Tree Survival and Growth on Graded and Ungraded Minesoil

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Compaction of soil by routine trafficking of earthmoving equipment during reclamation of surface-mined land can reduce tree survival and growth. Several species of trees-white pine (Pinus strobus L.), Virginia pine (P. virginiana Mill.), sugar maple (Acer saccharinum L.), sycamore (Platanus occidentalis L.), red oak (Quercus rubra L.), and black walnut (Juglans nigra L.) were planted on adjacent sites that were alike in all respects except that one was graded by standard reclamation procedures and the other had been abandoned in a semirough and uncompacted state. After 2 years, 42% of seedlings planted on the compacted site had survived, whereas 70% of those planted or, the uncompacted site survived. Trees grew taller on the uncompacted site. Thus, efforts to produce an aesthetically pleasing, smooth surface on reclaimed mined land may be counterproductive, adversely affecting tree survival and early growth. Tree Planters' Notes 41(2): 3-5 ; 1990.

Since passage of the Surface Mining Control and Reclamation Act of 1977, reclamation of mined land in the Appalachian coal region involves replacing the overburden to return the land to approximately the original contour and to restore the land to its original productivity. Slopes are reconstructed and graded smooth with heavy equipment. The final surface is usually "tracked-in" with bulldozers to create depressions (from the bulldozer treads) that can trap seed and water and promote germination of a uniform ground cover.

Unfortunately, traffic associated with grading the final surface and tracking-in a seedbed compacts the surface soil and makes it difficult to plant trees properly. Consequently, trees do not survive well, and when they do, their growth is retarded. Overall, the long-term productivity of sites with heavily trafficked mine soils is greatly diminished.

The effect of compaction on tree performance is not readily recognized by most reclamationists, partially because most species of herbaceous ground cover are not as seriously affected as trees, and because most miners are not familiar with the relationships between soil properties and tree establishment.

About 90% of the surface-mined land in Virginia is planted to trees, and much of the remaining land will return to trees via secondary succession. Given the importance of reforestation on surface mines in this region, this study was established to document the effect that compaction from grading and tracking-in has on tree survival and growth.

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Description of Study

The study was established on a partially reclaimed surface mine in Wise County, VA, in the central Appalachian coal fields. The test site was characterized by uniform overburden material of 2:1 sandstone/siltstone on a 30% slope that was 120 feet long and had a north aspect. Half of the site was graded, tracked-in, and hydroseeded, and the other was left rough-graded. The graded slope had already been operationally seeded with a hydroseed mix containing Kentucky-31 tall fescue and several clover species.

The ungraded slope had approximately 20% cover from volunteer forbs and grasses and was further hand seeded with a mixture of birdsfoot trefoil (10 pounds per acre), Appalow lespedeza (15 pounds per acre), and perennial ryegrass (10 pounds per acre). Fertilizer was applied to provide nitrogen (40 pounds per acre), phosphorus (100 pounds per acre), and potassium (50 pounds per acre). This "reforestation" mixture of legumes, grasses, and fertilizer has been demonstrated to produce a ground cover that is compatible with trees (2). The ground cover was established during June 1986, and trees were planted during April 1987.

Six species of trees were planted on each site: white pine, Virginia pine, black walnut, red oak, sugar maple, and sycamore. Each species was planted in rows from the bottom to the top of the slope. The number of trees per row varied from 10 to 15, depending on the length of the slope. Four rows of white pine *(Pinus strobus* L.) and Virginia pine (*P. virginiana* Mill.) were planted and two rows of black walnut *(Juglans nigra* L.), red oak *(Quercus rubra* L.), sycamore *(Platanus occidentalis* L.), and sugar maple (*Acer saccharinum* L.) were planted. All of the hardwood species were top-pruned to 1 foot before planting.

Table 1—*Tree survival and growth on a mine soil as affected by surface grading compaction*

	Survival (%)		Tree height (in)		
Species	Graded & tracked-in	Rough-graded	Graded & tracked-in	Rough-graded	Height response (%)
Black walnut	55	100	17	22	29
Red oak	40	62	17	17	0
Sugar maple	60	100	23	46	100
Sycamore	0	69	—	51	—
Virginia pine	63	42	18	22	22
White pine	37	43	10	17	70

Results

The greater amount of compaction on the smooth slope was immediately apparent during tree planting. Even during the early spring, when soils were relatively moist, it was very difficult to make a large hole with the dibble bar on the graded slope. On the rough-graded slope, however, planting holes were easy to open with a tree planting bar. After 2 years, more trees survived, and surviving trees were taller, on the rough-graded site than on the conventionally prepared tracked-in site.

Overall, tree survival on the rough-graded slope was nearly 70%, but it was only 42% on the compacted tracked-in slope (table 1). The effects of compaction were most severe on the hardwood species. All black walnut and sugar maple seedlings planted on the rough-graded slope survived, but only 50% and 60% of those planted on the smooth slope survived. On the rough-graded slope, 70% of the sycamores survived, and some grew to more than 4 feet tall in 2 years. On the compacted slope, however, none survived.

Compaction due to final grading reduced the rate at which the surviving trees grew. Most species were taller on the rough-graded slope. The effect was greatest for sugar maples, which grew twice as tall on the uncompacted site. The differences will probably become even more dramatic with time. Although trees have not yet been measured during the third year, it was observed that the oaks have added as much as 2 feet of new growth on the rough-graded slope, but only several inches on the compacted slope.

Tracking-in is usually done to obtain a more uniform ground cover. In this study, however, the best ground cover after 3 years occurred on the site that was not tracked-in. The cover on the graded slope was very lush during the first year, but subsequently has become sparse and appears to be unhealthy. On the other hand, the reforestation ground cover on the rough-graded slope was sparse during the first year (1986) but has subsequently become very dense.

The ability of certain tree species to tolerate adverse conditions was also noted. Virginia pine, a shallow-rooted species, survived much better than white pine on the compacted site. Observations at other sites indicate that Virginia pine is better suited to poor sites. Even at older ages when white pine typically grows much faster, Virginia pine will continue to outperform white pine on compacted soils (1). On the other hand, Virginia pine is very sensitive to shading, which it survived poorly on the rough-graded slope. The reforestation ground cover was designed to be sparse during the first year when trees are being established and then become dense when trees have grown above the cover. Poor survival of Virginia pine would not have occurred if seedlings had been planted during the year that the cover was seeded.

Early results from this comparison of ground cover and tree performance on these two differently prepared sites clearly demonstrate that normal reclamation grading practices to produce a smooth slope and uniform ground cover reduce tree survival and growth. This study also shows that good ground cover can be obtained without grading and tracking-in. The dense leguminous ground cover that resulted on the rough-graded slope is less likely to need follow-up work to meet legal requirements for mine reclamation. Leaving reclaimed sites in an uncompacted, semirough condition is less expensive and results in a more productive forest land use.

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Acorn Sowing Date Affects Field Performance of Blue and Valley Oaks

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Acorns of blue oak (Quercus douglasii Hook. & Arn.) and valley oak (Q. lobata Nee) were sown at monthly intervals from November until March. The earlier acorns were sown, the earlier seedlings emerged. First-year and second-year height growth and survival were also related to sowing date with the smallest growth and lowest survival for seedlings from the latest sowing. These results suggest that late acorn sowing reduces the chances for successful field establishment. Tree Planters' Notes 41(2): 6 -9 ; 1990.

During the last several decades there has been increasing concern about the management of native hardwoods in California, especially oaks. Part of the concern has resulted from recent reports that several species, including blue oak (*Quercus douglasii* Hook. & Arn.) and valley oak (*Quercus lobata* Nee), are not regenerating well in portions of their ranges (2, 5). In addition to regeneration problems, the total acreage of these species has been depleted in recent years because of residential and commercial development, agricultural conversions, and firewood harvesting (1).

To address these concerns about poor natural regeneration and develop techniques for successfully artificially regenerating these species, a number of studies have recently been initiated (3). The following study was designed to develop information on optimal sowing dates for directly seeding acorns.

Methods

In late October 1987, 200 acorns were collected from individual blue and valley oak trees. Acorns with holes, cracks, or spots were discarded. Those remaining were divided into 5 groups of 30 and placed in a refrigerator (2 to 4 °C) in 1.75-mil-thick zipper-lock plastic bags, as recommended by Rink and Williams (6). From early November to March, one group of 30 from each species was sown every month in a plot at the University of California's Sierra Foothill Range Field Station, 20 miles northeast of Marysville.

The acorn planting area consisted of 30 rows, each containing 10 planting spots. The rows were 3 m long and .38 m apart. On each sowing date, acorns from each species were sown in three randomly selected rows. Acorns were planted with a hand trowel, on their sides, 1 to 2-cm deep.

On the first three sowing dates, soil moisture was near field capacity. For the final two dates, however, the soil was crusty and dry. To minimize the effect of initial soil moisture on germination, each acorn sown in February and March was irrigated with 180 ml water. No additional water was provided. The plot was kept free of weeds by spraying glyphosate in early February, and hand weeding thereafter.

Beginning in February, the plot was evaluated periodically to determine the date of shoot emergence for each acorn. An acorn was counted as emerged when the shoot was visible at the soil surface. Assessments were made twice a week through April, and once a week in May and June.

At the end of both the first and second growing seasons, year-end seedling height and survival were recorded. In spring 1989, each seedling was also evaluated twice a week for bud burst (leaves emerging through the bud scales). All of these variables were averaged for each row and statistically tested according to the analysis of variance and least significant difference (LSD) tests. For calculating row averages of height growth, emergence and bud burst, dead or absent seedlings were treated as missing values so that survival differences did not influence the data. Differences reported as significant were at the $P < 0.05$ level.

Results

Seedling emergence was closely related to sowing date. Acorns that were sown earliest were the first to send up shoots (figs. 1 and 2). Subsequent sowings came up progressively later. The blue oaks began to emerge in mid-February, and the first valley oaks appeared 2 weeks later. There were significant differences in average emergence date between sowing dates and species.

There were also significant differences in first-year and second-year height growth among both sowing dates and species. Since there were no interactions, these values were averaged over species (table 1) and sowing dates (table 2). For both years, the least growth occurred in seedlings sown last, and the most in seedlings sown first.

Valley oaks grew almost twice as much as blue oaks during the first growing season. Although the valley oaks continued to grow significantly taller the second season, the magnitude of this difference was less than the first year. For blue oaks, average height growth the second year was approximately the same as it was the first year. For valley oaks, it was only about half.

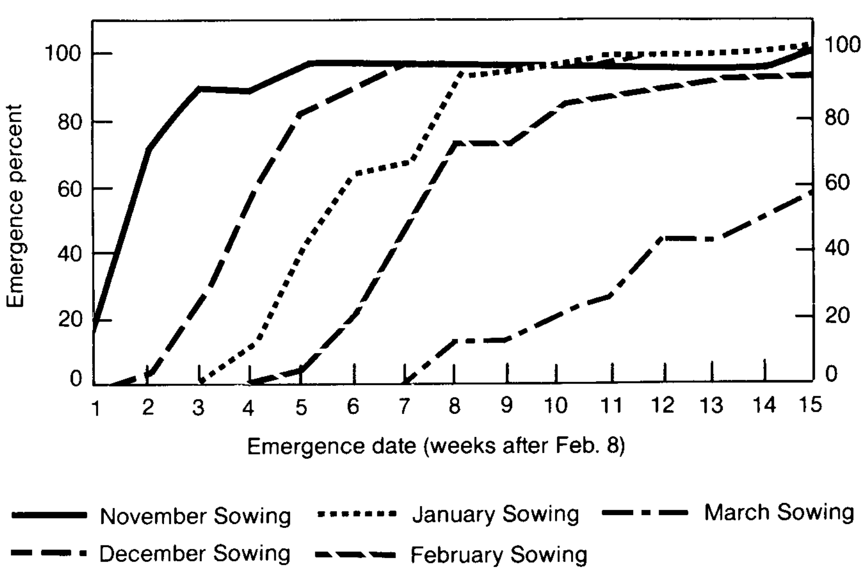

Figure 1—*Cumulative emergence rates of blue oak seedlings from acorns sown on different dates.*

Table 1—*First-year and second-year height growth of blue and valley oak seedlings from acorns sown in different months averaged for both species*

	Height growth (cm)	
Sowing date	1988	1989
November 10	28.0 a	21.0 a
December 11	25.0 a	16.2 c
January 12	27.7 a	18.0 bc
February 11	26.8 a	17.1 bc
March 10	19.9 b	15.5 c

In each column, values not followed by the same letter differ significantly ($P \leq 0.05$) by a least significant difference test.

Table 2—*First-year and second-year height growth of blue and valley oak seedlings averaged for all sowing dates*

	Height growth (cm)	
Species	1988	1989
Blue oaks	17.0 a	16.3 a
Valley oaks	33.9 b	18.9 b

In each column, values not followed by the same letter differ significantly ($P \leq 0.05$) by a least significant difference test.

There was little difference in 1989 bud burst date among any of the treatments. All seedlings that survived the first year began leafing out between March 20 and April 14. Averages among sowing dates and species varied by less than 3 days.

There were significant differences in first-year and second-year survival for both sowing dates and species. Since there were also significant interactions, the data are reported separately for each species (table

3). Survival was extremely high both years, averaging over 94% at the end of the first year, and 92% at the end of the second. In 1988, survival was significantly lower for the final sowing date for both species, and in 1989 for blue oak.

Discussion and Conclusions

Direct planting of acorns is a method of artificially regenerating oaks that has been used successfully in the South (4). However, it was not clear if this technique would work in California because theMediterranean-type climate and environment is harsh, with little or no rainfall

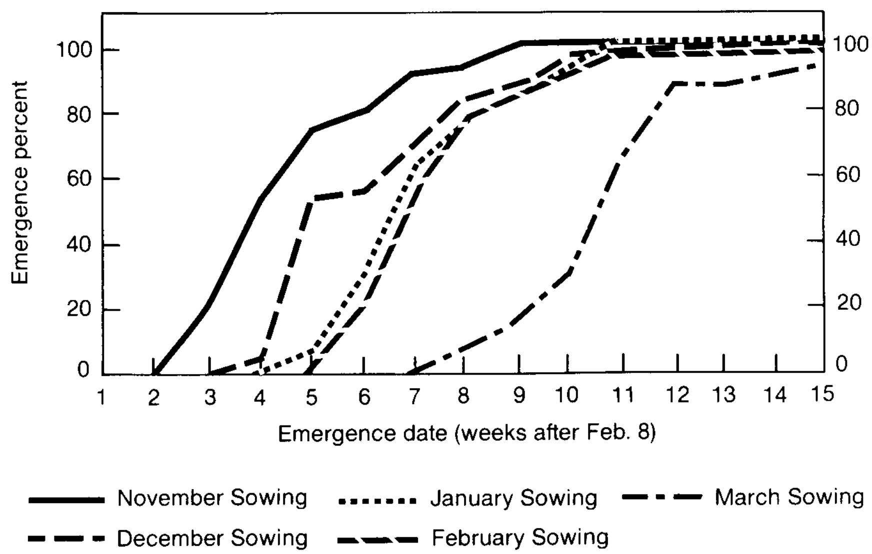

Figure 2—*Cumulative emergence rates of valley oak seedlings from acorns sown on different dates.*

Table 3—*Seedling survival from acorns sown in different months*

	Survival (%)	
Sowing date	1988	1989
Blue oak		
November 10	100 a	100 a
December 11	100 a	100 a
January 12	100 a	97 a
February 11	97 a	97 a
March 10	60 b	60 b
Valley oak		
November 10	100 a	100 a
December 11	97 ab	97 a
January 12	100 a	97 a
February 11	100 a	90 a
March 10	93 b	90 a

For each species, values in each column not followed by the same letter differ significantly ($P \leq 0.05$) by a least significant difference test.

between March and September. The results of this study indicate that direct seeding is a promising approach. Even without irrigation, fetilization, or augering, but with weed control, seedling survival after 2 years was over 90% and most plants had grown fairly vigorously.

It was clear from the study, however, that for both blue and valley oaks, sowing date can greatly impact field performance.

Late season sowing resulted in delayed emergence, smaller height growth, and lower survival. To promote early emergence and rapid growth, seedlings should be sown early in the fall, after the first fall rains have soaked the soil. Early emergence and growth of seedlings should help them become established during favorable growing conditions, before soil moisture becomes limiting.

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Stratification Not Required for Tree-of-Heaven Seed Germination

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Tree-of-heaven (Ailanthus altissima (Mill.) Swingle) seed germination and shoot dry weight were determined after stratifying samaras at 5 and 25 °C for up to 28 days. Seeds of samaras stratified at 5 °C for 0, 4, and 12 days showed 70, 77, and 96% germination, respectively, 7 days after sowing. Fourteen days after sowing, 90% of nonstratified seeds had germinated, but shoot weight of nonstratified seedlings was 16% less than that of seedlings from fruit stratified at 5 °C for 12 days. Germination 7 days after sowing was greater after stratification at 5 °C than at 25 °C, but the capacity for a large percentage of untreated seeds to germinate within 14 days after sowing indicates stratification is unwarranted for most growers. Tree Planters' Notes 41(1): 1012; 1990.

Tree-of-heaven grows rapidly in environments where other trees do not survive. Its capacity to resist urban microclimates and to produce biomass rapidly when planted on reclaimed land have been the focus of recent research *(1,2).* Reports of these studies indicate that tree-of-heaven seeds germinate without stratification.

However, plant propagation reference books suggest that tree-of-heaven seeds have dormant embryos and require a 30 to 60-day cold stratification before germination *(3-5).* The objective of this study was to quantify the effects of stratification on tree-of-heaven seed germination and seedling dry weight.

Materials and Methods

Samaras with dry, brown wings were harvested from a tree-of-heaven in College Park, MD, on October 28, 1988. The samaras were sealed in polyethylene and stored at 11.5 °C (±0.5 °C) until November 3, when 72 lots of 30 samaras were selected. Each lot was mixed with 125 cm^3 coarse vermiculite (Schundler Co., Metuchen, N)) in separate polyethylene bags, and 36 lots were assigned randomly to each of two pregermination treatments, cold stratification and nonstratified. Forty cubic centimeters of deionized water was added to 27 lots in the stratification group; bags with these lots were sealed and placed in a dark refrigerator at 5 °C. Twenty-seven bags with lots in the nonstratified group were sealed and returned to storage.

Seeds in the nine lots remaining from both treatments were sown on November 3 (day 0). Each lot of 30 samaras was arranged in a single layer on two Steel Blue Seed Germination Blotters (Anchor Paper Co., St. Paul, MN) saturated with deionized water.

A third moist blotter was placed on top of each lot of samaras. The 18 groups of blotters and samaras were enclosed in separate 127 x 133 x 33-mm acrylic germination trays (Hoffman Manufacturing Co., Albany, OR), and trays were arranged randomly in a dark seed germination incubator with alternating temperatures of 20 °C (-0.5 °C, 16 hr) and 30 °C (±0.5 °C, 8 hr). The number of germinated seeds in each lot was determined 7 and 14 days later by inspecting each samara for emergence of a radicle. After germination data were collected on day 14, all shoot tissue was removed from each lot, dried in an oven at 65 °C for 24 hours, and weighed. These procedures were repeated for nine lots chosen randomly from both groups on days 4, 12, and 28.

A second experiment began on December 22, when samaras were removed from storage, and 48 lots were prepared. Forty cubic centimeters of deionized water was added to 24 lots, and all bags were sealed. Twelve lots with water and 12 without water were placed in the refrigerator at 5 °C; all other lots were placed in a light-tight container in a laboratory at 25 °C. Twelve days

Scientific article 8129, Contribution A-5070 from the Maryland Agricultural Experiment Station.

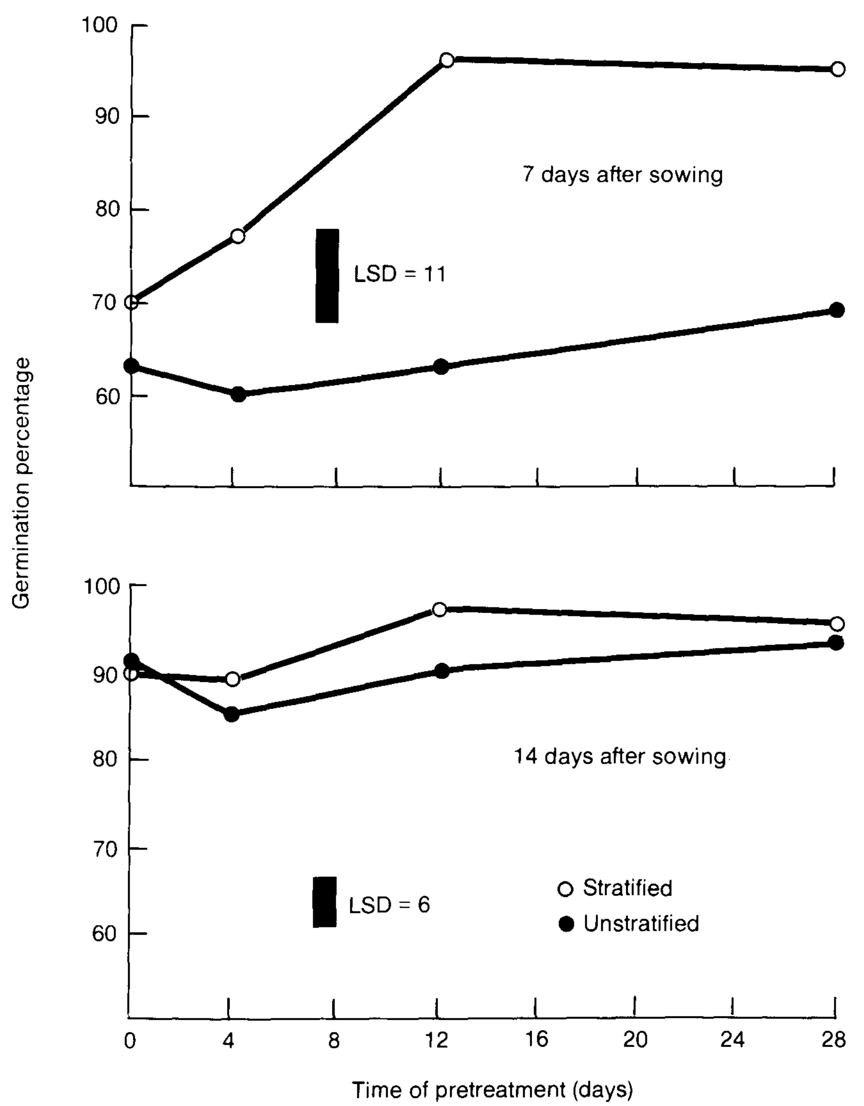

Figure 1—*Effect of moist stratification at 5 °C on germination of tree-of-heaven seeds. Each point is the mean of 9 replicates, each composed of 30 seeds. Vertical bars represent LSD (α = 0.01).*

later, the samaras were removed from bags and arranged on blotters in germination trays. The number of germinated seeds was determined after incubating the samaras for 7 days under the same conditions described for the first experiment.

Each lot of 30 seeds was considered an experimental unit for the statistical analysis of both experiments. Analysis of variance was used to determine the significance of stratification and time effects. Least significant difference (LSD) values were calculated for each dependent variable as described by Steel and Torrie (6) because interactions between main effects in both experiments were significant ($P < 0.05$).

Results

Stratification at 5 °C for as little as 4 days increased the percentage of tree-of-heaven seeds that had germinated 7 days after sowing, and germination increased with time of stratification up to 12 days (figure 1). Differences between percentage germination of stratified and nonstratified seeds were not statistically significant 14 days after sowing. However, the dry weight of seedling shoots at day 14 was greater for seedlings from samaras stratified 12 and 28 days than for seedlings from nonstratified samaras (table 1).

Results of the second experiment show that moist pretreatment at both stratification temperatures increased percentage germination 7 days after sowing (table 2). Among seeds treated with moist stratification, germination was significantly greater for seeds pretreated at 5 °C than for those pretreated at 25 °C.

Discussion

The results of this study show that stratifying tree-of-heaven seeds for as little as 4 days speeds germination, with maximum germination occurring after less than half the stratification time recommended previously (3-5). Mechanisms by which stratification hastened germination could include imbibition of seeds with water and the breakdown or leaching of chemical inhibitors. Data from the second experiment indicate that both mechanisms were involved because both moisture and low temperature were required for maximum germination. Despite the increase in germination 7 days after sowing for stratified seeds, stratification of tree-of-heaven seeds does not appear justified because high percentages of both stratified and nonstratified seeds had germinated within 14 days after sowing.

Although this study tested effects of stratification on the germination of seeds from a single tree in Maryland, the results are consistent with reports that tree-of-heaven seeds collected in New York (1) and Illinois and Massachusetts (3) germinated without pretreatment. Germination percentages and rates were not specified in these reports, however, so the degree to which tree-of-heaven seed dormancy varies with seed source is unknown.

Table 1-Shoot dry weight *of tree-of-heaven seedlings after seed stratification at* 5 °C *for up to* 28 *days*

Time of pretreatment (days)	Shoot dry weight (mg) Stratified	Nonstratified
0	162	168
4	168	154
12	153	194
28	169	197

Each value is the mean of 9 replicates; LSD = 21 (a = 0.01).

Table *2-Germination of tree-of-heaven seeds after moist and dry stratification at 5 and 25 °C*

12-day pretreatment	Percentage germination 7 days after sowing
Dry stratification	
5 °C	76
25 °C	75
Moist stratification	
5 °C	95
25 °C	84

Values are means of 12 replicates, each composed of 30 seeds; LSD = 8 (a = 0.01).

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Dry Site Survival of Bareroot and Container Seedlings of Southern Pines From Different Genetic Sources Given Root Dip and Ectomycorrhizal Treatments

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Survival rates of loblolly pine (Pinus taeda L.) seedlings from two seed sources and shortleaf pine (P. echinata Mill.) seedlings from one source were compared for the first 2 years after outplanting on drought-prone sites. Several combinations of stock type and preplanting treatments were examined: bareroot seedlings with no pretreatment, bareroot seedlings treated with Terra-Sorb® root dip, container seedlings, and container seedlings inoculated with ectomycorrhizae. Survival rates were not significantly different for seed sources. However, after two growing seasons, bareroot seedlings survived better than . container seedlings and container seedlings with ectomycorrhizae on the most severe sites. On a more moderate site, container seedlings showed the highest survival, and treated bareroot seedlings showed higher survival rates than untreated bareroot seedlings. Tree Planters' Notes 41(2):13-21; 1990.

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The western limit of the natural range of loblolly pine (*Pinus taeda* L.) is limited by a number of ecological factors (12). As this edge is approached, conventional reforestation techniques (for example, planting bareroot 1 + 0 seedlings) commonly fail. In Texas, this problem has become more critical as interest in reforestation of marginal agricultural lands has increased and accelerated urbanization has continued to decrease existing commercial forest acreage (2).

The objective of this study was to evaluate the influence of genetic sources, planting stock, and root treatments relative to pine seedling survival after outplanting. These evaluations were conducted over a 2-year period under site conditions common to the region and typical of sites being reforested.

The comparison of genetic material was made among two sources of loblolly pine seed and one source of shortleaf pine (*Pinus echinata* Mill.) seed. The planting stocks and root treatments were 1) bareroot seedlings; 2) bareroot seedlings with roots dipped in TerraSorb®, a hygroscopic starch, before planting; 3) container-grown seedlings; and 4) ectomycorrhiza-infected container-grown seedlings. The field sites were two recent clearcuts and an old pasture.

Materials and Methods

Genetic materials, preplanting growth regimes, and treatments. Seed sources were 1) loblolly pine selected for superior growth and form, 2) loblolly pine selected for drought resistance (30), and 3) shortleaf pine selected for superior growth and form (table 1).

All bareroot seedlings were produced by the Texas Forest Service using standard nursery techniques at their Indian Mound Nursery near Alto, TX. Seedlings were lifted while dormant, less than 1 month before planting. When graded according to the Wakeley guidelines (31), all planted bareroot seedlings were grades one or two. Mycorrhizal infection was not assessed.

Container seedlings were produced in temperature-controlled and supplementally lit greenhouse space on the Texas A&M University campus, College Station, TX. Seedlings were grown in Styrofoam planter flats with individual cells that were about 13 cm deep and 3 cm in diameter. The growing medium was a

1:1 mixture of peat and vermiculite. Fertilizer, water, and light regimes were generally manipulated for rapid growth, except for the ectomycorrhizal seedlings, which were fertilized at about half the nutrient level of the other seedlings. This lower application rate was used because high levels of nitrogen and phosphorus have been shown to inhibit ectomycorrhizal development (25, 28). All container seedlings were placed in a shadehouse 3 weeks before outplanting to harden seedlings (27) .

Mycorrhizal seedlings were inoculated with *Pisolithus tinctorius* (Pers.) Coker & Couch provided by the Institute for Mycorrhizal Research and Development (IMRD), Southeastern Forest Experiment Station, USDA Forest Service, Athens, GA. Inoculation and fertilization followed the institute's guidelines. Before outplanting, a subsample of 25 inoculated and 25 non-inoculated seedlings from each seed source were sent to the IMRD for assessment of ectomycorrhizal infection. The *Pisolithus tinctorius* (Pt) indices (17) for inoculated superior loblolly pine, drought-hardy loblolly pine, and shortleaf pine were 74, 83, and 69, respectively. A Pt index of at least 50 is believed necessary to significantly increase survival and growth of southern pine seedlings planted on reforestation sites (17). On non-inoculated container seedlings, infection by Pt was absent, but an average of 10% of the root tips were infected by other ectomycorrhizal species (primarily *Thelephora terrestris* (Ehrh.) Fr.).

Table 1--Genetic sources used in study

Genetic source	Description
Superior loblolly pine	Collected as bulk lot from first-generation seed orchard representing the continuous range of loblolly pine in East Texas
Drought-hardy loblolly pine	Collected as bulk lot from first-generation seed orchard representing western fringe of continuous range and material selected from "Lost Pines" (30) region in Texas
Shortleaf pine	Collected as bulk lot from first-generation seed orchard representing natural range of shortleaf pine in Texas

For container seedlings, mean seedling shoot and root mass were 665 and 250 mg, respectively. These seedlings fell within the acceptable size criteria for container stock (4). We observed that the non-inoculated seedlings, probably because they received more fertilizer, were slightly taller and had better nutritional appearance than inoculated seedlings.

Bareroot seedlings were planted with and without a root dip treatment. In the root dip treatment, a slurry of water and Terra-Sorb, a gelatinized, starchhydrolyzed polyacrylonitrile graft copolymer, was prepared in accordance with the manufacturer's instructions. Root systems receiving the treatment were dipped immediately before planting. To ensure equal care, roots of non-treated seedlings were dipped in water immediately before planting.

Study sites. The study was conducted on three sites (table 2) located near the western edge of the two species' natural range in northeast Texas (fig. 1). One study site, a clearcut, was in Henderson County near Athens (AthCC). The other sites were located in Bowie County near New Boston. One New Boston site was a clearcut (NBCC) and the other an oldfield pasture (NBOP). Soil moisture and organic matter characteristics are listed in table 3. Although soil nutritional status was evaluated for each site, that work is not reported here because all sites were nutritionally adequate and there were no major differences among sites.

On each site a standard U.S. Weather Service rain gauge was maintained during the first 9 months of the study. After that, weather information was obtained from the nearest U.S. Weather Service Station.

Figure 1—*Location of study sites in relation to natural ranges of loblolly and shortleaf pines.*

Experimental design. On each site, three complete replications were established. Each replication consisted of 12 numerically square plots, each containing all combinations of the four preplanting treatments and three genetic sources. Individual plots consisted of 49 trees from one combination of treatment and genetic source. Combinations were randomly assigned to plots within replications. Seedlings were planted on a 1.8 by 3.0 m spacing, with at least two border rows planted around each replication.

Planting, measurement, and analysis. All seedlings were hand planted in late February 1983 by Texas Forest Service planting crews using dibble bars. So that the influence of planters could be minimized, plots were planted one at a time, by at least three planters each. The recommendations of Owston and Stein (22) were followed as closely as possible in the care and planting of seedlings.

All seedlings were evaluated for survival about 2, 6, 9, 14, and 21 months after planting. For statistical analysis, the arcsine of the square root of average plot survival was used to transform data.

Soil sampling and analysis. To aid in interpretation of results, soil water retention characteristics and organic matter contents were characterized. For each plot, the first and second 15 cm of soil were sampled. Within each plot a minimum of four randomly selected points were sampled and composited for laboratory analysis.

Soil organic matter was determined by the Walkley and Black technique S (27), and available water-holding capacity was determined by the ceramic pressure plate extraction procedure using disturbed soil samples (24). Available water was assumed to be that water held between soil water potentials of -0.01 and -1.5 MPa (1 megaPascal = 10 bars).

Results

Sites were similar in temperature and total precipitation (fig. 2). However, there were

Table 2—*Description of study sites, treatments, and soils*

Site	Site description	Soil classification	Soil texture*
New Boston clearcut (NBCC)	Loblolly pine plantation, harvested December 1981, drum chopped, burned November 1982	Blevins series, fine silty, siliceous, thermic Typic Paleudult	Loam to sandy loam
New Boston old pasture (NBOP)	Dominated by *Andropogon* spp., burned December 1982	Rosalie series, loamy siliceous, thermic Arenic Paleudult	Sandy loam to loamy sand
Athens clearcut (AthCC)	Slash pine (*Pinus elliottii* Englem.) plantation, harvested spring 1982, hardwood cut or girdled, burned November 1982	Pickton series, loamy siliceous, thermic Grossarenic Paleudalf	Sand to sandy loam

*Texture of 0 to 30 cm soil depth.

marked differences among sites in the distribution of precipitation during the study. In the first 2 months, the NBCC and NBOP sites received 216 and 222 mm of precipitation, respectively, whereas the AthCC site received 99 mm. In contrast, the AthCC site, during the middle and late summer months (June-September 1983), received 25 to 100 mm more precipitation than the New Boston area sites, which had only a trace of precipitation during August 1983.

Contributing to the influence of precipitation, soils of the study sites were distinctly different from each other physically. Among sites, differences in available soil moisture-holding capacity were significant for both soil depths. For 0 to 15 and 15 to 30 cm soil depths, the NBCC site was 44 and 33% greater than that of the NBOP site and 185 and 197% greater than that of the AthCC (table 3). These differences primarily reflected differences in soil texture (table 2) but also appeared related to soil organic matter content (table 3). Organic matter content was significantly greater in the NBCC site than in the other sites, perhaps reflecting the finer soil texture and drum chopping of this site (table 2).

Seedling survival. An ANOVA table indicating statistical differences for the final (21 month) survival evaluations is presented in table 4. Over the study period there were no statistically significant differences in survival among genetic sources. Twenty-one months after planting, mean survival of genetic sources varied from 71 to 80% on the NBOP site, 70 to 73% on the NBCC site, and 52 to 58% on the AthCC site. Large pretreatment differences among the three sites were observed, leading to further analyses being performed on an individual site basis.

Preplanting treatments significantly influenced seedling survival, with the observed effects varying among sites. On the NBOP site (fig. 3), the site with highest overall survival, the container and Terra-Sorb-dipped bareroot (trsb-bareroot) seedlings showed significantly higher survival than bareroot seedlings. Also, container seedlings had significantly higher survival than ectomycorrhizal container seedlings (myco-container).

In contrast, on the NBCC site, the bareroot and trsb-bareroot seedlings had significantly higher survival than either the container or myco-container seedlings, and survival of the latter seedlings was significantly lower than in all other treatments. As shown in figure 3, the AthCC site exhibited a survival pattern similar to that of the NBCC site, but overall survival was markedly lower. After 21 months the myco-container seedlings again accounted for the lowest survival (39%) and the only significant difference between treatments.

As expected, mortality was generally greatest during the first growing season. However, while survival continued to decline in

Table 3—*Soil properties related to moisture retention on the study sites at two soil depths (0–15 cm and 15–30 cm)*

Site	Available soil moisture holding capacity (% of mass) 0–15 cm	Available soil moisture holding capacity (% of mass) 15–30 cm	Soil organic matter (% of mass) 0–15 cm	Soil organic matter (% of mass) 15–30 cm
New Boston clearcut (NBCC)	18.2 a	19.5 a	1.52 a	0.66 a
New Boston old pasture (NBOP)	12.6 b	14.6 b	0.88 b	0.42 b
Athens clearcut (AthCC)	6.4 c	4.9 c	0.72 b	0.39 b

Numbers in the same column followed by the same letter do not differ significantly at the 0.05 level.

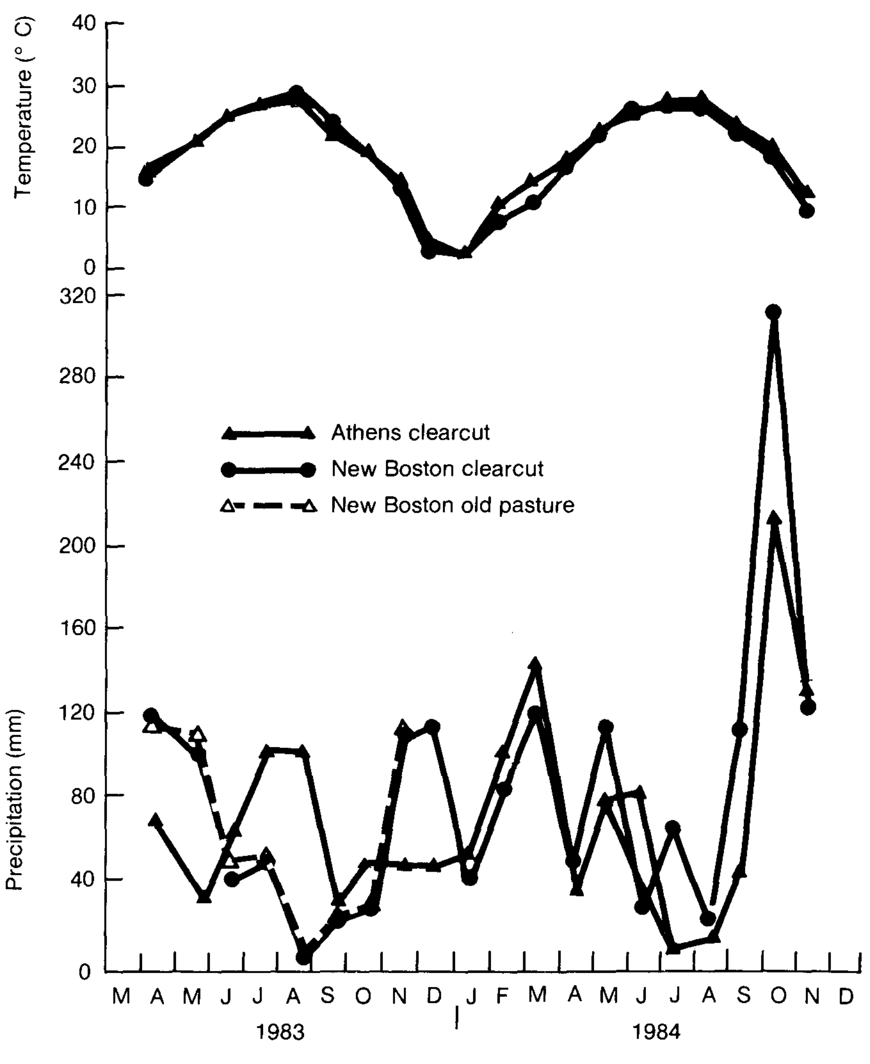

Figure 2—*Precipitation and temperature on study sites, New Boston old pasture, New Boston clearcut, Athens clearcut. Precipitation data after December 6, 1983, and all temperature data are from United States Weather Service Stations at New Boston and Athens, TX.*

the AthCC and NBOP sites over the first winter (December-April), the NBCC had little seedling loss during this period. Similarly, while all sites exhibited lower seedling mortality during the second growing season (May-November), the NBCC site had minimal additional seedling loss (fig. 3). The container and myco-container seedlings had very similar survival rates on the AthCC and NBCC sites in December 1983, but on the AthCC site, survival rates were dramatically lower 1 year later.

Discussion

Lack of significant differences in survival among seed sources was unexpected but perhaps reasonable. Shortleaf pine was chosen for its recognized ability to survive on xeric sites (6, 11, 31, 32). The drought-resistant loblolly pine seed source was composed of families that had been selected on the basis of seedling survival under severe drought, and similarly, the "superior" loblolly pine source, taken from the East Texas region, may have been adapted to more xeric conditions than families from more mesic portions of the species' range. However, it should be recognized that variability and lack of control of competing vegetation within sites may have contributed to statistical imprecision.

Among sites, differences in survival appeared related to soil, site history or preparation, and climatic conditions. After 21 months, seedlings on the AthCC site had the lowest overall survival. This site, with the coarsest soil texture and lowest available water-holding capacity (table 3), received only 100 mm of precipitation during the initial establishment period (2.5 months), a period of heavy mortality for all seedling treatments. Moreover, by May 1983, annual weeds were over 1 m tall and dense enough to inhibit movement by the crew. On the AthCC site this level of competition, coupled with the limited soil moisture retention capacity, appeared to be a dominant cause of sustained declines in survival throughout the study.

Similarly, the generally higher seedling survival on the NBCC site appeared related to its higher available soil moisture retention capacity (table 3) and higher rainfall in the initial establishment period (fig. 2). Declines in survival during the first growing season appeared due to intense competition from broadleafed annuals, which were nearly 1 m tall in May and over 1.8 m tall in October 1983. Seedlings that survived this period exhibited little additional mortality, suggesting good seedling establishment after initial water deficits declined.

Table 4—*Results of analysis of variance for survival after 21 months; percentages were transformed by the arcsine–square root method*

Source	df	Sum of squares	Mean squares	F value	Probability F*
Site (St)	2	3,626.04	1,813.02	32.51	.0001
Treatment (T)	3	2,772.94	790.98	14.18	.0001
Source (So)	2	15.17	7.58	0.14	.8731
St × T	6	2,097.56	349.59	6.27	.0001
St × So	4	391.09	97.77	1.75	.1478
T × So	6	693.08	115.51	2.07	.0672
St × T × So	12	1,763.28	146.93	2.63	.0056
Error	72	4,015.41	55.77		

*Indicates probability of a higher value.

Overall seedling survival was highest on the NBOP site. Again survival appeared related to soil and site conditions. Soil moisture retention capacity (table 3) was adequate; seedlings were planted in an undisturbed soil; and the site received relatively high rainfall during the initial establishment period (2.5 months) (fig. 2). These conditions favored good early establishment. Moreover, the pasture's grass cover, normally considered a potentially severe hindrance to pine establishment (6, 13, 26), was not a continuous sod and its moisture-conserving mulch layer had been only partially consumed by the control burn. Thus, level of competition for soil moisture and growing space appeared lower on this site.

On the AthCC and NBCC sites, lower survival of the container and myco-container seedlings was in contrast to their higher survival on the NBOP site (fig. 3). Numerous articles have indicated that container seedlings survive better than bareroot seedlings on severe sites (6). Likewise, high levels of mycorrhizal infection have enhanced seedling survival (15, 16). However, it has also been shown that there are numerous exceptions (23), and both seedling morphology and site preparation may strongly influence this relative survival ranking.

The lower survival of container stock may reflect a variety of factors. In considering loblolly pine bareroot seedlings, Barnett et al. (7) have concluded that, although larger seedlings are better able to survive in areas with heavy competition and better soil moisture conditions, smaller seedlings with lower shoot-root ratios are better suited for more xeric sites. Somewhat in contrast, larger

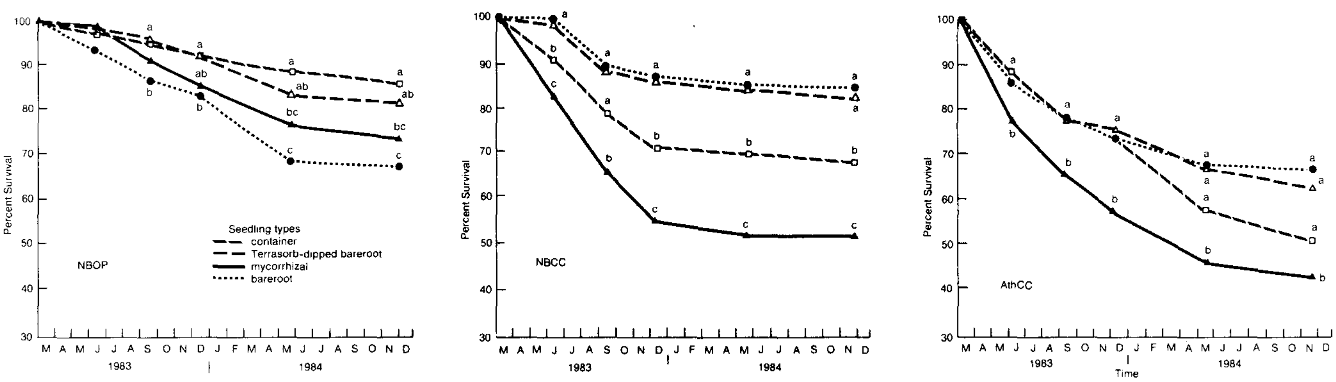

Figure 3—*Survival of pine seedlings, all species combined, on the New Boston old pasture, New Boston clearcut, and Athens clearcut, which received the following preplanting treatments: Terra-Sorb®-dipped–bareroot, bareroot, container, and mycorrhizal-container seedlings. For individual sites and times, treatments followed by a different letter differed significantly at the 0.05 level according to Duncan's multiple range test.*

container seedlings generally survive and perform better than smaller individuals (1, 3, 5, 9), but on the most severe sites, container stock size is less critical for survival than for seedling growth. Apparently on severe sites the intact root system of container seedlings results in good survival over a greater range in stock sizes (7). Considering the impact of mycorrhizae, Barnett (4) found larger non-inoculated container seedlings, which had received higher fertility in the greenhouse, performed better after outplanting than mycorrhizal seedlings produced at lower fertility.

The bareroot seedlings in this study, graded according to Wakeley's (31) criteria, were of good to excellent quality. In contrast, seedling size was not optimal for container seedlings. Although both inoculated and non-inoculated seedlings met minimum acceptable size criteria (5), their mean shoot mass (665 mg) was at the mid-range of shoot masses (228 to 1,249 mg) found associated with acceptable field success (4).

Coating of root systems with a hygroscopic substance is often done to reduce desiccation of seedlings during handling and shipment (8, 18). It has been suggested as a preplanting dip to reduce planting shock and mortality caused especially by short-term droughts during or immediately following planting (14, 19, 20). However, its effectiveness is uncertain and appears to decline with severity and duration of drought stress. Magnussen (14) found that coating the roots of seedlings planted on a site exposed to a brief 2-week drought improved survival by 24%. However, Tung *et al.* (29) and Dunsworth (10) working on more severe sites failed to show a significant long-term influence on survival. On the more moderate NBOP site, the early decline of control bareroot seedling relative to the trsb-bareroot seedlings and the container seedlings suggests the occurrence of a relatively short-term or more moderate post-planting drought against which the Terra-Sorb and other root treatments were effective and from which the seedlings derived long-term benefit in terms of establishment. The failure of this treatment to significantly enhance survival on the other, more severe sites is consistent with results previously discussed.

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Cone Maturation and Seed Yield of Ocala Sand Pine

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The serotinous nature of cones of the Ocala sand pine (Pinus clausa (Chapm. ex Englem.) Vasey ex Sarg. var. clausa) allows for their collection at any time of the year. For the development of Ocala sand pine seed orchards, better information was required on optimal collection dates. Data from the tests reported here indicate that to avoid lower seed yields and lower germination rates, collections should not begin before mid-October (depending on the order of clone ripening). Collections should be completed not more than about 3 months after maturation because seed yields per cone decrease with increasing time of exposure on trees. Tree Planters' Notes 41(2):22-26; 1990.

Sand pine (*Pinus clausa* (Chapm. ex Engelm.) Vasey ex Sarg.) is a minor species of southern pine with a restricted range in central and northwestern Florida (8). The two recognized varieties differ primarily in their cone characteristics. Variety *immuginata*, commonly known as Choctawhatchee sand pine, is native to northwestern Florida and has cones opening at the normal time, whereas variety *clausa* (11), known as Ocala sand pine, grows in central Florida and has serotinous cones.

The largest concentration of the species occurs on the rolling sandhills of central Florida in a block of some 200,000 acres of Ocala sand pine lying mostly within the Ocala National Forest. Experience has shown that sand pine is the best adapted of the southern pines to these local dry, infertile soils, and it is used to regenerate such sites. Seeds for reforestation have usually been obtained from harvesting operations, but a sand pine seed orchard is now in production and provides increasing proportions of the seed needs.

Collections of the serotinous cones have been made at almost any time of the year when logging operations are under way. However, it seemed worth investigating whether yield and quality of seeds could be improved by making collections during a restricted period after cone and seed maturation had occurred. No reliable data existed about times for either cone and seed maturation or optimal collection. Cone maturity has been related to cone color-ripeness is associated with development of light brown color (5). Specific gravity is the indicator most commonly used for cone maturity in southern pines generally (10), but it is not reliable for sand pines (5).

The present study was undertaken to determine times of maturation in cones and seeds and to identify optimal dates for cone collection from Ocala sand pine.

Methods

Tests were conducted in two successive years (1986 and 1987) in the Ocala sand pine seed orchard of the USDA Forest Service near Silver Springs, FL. First-year cone collections were made at 2-week intervals from late September to early December (6 collection dates). In the seed orchard, six clones that had good cone crops were selected for repeated collections. The clonal collections were from one ramet. Twelve cones were collected from each clone on each collection date. These were placed in Kraft paper bags, marked with clone number and date of collection, and held at ambient temperatures. The collections made from 6 clones on each of 6 dates resulted in 36 bags of 12 cones each.

Cone processing was delayed until all collections were completed. Previous tests had indicated that such cone storage of Ocala sand pine did not affect seed germination (2). The cones were shipped to Pineville, LA, for extraction and seed testing. Extraction began on December 30, 1986, by dipping the 12 cones from each Kraft paper bag individually into boiling water for 15 seconds to break the resinous seal of the cone scales (5). The cones were then dried in a forced-draft oven at 95 °F for 24 hours. Empty seeds were separated by flotation in 95%

ethanol. Full and empty seeds were counted.

All cones from a single clone had about the same number of seeds. The number of seeds extracted per clone was therefore determined for each date of collection to estimate the effect of collection date on seed yield. Studies with other species have shown that this technique works well (3).

A 200-seed sample (2 dishes of 100 seeds each) was tested for each clone and collection date. Germination counts were made 3 times weekly to evaluate the speed of germination. Tests were conducted for 28 days with unstratified seeds on a sand-peat medium.

In the second year, the same methodology was followed, but this time collections were made at 3-week intervals from early August to early January (8 collection dates). The same clones were used as in the earlier study and the cones were handled and processed in the same manner. At the time of three of these collections (August 10, October 13, and January 4), 1-year-old cones also were collected from each clone. Extraction began on February 8, with the full and the empty seeds separated as before. Samples of full and empty seeds of each clone were weighed to determine seed weight per clone for each collection date.

Results

Seed yields. *Year one*. Seed yield varied between clones; for example, clone 25 averaged 28 seeds per cone whereas clone 45 averaged 54 per cone (table 1). Collection dates (between September 22 to December 1) did not have an important effect on seed yield. Seed number per cone varied from 36 (November 17) to 46 (October 20), but variation was so great that the differences were not statistically significant (0.05 level); also they did not follow a logical progression.

The percentage of full seeds per cone averaged 64% and ranged from 53 to 79% (table 1). Results from other species indicate that these percentages are consistent from year to year within a clone (9).

Year two. In the second year, collection dates were extended from early August to early January and some collections of 1-year-old cones were made from the same trees. The longer collection period revealed trends in yield of both full and empty seeds. Cones collected on August 10 case-hardened, and no seeds were obtained. At the end of August, only 4 of 34 seeds extracted per cone (fig. 1) were full. The yield of full seeds increased on each later collection until November 23 and decreased thereafter.

Table 1—*Seed yields and germination rates of Ocala sand pine cones collected on six dates (year 1), based on 12 cones per clone*

	Clone number						
Collection dates	23	25	28	35	40	45	Avg.
Yield of full seeds (#/cone)							
September 22	39	32	30	22	60	47	38
October 6	28	16	18	47	44	60	35
October 20	44	26	49	44	57	55	46
November 3	30	23	31	44	53	58	40
November 17	40	24	44	42	51	57	36
December 1	32	45	39	19	47	49	38
Average	36	28	35	36	52	54	39
Total yield (#/cone)	62	44	66	58	66	74	61
Percent yield of full seeds	57	63	53	62	79	73	64
Percentage germination							
September 22	81	91	89	92	79	48	80
October 6	88	90	86	94	94	76	88
October 20	93	94	91	95	98	94	94
November 3	97	90	95	95	90	84	92
November 17	93	95	94	99	94	94	95
December 1	96	94	97	96	94	89	94

The trend after maturation toward decreasing numbers of full and increasing numbers of empty seeds was further illustrated by data on cones from the previous cone crop (fig. 1). These data confirmed the trend observed in the first year-the numbers of full seeds decreased with increasing exposure time on the trees. Average numbers of full seeds per cone decreased from about 40 at maturation to 5 fourteen months later. There was, of course, a corresponding increase in empty seeds. Although these data represent two separate years of cone initiation (fig. 1), they are believed to represent the trends realistically because the crops grew in consecutive years on the same trees.

As expected, clones varied considerably in seed yield and in the dates on which optimal yields were obtained.

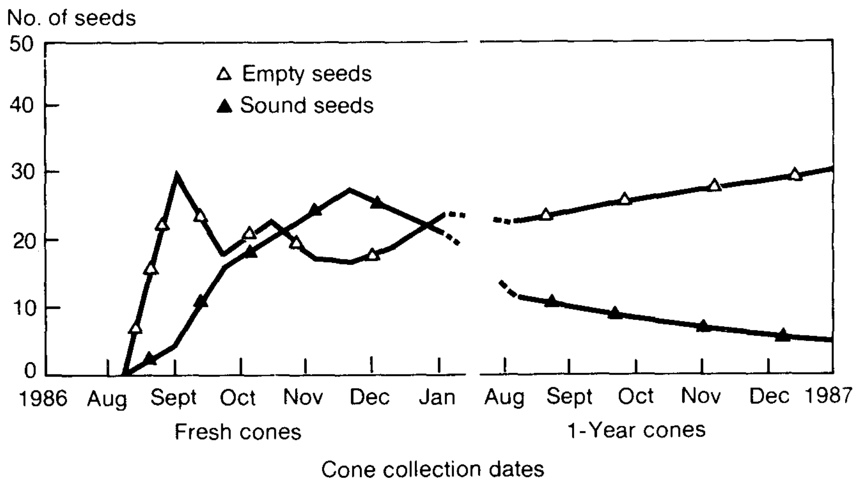

Figure 1—*Seed yield of Ocala sand pine cones on successive collection dates. Each plotted yield is an average from 36 cones (12 cones from each of 6 clones) collected on that date.*

Germination. *Year one*. Germination varied with cone collection date (table 1). Seeds from cones collected on the two earliest dates (September 22 and October 6) averaged 80 and 88% germination, respectively, whereas those for the four later collection dates averaged 94%. Germination also showed a statistically significant interaction with clones, so that some clones were more adversely affected than others by early collection. For example, for clone 45, seeds collected on September 22 yielded only 45% germination whereas, of those collected 4 weeks later (October 20), 94% germinated.

Germination speed expressed as the peak value of the maximum cumulative percentage germination divided by the number of days from sowing (6) followed the pattern for total germination. Seeds from the two earliest collections germinated slower overall, but there was considerable variation among clones. Clone 45, which had the lowest total germination, also had the slowest germination.

Year two. In the second year, trends in germination generally agreed with those in the first. Viability was low for seeds collected on August 31 and September 21 (table 2). Collections made in mid-October or later had relatively constant viability.

Seeds from the August 6 collection of 1-year-old cones had a lower total germination than those collected later. The reason for the low viability at this collection date is not clear.

Discussion

Cone collections did not begin early enough in the first year to decide when cone and seed maturity occurred, but the two earliest collections (September 22 and October 6) resulted in lower germination than later collections, particularly in some clones. Seeds that had lower total germination rates also germinated more slowly. In the second year, data confirmed that collections in August, September, and early October resulted in seeds with reduced viability. When seed yields also were considered, it became apparent that cones from Ocala sand pine should not be collected until November.

Collections of cones from the same 6 clones over a 2-year period indicated a marked reduction over time in numbers of full or sound seeds. Yields of full seeds from collections in the fall of 1986 averaged about 40 per cone. After a year on the trees, yields were less than 10 per cone. There was a corresponding increase in empty seeds per cone.

Insect damage seems the most logical explanation for the loss of full seeds over time. The damage to seeds in southern pine cones done by the shieldbacked pine seed bug (*Tetyra bipunctata* (Herrich-Schäffer)) is well documented (7), and continuous feeding on cones and seeds by various insect species has been reported to reduce the overall production and cost effectiveness of the Ocala sand pine seed orchard (1).

Earlier research has shown that such decreases in yield do not occur when cones are collected and stored in bags for 1 year at ambient temperatures (4), and that seeds from 1-year cones have lower germination (86%) than new cones collected at the same time (93%) (5). Only 56% of seeds from older cones (2 + years) germinated.

Table 2—*Ocala sand pine germination, based on yields from 12 cones per clone on eight collection dates (year 2) plus yields of some 1-year cones*

	Clone number						
Collection dates	23	25	28	35	40	45	Avg.
Current year's cones (% germination)							
August 10	0	0	0	0	0	0	0
August 31	0	54	0	59	77	0	63
September 21	87	68	79	50	68	60	69
October 13	85	67	82	87	84	84	83
November 2	85	80	62	81	90	88	81
November 11	96	86	86	88	86	88	88
December 14	91	88	76	89	78	86	84
January 4	85	73	66	96	94	88	84
First year's cones (% germination)							
August 10	84	70	78	86	73	60	75
October 13	80	83	78	89	91	69	82
January 4	IS	81	84	93	70	81	82

IS = Insufficient full seed for testing.

Recommendations

The tests indicate that general collections of the current year's cones of Ocala sand pine should not begin until November because both seed yield and viability are lower in earlier collections. For clonal material, it may be more efficient to base collection dates on clonal ripening dates; then, in selected clones, collection may begin in mid-October. The data also indicate that delays in cone collection for 6 months or longer may result in much smaller numbers of full seeds per cone. Thus, the optimal dates for collection of Ocala sand pine cones occur during the 3 months after maturation.

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Micropropagation of Valley Oak Shoots

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Stem segments of valley oak (Quercus lobata Nee) were placed on broad-leaved tree medium (BTM) and Gresshoff-Doy (GD) medium supplemented with 0.55 mg/l of 6-benzylaminopurine (BAP) to observe axillary bud elongation. Both media promoted bud and shoot development, but shoot initiation by explants on GD medium occurred earlier and more abundantly than on BTM medium. - Successful shoot development from axillary buds, with subsequent rooting, will permit production of valley oak planting stock from cryogenically preserved woody tissues of parent trees and this will contribute to the preservation of a species currently in decline throughout much of its native range. Tree Planters' Notes 41(2):27-30; 1990.

Regeneration of cryogenically preserved plant tissues in vitro is an alternative method for the reproduction of species with seeds lacking long-term storage capacity (16). Valley oak (*Quercus lobata* Nee), a riparian and upland alluvial white oak species indigenous to California (7), has significantly declined in number due to agriculture and urbanization (11). Many existing stands are failing to regenerate (6), and attempts to store acorns of the white oaks have often failed (2). Recently, valley oak has been listed as a "species of concern" in California, with the potential to be listed as "threatened" or "endangered" in the near future (14).

In vitro culture of species within the genus *Quercus* has been difficult (9), but some success has been demonstrated. Seckinger *et al*. (13) noted root formation on callus of northern red oak (*Q. rubra* L.) when cultured on Murashige and Skoog (MS) medium supplemented with a-naphthyleneacetic acid (NAA) and benzyladenine (BA). Also, calluses formed on pin oak (*Q. palustris* Muenchh.) cuttings placed on MS medium with various levels of NAA, but neither shoots nor roots were generated (8).

Successful shoot initiation in the oaks has generally been achieved by placing stem segments with one or more axillary buds on media supplemented with low levels of various hormones. Shoot development of northern red oak has been observed on Heller's medium with ammonium sulfate and Gresshoff-Doy (GD) medium (15), MS medium (4), and broadleaved tree medium (BTM) and woody plant medium (WPM) (3), each supplemented with low levels of 6-benzylaminopurine (BAP) or BA. Shoot growth of northern red oak and durmast oak (*Q. petraea* (Matt.) Liebl.) has also been observed on modified WPM and De Fossard medium and aspen culture medium (ACM) supplemented with various concentrations of the hormones BAP, KIN (kinetin), 2iP ([2-isopentenyl]adenine), NAA, indole-3-butyric acid (IBA), and gibberellic acid (GA_3) (10). Bennett and Davies (1) successfully propagated single-node stem segments of Shumard oak (*Q. shumardii* Buckl.) on liquid WPM with BA, and Sato *et al*. (12) propagated epicotyl segments of Sawtooth oak (*Q. acutissima* Carr.) on BTM medium supplemented with BAP.

This study was done to assess the suitability of GD and BTM media augmented with 0.55 mg/l of BAP to promote shoot development from axillary buds of valley oak. Shoot development, with subsequent rooting, will permit woody tissues of parent trees to be regenerated in vitro after cryogenic preservation, resulting in plantlets suitable for use in the production of valley oak planting stock.

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Materials and Methods

Stem sections approximately 5 cm in length were taken from vigorous 1-year-old greenhouse-grown valley oak seedlings and all leaves were removed. The sections were cleaned and sterilized ultrasonically and chemically for 3 minutes in a solution of 6% calcium hypochlorite and 0.05% Tween 80 with an adjusted pH of 6.0. The sections were then dipped in a solution of hydrochloric acid at pH 3.5, rinsed three times in sterile water, and cut into segments 1.0 to 1.5 cm long. Only stem tissues that appeared partially lignified were used, as preliminary trials indicated these were less susceptible to damage during sterilization than nonlignified tissues.

After sterilization, stem segments with one or more axillary buds were placed in 100 by 20mm petri dishes containing 35 ml of either GD medium (5) or BTM medium (3) supplemented with 0.55 mg/l of BAP. Each medium also contained 7 g/l of sucrose and 10 g/l of Difco Bacto-agar (Difco Laboratories, Detroit, MI), and the pH was adjusted to 6.0. Both media were autoclaved at 120 °C for 30 minutes before the explants were placed in them. Explants remained in the petri dishes for 1 week but were transferred away from their exudations once daily for the first 3 days.

The explants were then transferred to 22 x 175-mm test tubes containing 15 ml of one of the two culture media prepared as indicated above. One explant was placed in each test tube, 50 explants were set up per treatment, and the explants of each treatment were divided into 10 replications of 5 explants each. After 2 weeks in the test tubes, all explants were placed on fresh media. Explants were grown in an EGC M-12 growth chamber (EGC, Inc., Chagrin Falls, OH) under a combination of metal arc, high-pressure sodium vapor, and incandescent light. The light intensity was attenuated to approximately 300 μE/m^2/s (400 to 700 nm) with 50% shade cloth. The photoperiod was 12 hours, and day and night temperatures were set at 23 °C and 16 °C, respectively.

After 28 days, the number of buds induced and the number of shoots on each explant were recorded. The height of each shoot was also measured to the nearest millimeter. The arcsine transformation was performed on the percentages of explants with buds and with shoots, and all differences between treatment means were evaluated with the t test using the Statistical Analysis System (SAS Institute, Inc., Cary, NC).

Results

The bud and shoot productivity of the explants grown on the two media with 0.55 mg/l of BAP differed significantly after 28 days (table 1). Although a greater proportion of the explants grown on BTM medium had buds than those grown on GD medium, a greater proportion of explants on the later medium had produced shoots at this time. A total of 32 buds and 27 shoots were observed on explants cultured on BTM medium and 25 buds and 39 shoots on explants grown on GD medium. The minimum height of the shoots produced on both media was 1 mm, but the largest

Table 1-*Bud and shoot development of valley oak explants grown on broad-leaved tree medium (BTM) and Gresshoff-Doy (GD) medium with 0.55 mg/l of BAP after 28 days*

Medium	Explants With Buds (%)	Explants with shoots (%)	Shoot height (mm)
BTM	64	54	6
GD medium	50	78	6
Level of significance	*	**	NS

Differences between treatment means were evaluated with the t test; * and ** denote that the means differ at a level of significance of 0.001 <P<0.01 and P<0.001, respectively; NS denotes that P>0.05.

Figure 1—*In vitro shoot development from axillary buds of valley oak after 28 days.* **(A)** *Shoot development of explants cultured on broad-leaved tree medium (BTM) with 0.55 mg/l of BAP.* **(B)** *Shoot development of explants cultured on Gresshoff-Doy (GD) medium with 0.55 mg/l of BAP.* **(C)** *Shoot development of pruned, greenhouse-grown valley oak seedlings. Bars represent 5 mm.*

shoot produced on BTM medium was 19 mm whereas the largest on GD medium was 15 mm. Nevertheless, mean shoot height did not differ between the two treatments.

Overall, the buds and shoots of the explants grown on both media appeared to be healthy and vigorous. Shoots initiated on BTM medium (fig. 1A) and GD medium (fig. 113) were similar in stem diameter, internode length, and number of leaves to shoots originating as sprouts on pruned, greenhouse-grown valley oak seedlings (fig. 1C). Growth rates of shoots produced in vitro also approximated those of shoots occurring on greenhouse-grown seedlings. Leaves produced by shoots in culture were well formed and free of chlorosis and necrosis. In a preliminary test in which valley oak explants were placed on BTM and GD media without BAP, a single small leaf formed on each explant but characteristic shoot development was absent.

Discussion

After 28 days on BTM and GD media supplemented with 0.55 mg/l of BAP, shoot development of valley oak from axillary buds was more prolific on the GD medium. In other studies involving micropropagation of oaks, Vieitez *et al*. (15) reported successful shoot development from axillary buds of *Q. robur* on GD medium supplemented with BAP. However, Chalupa (3) reported BTM medium supplemented with BAP to be more suitable for axillary shoot development of this species than GD medium, as a greater number of taller and more vigorous shoots were produced on the BTM than on the GD medium. In addition, Sato *et al*. (12) found BTM medium with BAP to be satisfactory for shoot development of sawtooth oak. Given that buds were abundant and substantial shoot development also occurred on explants grown on BTM medium in this study, it is possible that this medium would have proven comparable to the GD medium in facilitating shoot development of valley oak if the experiment had been extended. Furthermore, lack of a significant difference between the mean height of the shoots cultured on the two media provides additional indication that both media may be suitable for shoot proliferation of this species in vitro.

The results of this study indicate that both BTM and GD media supplemented with BAP are suitable for the initiation of valley oak shoots from axillary buds, but that shoot proliferation occurs earlier on GD medium. Efforts are currently under way to devise and refine procedures to induce root system development of these shoots. Successful propagation of viable valley oak plantlets in vitro will permit production of planting stock from cryogenically preserved tissues of this species, and subsequently facilitate the preservation of a species currently in decline in its natural habitat.

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Preliminary Evidence of Genetic Variation in Winter Injury and Seedling Height of Paulownia Trees in New Jersey

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A progeny test of 9 paulownia trees (Paulownia tomentosa Sieb.) for height growth showed that the best open-pollinated family of seedlings averaged more than twice as tall as the poorest family after two growing seasons. Tree Planters' Notes 41(2) :31-33; 1990.

Paulownia tomentosa Sieb., a tree native to central China (2), was first brought to the eastern United States about 1834 (10) or 1845 (7) and has since become naturalized from Alabama to New Jersey (8). Known as *kiri* in Japan, it has been grown there for centuries to produce lightweight, check-resistant wood for *tansu* (bridal chests), *koto* (musical instruments), coffins, beams, ridgepoles, pillars, rice pots, sandals, spoons, and bowls. Its uses have become strongly linked to tradition. As the prosperity of the Japanese increased following World War II, demand for this resource soon outpaced the capacity of domestic sources. Plantation-grown wood from China, Korea, and Taiwan, able to satisfy some of this demand, was not considered to be of good-enough quality for use in the most expensive *tansu* (7).

In 1973, a Japanese importer discovered that paulownia had become common in the Eastern United States, and that trees here were larger than any he had seen before. Although widely dispersed, they could easily be found by helicopter search crews who spotted the lavender-blossomed crowns in spring. That year, 60 tons of logs were harvested and shipped to Japan. The Japanese determined that American-grown paulownia was of the highest quality, and exports climbed rapidly from 423,787 cubic feet in 1978 to 1,024,135 cubic feet in 1979, with prices as high as $1,500 per thousand board feet. By 1985, one Virginia tree farmer had sold 50 paulownia logs for $55,000 (5).

Winter injury and resulting crookedness are often seen in New Jersey, although the largest paulownia in the United States grows in nearby Philadelphia (1), and in that city huge paulownias flank the Museum of Art and surround Logan Circle.

In 1986, a small research project was initiated here to test the effects of varying fertilizer levels and spacing on the growth rate and stem form of paulownia. Different parent trees were included in the study, because New Jersey lies at the northern end of paulownia's naturalized range, and natural selection for greater winter hardiness may be occurring as a locally adapted land race develops.

Methods

In the summer of 1986, we enlisted the help of county agents and the New Jersey Bureau of Forest Management to search for the best timber-form paulownias. Selection criteria included size, straight trunks, and absence of winter dieback. Six trees were initially chosen (nos. 1, 2, 4, 5, 7, and 8 in table 1); later, three additional parents were added: the record tree in Philadelphia (no. 3) because of its size; a tree in Hainesburg, NJ (no. 6) representing the northern extreme of the land race; and a tree planted in 1925 at the New Jersey Botanical Garden, Ringwood, NJ, from a seed source in Ohio (no. 9). This latter tree had exceptionally large, dark green, glossy leaves, its trunk bore evidence of repeated dieback and winter injury, and we were curious to see if its progeny would be different. Seeds were collected from all 9 trees in November 1986.

In January 1987, seed of each lot was broadcast atop one full rack of Leach tubes (165 cm^3) filled with moistened 1:1 peatvermiculite mix. The soil mix

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contains vermiculite and sphagnum peat in equal proportions plus 589 g/m^3 5-10-5 fertilizer, 441 g/m^3 KNO_3, 441 g/m^3 $CaNO_3$, 589 g/m^3 triple super phosphate, 4,708 g/m^3 lime, 883 g/m^3 Micromax, 883 g/m^3 Aqua-gro, 589 g/m^3 $MgSO_4$, and 112 g/m^3 Oxamyl.

Because paulownia requires light in order to germinate (4), we covered the nine racks with clear plastic and placed them in a shaded area in the greenhouse. Kundt (6) outlines cultural practices for the germination and establishment of this species. When seeds had germinated, in 7 to 10 days, the plastic was removed and the racks were placed in the sun. No supplemental lighting was used. By March, seedlings were thinned to one per tube and in April, were transferred to 3.8 liter (1-gallon) containers.

Seedlings were outplanted in six randomized blocks at the Rutgers Horticulture Farm, New Brunswick, NJ, in June 1987. Each block contained 6 seedlings from each of the 9 parent trees. Three blocks were planted at 2.4 x 2.4 m spacing, and 3 at 1.2 x 1.2 m spacing. One wide-spaced and one close-spaced block received no fertilizer, one of each received a spot-treatment of 488 kg/ha of 10-10-10 (3.3 g/tree), and the balance received 977 kg/ha of 10-10-10 (6.6 g/tree). Application rates were derived from a previous study by Beckjord and McIntosh (3). Woodchip mulch was applied in a 0.6-m-diameter circle around each seedling to suppress weeds, and the plantation was irrigated as necessary through the first summer.

Table 1—*Number of paulownia seedlings in each seedlot (N = 36) killed to the ground after first winter and mean two-year heights of seedlots (N = 12) in two continuing plots*

Parent	No. of seedlings with winterkill	Height (m)
1. Salem, NJ	3	3.19 a
2. Kingston, NJ	3	2.86 ab
3. Philadelphia, PA	4	2.84 ab
4. Campbelton Drive, Princeton, NJ	6	2.83 ab
5. Faculty Drive, Princeton, NJ	1	2.59 abc
6. Hainesburg, NJ	3	2.44 abc
7. Wawa Lot, Princeton, NJ	9	2.05 bcd
8. University Place, Princeton, NJ	9	1.79 cd
9. Ringwood, NJ	12	1.26 d

Means bearing the same letter do not differ significantly, Duncan's multiple range test, $P < 0.0001$.

In May 1988, surviving seedlings were tallied, and stems were lightly pruned by removing large branches. Unacceptably crooked or leaning stems were cut to the ground. Occasional girdled stems and clipped roots that appeared to be damage from mice were observed. Because extreme variation in site quality (later found to be due to difference in soil moisture, with the poor plots excessively dry and gravelly) had become apparent following the first summer (trees in one closed-spaced and one wide-spaced plot grew tall, while those in the remaining plots grew very poorly), we abandoned the poor plots and fertilized 3 trees of each seedlot in the remaining wide-spaced plot on a randomized basis. Each fertilized tree received 90 g of 10-10-10 (977 kg/ha), broadcast in a 1.25-m-diameter circle around the stem. The remaining 3 trees of each seedlot in the same plot received no fertilizer. The 1.2 x 1.2 m block was not fertilized.

In November 1988, tree heights were measured in the two continuing plots. An analysis of variance was conducted to test the significance of height differences among seedlots and between fertilization levels. Duncan's multiple range test ($P = 0.05$) was used to identify significantly different levels of seedling growth when F values proved significant (9); the Pearson product-moment correlation was run between family mean heights and numbers of seedlings of each family that were killed to the ground during the first winter.

Results

Stem survival through the first winter varied among seedlots, with 12 of 36 seedlings of the most tender lot killed to the ground, and only 1 of 36 of the hardiest lot killed (table 1). Stem survival (not killed to the ground) was 85%. No dieback other than winterkill to the ground was apparent as the trees began their 1988 growth. Mean height at the end of the second season was 2.20 m, with the tallest tree in the plantation measuring 4.87 m. Differences among the 9 seedlots were highly significant ($P < 0.0001$). Winterkill was inversely correlated with second-season height ($r = -0.84$, $P < 0.005$).

Mean height of fertilized trees in the wide-spaced plot was 2.15 m, and unfertilized trees in the same plot 1.94 m; the difference was not significant (perhaps because of the low power of the test). Trees in the 1.2 x 1.2 m block averaged 2.71 m, while those in the 2.4 x 2.4 m block averaged 2.05 m, but because of lack of replication of these blocks, we cannot state whether the difference was due to spacing or to site variation.

Discussion

The inverse correlation between first-year winterkill and second-year height in trees growing at the north end of paulownia's naturalized range in North America is not surprising. The poor showing in both winter hardiness and second-year height of progeny of the Ringwood tree (no. 9) shows that phenotypic selection for winter hardiness might be worthwhile with paulownia, and should result in faster growing trees. It could also result in straighter trees, because the poor tree form so common in this area appears to be due at least in part to winterkill.

In view of the differences in height and winter injury found among open-pollinated families of paulownia in this small study, it appears that hardier, faster growing strains could be developed for the northern portion of this species' naturalized range in North America.

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