

**Nurseries/Seed and
Seedlings**

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IMPROVING LONGLEAF PINE SEEDLING PRODUCTION BY CONTROLLING SEED AND SEEDLING PATHOGENS

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Abstract—The demand for container longleaf pine (*Pinus palustris* Mill.) planting stock is increasing across the Lower Gulf Coastal Plain. Poor-quality seeds and seedling losses during nursery culture further constrain a limited seed supply. Improved seed efficiency will be necessary to meet the need for increased seedling production. Seed presowing treatments and seedling fungicidal applications were evaluated in container longleaf pine seedling operations to determine if efficiency of seedling production could be improved. Application of treatments to reduce pathogenic fungi on seed and in seedling culture significantly increased plantable container stock.

INTRODUCTION

With a tenfold increase in seedling production occurring in the last few years, interest in the production and planting of longleaf pine (*Pinus palustris* Mill.) seedlings has reached an all time high. A limitation in producing even more seedlings is lack of high-quality seeds that not only germinate well, but result in plantable stock. Earlier results have shown that longleaf seed coats carry pathogenic fungi that not only reduce germination, but also result in significant seedling mortality (Barnett and others 1999). Pawuk (1978) and Fraedrich and Dwinell (1996) found that *Fusarium* spp. are commonly found on longleaf pine seeds and cause longleaf seedling mortality. Tests have shown that treating longleaf seeds with a sterilant or fungicide prior to sowing can improve both germination and seedling establishment (Barnett 1976, Barnett and Pesacreta 1993, Littke and others 1997). However, the effects of using both seed pretreatments to control seed-coat pathogens and fungicides to minimize seedling losses during the cultural period have not been reported. Our objectives were to develop recommendations for presowing treatments and fungicidal applications that will improve the efficiency of seedling production.

METHODS

The seeds used originated from bulked seed orchard lots of longleaf pine adapted to the Western Gulf Coastal Region. Seedlings were grown at the Southern Research Station's facility at Pineville, LA, following guidelines for producing longleaf pine container stock (Barnett and McGilvray 1997).

All seedlings were grown in Multipot 3-96a containers, and both seed presowing treatments and seedling fungicidal applications were evaluated. The presowing treatments were a control and a hydrogen peroxide application—1-hour soak in 30-percent hydrogen peroxide (Barnett 1976, Barnett and McGilvray 1997). The seedling fungicide treatments included: (1) a control, and applications of

(2) Benlate®, (3) Fungo-flo®, and (4) Fungo-flo® plus Subdue®. The fungicides were applied biweekly after seed germination was complete. The recommended concentrations of the fungicides used are: Benlate® 50WP (benomyl)—1 rounded teaspoon per gallon of water; Fungo-flo® (46.2 percent a.i. thiophanate-methyl)—0.2 fluid ounce per gallon of water; Fungo-flo® plus Subdue® 2E (metalaxyl) at 10 ppm active ingredient (0.15 ml per gallon of water).

The study was a randomized experiment with three replications of three trays each for each treatment replication. A total of 72 trays of 96 seedlings each was included in the study. The seeds were sown in late April 1999; seedling counts were made in December 1999 to determine the percentage of plantable seedlings (number of cavities with a plantable seedling divided by number of cavities with a germinant). Plantable seedling percentages differ from germination percentages in that losses of germinants due to disease are taken into consideration.

RESULTS

Both the seed and seedling treatments had a significant effect on plantable seedling percentages at the end of the study, and there were no statistical interactions between the presowing and fungicidal treatments (table 1). The hydrogen peroxide treatment of seed increased plantable seedlings from 85 to 93 percent; fungicide treatment of controls increased plantable seedlings from 78 to 92 percent. When the hydrogen peroxide seed treatment was used, 92 percent of the seedlings that did not receive fungicides were plantable. Fungicide applications to seedlings only improved average plantable stock by 2 percent.

Seedlings grown from seed that did not receive the hydrogen peroxide presowing treatment had a plantable seedling percentage of only 78, compared to 88 for those treated with fungicides during culture. There were no significant differences in the effectiveness of fungicides.

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Table 1—Longleaf pine plantable seedling percentages resulting from seed and seedling treatments^a

Seedling treatment	Seed treatment		
	Control	Hydrogen peroxide	Average
	----- Percent -----		
Control	78	92	85a
Benlate®	85	93	89b
Fungo-flo®	88	94	91b
Fungo-flo® plus Subdue®	90	94	92b
Average	85a	93b	

^a Plantable seedling percentages (numbers plantable in November divided by numbers with an initial germinant) are averages for 288 seedling cavities for each of 3 replications. Averages within columns and across rows followed by the same letter are not significantly different at the 0.05 level.

DISCUSSION

Results from this study demonstrate the effectiveness of reducing fungal populations on longleaf pine seed coats before they are sown in containers. Elimination of pathogenic fungi from seed coats increases seedling establishment and reduces sources of disease infestation later in the nursery cultural period. Although 30-percent hydrogen peroxide was used in this study and is labelled as a stimulant of pine seed germination, earlier research has shown that a 10-minute Benlate® seed drench was equally effective and is a safer means of reducing seed coat pathogens (Barnett and others 1999). Benlate® has been labelled for conifer seed treatment in most of the southern States. Other fungicidal chemicals or methodologies also may be effective if they are not phytotoxic to seed germination.

There were no statistical differences among the fungicides used to reduce seedling losses during the nursery growing period. Because a great deal of research has

demonstrated its effectiveness in controlling *Fusaria*, Benlate® was used as a kind of fungicidal standard. However, the label for this fungicide has been withdrawn for conifer nursery use. Fungo-flo®, a labelled replacement for Benlate®, is equally effective in controlling pathogens of longleaf seed and seedlings. Subdue® is normally added to the fungicidal application because it broadens the spectrum of disease protection to include other pathogens such as *Pythium* and *Rhizoctonia*.

Combing presowing seed treatments to reduce pathogenic fungi on the seed coats with the application of appropriate fungicides to seedlings during the growing season to control pathogenic fungi greatly increases the efficiency of container seedling production.

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RECALCITRANT BEHAVIOR OF TEMPERATE FOREST TREE SEEDS: STORAGE, BIOCHEMISTRY, AND PHYSIOLOGY

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Abstract—The recalcitrant behavior of seeds of live oak (*Quercus virginiana* Mill.), and Durand oak (*Quercus durandii* Buckl.) was examined after hydrated storage at two temperatures, +4° C and -2° C for up to 1 year. Samples were collected and analyses performed at monthly intervals. At each sampling time, seeds were tested for viability and moisture content. Red buckeye (*Aesculus pavia* L.) seeds were similarly stored but analyzed at intervals of 3 months, while those of cherrybark oak (*Quercus pagoda* Raf.) and water oak (*Quercus nigra* L.) were tested yearly. Durand oak, live oak, and red buckeye seeds stored at -2° C maintained higher viability for a longer period of time than did those stored at +4° C. However, live oak acorns were damaged by the colder storage temperature. Sprouting during storage occurred at the higher storage temperature, but not at -2° C. After 2 years, water oak and cherrybark oak acorns which had been dried prior to refrigeration had lower viability than those stored fully hydrated. The damage was especially apparent in cherrybark acorns, with viability reduced after 1 year to 22 percent in those dried and then stored at -2° C and to 5 percent in those stored at +4° C. It is suggested that all precautions against desiccation be taken when collecting cherrybark and water oak acorns that are not for immediate use. Unless the acorns are collected when fresh and maintained in a fully hydrated state, severe losses can arise when stored for only 1 year. Fourier transform infrared spectrometry (FT-IR) studies have shown that cherrybark acorns subjected to severe desiccation exhibit irreversible changes in membrane lipid and protein secondary structure. This change was the most sensitive indicator of viability loss as yet encountered in these experiments. Future studies will examine the role of protein denaturation in seed deterioration.

INTRODUCTION

Early studies on low temperature storage of hardwood tree seeds resulted in the division of seeds into two storage behavior classes (Roberts 1973): 'Orthodox' seeds undergo a period of desiccation before being shed from the tree and can easily be stored at low temperatures for long periods of time at moisture contents of less than 12 percent. Temperate 'recalcitrant' seeds, however, do not undergo this final maturation drying and are thus very sensitive to moisture loss, making storage for any useful period extremely difficult. Immediate causes of seed viability loss are attack by pathogens and premature germination. Recent work has modified both Roberts' initial definition of recalcitrance and our perspective of the nature of recalcitrance. Pammenter and others (1994) and Berjak and Pammenter (1997) recognized the damage caused by aberrant metabolic processes while seeds are in hydrated storage and as water is lost. Thus, while much progress has been made in understanding the nature of recalcitrance, the storage of some recalcitrant tree seeds over a long period remains an insurmountable problem. North American genera containing species with recalcitrant seeds are *Castanea* (Pritchard and Manger 1990), and some *Acer*, *Aesculus*, and *Quercus* (Bonner 1990).

Acorns of the red oaks and of *Quercus robur* have reportedly been stored at -1° C or -2° C for periods up to 5 years in Europe (Suszka and Tylkowski 1980, 1982). Experiments here have been less successful, suggesting a varying degree of dormancy between European and U.S. species. We are reporting the results from three studies: (1) a 1 year storage study of Durand oak (*Quercus durandii* Buckley), live oak (*Quercus virginiana* Mill.), and red buckeye (*Aesculus pavia* L.) at 2 temperatures; (2) second year results of a water oak (*Quercus nigra* L.) and cherrybark oak (*Quercus pagoda* Raf.) acorn storage experiment at 2 temperatures and 2 moisture contents; and (3) a Fourier transform infrared (FT-IR) spectroscopy study of desiccating cherrybark oak acorns.

METHODS

Durand oak and red buckeye seeds were collected locally in Oktibbeha County, MS. The water oak and cherrybark oak acorns were purchased from a local supplier, while the live oak acorns were collected in Washington County, MS. All seeds were cleaned by floatation, soaked overnight, and then stored at 4° C until the start of the experiment. Original moisture contents for each drying regime were determined by drying 2-4 samples of seeds at 105° C for 16-17 hours. In preparation for germination tests, acorns were cut in half horizontally. The seed coat was removed from the half

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containing the embryo, and the half with the cup scar was discarded. Buckeye seeds were germinated intact. Germinations were conducted on moist Kimpak at an alternating temperature regime of 20° C for 16 h in the dark and 30° C for 8 h with light. Since sprouting in storage can be a common problem, counts were made at the start of each germination test of the number of seeds in a sample which had sprouted during storage. Experiments were conducted as follows:

Experiment 1

This experiment was conducted on tree species with highly recalcitrant seeds. Samples of 250 fully hydrated acorns of Durand oak and live oak were stored in plastic bags at either 4° C in a Lab-Line Ambi-Hi-Low Chamber or at -2° C in a modified chest freezer. Percent germinations and moisture content were determined for the fresh acorns and every 30 days thereafter for one year, as acorn supplies permitted. A subsample of acorns was dissected, and the embryos and cotyledons cryostored for future chemical and FT-IR spectroscopic analyses. Acorns were germinated as two replications of 50 seeds each per sampling period and were rehydrated overnight in tapwater prior to germination testing. Red buckeye seeds were stored as above; however, they were tested only at fresh, 90-, 180-, and 360-day intervals and were stored in batches of 59 seeds per bag. Germination tests consisted of 2 replications of 15 seeds each per sampling period.

Experiment 2

High and low moisture levels for water and cherrybark oak acorns were imposed by either soaking in tapwater for 16 hours or by drying on a lab bench for 48 hrs. Lots consisting of 110-120 acorns were stored in 4-mil polyethylene bags at either 4° C or at -2° C as described above. Original percent germinations and moisture contents were determined for fresh acorns and thereafter at yearly intervals. Acorns were germinated as two replications of 50 seeds per sampling period and were soaked overnight in tapwater prior to germination testing.

Experiment 3

Cherrybark oak acorns collected in 1999 were spread on blotter paper in a single layer on the lab bench. Cotyledon samples of fresh seeds and those that had been dried for 2, 4, 6, and 8 days were analyzed by FT-IR spectroscopy as follows: Thin slices of cotyledon tissue were placed between CaF₂ windows of a demountable transmission cell. For each spectrum, 512 scans at 2/cm resolution were collected on a Nicolet 20 DXB spectrometer using an MCT-A detector. Single beam spectra were ratioed against an open beam background to yield transmission spectra. Sampling continued until seed moisture content dropped below 15 percent, and samples were analyzed for changes in macromolecular structure that might occur during drying and during rehydration. The experiment was replicated on acorns collected in 2000.

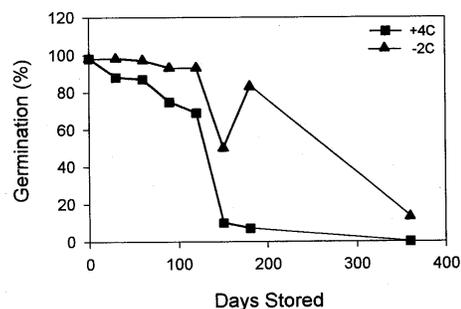


Figure 1—Viability of Durand oak (*Quercus durandii* Buckley) acorns stored for 1 year at 4° C and at -2° C.

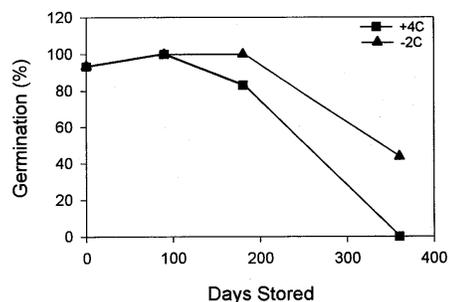


Figure 2—Viability of red buckeye (*Aesculus pavia* L.) seeds stored for 1 year at 4° C and at -2° C.

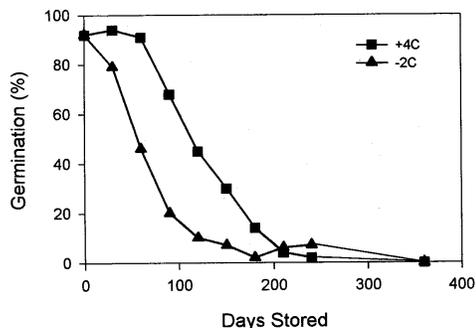


Figure 3—Viability of live oak (*Quercus virginiana* Mill.) acorns stored for 1 year at 4° C and at -2° C.

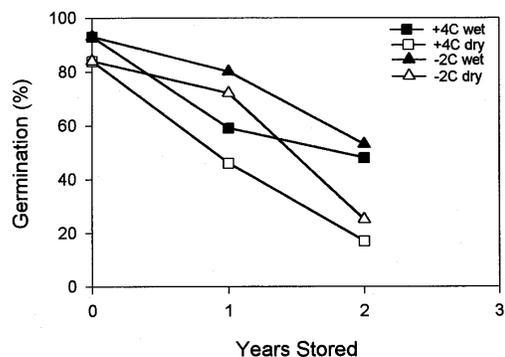


Figure 4—Water oak (*Quercus nigra* L.) acorns stored at two moisture contents and two temperatures.

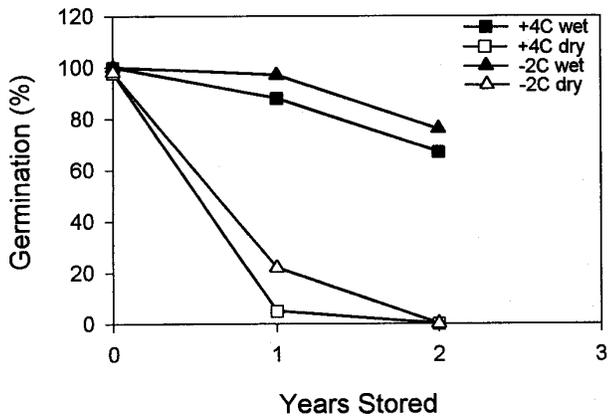


Figure 5—Cherrybark oak (*Quercus pagoda* Raf.) acorns stored at two moisture contents and two temperatures.

RESULTS

Experiment 1

Durand oak acorns stored at -2°C had significantly higher viability than those stored at 4°C in as little as 30 days (figure 1). After 210 days, acorns stored at -2°C averaged 83 percent viability, while only 6 percent of those stored at 4°C survived. Red buckeye seeds also remained viable longer if stored at -2°C (figure 2). The differences in viability did not occur, however, until after 90 days in storage. Acorns of live oak were the only ones tested to date that survive longer if stored at 4°C (figure 3). Storage at -2°C resulted in significant damage to the acorns. Fresh moisture contents were 38.1, 60.6, and 56.6 percent for Durand oak, red buckeye, and live oak, respectively, and did not change greatly during storage.

Experiment 2

Water oak acorn moisture content was 30.5 percent (on a fresh weight basis) for the fresh acorns and 25.6 percent for those dried 2 days prior to storage. Drying reduced initial acorn viability by 9 percent (figure 4). After 1 year, temperature of storage had a greater effect on seed viability than did initial moisture content. Both fully hydrated and

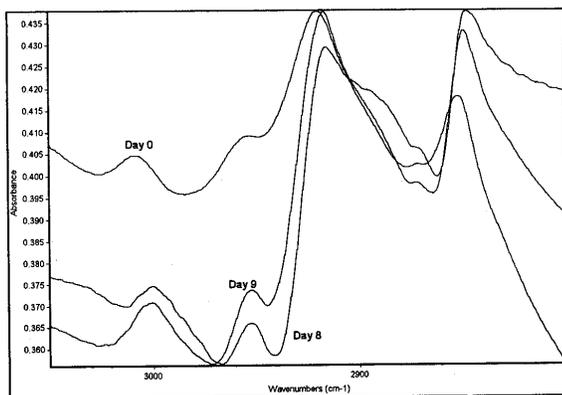


Figure 6—Membrane lipid vibrations in fresh (day 0) cherrybark acorn cotyledon tissue dried for 8 days, and then rehydrated (day 9). Peak frequencies are at 2850.9, 2847.2, and 2848.8 cm^{-1} .

dried acorns stored at -2°C maintained a higher viability than those stored at 4°C . This was not the case after 2 years of storage, when moisture content was the more important factor. Acorns which had been dried prior to refrigeration had lower viability than those stored fully hydrated. Moisture content did not change significantly throughout the course of the experiment.

Cherrybark oak acorn moisture content was 29.6 percent for the fresh acorns and 19.9 percent for those dried 2 days. However, drying reduced initial viability by only 2 percent (figure 5). Unlike water oak acorns, moisture content, and not temperature, was the important factor after both 1 and 2 years of storage. Only acorns stored in the fully hydrated condition retained high viability; dried acorns experienced significant losses in viability, after only 1 year in storage and were dead after 2 years. Changes in moisture content during storage were not significant.

Experiment 3

Cherrybark acorn germination was highly dependent on moisture content, and severely declined when seed moisture dropped below 17 percent (table 1). Changes in molecular structure due to drying and rehydration were measured by changes in the frequency (and bandwidth) of the infrared absorbance of lipid and protein functional groups. Membrane lipid structure was measured by the frequency and bandwidth of the symmetric CH_2 stretch at 2850/ cm (Sowa and others 1991). An increase in vibrational frequency corresponds to increased fluidity (phase change from gel to liquid crystalline). In the liquid crystalline phase, membranes are fluid and in their normal state; when in the gel phase, membranes may leak cell solutes and cause irreparable damage to seeds. In this experiment, fresh tissues exhibited reversible shifts between gel and liquid crystalline phases upon drying and rehydration in the cotyledon tissue (figure 6). After drying for 8 days, membrane lipids changed to gel phase and did not recover their fluidity upon rehydration.

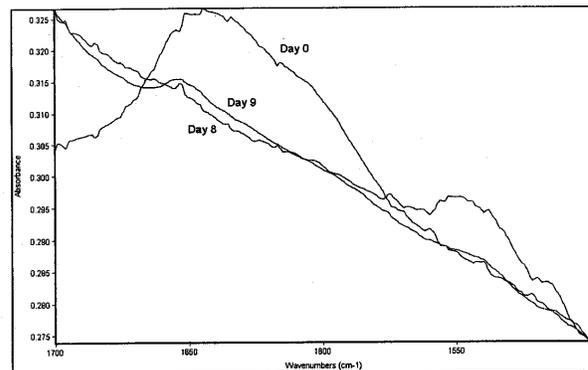


Figure 7—Amide protein vibrations in fresh (day 0) cherrybark acorn cotyledon tissue and in tissue dried for 8 days and then rehydrated (day 9).

Protein secondary structure was measured using the amide I and II vibrations near 1650 and 1550/cm (Sowa and others 1991). Changes in amide frequency correspond to changes in secondary structure. Alpha-helix structures absorb at higher frequencies, while beta-sheets absorb near 1630/cm; denatured protein typically exhibits extended beta-sheet conformation, with infrared absorbances common at frequencies less than 1630/cm. Irreversible changes in the protein secondary structure, illustrated by shifts in the amide absorbance near 1650/cm, occurred in the cherrybark acorn cotyledon tissue (figure 7). Secondary structure was completely lost upon dehydration (day 8) and remained so upon rehydration of these samples (day 9).

DISCUSSION

No one single temperature was best for storage of recalcitrant seeds. In previous experiments, chinkapin (*Quercus muehlenbergii* Engelm.), northern red (*Quercus rubra* L.), and Shumard (*Quercus shumardii* Buckl.) oak acorns favored the lower storage temperature of -2° C (Connor and Bonner 1999). While Durand oak acorns and red buckeye seeds exhibited significantly higher viability when stored at -2° C, live oak acorns were harmed by the low temperature. Also, sprouting during storage was a problem in red buckeye (17 percent after 180 days), live oak (18 percent after 120 days), and Durand oak seeds (16 percent after 120 days) stored at +4° C. Sprouting remained below 2 percent in seeds stored at -2° C for the same lengths of time.

Both water oak and cherrybark oak acorns retained high viability after 2 years when stored fully hydrated. To date, sprouting and changes in moisture content are not factors in the successful storage of either species. However, unlike a previous report (Connor and Bonner 1999), we did not find significant differences in viability caused by temperature of storage (figs. 4,5). Also, while drying of water oak and cherrybark acorns for 2 days before storage did not affect original viability, the damage was significant in water oak acorns stored for 1 year at +4° C and in cherrybark oak acorns after 1 year at either storage temperature. It is therefore strongly suggested that all precautions against moisture loss be taken when collecting acorns of these species that are not for immediate use. Unless the acorns are collected when fresh and maintained in a fully hydrated state, severe losses can arise when stored for only 1 year. Orchard managers and seed processors must place emphasis on careful handling of acorns during the collection process. Also, the sooner acorns can be collected after dropping from the tree, and placed under refrigeration, the higher the probability of successful long-term (1 year) storage.

Membrane lipids changed phase from liquid crystalline to gel upon drying and did not recover upon rehydration as viability was lost. Ions can pass indiscriminately through cell membranes in the gel phase, and this loss of selective permeability ultimately results in seed mortality. In this experiment, the change occurred first in the cotyledon tissue and then in the embryos; since embryos in recalcitrant seeds maintain a fairly high water content (Connor

and others 1996, 2001), this was not unexpected. It was interesting to note that after severe desiccation, rehydration did not restore membranes to their original fluid state.

Changes in protein secondary structure occurred in cotyledons as moisture was lost. Secondary structure was completely lost upon dehydration and remained so upon rehydration of nonviable samples. This evidence of protein denaturation occurring in the cytosol and/or cellular membranes was the most sensitive indicator of viability loss as yet encountered in these experiments. It is also contrary to behavior observed in orthodox seeds using infrared techniques (Golovina and others 1997) and will be addressed in future investigations.

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EFFECTS OF LIFTING METHOD, SEEDLING SIZE, AND HERBACEOUS WEED CONTROL ON FIRST-YEAR GROWTH OF LOBLOLLY PINE SEEDLINGS

Jason P. Reynolds, Thomas A. Greene, and John R. Britt¹

Abstract—In fall, 1999, an experiment was installed to measure the effects and interactions of lifting method, seedling size, and weed competition on growth of loblolly pine (*Pinus taeda*) seedlings during the first two growing seasons. Loblolly pine seedlings grown at two bed densities and lifted either by hand or machine were planted in southwestern Georgia and either given complete weed control or no weed control. The treatments were arranged in a 2 x 2 x 2 factorial and replicated three times. Mean root collar diameter was 5.7 mm for seedlings grown at 301/m² and 7.6 mm for seedlings grown at 161/m². Total height of all seedlings was measured after planting and at the end of the 1st growing season. Ground line diameter was also measured at the end of the first growing season. This paper will present the main effects and their interaction on height and volume after the first growing season.

INTRODUCTION

There has been an increased interest from the forest industry throughout the South in large, high vigor seedlings. The industry's desire is to produce high quality pine seedlings, which not only survive planting, but begin growth during the first growing season. Seedling quality research has shown that variables such as root system size, stem caliper, and root/shoot ratio affect growth and survival of pine seedlings (South and others, 1995). The method of lifting seedlings from the nursery beds has also been shown to influence growth and survival (Greene & Danley, 1999, South & Stumpff, 1990). As part of a continuing series of seedling quality studies, an experiment was designed to look at the main effects and interactions of three factors that affect seedling performance. These factors are as follows:

- 1) Seedling size (large or small) controlled by seed bed density at the nursery;
- 2) The method of lifting (hand or machine); and
- 3) The presence or absence of herbaceous competition during the first growing season.

METHODS AND MATERIALS

This study was installed to look at the effects and interactions of the treatments throughout the first two growing seasons. The study design was a 2 X 2 X 2 factorial randomized complete block with three replications. The seedlings were planted in row plots at a 1.2-m x 3-m spacing. A total of 840 measurement trees were measured for the variables of interest.

The seedlings for this study were grown at two densities (301/m²) and (161/m²) at a nursery in Marion County, Georgia. The seedlings were lifted either by hand or by a two-row Mathis belt lifter, stored under refrigeration for two

days and planted by researchers. The study was installed on a small field dominated by bermudagrass (*Cynodon dactylon*) and bahiagrass (*Paspalum notatum*), which provided a high level of uniform competition throughout the growing season. The study plots either received total weed control throughout the growing season or no weed control. Planted heights and root collar diameters were measured at the time of planting. At the end of the first year, survival, height, and ground line diameter were measured.

In addition to the planted seedlings, 120 seedlings were destructively sampled for morphological characteristics; thirty trees from both densities and lift methods. Measurements taken from these seedlings included root collar diameter, dry shoot weight, and dry lateral and taproot weight.

RESULTS AND DISCUSSION

Seedling Sample Results

Seedling caliper was positively affected by lower seedbed density. Mean root collar diameter (RCD) was 5.7 mm for the seedlings grown at the higher seed bed density while the lower density seedlings had a mean diameter of 7.6 mm. The lifting method had a significant effect on lateral root weights where the hand lifted seedlings had greater dry weight than did the machine lifted seedlings. Total root weight was also affected by density where high-density seedlings had significantly less total root weight than did the low-density seedlings. Seedling diameters and weights by bed density and lifting method are presented in table 1. Significance levels for the treatment effects are presented in table 2.

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Table 1— Dry weight means (grams) for the treatments from 120 seedlings sampled for morphology

Seedbed Density	Lifting method	Shoot weight (g)	Taproot weight (g)	Lateral root weight (g)	Total Root weight (g)	Shoot:Root ratio	RCD (mm)
Low	Hand	6.25	1.21	0.84	2.05	3.23	7.67
Low	Machine	5.71	1.21	0.74	1.94	2.98	7.61
High	Hand	3.45	0.57	0.72	1.29	2.74	5.61
High	Machine	3.25	0.57	0.47	1.04	3.38	5.71

Table 2— Significance levels for treatment terms in ANOVA models for the dry weights of the dependent variables from 120 seedlings sampled for morphology

Treatments	Shoot	Tap root	Lateral root	Total root	Shoot:Root	RCD
Significance	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F	Pr >F
Bed Density	0.000	0.000	0.009	0.000	0.726	0.000
Lift Method	0.25	0.979	0.014	0.143	0.185	0.798
Density X Lift	0.59	0.936	0.291	0.561	0.003	0.213

First Year Growth Effects

Seedbed Density—Although the lower seedbed density did produce larger seedlings, density was not a significant predictor of survival or height growth through the first year. This does not concur with previous experience or with published data (South and others 1995). Seedbed density was, however, a significant predictor of first-year volume and height. Further analysis was done using root collar diameter as a covariant to determine if there were any additional affects from seedbed density beyond seedling caliper differences. No additional variance was explained by bed density.

Lifting Method—Lifting method significantly improved first year survival and growth of seedlings. This affect can be explained by the greater retention of lateral root mass by the hand lifted seedling over that of the machine lifted seedlings. Table 1 shows that high density seedlings lifted by hand had nearly the same weight in lateral roots as did the machine lifted low density seedlings.

Herbaceous Weed Control—As expected, herbaceous weed control (HWC) significantly improved first year height growth, ground line diameter, survival, and volume index. The interaction of lifting method X HWC on volume index was significant at a 9 percent level of confidence. This interaction showed a greater positive response by hand lifted seedlings to weed control than those which were machine lifted, implying a more favorable response to silviculture from seedlings with more lateral root mass.

Significance levels for the treatments as they relate to the study plots are shown in table 3. First-year means for height and volume index are presented in figures 1 & 2 respectively. Table 4 presents the main effect means for the three randomized complete blocks. The means for all combinations of treatments are listed in table 5.

Table 3 – Significance levels for dependent variables for seedlings in three randomized complete blocks in South West, GA

Source of Variation	Percent survival	Volume index year 1 (cc)	Year 1 height growth (cm)	Year 1 height (cm)	GLD year 1 (mm)
Block	0.9911	0.8191	0.8211	0.9860	0.9851
Bed density	0.4091	0.0398	0.9231	0.0401	0.0068
Lift Method	0.0473	0.0254	0.0298	0.0162	0.0496
Weed Control	0.0473	0.0001	0.0003	0.0003	0.0001
Density X Lift	0.3159	0.4106	0.4652	0.3190	0.6555
Method					
Density X	0.4091	0.9485	0.1564	0.1943	0.5189
HWC					
Lift Method X	0.4091	0.0974	0.1448	0.1293	0.2771
HWC					
Density X Lift	0.7808	0.8004	0.8670	0.9221	0.9309
X HWC					

Table 4—Main effect means of survival, first flush length, height increment, and end-of-season height, ground line diameter, and volume index for seedlings planted in three randomized complete blocks in Southwest, Georgia in 2000

Level of Main effect	Survival (percent)	First flush length (cm)	Volume index year 1 (cc)	Height growth year 1 (cm)	Height year 1 (cm)	Ground line diameter year 1 (mm)
<u>DENSITY</u>						
LOW	92 a	15 a	14 a	31 a	54 a	9.3 a
HIGH	95 a	13 b	11 b	31 a	49 b	8.3 b
<u>LIFTING</u>						
HAND	97 a	15 a	14 a	34 a	54 a	9.4 a
MACHINE	89 b	13 b	11 b	29 b	49 b	8.4 b
<u>HWC</u>						
YES	97 a	14 b	18 a	36 a	56 a	10.7 a
NO	89 b	15 a	7 b	26 b	47 b	6.9 b

A difference in letters indicates significant difference at p=0.05 from Duncan's multiple range test.

Table 5 – Year 1 means for all treatments from seedlings planted in Southwest, Georgia in 2000

Seedbed Density	Lift method	HWC	Survival (percent)	Height growth	Height	Diameter	Volume index
high	hand	no	93	25	43	6.3	5
high	hand	yes	100	41	59	10.7	19
high	machine	no	85	25	44	6.2	5
high	machine	yes	100	34	52	9.8	14
low	hand	no	96	30	53	7.7	9
low	hand	yes	99	40	62	11.7	24
low	machine	no	83	26	48	7.3	7
low	machine	yes	89	30	52	10.5	16

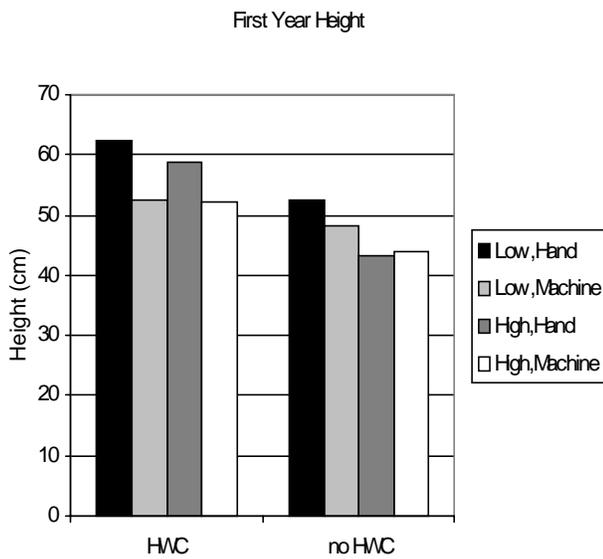


Figure 1— First year incremental height growth of the various treatments. Significant at p = 0.05 level on lifting method and weed control.

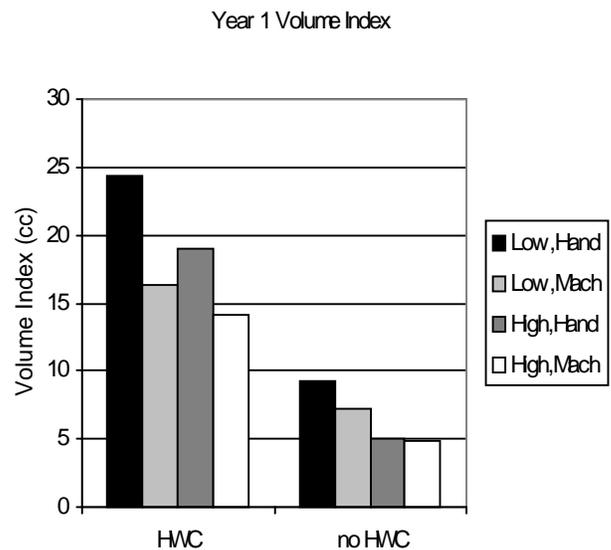


Figure 2—First year volume index of the various treatments. Significant at p = 0.05 for all treatments.

CONCLUSIONS

The results from this study indicate that seedling quality has a significant impact on first year performance of loblolly plantations. Larger seedlings have greater volume through the first year than do smaller seedlings. Hand lifting seedlings improves lateral root mass retention. As a result, hand lifted seedlings have greater first year growth and survival than machine lifted seedlings. Herbaceous weed control during the first year improves survival and growth of seedlings. Furthermore, there is some evidence to suggest that high quality, vigorous seedlings, respond more favorably to the silvicultural treatment of weed control.

Therefore, nursery practices that produce large caliper, vigorous seedlings, and lifting methods which limit damage to the seedling's stem and root system are encourage due to the seedling's superior performance in the field. In order to realize the full benefit of the investments of silvicultural treatments in a plantation, the use of high quality seedlings that are cared for properly should be considered a key tool to plantation success.

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EFFECTS OF FLOOD DURATION AND DEPTH ON GERMINATION OF CHERRYBARK, POST, SOUTHERN RED, WHITE, AND WILLOW OAK ACORNS

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Abstract—Effects of flood duration (0, 10, 20, and 30 days) and depth (10 and 100 centimeters below a water surface) on acorn germination were tested for two bottomland oaks (cherrybark oak [*Quercus pagoda* Raf.] and willow oak [*Q. phellos* L.]) and three upland oaks (post oak [*Q. stellata* Wang.], southern red oak [*Q. falcata* Michx.], and white oak [*Q. alba* L.]). The study was a 4 x 2 factorial with a completely randomized design. Acorns of the five species were collected in November 1995 in Drew County, Arkansas and stored in a refrigerator at 4 degrees Centigrade until stratification. Acorns were stratified for 45 days in plastic germination flats with 20-cubic centimeter cells filled with a silt loam soil and then flooded in a small pond from March 19 to April 18, 1996. After flooding, acorns were germinated for 60 days. Flood depth did not significantly affect germination of any species, but flood duration affected germination of the three upland species. There was no interaction between flood duration and depth for any species. Among the upland species, germination of white oak acorns with 20 days or more of flooding was almost totally prohibited, while germination of southern red oak acorns gradually decreased as flood duration increased. Although germination of post oak was significantly reduced by 20 and 30 days of flooding, more than 65 percent of the acorns germinated. Results of our study indicate that the effects of flooding on the species composition of bottomlands begin with the germination process.

INTRODUCTION

Seasonal flooding frequently occurs in bottomlands and is a principal factor in determining tree species distribution (Hodges and Switzer 1979). Flooding may affect tree growth by displacing soil air and limiting root respiration along with other effects, and extended flooding can kill flood-intolerant trees (Kramer and Kozlowski 1979). Flood tolerance of the major bottomland hardwood species, including many oaks, has been summarized (Hook 1984; Allen and Kennedy 1989), but little is known about the flood tolerance of tree seeds. For instance, some species can develop aerenchymatous tissue to facilitate transport of oxygen to the roots, but this is not possible for seeds (Norton 1986). For the oaks, acorns of some species may be damaged by extended flooding, and damaged acorns may not be able to germinate or produce vigorous seedlings. There is some indication that acorns of the bottomland oaks can tolerate more flooding than upland species. For instance, 15 days of flooding severely reduced acorn germination of white oak (*Quercus alba* L.) (Bell 1975), but acorns of Nuttall oak (*Q. nuttallii* Palmer) were not affected by 34 days of flooding (Briscoe 1961). Guo and others (1998) found that spring flooding significantly reduced epicotyl emergence of black (*Q. velutina* Lam.) and northern red oak (*Q. rubra* L.) acorns but did not affect cherrybark (*Q. pagoda* Raf.) or water oak (*Q. nigra* L.).

Water depth may vary greatly during flooding based on location within the floodplain and intensity of the flood. Water depth may affect aeration, temperature, and pressure, which may influence acorn viability. The effect of flood depth on acorn germination has not been studied. Therefore, the objective of this study was to test the effects of flood duration and depth on acorn germination of five oak species common to the southern United States. The species were two bottomland oaks (cherrybark and willow oak [*Q. phellos* L.]) and three upland oaks (post oak [*Q. stellata* Wang.], southern red oak [*Q. falcata* Michx.], and white oak).

METHODS

In November 1995, acorns from an individual tree of the five oak species were collected in Drew County, AR. After conducting a float test, acorns were air dried overnight and stored in polyethylene bags at 4 degrees Centigrade. A silt loam soil (Typic Ochraquults) was collected in Drew County, AR. The soil was air dried and hand-processed to pass a 5-millimeter sieve. Plastic germination flats with sixty 20-cubic centimeter cells per flat were filled with soil, and twelve acorns of each of the five species were buried 1 centimeter below the soil surface with one acorn per cell. Acorns were buried in soil because small mammals commonly bury acorns and survival of acorns on the forest floor is generally low (Bowersox 1993). After sowing, the soil was saturated with distilled water, and flats were

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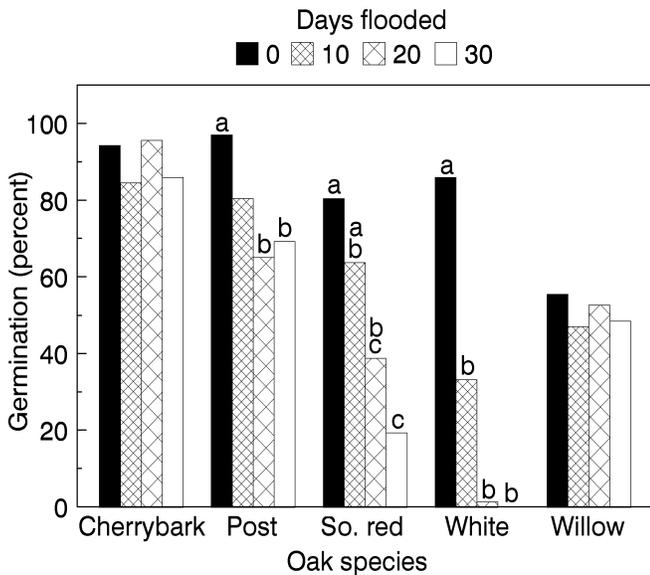


Figure 1—Effects of flood duration on germination of cherrybark, post, southern red, white, and willow oak acorns. Bars of a cluster with different letters differ at $\alpha = 0.05$.

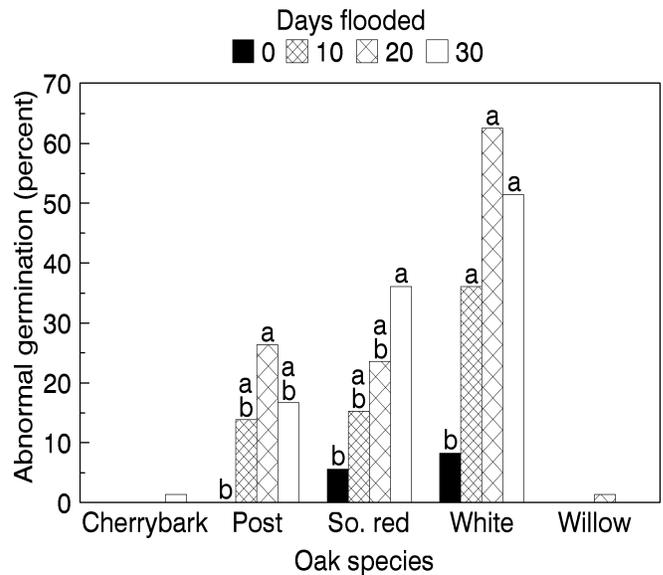


Figure 2—Effects of flood duration on the abnormal germination (a radicle or roots produced with no accompanying epicotyl or shoot) of cherrybark, post, southern red, white, and willow oak acorns. Bars of a cluster with different letters differ at $\alpha = 0.05$.

stored for 45 days at 4 degrees Centigrade to stratify acorns, assuring a uniform state of dormancy.

The study design was a 4 x 2 factorial with a completely randomized layout with treatments of flood duration and depth. There were four flood durations: 0 (control), 10, 20, and 30 days, and two flood depths: 10 and 100 centimeters below a water surface. Each treatment combination was replicated three times with 12 acorns per replicate. Flooding was conducted in a 0.2-ha pond in Drew County, AR from March 19 to April 18, 1996. Plastic germination flats with the 10-centimeter depth were protected with a wire screen (1.2-centimeter mesh) to keep out seed-consuming animals. A maximum-minimum thermometer was submerged with germination flats, and water temperature was recorded every 10 days. Flooding of replicates of the 10- and 20-day treatments was staggered in time so that environmental gradients occurring over the 30-day period could affect all treatment levels. Plastic germination flats awaiting flooding and those with completed flooding treatments were drained and stored at 4 degrees Centigrade until day 30 when all germination flats were collected. Minimum water temperatures for the 10-centimeter depth were 10.1, 11.0, and 14.6 degrees Centigrade, respectively, for the three 10-day flooding periods, and maximum temperatures were 17.4, 19.3, and 22.3 degrees Centigrade. Corresponding minimum temperatures for the 100-centimeter depth were 10.6, 11.9, and 14.6 degrees Centigrade, and maximum temperatures were 14.4, 16.8, and 18.2 degrees Centigrade.

For germination tests, the plastic germination flats were placed in a laboratory with a bay of south-facing windows, exposing flats to diffuse sunlight. The germination flats were periodically irrigated with distilled water to keep the

soil moist. Temperature in the laboratory was maintained at 20 degrees Centigrade. Epicotyl emergence of each acorn was recorded weekly over an 8-week period when length exceeded 2 centimeters. Seedlings were allowed to continue development in the germination flats after acorns were recorded as germinated. To assess possible germination activity at the time of flooding, subsamples of acorns were established that were identical to the unflooded control, except that they were removed from soil and examined at the beginning of the germination test; the four activity classes were none, acorn split, radicle ≤ 5 millimeters, and radicle >5 millimeters. At the end of the germination test, all ungerminated acorns were examined for decay and abnormal germination (a radicle or roots exceeding 2 centimeters but no corresponding shoot).

Germination results were analyzed by GLM of SAS (SAS Institute Inc. 1986). Significance was accepted at $\alpha = 0.05$. Means were separated by the Ryan-Einot-Gabriel-Welsch multiple range test at $\alpha = 0.05$.

RESULTS

Flood duration significantly affected germination of post ($P = 0.03$), southern red ($P < 0.01$), and white ($P < 0.01$) oak acorns, but did not affect cherrybark ($P = 0.12$) and willow ($P = 0.89$) oak acorns. Flood depth did not significantly affect germination of any species, and there was also no significant interaction between flood duration and depth. Temperatures averaged 15.8 degrees Centigrade for the 10-centimeter depth and 14.4 degrees Centigrade for the 100-centimeter depth, but the slight lowering of average temperature with increasing water depth was apparently not enough to affect flood damage.

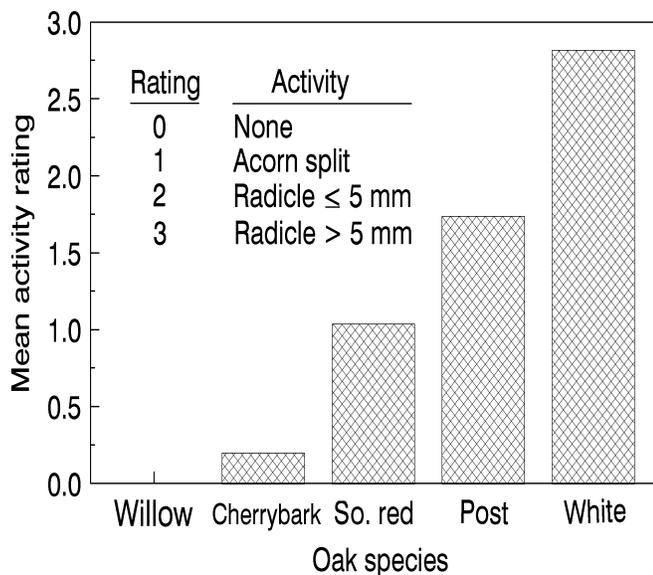


Figure 3—Activity rating of unflooded acorns of cherrybark, post, southern red, white, and willow oaks determined after 75 days of stratification which coincided with the end of the flooding treatments.

Germination rate of the control for post oak acorns was 97 percent, which did not differ from the 10-day flooding (81 percent) but was significantly greater than the 69 and 65 percent of the 20- and 30-day flooding treatments (figure 1). No difference was found among the three flood duration treatments. For southern red oak acorns, germination rate of the control was 81 percent. That was not different from the 10-day flood treatment at 64 percent but was significantly different from the 20- and 30-day treatments (39 and 19 percent). There were no differences between the 10- and 20-day treatments and between 20- and 30-day treatments. Compared to post and southern red oak, acorns of white oak were more severely affected by flooding. Germination rate of the control was 86 percent. With 10-day flooding, germination was reduced to 33 percent, which was significantly different from the control. Almost no germination occurred with the 20- and 30-day durations.

Cherrybark oak germination varied from 95 to 85 percent with the greatest germination rate occurring with 20-day flooding. Willow oak acorns varied within a narrow range (47-55 percent), but the germination rate was much lower than that of cherrybark oak.

Germination failures of post, southern red, and white oaks were mostly accounted for by abnormal germination, where radicles or roots developed without accompanying epicotyls or shoots (figure 2). Abnormal germination generally increased for these species with increasing flood duration. In contrast, abnormal germination was nearly nil for cherrybark and willow oak. For willow oak, most of the acorns that did not germinate were classified as being decayed, averaging 42 percent across all durations with no significant treatment effects.

The activity of unflooded acorns at the end of 75 days of stratification was considerably less for bottomland species than for upland species (figure 3). Willow and cherrybark oak acorns showed almost no activity. Most of the southern red oak acorns were split, but no radicles had emerged. In contrast, most post oak acorns had radicles less than 5 millimeters long, while most radicles of white oak were longer than 5 millimeters.

DISCUSSION

Among the five tested species, post, southern red, and white oaks are upland species, while cherrybark and willow oaks are bottomland species. However, cherrybark oak is seldom abundant on wet or swampy soils, and it grows best on loamy sites on the first bottom ridges (Krinar 1990). Willow oak is found on ridges and high flats of first bottoms of major streams, and on ridges, flats, and sloughs on second bottoms, but it grows best on clay loam ridges of new alluvium (Schlaegel 1990).

Cherrybark oak acorns are tolerant to flooding at least up to 30 days (Guo and others 1998). This study further shows that flooding in deep water in spring does not affect acorn germination of the species. Germination rates of the acorns were high, ranging from 81 to 97 percent across the treatments. In contrast, germination of willow oak acorns was only around 50 percent, including the control. It is not clear why the willow oak acorns had such low germination rates. Bonner (1974) found different germination rates for willow oak acorns collected at different dates; acorns collected on October 6 had a germination rate of 59 percent, compared to 86 percent on October 18, and 96 percent on November 1. Although our acorns were collected in November, they could have possibly fallen to the ground earlier.

For the three species affected by flooding, post oak showed considerable tolerance to flood damage. Even after 30 days of flooding, more than 65 percent of the post oak acorns germinated normally. Thus, flooding damage to acorns is probably not a significant factor limiting the distribution of post oak. Southern red oak also showed some tolerance to short-term flooding; the germination rate was more than 50 percent for 10 days of flooding. Thus, a short flooding period about 10 days is not likely to substantially reduce southern red oak acorn establishment. Compared to post and southern red oak, however, white oak acorns are very sensitive to flooding. Ten days of flooding reduced germination appreciably, and 20 days of flooding almost eliminated any possibility of germination. Bell (1975) also found that acorn germination of white oak was severely limited by 15 days of flooding. This sensitivity may be caused by the characteristic that white oak acorns germinate soon after they fall to ground. In this study, most acorns germinated during stratification. Increased metabolism within the acorns apparently made them susceptible to flooding. Martin and others (1991) pointed out that increased anaerobic metabolism can damage seeds through the buildup of toxic materials.

Although germination of southern red oak acorns was reduced significantly after 20 days of flooding in this study, different results were reported by Larsen (1963) who tested

the effects of water soaking for up to 8 weeks on acorn germination of southern red oak, willow oak, laurel oak (*Q. laurifolia* Michx.), and overcup oak (*Q. lyrata* Walt.). Flooding did not affect germination of southern red and willow oak acorns, and both species had germination rates between 40 and 45 percent. The response of willow oak acorns to flooding is similar to that found in this study. However, the germination of southern red oak acorns without flooding in Larsen's study was much lower than that in our study, which may indicate considerable variation among the seed lots.

For upland species, the embryo axes of acorns were most severely damaged by flooding. Guo and others (1998) found similar damage for black and northern red oak acorns. However, radicles or roots often developed from the connective tissue between the embryo axis and the cotyledons, especially for white oak acorns. Some of the radicles and roots were still alive after 30 days of flooding and the 60-day germination test even though the embryo axes were apparently dead. However, no seedlings developed from the flood-damaged acorns because of the dead embryo axes.

One factor that affects distribution of species is flooding on alluvial sites. Tree seeds must be able to withstand flooding before seedlings can occupy alluvial sites. Cherrybark and willow oaks apparently have no problem becoming established on sites with spring flooding of up to 30 days. They may withstand additional flooding but further research is needed to confirm this. An interesting finding of our study is the tolerance of the post oak acorns to flooding. Post oak typically grows on dry sites on upper slopes (Stransky 1990), yet its acorns showed a fairly high tolerance to flooding. It is likely that the exclusion of this species on alluvial sites is due to some other factor than damage of acorns by flooding.

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