Presowing Treatments Affect Shortleaf Pine Seed Germination and Seedling Development

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Two experiments using 6 half-sib families of skortleaf pine (Pinus echinata Mill.) *were conducted to provide better information on seed stratification needs. Results from laboratory and nursery germination tests and evaluations of seedling development in the nursery indicate that stratification for 15 days was adequate when germination conditions were nearly ideal. However, results from the studies indicate that the speed of germination was much slower when germination temperatures and daylengths were lower than optimum. Under these conditions, 45 days of stratification were needed to achieve uniform rates of germination. The more adverse the germination conditions were, the more important it was that stratification was longer. Seeds that germinated early produced larger stock than those that germinated later. Predictions of seed gerurination in nurseries can be improved by determining the typical environmental conditions at the time of sowing and by requesting that germination tests be conducted under corresponding conditions.* Tree Planters' Notes 44(2):58-62; 1993.

Shortleaf pine *(Pinus echinata* Mill.), the most widely distributed southern pine species, is planted extensively in Arkansas and Oklahoma. Little research has focused on the production of high-quality seeds, and much less information is available on appropriate technology for producing shortleaf pine than for loblolly pine (P. *taeda L.)* seeds. Because information on shortleaf pine seed quality, nursery culture, and regeneration techniques has been limited, the gaps have been filled by using data from loblolly pine (Barnett et al. 1986). The research reported here is part of an effort to develop information specific to shortleaf pine.

Prompt, uniform germination and early establishment are essential to producing shortleaf pine seedlings of consistently high quality. Overcoming seed dormancy is one of the major steps toward ensuring prompt, uniform germination. Typically, stratification (moist prechilling) of conifer seeds is done after an 8- to 24-hour period of moisture imbibition. However, recent studies show that length of water soaking may affect germination of species such as longleaf pine (P, *palustris* Mill.) (Barnett and Pesacreta 1990). Fully imbibed seeds are nor many placed in polyethylene bags and held at temperatures of 1 to 4 /C (34 to 38 /F). The length of stratification varies according to the extent of dormancy in the seeds.

Although stratification treatments are routinely applied to shortleaf pine seeds, few studies provide specific guidelines. Seidel (1963), working a single seed lot, found that germination speed increased progressively with duration of stratification up to 60 days. Barnett and McGilvray (1971) tested 16 separate lots representing various geographic sources and years of collection. They found that freshly collected seeds were much less dormant than stored ones. In these tests, germination speed of stored seeds continued to increase through 56 to 70 days of stratification.

The objectives of this study were to determine the effects of various pregermination treatments on germination of shortleaf pine seeds tested under optimum laboratory and more adverse laboratory and seedbed conditions, and on seedling develop ment at the time of lifting.

Materials and Methods

General protocol. The general protocol for the two experiments reported here was to use 6 half sib families from the USDA Forest Service seed or chard at Mt. Ida, Arkansas. Shortleaf pine seeds were stored at - 4 /C (25 /F) before use, and all empty seeds were removed by flotation in ethanol (Barnett 1971). In addition to evaluating a range of pretreatments (after 16 hours of water imbibition), seeds were tested under 3 conditions:

1. Ideal conditions-22 /C (72 /F) and 16-hour photoperiod, as indicated by standard germina tion tests (Association of Official Seed Analysts 1980).

2. Adverse laboratory temperatures-15 /C (60 /F) in test 1 and 18 /C (65 /F) in test 2-and a 12-hour photoperiod.
a. Field nursery conditions.

The seeds were germinated and tested at the Alexandria Forest Center forest nursery near Pineville, Louisiana. Experimental design in the nursery was modified to reflect operational practices and consisted of two replications of 4 rows each (50 seeds per row) oriented across 1.2-m (4-foot) beds and spaced 15 cm (6 inches) apart; the result was a sowing density of 269 seeds per mz (25 seeds per square foot). Sowing was done during the first week of April. Depending on latitude and annual climatic variation, nursery beds in early April more nearly approximate the 15 /C (60 /F) and 12-hour photoperiod conditions than standard laboratory conditions.

Test 1. Seeds from the 6 half-sib families were subjected to 0, 30, and 60 days of stratification. A fourth treatment was 60 days of stratification plus a subsequent 3-day aerated water soak. Germination of 200-seed samples for each of the 6 families, 4 treatments, with 3 locations of 3 replications each, was measured 3 times a week for 28 days. Responses to treatments were evaluated by determining germination percentages and values. Germination values reflect the speed of germination and are expressed as the peak value of the maximum cumulative percentage of germination divided by the number of days from sowing (Czabator 1962). Germination of the seeds sown in the nursery was measured weekly. Those that germinated during each 1-week period were marked with plastic rings of a specified color so they could be identified and remeasured later on in the growing season. Germination counts in the nursery were made weekly until germination was complete. Percentages of nursery trees were computed by taking the number of germinants produced 60 days after sowing in the nursery relative to the total seed sown for each nursery plot. In addition to germination, seedling mortality that occurred up to early June and seedling heights and diameters at lifting in the following winter were measured and development was related to the time of germination by tracking the plastic ring color of individual seedlings.

Test 2. Seeds from the same half-sib families were evaluated as in test 1, but from different samples. Three replications of 200-seed samples were subjected to 8 treatments: 0, 15, 30, and 45 days of stratification with and without 3 days of aerated water soaks following stratification in 3 locations. Aerated water soak treatments were combined with each length of stratification in this test to better evaluate this combined treatment at shorter periods of stratification. Germination tests were conducted under the same 3 sets of conditions as described in test 1, except that the germination test temperature was increased to 18 /C (65 /F)-from 15 /C (60 /F) in test 1-in the less favorable laboratory testing environment. Post germination seedling development in the nursery was not followed in this test.

Differences in germination response and seedling development resulting from the treatment applications were tested for statistical significance at the 0.05 level by analyses of variance. Orthogonal single degree of freedom tests were conducted to evaluate differences among locations. Germination data were averaged across locations to evaluate differences among stratification regimes.

Results and Discussion

Results indicate that the seed lots tested were not nearly as dormant as those reported in earlier studies by Seidel (1963) and Barnett and McGilvray (1971). Data from the previous studies indicated that stratification for at least 56 days was needed to obtain prompt germination. The 6 half-sib seed lots used in the current study did respond differently to treatments. Some families germinated well without stratification while others needed considerable prechilling. Because there was no logical explanation for response differences among seed lots, the lots were averaged to get a representative sample. Seeds tested under standard laboratory conditions and in nursery beds did not respond in total germination to periods of stratification beyond 30 days in this study (table 1). Germination percentages of seeds tested at 15 /C (60 /F) temperatures and shorter photoperiods indicated that they did benefit from the longer prechilling treatments. Germination in both of the less favorable environments (15 /C [60 /F] and nursery) was lower without stratification than the 22 /C (72 /F) condition, and the germination tests indicate the environmental conditions of 15 /C (60 /F) were more adverse than those occurring in the nursery seed beds. Data from other tests show that if the field conditions had been colder, seeds would have lower germination rates in treatments providing shorter stratification (McLemore 1969). The additional aer-

Table 1-Germination percentages and values for shortleaf pine seed lots subjected to different germination conditions and various lengths of stratification (0 to 60+ days)

	Germination %					Germination value				
Germination conditions	Day 0	Day 30	Day 60	Day 60+	Ave.	Day 0	Day 30	Day 60	Day 60+	Ave.
Ideal lab (22 /C)	80	91	90	88	87 c	14.9	31.3	34.2	25.7	26.5 c
Adverse lab (15 /C)	0	17	62	70	37 a	0	0.6	5.7	8.0	3.3 a
Nursery	45	79	76	77	67 b	2.1	9.6	11.4	12.6	8.9 b
Average	42 a	62 b	76 c	78 c		5.9 a	13.8 b	17.1 d	15.4 c	

The germination data are means of two 100-seed samples from 3 replications of 6 different half-sib families. The 60+ treatment consists of 60 days of cold stratification (2 /C) plus 3 days of aerated water soaks at 24 /C, Means within and across germination percentage and value columns followed by the same letter are not statistically different at the 0.05 level.

ated water soak treatment following stratification did not improve total seed germination over that of 60-day stratification, except when tested at 15 /C (60 /F).

In the nursery, germination peaked during the second week after sowing, but seedling mortality continued to increase as the seeds germinated later in the season (table 2). Mortality of seedlings that germinated in the fourth and fifth weeks averaged 27 and 56%, respectively. It is also interesting to note that seedlings lifted from the nursery during this late germination period were considerably smaller than, and were never able to compete with, those from early-germinating seeds. Seed stratification did result in stock at the end of the growing season that was statistically larger (156 versus 118 mm in height and 3.9 versus 3.2 mm in diameter) than from untreated seeds (0 days of stratification), but there were no size differences due to varying lengths of stratification pretreatments.

In test 2, treatments of 0, 15, 30, and 60 days of stratification were evaluated with and without a subsequent aerated water soak treatment (table 3). The seed lots used in the study showed little additional response in total germination to stratification beyond 15 days, although response did vary by seed lot. Apparently the seed orchard seeds evaluated in these tests were less dormant than the of stratification, although this dormancy may have been influenced by storage. Seeds from orchards are generally larger, and studies with loblolly pines indicate that large seeds tend to germinate faster than small ones, probably because they are less dormant as a result of less seed coat constraint (Barnett 1991, Dunlap and Barnett 1983).

Germination of unstratified seeds was better at the ideal laboratory conditions of 22 /C (72 /F) and 18-hour photoperiod than at more adverse laboratory conditions of 18 /C (65 /F) and 12-hour photoperiod or at nursery conditions. In this test, the more adverse laboratory and nursery conditions were about equal in effect, that is, they reduced germination by about 18 percentage points in the 0-day stratification treatment (table 3). This similarity in response reflects the similarity of the lab oratory conditions (18 /C; 65 /F) to the temperature of the seed beds. The 3 additional days of aerated water soaking improved overall germination of seed of the 0-day treatment by 7 percentage points over the regular stratification treatments, but this difference was not statistically significant (table 3).

Speed of germination of stratified seeds, as determined by germination values, continued to increase with additional lengths of treatment (table 3). Al though total germination percentage did not in crease with longer periods of stratification, germination values did improve, reflecting an increasing rate of germination. Germination values were con sistently greater for the tests conducted at 22 /C (72 /F) than for those conducted at 18 /C (65 /F).

One interesting result of these tests is the deter mination that predictions of performance on nurs

Table 2-Mortality and seedling size of shortleaaf pine seedlings at lifting as related to the time of germination,

Time after sowing Days Week	Germination per week (%)	Mortality of germinants (%)	Seeding size (mm) Height Diameter
8-10 1	6	12	157 4.1
13-17 2	52	13	160 4.0
20-24 3	30	18	144 3.5
27-31 4	10	27	118 3.1
34-38 5	2	56	115 2.7

Seeds were sown April 2, 1985, m two replications of four 50-seed rows for each of six half-sib families. Size measurements were made in early January 1986.

Table 3-Germination percentages and values for shortleaf pine seed lots of test 2 subjected to different germination conditions

Germination Conditions	Days of seed pretreatment 0	15	30	45	0+	15+	30+	45+	Ave.
Germination percentage									
Ideal lab (22 /C)	61	86	92	94	68	88	92	95	84 b
Adverse lab (18 /C)	44	92	92	94	45	87	92	94	80 ab
Nursery	42	80	87	83	54	77	86	85	74 a
Average	49 a	86 c	90 d	90 d	56 b	81 c	90 d	91 d	
Germination values									
Ideal lab (22 /C)	8.7	33.4	42.6	55.4	16.1	44.3	59.1	91.7	43.8 b
Adverse lab (18 /C)	4.9	22.6	28.3	33.2	8.6	25.7	34.8	71.8	28.7 a
Average	6.8 a	28.0 c	35.4 d	44.3 a	12.3 b	35.0 d	46.9 a	81.8 f	

The germination data are means of two 100-seed samples of 3 replications of 6 different half-sib families. The length of pretreatment followed by (+) indicates stratification followed by 3 days of aerated water soaking. Means within and across columns followed by the same letter are not significantly different at the 0.05 level.

ery beds based on germination tests can be improved by measuring the environmental conditions at the time of sowing and then closely duplicating those in the seed testing laboratory. Soil-temperature data can be accumulated over several years with relatively inexpensive monitoring equipment. With this background information, germination tests can be conducted under conditions that reflect typical seed bed conditions.

Previous studies indicate that soaking seeds in aerated water is a short-term substitute for stratification. Soaking shortleaf pine seeds in continuously aerated water at 10 /C (50 /F) stimulated germination as much as colder soaks and did so more quickly (Barnett 1971). This technique provides a rapid stimulatory effect on shortleaf pine seeds, but it is not a replacement for more effective stratification treatments. There was no demonstrated advantage of using aerated soaks after lengthy periods of stratification.

Conclusions

Stratification is essential to obtain prompt and uniform germination. The length of treatment varies by seed source and length of storage, but the total germination of orchard collections of shortleaf pine seeds did not respond to stratification beyond 15 days. However, speed of germination continued to increase with stratification up to 45 days. There were major differences in seed performance due to the environmental conditions under which they germinated. Seeds without stratification germinated very poorly under the more stressful conditions of nursery beds or lowered laboratory temperatures and daylengths. These data confirm other studies showing that the optimum length of stratification increases as germination conditions become more adverse, that is, 15 and 18 /C (McLemore 1969). Temperatures of 22 /C continue to be the most optimum for germination.

Seeds that germinated first in the nursery produced larger seedlings than those that germinated later. Late germinants were typically small; they could not compete with those that germinated earlier and many died or became culls.

In spite of many attempts to find better predictors, germination percentages from laboratory tests conducted under optimum conditions are normally used to predict seed performance in nurseries (Barnett and McLemore 1984). Nursery managers who have difficulty in predicting seed germination can improve their estimates on their nursery beds by determining typical environmental conditions at the time of sowing and by requesting that their seed germination tests be conducted under those conditions as well as standard laboratory ones.

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