

# LONGLEAF PINE SEED PRESOWING TREATMENTS: EFFECTS ON GERMINATION AND NURSERY ESTABLISHMENT<sup>1</sup>

James P. Barnett,<sup>2</sup> Bill Pickens,<sup>3</sup> and Robert Karrfalt<sup>4</sup>

**ABSTRACT**—Longleaf pine (*Pinus palustris* Mill.) seeds are sensitive to damage during collection, processing, and storage. High-quality seeds are essential for successful production of nursery crops that meet management goals and perform well in the field. A series of tests was conducted to evaluate the effect of a number of presowing treatments, e.g., soaking, stratification, and coat sterilization on performance of longleaf pine seeds in the laboratory and nursery. The results of these tests that were installed to determine if presowing treatments improved seed performance are reported here.

## INTRODUCTION

Interest in restoring many sites in the South to longleaf pine (*Pinus palustris* Mill.) has increased dramatically in the last 10 years. One of the limitations in producing the quantities of seedlings needed for this reforestation effort is the lack of high-quality seeds. The quality of longleaf pine seeds has been a problem across the South since the quantities collected and produced have markedly increased. Part of the problem of low quality relates to level of maturity at time of collection and to difficulties in cone storage and processing (Barnett and Pesacreta 1993). Handling of large amounts of cones and seeds results in loss of seed quality because all of the recommended criteria for maintaining high quality cannot be met. Nursery managers have looked for seed treatments that may improve performance of such longleaf pine seeds. Treatments in use vary from stratification to soaks in hydrogen peroxide or a fungicide and specific use recommendations vary. At the suggestion of Selby Hawk of the North Carolina Forest Service, a cooperative study among personnel of the Claridge Nursery at Goldsboro, NC, the National Tree Seed Laboratory (NTSL) at Dry Branch, GA, and the Seed Testing Facility (STF) of the Southern Research Station at Pineville, LA, was initiated to evaluate some of the currently used treatments. The objective of the study was to develop recommendations for presowing treatments that will improve performance of longleaf pine seeds.

## METHODS

Treatments were applied to the seeds in late April of two separate years—1997 and 1998. Germination tests were conducted at the NTSL, the STF at Pineville, and at the Claridge Nursery.

### 1997 Tests

The presowing treatments were: (1) control, (2) 1-hour (hr) 3 percent hydrogen peroxide (HP) soak, (3) 1-hr HP soak plus 16-hr water soak (WS), (4) 1 -hr HP soak plus 16-hr WS plus 14-day stratification (ST), (5) 16-hr WS plus 14-day stratification, and (6) 16-hr water mist plus 14-day stratification. The 1 -hr soak in 38 percent HP was based on

earlier research (Barnett 1978) and is labeled as a stratification treatment. It is used operationally at the Claridge Nursery (Barnett and McGilvray 1997). The 14-day stratification treatment is recommended for longleaf pine seeds by the NTSL (Barbour 1998, Karrfalt 1988). These responses to stratification are based on seed imbibition on the germination medium. Other tests of stratification at the Pineville Lab (STF) indicated that the 16-hr WS as conducted for operational stratification may reduce germination by 10 percentage points (Barnett and Pesacreta 1993). So, a mist imbibition treatment (misting 1 of every 10 minutes) was included to compare this technique with the water imbibition soak commonly used at nurseries to prepare seeds for stratification. It was felt that the rapidity of water absorption of longleaf pine seeds might be adversely affecting resulting performance (Barnett 1981) and that an intermittent mist might slow imbibition and have a less negative affect on germination.

Three seedlots were selected for the study. One high viability lot was provided by the STF and the two other lots were selected as medium and low quality by Claridge Nursery personnel. Five dishes of 50 seeds each were used for testing in the laboratories: 10 trays of 98 cavities each were used for testing in the nursery. The NTSL applied the presowing treatments to the seeds tested at NTSL and Claridge Nursery. The STF personnel applied the treatments to the same seedlots that were tested at Pineville.

Laboratory germination tests followed the Association of Official Seed Analysts (AOSA) guidelines. Germination counts were made at 2- to 3-day intervals at STF in order to determine peak day or the speed of germination. Counts at NTSL and Claridge Nursery were made at 7-day intervals. In all cases, germination was complete within 28 days.

A determination of seedling establishment or percent stocking was made at the Claridge Nursery 3 months after sowing. This evaluation was made to determine if some treatments were more effective than others in protecting seeds from damping-off diseases during germination and early seedling development.

<sup>1</sup>Barnett, J.P.; Pickens, B.; Karrfalt, R. 1999. Longleaf pine seed presowing treatments: effects on germination and nursery establishment. In: Landis, T.D.; Barnett, J.P., tech. coords. National proceedings: forest and conservation nursery associations—1998. Gen. Tech. Rep. SAS-25. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southern Research Station: 43-46.

<sup>2</sup>USDA Forest Service, Southern Research Station, 2500 Shreveport Hwy., Pineville, LA 71360; TEL: 318/473-7216.

<sup>3</sup>North Carolina Department of Environment and Natural Resources, Division of Forest Resources, P.O. Box 29581, Raleigh, NC 27525.

<sup>4</sup>USDA Forest Service, National Tree Seed Laboratory, Route 1, Box 182B, Dry Branch, GA 31020; TEL: 912/751-3552.

Table I-Germination and seedling stocking of **longleaf** pine seed treatments tested in 1997 under laboratory and nursery conditions'

Treatments	Peak day	Germination				Stocking	
		STF	NTSL	Nurs. 1	Nurs. 2	Nurs. 1	Nurs. 2
	No.						
Control	<b>7.0ab</b>	76b	71c	<b>75bc</b>	<b>72c</b>	66bc	64b
Hydrogen peroxide (HP)	<b>7.2a</b>	84a	<b>84a</b>	70d	81ab	70b	78a
HP + <b>16-hr</b> water soak (WS)	<b>6.0bc</b>	<b>71b</b>	74c	<b>84a</b>	85a	<b>81a</b>	<b>80a</b>
HP + WS + <b>14-day</b> strat.	<b>4.4d</b>	76b	78b	79abc	<b>85a</b>	77a	82a
WS + <b>14-day</b> strat.	<b>4.0d</b>	85a	84a	79bc	77bc	54d	50c
Mist + <b>14-day</b> strat.	<b>5.0c</b>	86a	82 a b	<b>80ab</b>	76bc	<b>62c</b>	65b

Germination 28 days after sowing in the **Claridge** Nursery (two **separate** tests of the same treatment **applications** sown 2 weeks apart) and Pineville (STF) and Dry **Branch** (NTSL) **Laboratories**. Peak day **represents** the **time** when maximum **daily germination** occurs and is a measurement of speed of germination. Seedling **stocking** is expressed as the percentage of seeds that become established 90 days after sowing. **Averages** within **columns** followed by the same letter are not **significantly** different at the 0.05 level.

### 1998 Tests

The study was essentially repeated in the second year. The treatments differed from the previous year by dropping the water mist-imbibition and stratification evaluation that did not germinate in the laboratory significantly different to the more conventional water soak-stratification treatment. Added in its place was a 10-minute benomyl drench (0.05 percent solution of **benomyl 50WP** or 227 grams per 12 gallons water). This treatment was based on research of Weyerhaeuser Company that demonstrated the **efficiency** of the benomyl seed-dip treatment for controlling seedborne **Fusarium** and was the basis for registration in **North Carolina** (Littke and others 1997).

Three **seedlots** were again used in this study (high, medium, and low viability). A replication in time was a component of this test. All treatments were applied at the Pineville lab and shipped to the NTSL and Claridge Nursery for testing beginning in late April and were repeated again 2 weeks later. Germination counts were made at **7-day** intervals at the three testing locations. The other aspects of the study were the same as in the 1997 test.

### RESULTS AND DISCUSSION

Although essentially the same treatments were evaluated in the two separate years of testing, there are sufficient differences in procedures to discuss the results separately by year.

#### 1997 Tests

The **seedlots** were selected to provide an evaluation of the treatments on different seed qualities; lots **1, 2, and 3** were selected to represent low, medium, and high qualities. All tests showed consistent differences among seedlots. For most analyses, there were statistically significant (**0.05**-percent level) interactions between **seedlots** and treatments. These interactions reflect that usually the lower quality lot responded more positively to the presowing treatments than the high-quality lot.

A tabulation of responses to the seed treatments is shown in table 1. There were some major differences among testing locations in the results obtained, e.g., in Claridge

Nursery test **#1**, the HP treatment performed consistently lower than in test **#2** at the Nursery or at either the Pineville or Macon laboratories. This treatment, which did poorly in Nursery test **#1**, was equal to the best responding treatments in the other tests. The HP plus **16-hr** soak treatment performed best in Claridge Nursery Test **#1**, but performed worst in the laboratory tests. One possible rationale for the differences in performance of the HP treatment in the nursery tests is that the treatment labels were switched during the test **#1** seeding process. At any rate, it is fortunate that two evaluations were conducted at the nursery.

A flaw may have occurred in the Claridge test **#1** study related to the HP treatment. In Nursery test **#2**, the HP treatments were superior to the control and equal to the stratification ones. The laboratory tests at Macon and Pineville showed that the HP soak and the **14-day** stratification treatments (both soak and mist) performed best. So, there seems to be some differences between the nursery and the labs.

A determination of percent stocking in the nursery containers was done on July **15, 1997**, about 3 months after sowing. In both nursery tests, the treatments that involved HP produced better stocking than the control or stratification treatments. Stocking resulting from the water **soak**-stratification treatment was significantly poorer than in the mist-stratification treatment. So, even though water imbibition occurred at comparable rates in the water soaking and misting treatments, there may be merit in evaluating misting approaches that would result in slower rates of absorption.

#### 1998 Tests

The differences in germination due to seedlots, presowing treatments, and their interactions were statistically significant at the 0.05 level in each separate test (table 2). To make evaluations of the responses due to the measurement variables more straightforward, germination is presented by presowing and **seedlot** treatments and by presowing treatments and testing locations.

The **effects** of presowing treatments and **seedlot quality** indicate limited response to treatments in the **seedlot** of highest quality (table 3). Germination at 28 days ranged from 85 percent for the control to 93 percent for the benomyl drench. However, when the medium- and low-quality lots were evaluated, there were major differences in response among the presowing treatments. The HP and benomyl treatments resulted in increases in germination over that of the control with performance of the lower quality lot increasing by 20 percentage points with the HP treatment and 15 percentage points for the benomyl drench. Treatments that included a **16-hr** water soak reduced overall germination from 10 to 30 percentage points.

The responses to treatments followed similar trends at each testing facility and between the two replications in time (table 4). As expected, germination in the nursery was somewhat lower than in the laboratories. However, the HP soak and benomyl drench consistently improved germination over that of the control in all situations.

The tests in both 1997 and 1998 indicate that a significant problem in **longleaf** pine seed performance results from the pathogens carried on the seedcoats. Fraedrich and Dwinell (1998) recently reported that the pitch canker fungus (*Fusarium subglutinans* [Wollenw. & Reinking] Nelson, Toussoun & Marasas f. sp. **pini**) is a cause of significant mortality of **longleaf** pine germinants. Our results show that the treatments that reduce microorganisms on the seedcoats improve germination of moderate and **low**-quality seedlots. The HP soak improved seedling establishment at 90 days in the nursery in the 1997 study by a significant amount (14 percentage points). In the 1998 tests, both the HP and benomyl treatments improved performance of lower quality seedlots. The high viability lot was largely unaffected by presowing treatments.

## CONCLUSIONS

The results of both yearly tests indicate that the maximum improvement in **longleaf** pine seed performance can be obtained by using treatments that reduce **seedcoat**

Table 2—Germination of **longleaf** pine seed presowing treatments tested in 1998 under laboratory and nursery conditions<sup>1</sup>

Treatment	Seed quality	Test 1			Test 2		
		STF	NTSL	Nurs.	STF	NTSL	Nurs.
Control	H	91	84	83	82	85	80
	<b>M</b>	85	73	52	66	70	60
	L	58	52	49	58	49	58
Avg.		71	70	61	89	88	66
Hydrogen peroxide (HP)	H	92	92	76	90	92	86
	<b>M</b>	80	71	61	72	70	70
	L	78	75	60	81	72	83
Avg.		83	79	66	81	78	73
HP + water soak (soak)	H	84	88	88	74	85	88
	<b>M</b>	21	36	46	45	32	29
	L	48	21	44	42	30	21
Avg.		50	48	59	54	49	46
HP + soak + stratification	H	89	84	91	90	95	92
	<b>M</b>	25	40	33	49	27	52
	L	24	15	23	46	45	44
Avg.		46	46	49	62	56	63
Water soak + stratification	H	92	93	89	94	94	87
	<b>M</b>	56	50	34	61	57	60
	L	41	43	39	53	39	40
Avg.		63	62	54	69	63	82
Senomyl drench	H	92	93	91	92	95	92
	<b>M</b>	79	80	69	72	80	74
	L	70	65	71	64	70	64
Avg.		80	79	77	76	82	77

<sup>1</sup>Data are averages of the 5 replications of 50 seeds each. Highest germination in the nursery may have been at 7, 14, or 21 days; counts were lower on 13 of the 18 seedlot-treatment combinations due to damping-off losses before the final count at 28 days. Differences due to treatments, seedlots, and their interactions were statistically significant at the 0.05-percent level for each separate test.

Table 3—Germination of **longleaf** pine seeds tested in 1998 by presowing treatment and seed quality conditions

Treatment	Seed quality condition			
	High	Medium	Low	Average
Control	84	84	54	87
Hydrogen peroxide (HP)	88	71	71	77
HP + water soak (soak)	84	35	34	51
HP + soak + stratification	90	38	33	55
Soak + stratification	92	53	42	82
Benomyl drench	92	78	87	78

Table 4—Germination of **longleaf** pine seeds by presowing treatments and tested two times in 1998 in the laboratory and nursery

Treatment	Test 1			Test 2		
	STF	NTSL	Nurs.	STF	NTSL	Nurs.
Control	71	70	81	89	88	88
Hydrogen peroxide (HP)	83	79	88	81	78	73
HP + water soak (soak)	50	48	59	54	49	48
HP + soak + stratification	48	48	49	82	58	83
Soak + stratification	83	82	54	89	83	82
<b>Benomyl</b> drench	80	79	77	78	82	77

contamination. Both the **1-hr** soak in **30-percent** HP and the 10-minute benomyl drench were effective in increasing germination of medium- to low-quality seedlots. High-quality lots were little affected by any presowing treatment. Although operational stratification increases the speed of germination of many **seedlots** by about 3 days, total germination of less than high-quality **seedlots** is usually reduced by the treatment. The data confirm results of earlier tests that showed that the overnight soaking of **longleaf** seeds as done in operational stratification may reduce total germination (Barnett and Pesacreata 1993). Data from the 1997 test (the 1998 data are not yet available) that determined the effect of presowing treatments on nursery stocking show that use of treatments that reduce **seedcoat** contaminants can markedly improve establishment of germinants in the nursery. So, an additional gain in the nursery can be obtained from the use of treatments that control the **seedcoat** pathogens that are common on **longleaf** pine seeds. The **10-minute** benomyl drench was equally effective as the **30-percent** HP soak and it presents a less expensive and safer treatment for nursery managers. We should seek additional labeling of this benomyl treatment because it provides an excellent opportunity to improve **performance** of **longleaf** pine **seedlots** of typical quality.

## REFERENCES

Barbour, J. 1996. **Longleaf** pine cone collection and seed conditioning guidelines. Revised. Dry Branch, GA: U.S. Department of Agriculture, Forest Service, National Tree Seed Laboratory. 7 p.

Barnett, J.P. 1976. Sterilizing southern **pine** seeds with hydrogen peroxide. *Tree Planters' Notes*. **27(3):17-19, 24**.

Barnett, J.P. 1961. Imbibition temperatures affect seed moisture uptake, germination, and seedling development. In: Proceedings of the 1st biennial southern silvicultural research conference. Gen. Tech. Rep. **SO-34**. New Orleans: U.S. Department of Agriculture, Forest Service, Southern Forest Experiment Station: **41-45**.

Barnett, J.P.; McGilvray, J.M. 1997. **Practical** guidelines for producing **longleaf** pine in containers. Gen. Tech. Rep. SRS-14. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southern Research Station. 26 p.

Barnett, J.P.; Pesacreata, T.C. 1993. Handling **longleaf** pine seeds for **optimal** nursery performance. *Southern Journal of Applied Forestry*. **17: 180-187**.

Fraedrich, S.W.; Dwinell, L.D. 1996. Mortality of **longleaf** pine seedlings caused by *Fusarium subglutinans* and an evaluation of potential inoculum services. In: Proceedings of the 3rd meeting of IUFRO Working Party **S7.03.04**; 1996 May 19-24; Orlando-Gainesville, FL: **48-54**.

Karrfalt, R.P. 1966. Stratification of **longleaf** pine. In: Proceedings southern forest nursery association meeting, southern forest nursery **association**; Columbia, SC: 4649.

Littke, W.; Miller, H.; Millican, D. [and others]. 1997. The efficacy of **Benlate** seed-dip treatment to control **seedborne** *Fusarium* disease in containerized **longleaf** pine (*Pinus palustris*). Weyerhaeuser Research and Development Rep. **050-1422-97.1**. Centralia, WA: Weyerhaeuser Forestry Laboratory. 22 p.