experiment. It appears that the poorly growing trees had as much, or nearly as much, foliar copper as the fastest growing trees, and that the very worst trees may have been deficient. Again, whether this is due to the interaction of other elements or solely due to copper unavailability at the highest pHs is unknown.

Several micronutrients that are considered essential were not analyzed for their presence in the foliage, largely because under the conditions of the experiment, it was unlikely that they were responsible for any of the problems. These elements are boron, molybdenum, chlorine, and sulfur.

Having reviewed the concentrations of the various elements that were found in the foliage of the pitch pine trees growing in various pH organic soils it appears that all of the macro-nutrients, N, P, K, Ca, and Mg were present in sufficient quantities over the range of pH that they were probably not limiting growth. Questions begin to arise when looking at the concentrations of the micro-nutrients, especially in the seedlings growing in the high pH ranges. The addition of dolomitic limestone to any soil has a

direct effect on the availabilities of the nutrients Fe, Mn, Zn, and Cu, and induced deficiencies may result. These elements, especially manganese, were all present in the pitch pine foliage at their lowest concentrations at the highest pH ranges. This may account for the apparent toxicity of calcium to pitch pine, a calcifugous species. We must also consider the interactions between elements. The work by Shear et al. (1948) showed that the presence of more or less of one element can influence the balance of other elements present, and the amount of an element required for a plant to function normally. He concluded that the presence of excess Mg and Ca not only lowered Mn content, but influenced K content, which in turn exacerbated Mn deficiency, thereby increasing the quantity of Mn required to prevent deficiency symptoms. It appears that the absolute concentration of an element in a leaf is not necessarily related to its functional concentration.

The pitch pine seedlings growing in the higher pH ranges also had elevated levels of Mg and Ca in their tissue, along with reduced levels of Fe, Mn, Zn, and Cu. K levels were consistent among all treatments, and showed no trend. The rising levels of Mg and Ca may have had an effect on the functional ability of the micro-nutrients, and exacerbated deficiencies. The fact that Mn levels were very low, regardless of any interactions, might have also been responsible for the stunting and chlorosis of the seedlings in the high pH soils. There is a simple method of testing whether Mn deficiency alone is the cause of the stunting and chlorosis of the seedlings in the higher pH soils. By applying either the inorganic ($MnSO_4$) or organic (MnEDTA) to the foliage of the seedlings in the high pH soils, we can measure whether or not there are improvements in growth. This would give us further insight, but still does not tell us if the deficiency is due to an inability of the plant to obtain Mn from the soil, or because of interactions of other plant nutrients that decrease the functional ability of Mn within the plant.

The second aspect of this experiment that need be addressed is the relationship between soil pH's and the incidence of damping-off. The general relationship between soil reaction and damping-off has been alluded to previously in this paper, by authors of the past (Illick and Aughanbaugh 1930, Perry 1930, Retan 1918). More recent evidence agrees with these past observations. Filer and Peterson (1975) state that soil pH is significant, losses are usually greatest at high pH and minimal near pH 5.5. Pawuk (1981) states that seedling loss during containerized production of longleaf and shortleaf pines is related to drainage and pH, and is greatest in commercial media with pH above 6.0. This is in agreement with the findings of this study. Out of 216 seedlings that died during the course of the experiment, 79% of the mortality occurred in the soils that had pH's of 6.0 and above. The probable pathogen responsible for the death of the seedlings in this experiment was a Fusarium spp., as it was isolated from 14 out of 14 dying seedlings. However, Pythium infected tissue are often overgrown by Fusarium, and so Fusarium may be incorrectly implicated in some cases (Peterson 1974). Smith (1974) states that Fusarium root disease is one of the most common diseases of conifer seedlings in the world. James (1985) lists several diseases associated with containerized conifer seedlings and suggests that the most common is Fusarium. He adds that Fusarium disease development may be restricted if soil mix is kept acidic, below pH 6.0. Other authors (Horst 1979, Pirone 1978) implicate species of Fusarium as an important contributor to damping-off problems for conifer seedlings.

The pathogen may be introduced via several sources. In a study by Pawuk and Barnett (1974) to locate the source of contamination, no damping-off organisms were recovered from their water source or the potting mix. In general most potting mixes are free of pathogens (Tinus and McDonald 1979) due to their acidity (peat) or previous sterilization (vermiculite and perlite). They did find Fusarium in the soil surrounding the greenhouse, which could provide a source of contamination. In a related paper,

Pawuk (1978) found that between 4% and 20% of longleaf pine seed, from various seed lots, were infested with Fusarium. Various isolates of this pathogen caused reduced germination, reduced seedling growth, and increased post-emergence damping-off. Peterson (1974) agrees, and states that pathogens are probably introduced on the seed or debris accompanying the seed. It seems that the presence of the pathogen on the seed coat is the most likely source of contamination. So, an effective starting point to eliminating Fusarium in the nursery would be to surface sterilize the seed coats prior to seeding, this should initially eliminate the pathogen, however it would provide little protection following germination. Barnett (1976) found that the seeds of the southern pines (loblolly, slash, shortleaf, and longleaf) could be effectively surface sterilized by soaking them in 30% hydrogen peroxide solution for 30-60 mins., without adversely affecting germination. Another method that is commonly used is dressing the seed with fungicides. This also produces effective control, but may (Pawuk 1979, Perkins 1981), or may not (Ghosh et al. 1981) interfere with germination depending on the species and the fungicide.

The next step in providing a pathogen free environment for the germinating seedlings is to sterilize the growing medium. The basic ingredients for potting mediums (peat, vermiculite, perlite), as previously mentioned, are relatively free of pathogens. An effective method of insuring that they are pathogen-free is through the use of steam pasteurization, which is commonly employed by nursery operators. Raising the temperature of the potting medium to 82° C (180° F) for 30 minutes is the recommended treatment. A temperature of 71° C (160° F) will kill most plant pathogenic fungi (Nelson 1985). Prior to steam pasteurization soils should be moistened and loosened to allow the heat to penetrate evenly.

There are several considerations one must take into account when constructing a potting medium, which may impact on disease development. The high water holding

capacity of peat-vermiculite mixes is beneficial in the small containers used for containerized tree seedlings because they are otherwise prone to rapid drying, however, this moist environment creates conditions that are favorable to the development of disease (Filer and Peterson 1974). With the addition of a fraction of sand to a peat/vermiculite mix, we can improve the structural composition of the potting medium. It will still retain its water holding capacity, but will have better drainage and aeration than a strictly peat/vermiculite mix. Tinus and McDonald (1979) state that it is especially crucial for conifers to have a potting medium that is porous and well aerated; although they do not state exactly why this is, I suspect they are referring to the prevention of disease. The sand also promotes "wetting" of the peat fraction which can be exceedingly difficult to re-wet once it has dried out. If sand is to be incorporated into the mix, then a coarse textured "concrete-grade" should be used (Nelson 1985). One drawback of sand is the additional weight, however, it is felt that this is offset by the improved characteristics of the potting medium, especially in a small scale operation such as might be used by Park Service personnel.

The general stress-disease relationship is well put by Filer and Peterson (1974) who state that, "any condition that reduces seedling growth and vigor usually causes an increase in infection." The stunting and chlorosis that was common among the trees in the higher pH soils likely contributed to the higher incidence of damping-off that occurred there. Another contributing factor may be the improved growth rate of the fungus at higher pH. From a study of the growth rate of an isolate of Fusarium, from one of the diseased seedlings in this experiment, on varying pH's of PDA, it was found that there was a significant increase in the growth of this isolate at the highest pH ranges. In an analysis of variance of the growth rates, the fungus grew significantly ($p < 0.05$) faster on PDA with the pH's 7.0 and 6.5, than on PDA with the pH's 4.5 and 5.0. Although there are considerable environmental differences between a sterile plate of

nutrient agar and a nursery potting soil, it does appear that the fungus seems to perform the best on both of these substrates at the highest pH ranges. This seems to indicate that much of the "blame" for the disease problems in the higher pH soils is not so much due to the better growth rates of the pathogen, but due to a combination of the two factors, stressed seedlings and a very favorable environment for the pathogen, which culminated in excessive seedling mortality.

Damping-off problems are a major concern to those producing container grown conifer seedlings, as evidenced by the wealth of literature that is dedicated to the prevention and control of these diseases. Many of the difficulties encountered could be easily circumvented if growers would pay closer attention to the potting soils and cultural techniques that they use. Pitch pine seedlings grown in a previous experiment, suffered over 33% mortality due to the fact that a standard potting medium was used, which though excellent for most species of plants, was not specifically constructed for conifers. It is apparent that others have also fallen into this trap. We are often so concerned with other aspects of an experiment that we may fail to pay attention to the basics. One author, W.H. Pawuk has published several articles concerned with the problems associated with damping-off of containerized conifers. It is interesting to follow the progression of his articles. In his first publication (Pawuk and Barnett 1971) he is interested in locating the sources of contamination, a logical starting point, and also testing fungicides for controlling the disease. He found that the seed coat is the major source of contamination, with the greenhouse a contributing factor. He was also able to obtain some control of disease with fungicides, however he found that fungicides were not effective once the disease had become established. Later (Pawuk 1981), in a much simpler experiment, grew conifer seedlings in 12 different potting medias (3 commercial, 9 blended in the greenhouse) and attributes smaller seedlings and the greatest disease losses to high pH of the media. Seedlings grew best in equal parts of peat

and vermiculite with a low pH. We can see that he too has found that by simply modifying the growing medium he can improve growth and decrease disease incidence, eliminating the need to apply fungicides or pre-treat the seed. Smith (1975) in a review of Fusarium problems associated with conifers states that, "on the whole, cultural methods have not been effective in controlling this disease". Results presented here contradict this and suggest that damping-off problems can be alleviated by growing conifer seedlings in a medium that is designed to prevent excessively moist conditions, along with being decidedly acidic. Obviously our results apply specifically to pitch pine, but as noted earlier, most conifers prefer an acidic medium for growth. If growers pays more attention, and do not blindly add liming amendments as a matter of course, but limit it to raise the pH only enough to increase the efficiency of the nutrients, then they will be providing an ideal environment for their container grown conifer seedlings, while at the same time culturally inhibiting pathogens.

TABLE 6. Grams of dolomitic limestone added to the various treatments

Treatment #	Grams/1kg Soil	Grams/9.3kg Soil
0)	0	0
1)	1	9.3
2)	2	18.6
4)	4	37.2
6)	6	55.8
8)	8	74.4
10)	10	93.3
15)	15	139.5
20)	20	186.6
25)	25	232.5
30)	30	279.0
40)	40	372.0

TABLE 7. Means of measurements of pitch pine seedlings grown in potting soil with increasing increments of added lime.

Treatment	Height cm	Diam. mm	Volume cc	Shoot wt.g	Root wt.g	Total wt.g
0	19.03 bc	2.01 cb	78.27 cb	.56 ba	.096 a	.65 ba
1	19.93 ab	1.94 cbd	84.30 b	.63 a	.100 a	.73 a
2	21.13 a	2.22 a	107.87 a	.59 a	.106 a	.69 a
4	18.20 ecd	2.04 b	77.47 cb	.55 ba	.102 a	.66 ba
6	18.74 bcd	1.86 cebd	66.72 cd	.49 bc	.097 a	.59 bc
8	17.26 ed	1.75 efd	54.87 edf	.45 c	.092 a	.55 dce
10	17.83 ecd	1.85 cebd	64.97 cd	.45 c	.096 a	.55 dc
15	16.70 ef	1.83 ced	60.54 ed	.42 dc	.093 a	.51 dfce
20	16.77 ef	1.72 ef	51.63 edf	.36 de	.091 a	.45 fg
25	16.57 ef	1.74 ef	51.99 edf	.36 de	.096 a	.45 fge
30	15.47 gf	1.67 ef	47.81 ef	.37 de	.098 a	.47 dfge
40	14.13 gf	1.61 f	41.52 f	.32 e	.090 a	.41 g

Treatments are grams of dolomitic limestone/ kg potting soil.

Note that in any one column, the means with the same letter in the suffix are not significantly different.

Table 8. Results of foliar tissue analysis of pitch pine seedlings.

	----------%----------					----------PPM----------				
pH	N	P	K	Mg	Ca	Al	Fe	Zn	Mn	Cu
4.26	2.2	0.34	1.28	0.08	0.64	292	88	76	408	9.4
4.60	2.1	0.31	1.17	0.08	0.66	158	92	60	409	11.4
4.78	2.1	0.36	1.19	0.10	0.67	107	149	95	486	7.5
5.22	1.8	0.30	1.16	0.08	0.67	48	78	79	252	10.4
5.60	1.1	0.21	0.71	0.03	0.47	48	60	101	169	5.5
5.74	1.8	0.28	1.22	0.09	0.78	40	151	94	115	8.4
5.93	1.5	0.23	1.12	0.07	0.71	32	121	86	81	6.5
6.19	1.6	0.24	1.33	0.13	0.86	40	92	72	64	8.4
6.28	1.6	0.25	1.24	0.11	0.85	48	171	74	58	7.4
6.52	1.5	0.21	1.34	0.11	0.8	57	119	52	62	6.4
6.51	1.3	0.18	1.20	0.12	0.76	32	52	50	47	4.4
6.60	1.5	0.19	1.54	0.16	0.87	40	48	54	46	4.6

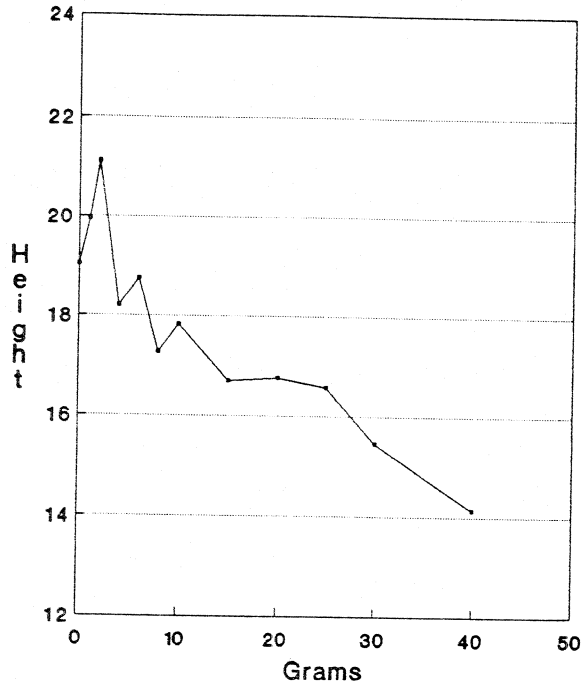


Fig. 5. Stem height (cm) versus grams dolomitic limestone/1000 grams potting soil.

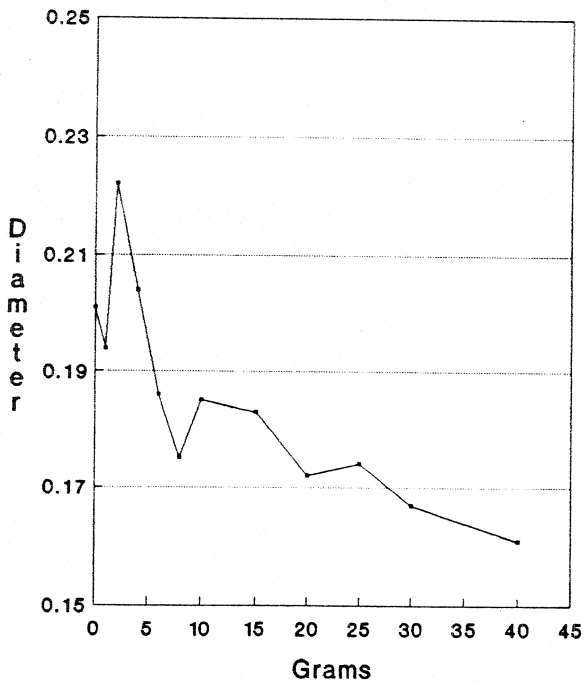


Fig. 6. Stem Diameter (cm) versus grams dolomitic limestone/1000 grams potting soil.

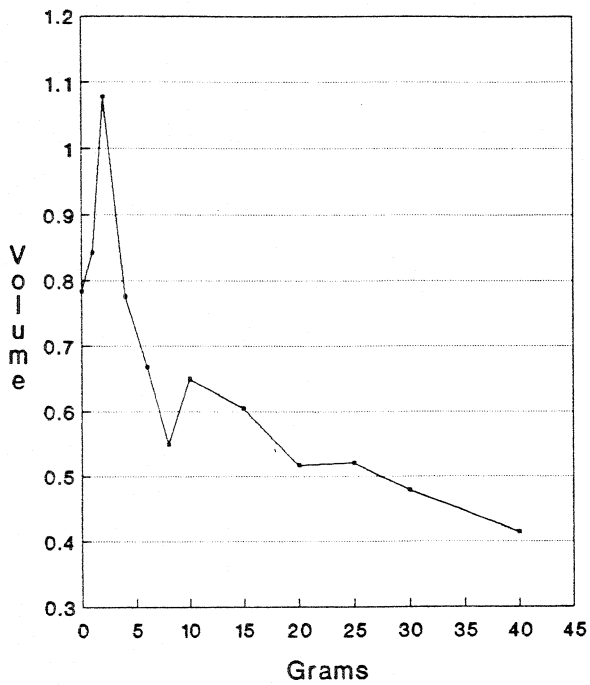


Fig. 7. Stem volume (cc) versus grams dolomitic limestone/1000 grams potting soil.

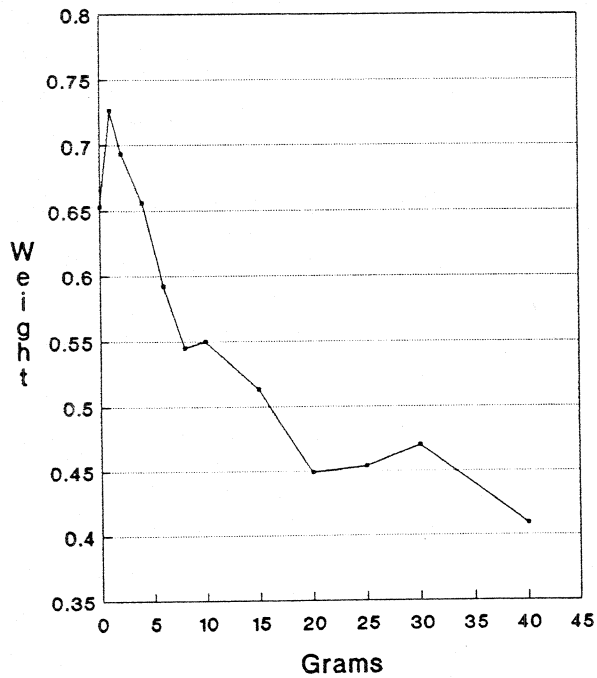


Fig. 8. Total dry weight (g) versus grams dolomitic limestone/1000 grams potting soil.

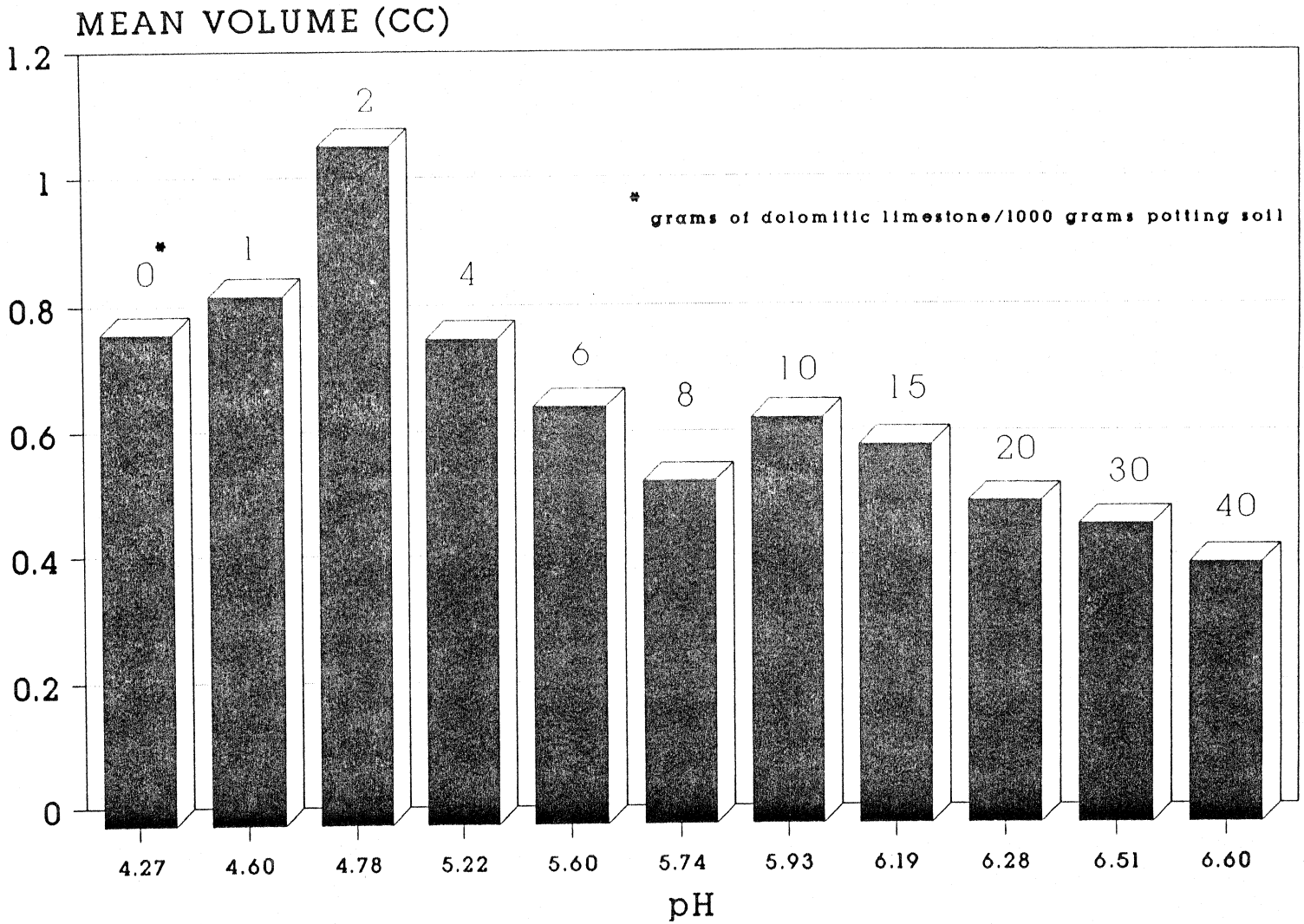


Fig. 9. Mean volume by soil pH.

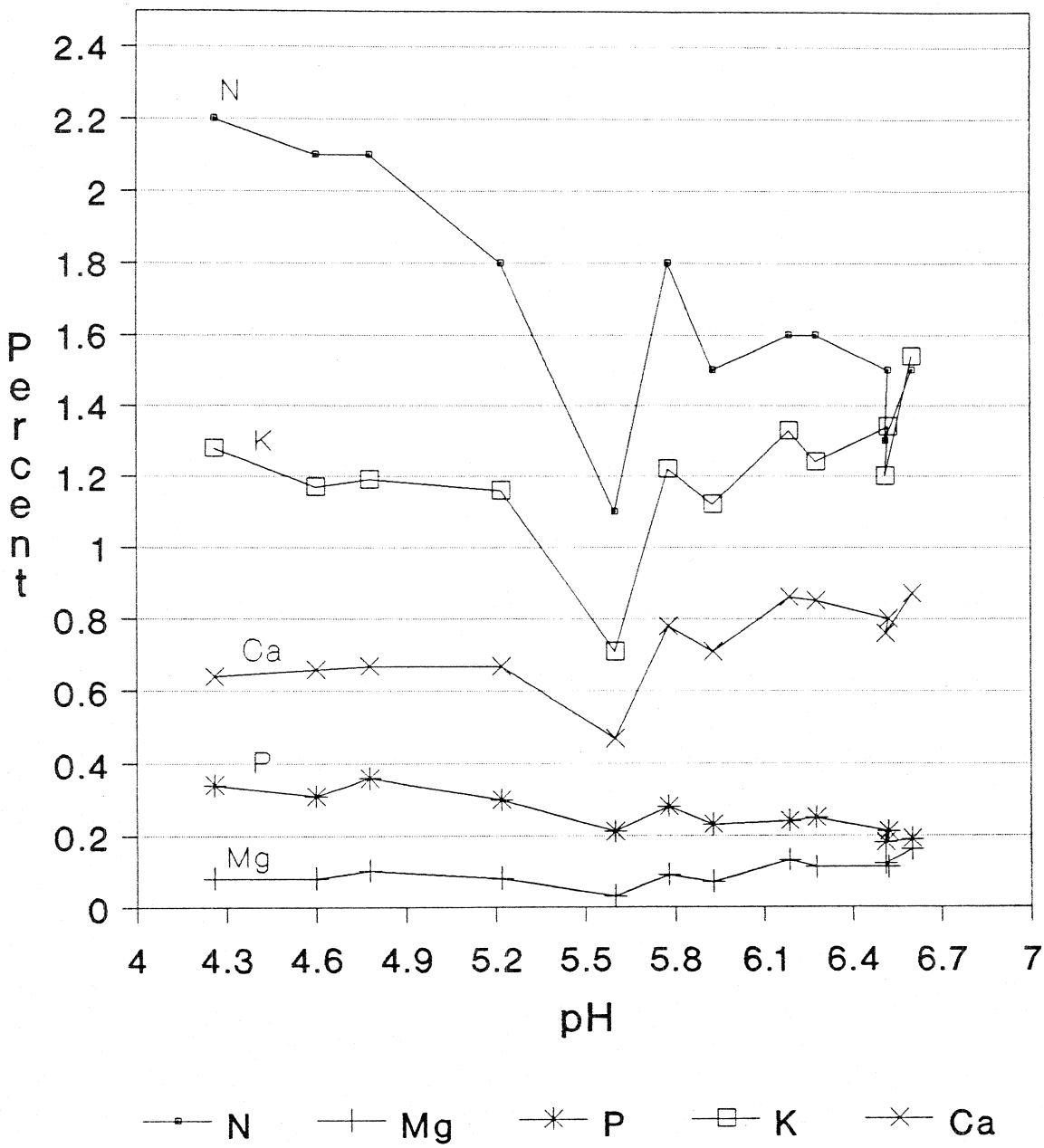


Fig. 10. Results of foliar tissue analysis of pitch pine seedlings.

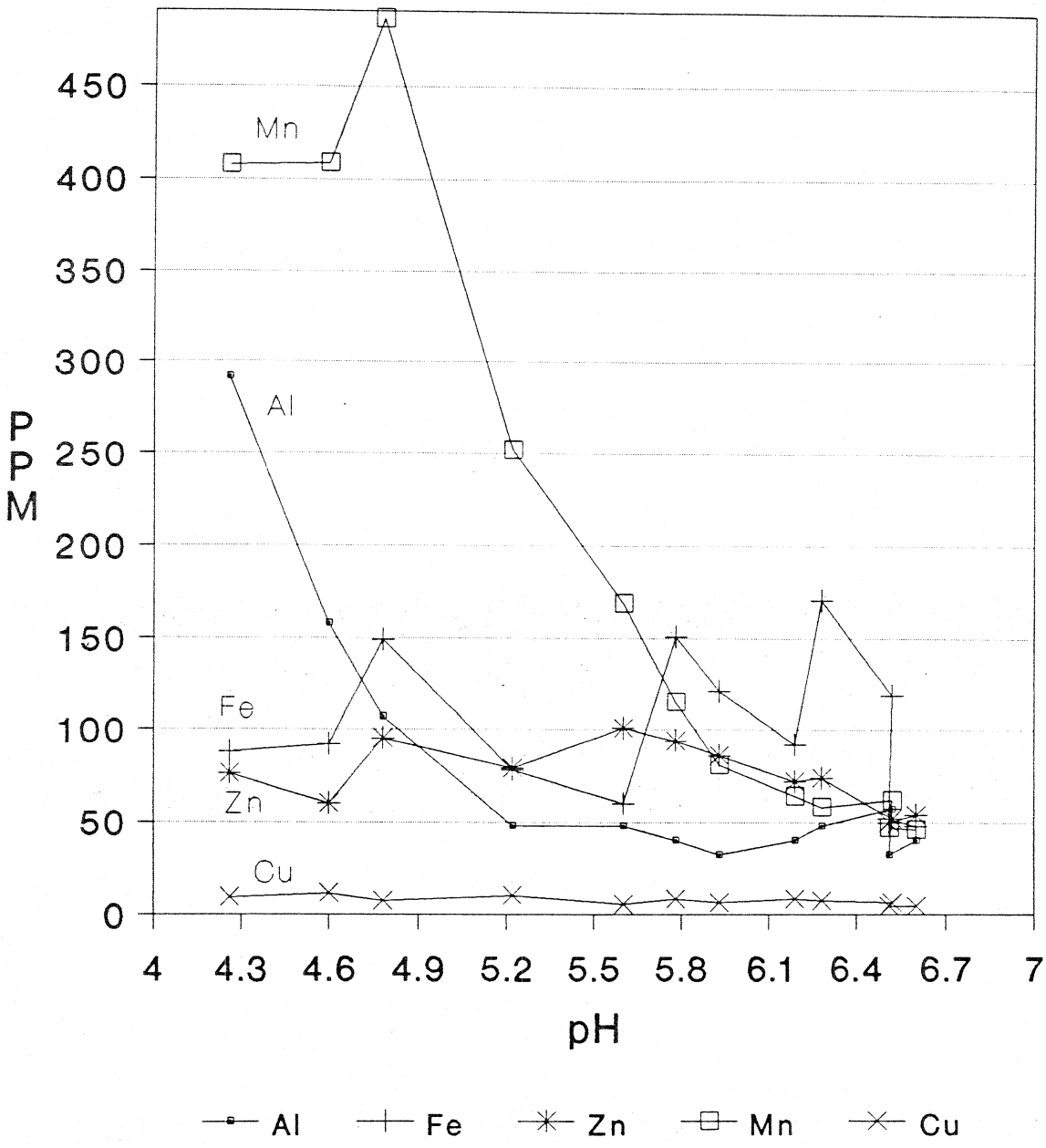


Fig. 11. Results of foliar tissue analysis of pitch pine seedlings.

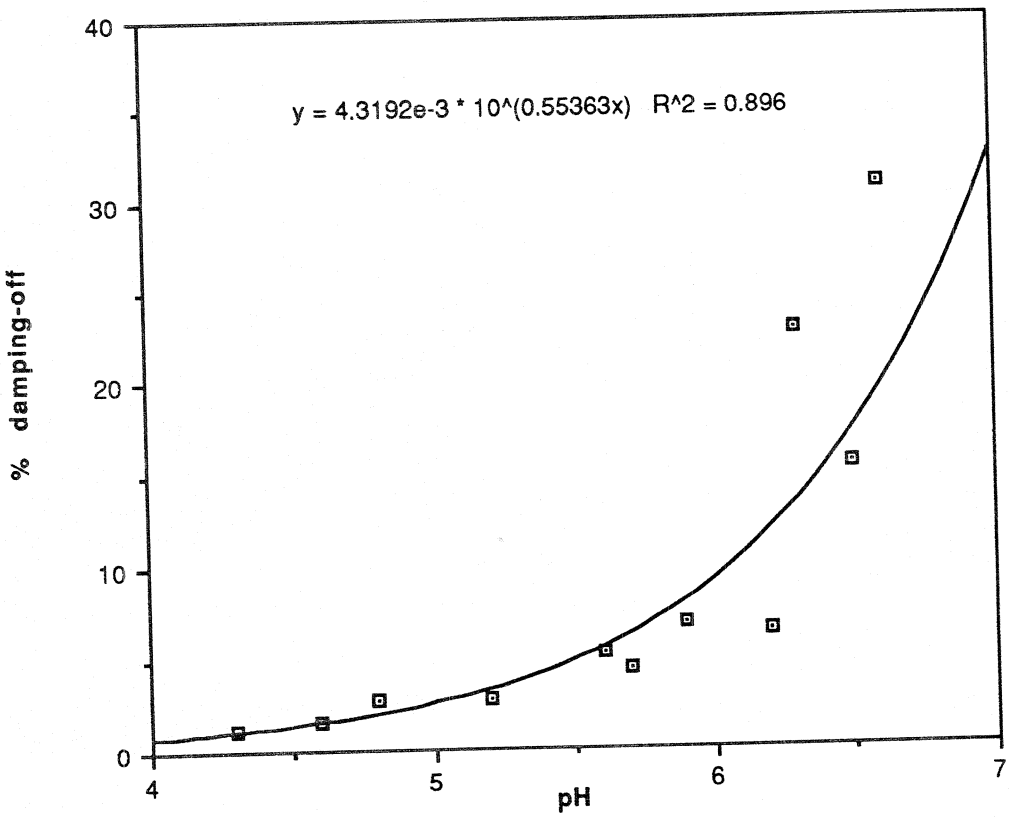


Fig. 12. Percent of pitch pine seedlings damping off as a function of soil pH.

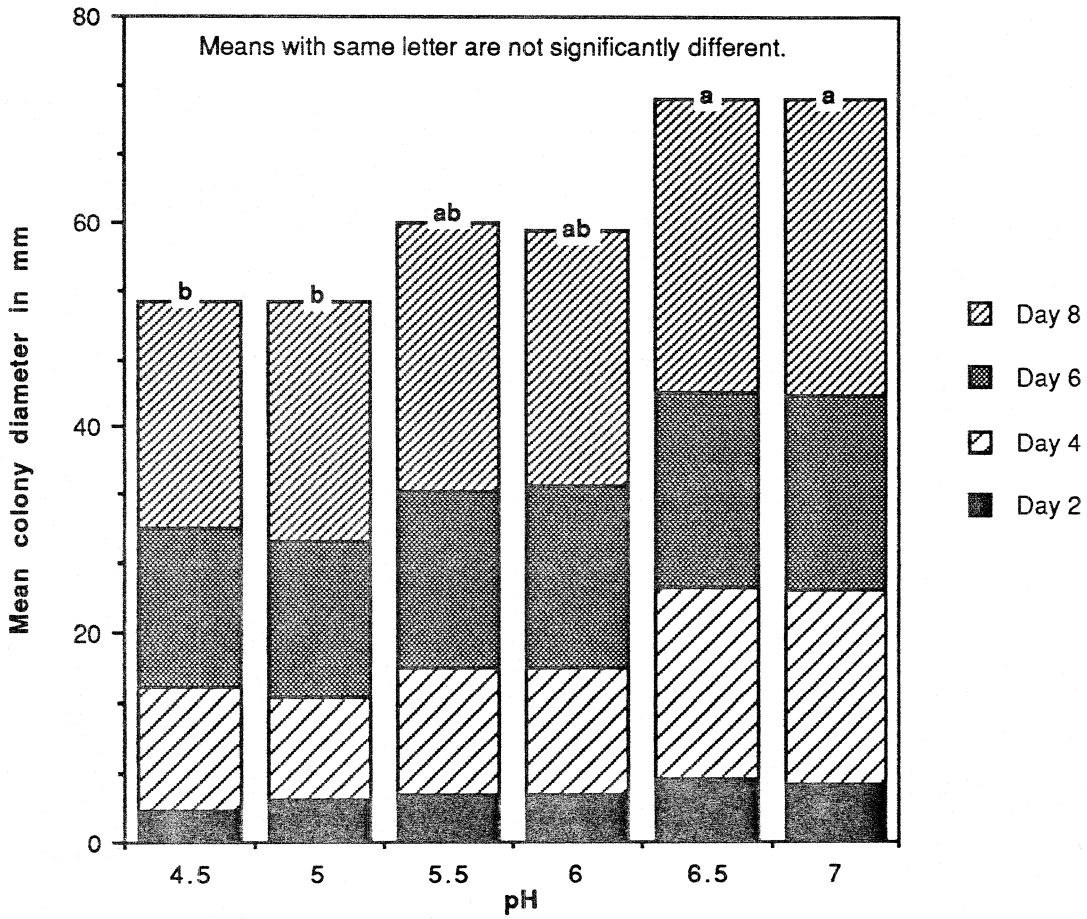


Fig. 13. Growth of Fusarium in culture as a function of pH.

APPENDIX 1

Preliminary Studies with outplanting of pitch pine seedlings

INTRODUCTION

Floyd Bennett Field occupies a man-made island of landfill. The woody vegetation consists of planted exotics, Austrian (Pinus nigra Arnold) and Japanese black pine (P. thunbergii Parl.), some naturalized pioneers such as mulberry (Morus rubra L.) and ailanthus (Ailanthus altissima (Mill.) Swingle), surrounded by expanses of Phragmites australis (Cav.) Trin..

Because Floyd Bennett Field is built on landfill, and does not support a large population of ectomycorrhizal hosts, it is likely that a deficiency of ectomycorrhizal propagules may exist. Despite the fact that these two species of Pinus are present, they may not necessarily support mycorrhizae that are compatible with pitch pine. On an adverse site, such as this, pine seedlings pre-treated with Pisolithus tinctorius (Pt) ectomycorrhizae can be expected to show increased tolerance to the extremes that this landfill soil might impose (Marx et al. 1976). Outplanting survival and growth of mycorrhizal seedlings significantly exceeds that of non-mycorrhizal seedlings under most circumstances (Trappe 1977). Studies have shown that pine seedlings mycorrhizal with Pt are better able to withstand high soil acidity (Marx 1975), are conferred protection from methane gas (Sherwood and Klarman 1980), may reduce the absorption of toxic elements (Berry 1982), and can better resist high soil temperatures (Marx and Bryan 1971). Many authors have used mycorrhizae to improve the growth and survival of a variety of pines, including pitch pine, on coal spoils and reclaimed surface mines (Schoenholtz et al. 1987, Berry 1982). These attributes of mycorrhizal seedlings make them likely to do better at Floyd Bennett.

Pitch pine seedlings established on strip-mined areas have been shown to respond positively to fertilizer amendments (Woods et al. 1984). Slow-release fertilizers have been shown to be compatible with mycorrhizal colonization (Maronek et al. 1982), and even improved it in one case. (Schoenholtz et al. 1987).

The objective of this study is to determine which treatment, or combination of treatments will aid in the establishment, survival, and growth of outplanted pitch pine seedlings on these inauspicious surroundings.

METHODS AND MATERIALS

The plantation at Floyd Bennett Field was planted in October 1987 under four different planting schemes. These were: (1) control; (2) fertilizer; (3) Pt mycorrhizal inoculation; and (4) fertilizer and Pt mycorrhizal inoculation. Each planting scheme is represented by 12 seedlings from each of 10 parents, for a total of 480 trees.

The fertilizer amendment was a combination consisting of 70% 5-20-20 (available inorganic) and 30% 38-0-0 (slow release nitroform). It was applied at a rate of 100 grams (approx 1/4 lb.) per tree. This combination of fertilizers and rate was recommended

(R. Flannery, personal communication). The 100 grams of fertilizer was divided into equal portions and placed in each of 4 holes punched 10-12 cm. from the base of each tree.

The mycorrhizal inoculant used was basidiospores of Pt purchased from the International Forest Seed Co., Odenville, AL. These spores come in a pelletized form of clay and adhesive. The seedlings were inoculated with spore pellets, at transplanting time, at a rate of .5 grams/tree (there are about 1.1×10^6 basidiospores of Pt per mg (Marx and Kennedy 1982)). The pellets were pushed into the soil, at the root ball/existing soil interface, to a depth of 3-6 cm.. It is necessary to place the spores in direct contact with the growing roots, because these fungi are obligate biotrophs, and cannot grow until they contact a root.

The plots were treated with the herbicide glyphosate at a 2% rate, when the trees became dormant in the fall but weeds were still active. As a precaution, the trees were

individually covered with pots prior to spraying. This cultural treatment has no effect on ectomycorrhizal colonization (Schoenholtz et al. 1987). The trees were measured in the spring of 1987, and at this time it was too early to tell whether the cultural treatments would have an effect.

DISCUSSION

The mycorrhizal inoculant Pt was used because it colonizes pitch pine (Marx and Bryan 1969), and is known to produce a positive growth response with pitch pine seedlings (Lee and Koo 1983, many others). Basidiospores of Pt are a practical alternative to the vegetative inoculum for several reasons. They do not require a growth phase under aseptic conditions. The basidiocarps are easily identified and under the proper circumstances can be collected in large quantities. The spores do not require special storage conditions, are easily extracted, take up little space, and remain viable for extended periods. I was able to collect several sporophores of Pt in the New Jersey Pine Barrens. However, the best method of producing seedlings colonized with a specific mycorrhizal host is to use the vegetative inoculum, rather than basidiospores (Mikola 1973). Basidiospores of the same fungus take 3-4 weeks longer than the vegetative mycelium to produce mycorrhizae (Marx et al. 1977).

There are three times when seedlings can be inoculated with mycorrhizae: i) before seeds are sown, ii) at the time seeds are sown, and iii) after seedlings emerge. Ruehle (1980) inoculated Pinus taeda seedlings with Pt basidiospores at 0, 2, 4, 6, and 8 weeks after planting. He found that he had the greatest degree of Pt colonization on the seedlings inoculated 0 and 2 weeks after planting, with lesser amounts on all other seedlings. There is little or no competition for colonization sites on newly emerged radicles, so introducing the mycorrhizae at this time gives them a competitive advantage. We must keep in mind that two types of roots occur on pines, long roots, of which the

radicle is one, and short roots, which are the only sites where mycorrhizae will form (Hatch and Doak 1933). So, no mycorrhizal colonization will occur until the radicle begins to produce "short roots", which will occur soon after the radicle is established. Seedlings inoculated and colonized earlier in their lives derive the benefits of the mycorrhizae for that much longer. In retrospect, perhaps it would have been better to have inoculated the seedlings in the greenhouse, at the time of seeding, rather than in the field when the seedlings were 8 months old.

Another factor influencing mycorrhizal development is competition with other microorganisms that occur in planting substrates. Nursery soils should be steam sterilized or fumigated prior to introducing mycorrhizal fungi. This eliminates root pathogens (fungi, bacteria, nematodes) that may damage or destroy the sites (nonlignified short roots) where mycorrhizae normally form (Marx et al. 1976). This will also eradicate indigenous fungal symbionts that would compete for root colonization space.

The seedlings that were outplanted had been growing in unsterilized nursery soil that was 1/10 New Jersey Pine Barrens soil for 8 months. These seedlings were undoubtedly colonized with a variety of rhizosphere-inhabiting fungi as exhibited by the presence of sporophores of the common mycorrhizal contaminant, Thelephora terrestris, which were observed growing with many of the seedlings in the greenhouse. Marx and Bryan (1975) added basidiospores of Pt to the root zone of two month old Pinus taeda that were already mycorrhizal with naturally occurring fungi, as in this study. After 8 months of growth the Pt basidiospore inoculum had colonized the seedlings and comprised approximately half of the ectomycorrhizae present. The height growth of these seedlings was similar to that of non-inoculated control seedlings. Outplanting trials with containerized pines show that 50-60% of the root system needs to be colonized in order to obtain positive growth response and improved survival (Ruehle

colonized in order to obtain positive growth response and improved survival (Ruehle 1980).

The conventional regimens of high fertilization in the nursery can inhibit mycorrhizal development. Maronek et al. (1982) found that slow-release Osmocote (18N-2.5P-10K) at rates of 2.3 - 4.5 kg /m^3 of potting mix, in combination with mycorrizal inoculation, produced pitch pine seedlings heavily infected with mycorrhizae and of a size comparable to that of seedlings grown with soluble fertilizer, which had little or no mycorrhizae. The seedlings grown for this experiment were fertilized with the traditional (heavy) regime of fertilizer that containerized conifers require, Peters 20-20-20, at two week intervals. This may have, in effect, kept the root systems "cleaner" than they otherwise would have been, though as stated earlier, they were undoubtedly colonized by a myriad of root inhabiting organisms.

As a review, there are several cultural considerations that will enable one to better produce mycorrhizal seedlings. First, it would be preferable to use the vegetative inoculum over the basidiospores, at or just following seeding time. Second, traditional fertilization schemes should be altered and a slow release Osmocote used instead. Third, steam pasteurized "soil-free" potting mix should be used; and fourth, flats or pots with multiple plants, rather than tubes or containers with individual seedlings should be used. Small containers of 200cc (12.2 cu.in.) or less quickly influence root growth and may so stress root systems, that they inhibit ectomycorrhizal formation (Sinclair and Marx 1982). The containers used in this experiment are Leach 164 cc (10 cu.in.) super-cells, and while these may be suitable for initial mycorrhizal formation, prior to out-planting, they are too small for evaluating growth responses to mycorrhizae in itself. The restricted root environment dominates seedling growth.

Following these simple cultural procedures should produce a seedling that will be above the minimum size for outplanting and be "pre-adapted" with an appropriate

mycorrhizal fungus. This type of seedling should better survive in the adverse surroundings of Floyd Bennett Field. Despite the shortcomings of this experiment, seedling roots will undoubtedly begin to form mycorrhizae with the introduced spores, though probably not in great enough percentages that we will see measurable results. Once the symbiosis is established the Pt will eventually begin to fruit in this plantation. As these trees are moved to various parts of Gateway they will be carrying this beneficial fungus with them. In this way, we will have effectively introduced this fungus to many places where it is probably not present. As the trees grow, and begin to bear seed, the offspring will likely encounter this fungus and should benefit from its presence.

The fertilizer application, on its own and in combination with the Pt, should begin to produce results in the near future. If it does stimulate growth, as it ought to (Woods et al. 1984), then it is a procedure that should be incorporated into future transplanting schemes. The cost of fertilizer, though it can be prohibitive in timber growing situations, should be well worth the nominal investment for small scale revegetating by the Park Service. There is little added labor, the fertilizer can worked into the surface soil in a ring around the plant, or a fertilizer tablet can be placed 10 cm. deep within 10-15 cm of the base of the transplant. Any method of improving growth rates of the seedlings can only serve to benefit. Larger seedlings are less apt to be killed outright by animals or insects, more resistant to drought (as they sink a deeper tap root), less likely to be stolen or uprooted, and so, generally have overall better survival.

The test plantation should be evaluated in the fall of 1989. After two growing seasons differences among treatments might be discernable. Measurements should be taken on height to the apical bud, and root collar diameter, just above the soil line. The root systems of seedlings growing under the mycorrhizae and mycorrhizae/fertilizer

treatments can be analyzed for the presence of Pt mycorrhizae, and should be compared to the root systems of control and fertilizer only seedlings, as well as to each other. It is unlikely that there will be a measurable growth response due to the mycorrhizae, but it would prove interesting to see just how much infection did occur.

APPENDIX 2

Preliminary Mycorrhizal Studies

INTRODUCTION

The mutualistic relationship between plants and mycorrhizae is a common, well studied phenomenon. The fungi are important in the uptake and transfer of nutrients to the plant, in return, they are provided with carbohydrates from the vascular plant. In addition, they may also deter root pathogens, protect seedlings from root toxins, and promote seedling growth through the production of growth regulators. They play an important role in the health of the plant, and in many cases, the plant may not grow or develop normally without them. Seedlings of many trees, especially the pines, will fail to survive the first growing season without mycorrhizae. This is particularly true on droughty or infertile soils.

Pitch pine is a versatile, highly adaptable species that ranges from coastal northern Maine to interior sections of Georgia. It is intolerant of shade and a poor competitor, but extremely hardy, so it can be found growing where other trees are unable to survive. It may be found growing directly behind the barrier dunes, subjected to salt spray, but attains its best form on sandy loam soils with a moderate amount of moisture (Harlow et al. 1979). Specimens can range from 1-2 m tall with a 15 m "crown" spreading along the ground, to a 30 m tall forest tree, suitable for timber use. The species has been much maligned in the past due to its often grotesque (ornamental) form and frequent "choice" of poor sites. Recently, this underutilized species has been recognized for its ability to grow on these same poor sites. Researchers are using it for revegetating mine spoils where little else will grow. Its winter hardiness and tolerance of poor, droughty soils has been combined with the excellent form and growth of loblolly pine, in pitch-loblolly hybrids (Little and Trew 1979), in an attempt to create a cold-hardy form of loblolly. A pitch pine timber tree improvement program is now under way in New Jersey (J. Kuser, personal communication). This recent surge in interest in this adaptable tree could benefit from increased knowledge of its mycorrhizal

in this adaptable tree could benefit from increased knowledge of its mycorrhizal counterparts.

Pitch pine is reported by Marx and Bryan (1970) to form ectomycorrhizae with Pisolithus tinctorius (Pers.) Coker and Couch, and Thelephora terrestris (Ehrh.) Fr.. Doak (1934) reported that Boletus bicolor (Peck), Suillus brevipes (Pk.) Kuntze subsp. brevipes, Boletinus pictis (Pk.) Pk, and Russula lepida all formed ectotrophic mycorrhizae with pitch pine in culture media. Growth studies on pitch pine inoculated with mycorrhizae have been limited to Pisolithus tinctorius and Thelephora terrestris (Berry 1982, Lee and Koo 1983, Maronek et al. 1982). There is a limited information concerning pitch pine and the benefits that may be derived from its mycorrhizal associates.

Mycorrhizal fungi vary in their ability to colonize and benefit tree species; even ecotypes within a species vary in this respect (Trappe 1977). Some fungi will outperform others only under certain environmental conditions (Trappe 1977). Pisolithus tinctorius (Pt) is one that will outperform most others, especially under droughty conditions, and is generally accepted as the standard by which all other mycorrhizae are measured. It is common, easily cultured, and mass production techniques have been developed. However, in a recent study (Ford et al. 1985) it was found that Scleroderma aurantium, a common gasteromycete, was far superior in eliciting a growth response from loblolly pine than Pt, or two other common fungi, while growing under stressful conditions. Yet, in 1987 over 5 million pine seedlings were commercially inoculated with Pt (Cordell et al. 1987). Now that much of the basic technology for mass producing inoculum has been developed, shouldn't we now try to match the most appropriate fungus (or fungi) with host to elicit the greatest response?

A simple way to discover new possible mycorrhizal symbionts of a desired tree species is to go into the field and collect sporophores of fungi, at the appropriate time of year, in the proximity of the desired tree species. A simple method (Fortin et al. 1980) of combining the desired tree and fungus species enables us to rapidly observe if mycorrhizae do indeed form. This allows us to readily identify potential fungal candidates for further testing of inoculum for growth and survival response of the host.

METHODS AND MATERIALS

In September and October of 1987, fresh sporophores of Pt, S. pinorigida (Sp), Boletinus decipiens (Bd), Boletellus projectellus (Bp), Tylopilus felleus (Tf), Leccinum aurantiacum (La), Russula foetens (Rf), Russula delica (Rd), and a Rhizopogon species (R spp.) were collected from beneath pitch pine trees. The fruiting bodies were brought back to the lab, cleaned of excess debris, and lightly sponged clean with a 10% clorox solution. The sporocarps were gently torn apart and isolations were aseptically taken from the tissue in the pileus. Tissues were plated onto MMN agar (Schenck 1982) and incubated at 22 C. All fungi grew successfully, though some grew better than others. Notably, Pt, Sp, Bd, Rd, and R spp. grew rapidly, while the others all grew relatively slowly. Fungi were transferred every 3-4 weeks to fresh agar. Stock culture slants were made and maintained on MMN agar at 2 C.

Seeds from pitch pine trees were collected in Ocean County, NJ in November 1987. Selected seeds were surface sterilized and germinated on potato dextrose agar (PDA) to reveal contaminants, if present. The sterilization procedure was: (1)agitate seed in 200ml distilled water with 1 ml Tween 20 added for 30 mins. (2)agitate seed in .5% (by weight) solution of distilled water and $Ca(OCl)_2$ for 30 mins. (3) soak seed for 10 mins. in sterile water bath. (4) Transfer seed to PDA. The seeds are allowed to germinate and observed for contamination. Contaminated seeds are removed as soon as

noted. After 7 days the "clean" seeds are aseptically transferred into 100 x 80 mm pyrex storage dishes (no.3205) that contain 250ml of screened vermiculite (to remove the finest particles) and 120 ml of a nutrient solution (Piche and Fortin 1981). At 14 days the seedlings were just beginning to lose their seed coats from the tips of the cotyledons, and were aseptically transferred into sterile growth pouches. A 3mm hollow glass rod was inserted into the pouches, along one side to facilitate watering. Holes were punched along the top at either end, and a glass rod was inserted to support the pouches. Three paper clips were used to close the pouches along the top, and keep the watering tube and seedling in place. The seedlings wilted following transfer, but had regained turgor within 24 hours.

The pouches were suspended inside a plexiglas chamber with a glass top, that was designed and constructed by Dr. Bedker and myself specifically for this experiment. The chamber was surface sterilized before the pouches were placed inside it, and it is only opened within the laminar flow hood. Lighting is provided by a 40 watt fluorescent and a 40 watt gro-lite suspended above the chamber.

To prepare the fungi for transfer into the pouches they are grown out on fresh agar 2-3 weeks prior to the expected transfer date. Three days before transferring, 7mm plugs are cut with a cork borer and these are laid on fresh agar, mycelium up, to allow the hyphae to regenerate. The seedlings were inoculated when short lateral roots along the axis of the main tap root were observed. In a preliminary trial, 4 seedlings were inoculated with plugs of each of 4 different fungi, Sp, Pt, Bd, and R. Characteristic mycorrhizae formed on roots of seedlings inoculated with Pt and Bd, while webs of hyphae of the Sp and R grew around root tissue, but did not form a mantle. It should be noted that presence or absence of a Hartig net is the only reliable index of mycorrhizal development, and that the presence of a fungal mantle is a less reliable and variable factor (Wilcox 1982). Root tissue was not examined for a Hartig net, so although it

appeared in several cases that mycorrhizae did indeed form, I can not justifiably make this claim. The methods for checking for the Hartig net are relatively easy. Freehand sections can be mounted on a slide in lactic acid or lactophenol with a trace of cotton blue, and observed for infection (Wilcox 1982).

Another method of inoculating pine roots is through the use of spores. Harley (1959) reviews work by Robertson in which seedlings subjected to spore "rain" developed mycorrhizae on their roots, "which leaves no doubt as to the efficacy of spores as infective agents". To explore this method mature sporophores of 7 different species of fungi were collected from beneath pitch pine trees in the Greenwood Wildlife Preserve, Burlington Co., New Jersey. These fungi were R. dissimulins, R. foetens, Boletinus decipiens, S. pinorigida, T. felleus, Boletellus projectellus, and P. tinctorius. The stipes were removed from the pileus, and the fungi were placed on layers of waxed paper and transported back to the Floriculture Greenhouse, Cook College. Previously, racks of 49 tubes of Leach super-cells had been sterilized in a 15% clorox bath for one half hour, and rinsed with water. These were then filled with peat/vermiculite/sand (1/1/1) that had been steam pasteurized. The racks of filled tubes remained covered until ready for use. The sporophores of the individual species of fungi were supported by coarse screening, 1 sq. in. mesh, approx. 15 cm. above a rack of filled tubes and then covered with a box. By covering each rack and its sporophores I was hoping to prevent the spores of one species from landing on any soil except of that directly beneath them. The spores were allowed to drop onto the soil surface for several days and the sporophores were removed once they began to dry out. Seeds of pitch pine were surface sterilized in 10% clorox for 5 minutes and 2 were placed in each planting tube. These were then covered with 1 cm more of pasteurized soil and the boxes were replaced over the individual racks. Approximately 10 days following seeding, when the seeds had germinated and began to require sunlight the boxes were permanently removed. Keeping

the racks covered until the last possible moment was intended to prevent as much inadvertent contamination as possible. After the boxes were removed, nothing more was done to prevent contamination. The seedlings were thinned to one per container one month after germination. No fertilizer or fungicide was ever applied to any of the racks. One rack of "control" seedlings was treated in exactly the same manner as all of the spore "rained-on" racks, except it received no fungal inoculant. One rack of P. tinctorius basidiospore infested soil was also produced. Because of the nature of the sporophore, the spores were hand mixed with a small quantity of vermiculite and sprinkled in a regular fashion onto the soil surface at the same time that the other racks were receiving their spore "rain". Five seedlings were pulled from each rack in January, after 4 months of growth, and the roots were washed and observed for the formation of mycorrhizae. At this time, no mycorrhizae were observed. In June of 1988, 5 more seedlings from each of the 7 racks were pulled. Of the seven, 3 showed consistent and distinctive mycorrhizal infection. The most striking was the infection of the seedlings that had received spores from Boletinus decipiens. All 5 seedlings exhibited good inoculation rates. The mycorrhizae were a very bright peach color, and formed clusters of bulbous nodules at the ends of the short roots. The seedlings that received a "rain" of S. pinorigida spores also showed good infection rates. Again all five of the seedlings observed were infected with a distinctive appearing mycorrhizae. These mycorrhizae were a consistent tan-light brown color, and were arranged in "finger-like" sprays at the ends of the short roots. These are profusely branched mycorrhizal short roots, termed coralloid structures. The third group of seedlings that exhibited mycorrhizal infection were the seedlings that received the

P. tinctorius spores. In this case infection rates were very low, with only one or two infection sites per seedling. The mycorrhizae that formed were isolated bifurcate or pinnate short roots wrapped in a tan to yellow mantle that was closely appressed to the

infection sites per seedling. The mycorrhizae that formed were isolated bifurcate or pinnate short roots wrapped in a tan to yellow mantle that was closely appressed to the root tissue.

DISCUSSION

Wilcox (1982) states that mycorrhizae may display a wide variety of color, structure and size depending on the fungal species and host. He also says that in addition to these distinctive mycorrhizae, there are many inconspicuous mycorrhizae which possess no characteristic color or shape, meaning they may appear relatively similar to the naked eye. Casual observation could not distinguish differences among the 4 other spore "rained on" seedlings that were inconsistent with the control seedlings. They all showed some degree of mycorrhizal infection, mostly appearing as single or bifurcate short roots covered with a dark mycorrhizae. The fact that these seedlings did not exhibit any peculiar mycorrhizal formations does not mean that they did not form mycorrhizae with the spores, just that if they did, then I was unable to tell.

The consistency and flamboyance of the mycorrhizae which were produced on the roots of the seedlings "rained-on" by the spores of either Boletinus decipiens or S. pinorigida suggests that they were the propagules of the infection.

My experimentation with these different mycorrhizae and methods of inoculation were performed out of personal curiosity. They were rather preliminary in nature, and meant to bring me up to a point where I might be able to perform a proper experiment between these two symbionts. Unfortunately time constraints prevent me from pursuing this subject matter any further. I greatly appreciate the advice and assistance of Dr. Peter Bedker and the support of Dr. John Kuser in allowing me the freedom to study this unique symbiotic association.

TREE LOCATIONS FOR PROGENY TEST

All trees were permanently marked with a 2"x 2" aluminum tag nailed to the base, unless otherwise noted by an *. These latter trees were replacement trees.

Marconi trees were selected from the area near the parking lot at Cape Cod National Seashore, Marconi area. Trees # 2, 3, 6, and 7 are untagged (*) replacement trees. Trees from this area are designated Marconi (M) 1-7.

M1: Approx. 70 m from the parking lot, down the right fork of the Cedar Swamp trail, on the right side. 3.5 m tall, 4.5 m wide, 20 cm dbh., closed cones.

M2*: Approx. 150 m from parking lot, down right fork of cedar swamp trail. on the left side. 4 m tall, 2.5 m wide, 10 cm dbh, open cones.

M3*: Next to tree #2, toward parking lot. 2.5 m tall, 2 wide, 10 cm dbh, small closed cones.

M4: On the left fork of the Cedar Swamp trail, just past the first sharp bend to the right, 8 m from the Bear oak sign, on left hand side. 2.5 m tall, 3 m wide, 13 cm dbh, open and closed cones, very crooked.

M5: located about 35 m down trail from tree #4, 3 m off of trail on the left. 4 m tall, 2 m wide, 15 cm dbh, closed cones.

M6*: Located about 6 m from the southwest corner of the public restrooms. 3.5 m tall, 5 m wide, 10 cm dbh, two main stems, open and closed cones.

M7*: Open grown tree, located 100 m northwest of the public restroom in the adjacent field. 3.5 m tall, 5 m wide, 13 cm dbh, opencones.

The parent trees selected from High Head come from the groves that are located down High Head road, beyond the bicycle parking lot, 1/4 mile past the oversand vehicle entrance. All trees from this area are designated High Head windswept (HHWS) 1-8. Trees # 2, 4 and 5 are untagged (*) replacement trees.

HHWS 1: Located in the first stand of trees encountered on the right hand side. 4 m tall, 5 m wide, 12 cm dbh, open cone.

HHWS 2*: Located approx. 30 meters southwest of tree # 6. 6 m tall, 4 m wide, open cones with a flat spreading top. This tree was eliminated following poor seed germination 2%).

HHWS 3: 80 m south of tree # 1. 2 m tall, 2 m wide, 10 cm dbh, open cone with a good cone crop.

HHWS 4*: 35 m southwest of tree # 6, or 5 m southwest of tree # 2. 5 m tall, 3.5 m wide, 15 cm dbh. open cones with a flat, windswept top.

HHWS 5*:A nice straight tree located 5 m north of tree # 6. 5 m tall, 3.5 m wide, 13 cm dbh. open cone

HHWS 6: A very nice, straight bushy tree located on the east side of the north/south sand path, 40 m north of the vehicle sand road. 6 m tall, 2.5 m wide, 16 cm dbh. The best tree form seen yet.

HHWS 7*:Closest tree to new house, 75 m south of the sand vehicle road. 3 m tall, 10 m wide, 15 cm dbh. A low spreading tree, prolific open coner. Great form for revegetation purposes.

Parent trees selected from the Provincelands sheltered area are all located along Airport road, south of the visitors center, and opposite the airport. All trees from this area are designated Provincelands sheltered (PS) 1-12. Trees # 2, 10, 11, and 12 are untagged (*) replacements.

PS1: Located south of the Provincelands Visitors' Center entrance on the slope facing south. 30 to 40 m southeast of a stand of dead trees. 4 m tall, 2 m wide, 10 cm dbh, open and closed cones. The tree appears very decrepit and is probably on the way out.

PS2*: 10 m east of the No Parking sign the bike path crosses the road . On the north side of the road, the tree is located 100 m down east of the bike path entrance. 2 m tall, 3.5 m wide, 5 cm dbh, open cones.

PS3: Directly across from airport employee entrance, next to # 4. 3.5 m tall, 2.5 m wide, 15 cm dbh, open cones, excellent crop.

PS4: Directly across from airport employee entrance, next to # 3. 3.5 m tall, 2.5 m wide, 12 cm dbh, open cones.

PS9: Located down the bike path along the left side 10 m from the path. 3.5 m tall, 1 m wide, 3 cm dbh. excellent crop of open cones.

PS10*:Located 20 m northeast of tree # 9. 4 m tall, 3 m wide, 16 cm dbh, open cones.

PS11*:Located 20 m northwest of tree # 9. 2.5 m tall, 1 m wide, 8 cm dbh. A vigorous young tree with open cones.

PS12*:Located on the south side of the road. 12 m east of the bike path entrance, along the south side of the path. 7.5 m tall, 3 m wide, 16 cm dbh, open cone.

Parent trees for the Fire Island plantation were selected from two sites. The first selection site is in the Sunken Forest. These trees are designated (SF) 1-11. There are two untagged (*) replacement trees, #'s SF4, and SF7.

SF1: 200 m west of (SF) dune crossing, and 100 m north of Burma rd. 3 m tall, 2 m wide, 8 cm dbh, closed cones.

SF4*: 300 m west of dune crossing, near trapline. 3.5 m tall, 2 m wide, 11 cm dbh, closed cones.

SF5: Near the site of the deer trap station #1. 2.5 m tall, 2 m wide, 7 cm dbh., closed cones.

SF6: 50 m northwest of deer trap station # 1. trees # 7 and 8 are nearby. 2.5 m tall, 2 m wide, 11 cm dbh, closed cones.

SF7*: This untagged tree is 1 m northwest of tagged tree # 7, which is near trees # 6 and 8, 50 m northwest of trap station # 1. 2.5 m tall, 1 m wide, 9 cm dbh, closed cones.

SF9: 100 m east of Sunken Forest dune crossing , near the fence north of the Burma Rd. 2 m tall, 5 m wide, 18 cm dbh, open cones. This tree and the subsequent two are broad, low, spreading trees.

SF10: Adjacent to # 9, further east. 2.5 m tall, 6 m wide, 16 cm dbh, open cones.

SF11: Just behind tree # 10. 2.5 m tall, 8 m wide, 14 cm dbh, open cones.

Parent trees from the Wilderness Area are were selected in the an area along the Burma Road, east of the Long Cove gate. Trees from this area are designated (WA) 1-9.

WA 1: In the first clump of trees on the left as you head east down the trail. Trees # 2 and 3 are adjacent. 2 m tall, 1.5 m wide, 8 cm dbh, open and closed cones.

WA 2: Near trees # 1 and 3, in the first clump on the left. 2.5 m tall, 2 m wide. 11 cm dbh, small open cones.

WA 3: Near trees # 1 and 2, in the first clump on the left. 3 m tall, 2.5 m wide, 7 cm dbh, small closed cones.

WA 4: 200 m farther on from the clump of trees # 1, 2, and 3. 4 m tall, 3.5 m wide, 13 cm dbh, small open cones.

WA 5: Small leaning tree near tree #4. 2.5 m tall, 1.5 m wide, 8 cm dbh, small open cones.

WA 6: Down the road from trees #4 and 5. 4 m tall, 3 m wide, 9 cm dbh, closed cones.

WA 7: Near tree # 6, a little farther out. 4 m tall, 3 m wide, 8 cm dbh, hundreds of closed cones.

WA 8: In a windblown grove of trees 200 m behind the primary dune and 1/4 mi. past Long Cove gate, south of Burma Rd. 3.5 m tall, 3 m wide, 14 cm dbh, large closed cones.

WA 9: Near tree # 8, in the same grove. 4 m tall, 3 m wide, 11 cm dbh, open cones.

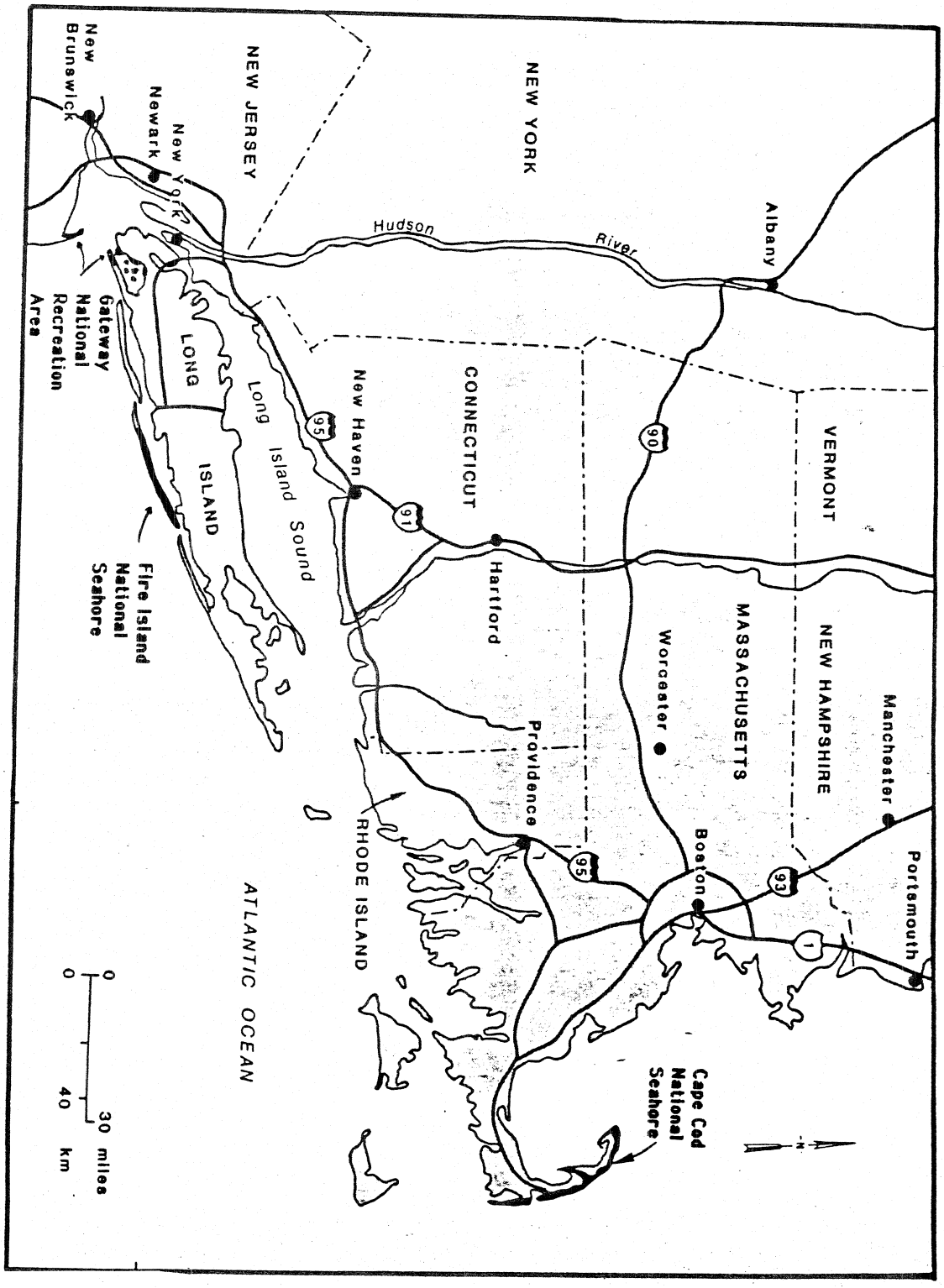


Fig. 14. Map showing locations of 3 research areas

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