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IMPROVING LONGLEAF PINE SEEDLING ESTABLISHMENT IN THE NURSERY BY REDUCING SEEDCOAT MICROORGANISMS'

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Abstract-Longleaf pine (*Pinus palustris* Mill.) seeds are sensitive to damage during collection, processing, and storage. Highquality seeds are essential for successful production of nursery crops that meet management goals and perform well in the field. We conducted a series of tests under laboratory and nursery conditions to evaluate what effect a number of presowing treatments, e.g., soaking, stratification, and coat sterilization, had on the performance of longleaf pine seeds of varying qualities. Test results indicate that removal of fungal contamination from the seedcoats will markedly improve seed germination and seedling establishment in the nursery. Both a l-hour soak in 30-percent hydrogen peroxide and a IO-minute drench in a 2.5 percent a.i. per liter benomyl solution improved longleaf pine seedlot performance, particularly in seedlots of low to medium quality. Based on seedling establishment 3 months after sowing, other treatments, which included water soaking and stratification, were less effective than sterilants. Because the benomyl drench was as effective as the hydrogen peroxide soak, it is the preferred treatment for controlling seedcoat contamination: it is both more economical and safer for the nursery manager to use. An effort is underway to extend the registration for this seed treatment to Southern States other than North Carolina, where it is currently labeled.

NTRODUCTION

Interest in restoring longleaf pine (*Pinus palustris* Mill.) to many sites in the South has increased dramatically over the last 10 years. One of the limitations of producing the quantities of seedlings needed for this reforestation effort is the lack of high-quality seeds. Following a marked increase in the quantity of seed collected and seedlings produced, the quality of longleaf pine seeds has been a problem across the South. Part of this problem relates to seed maturity at the time of collection and to difficulties in cone storage and processing (Bamett and Pesacreta 1993). Handling of large amounts of cones and seeds results in diminished seed quality, because many of the recommended criteria for maintaining high quality cannot be met. Nursery managers have looked for seed treatments that would improve seed viability and seedling performance. Treatments range from stratification to soaks in hydrogen peroxide or a fungicide; and specific use recommendations vary. 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 current treatments. The study's objective was to develop recommendations for presowing treatments that will improve longleaf pine seed performance in the nursery.

METHODS

Treatments were applied to the seeds in late April of both 1997 and 1998. Germination tests were conducted at the NTSL, the STF, and at the Claridge Nursery.

1997 Tests

The presowing treatments were: (1) control, (2) a l-hour, 30-percent hydrogen peroxide (HP) soak, (3) l-hour HP soak and a 16-hour water soak (WS), (4) l-hour HP soak, a 16-hour WS, and a 14-day stratification (ST), (5) 16-hour WS and a 14-day ST, and (6) 16-hour water mist, and a 14-day ST. The l-hour soak in 30-percent HP was based on earlier research (Bamett 1976) and is labeled as a seed ST. This procedure is used operationally at the Claridge Nursery (Barnett and McGilvray 1997). The NTS recommends the 14-day ST treatment (Barbour 1996, Karrfalt 1988). Responses to ST are based on seed imbibition on the germination medium. Other tests of ST at the STF indicated that the 16-hour water soak, as it was conducted for operational ST may reduce germination by 10 percentage points (Bamett and Pescreata 1993). We, therefore, included a mist imbibition treatment (misting 1 of every 10 minutes) to compare with the water imbibition soak commonly used at nurseries to prepare seeds for ST. We felt that the rapidity of water absorption by longleaf pine seeds might be adversely affecting performance (Barnett 1981, Taylor and others 1992) and that an intermittent mist might slow imbibition and improve germination.

Three seedlots were selected for the study. One high-viability lot was provided by the STF, and Claridge Nursery personnel selected the two other lots as medium and low quality. Five dishes of 50 seeds each were used for laboratory testing; 10 trays of 96 cavities each were tested in the nursery. The NTSL applied presowing treatments to those seeds tested at NTSL and the Claridge Nursery. Seed Testing Facility at Pineville personnel applied treatments to the same seedlots that were tested at STF. Laboratory germination tests followed the Association of Official Seed Analysts (AOSA) guidelines. In order to determine peak day or the speed of germination, germination counts were made at 2- to d-day intervals at STF. 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.

[1] Paper presented at the Tenth Biennial Southern Silvicultural Research Conference, Shreveport, LA, February 16-18, 1999.

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1998 Tests

The study made in 1997 was repeated in 1998. However, treatment was dropped because the germination of these seeds in the laboratory did not differ significantly from those in the more conventional WS-ST. Added in its place was a IO-minute benomyl drench (0.05 percent solution of **benomyl 50WP [2.5** percent **a.i.],** or 227 grams per 12 gallons water). This treatment was based on Weyerhaeuser Company research (Littke and others 1997) that demonstrated the **efficacy** of the benomyl seed-dip treatment for controlling seedbome ***Fusarium*** and was the basis for registration in North Carolina.

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 STF. Some of the seeds were then shipped to the NTSL and Claridge Nursery for testing that began in late April and was repeated 2 weeks later. Germination counts were made at **7-day** intervals at the three testing locations. Seedling stocking was determined approximately 90 days after sowing. The other aspects of this test were the same as in the 1997 test.

Table l-Germination of longleaf pine seed and seedling stocking following treatments in 1997 under laboratory and nursery conditions[a]

Treatments	Peak day	Germination				Stocking	
		STF	NTSL	Nurs.1	Nurs.2	Nurs.1	Nurs.2
	No.	Percent					
Control	7.0ab	76b	71c	75bc	72c	66bc	64b
Hydrogen peroxide (HP)	7.2a	84a	84a	70d	81ab	70b	78a
HP + 16-hr water soak (WS)	6.0bc	71b	74c	84a	85a	81a	80a
HP + WS + 14-day stratification	4.4d	76b	78b	79abc	65a	77a	82a
WS + 14-day stratification	4.0d	85a	84a	79bc	77bc	54d	5oc
Mist + 14-day stratification	5.0c	86a	82ab	80ab	76bc	62c	65b

[a] 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 became viable seedlings 90 days after sowing. Averages within columns followed by the same letter are not significantly different at the 0.05 level.

RESULTS AND DISCUSSION

Although essentially the same treatments were evaluated in 2 years of testing, sufficient differences in procedures necessitate discussing the results separately.

1997 Tests

Seedlots were selected to determine how different seed qualities were affected by the treatments; lots **1, 2,** and 3 represented low-, medium-, and high-quality seeds. All tests showed consistent differences among seedlots. Most analyses showed statistically significant (0.05 percent level) interactions among **seedlots** and treatments. These interactions demonstrated that lower quality **seedlots** usually responded more positively to presowing treatments than the high-quality **seedlot.**

Table 1 shows seed responses to five treatments and a control. Some major differences in the results obtained occurred among testing locations, e.g., in Claridge Nursery Test **1,** the hydrogen peroxide (HP) treatment performed consistently lower than in Test 2 at the Nursery or at either the STF or NTSL laboratories. However, HP treatment results in Nursery Test 1 were equal to the best response to treatments in the other tests. The HP plus **16-hour** soak treatment performed best in Claridge Nursery Test **1,** but performed worst in the laboratory tests. One possible reason such performance differences were noted in the nursery tests is that the treatment labels were switched when Test 1 seeds were sown. Nonetheless, 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. Laboratory tests at NTSL and STF showed that the HP soak and the **14-day** ST treatments (both soak and mist) performed best. Thus, there seem to be some differences between the nursery and the labs. A determination of percent stocking in the nursery containers was done about 3 months after sowing on July 15, 1997. In both nursery tests, treatments with HP produced better stocking than the control or ST treatments. Stocking resulting from the WS-ST treatment was significantly poorer than that resulting from the mist-stratification treatment. Therefore, even though water imbibition occurred at comparable rates in the water soaking and misting treatments, it may be helpful to evaluate misting approaches that would result in slower rates of absorption.

1998 Tests

Differences in germination among seedlots, presowing treatments, and their interactions were statistically significant (at 0.05) in the individual tests (table 2). To make more straightforward evaluations of our responses resulting from measurement variables, we presented germination by presowing and seedlot treatments, and by presowing treatments and testing locations.

The effects of presowing treatments showed limited response in the highest-quality seedlot (table 3); germination at 28 days ranged from 84 percent in the control to 92 percent in the benomyl drench. In the medium- and low-quality lots, however, there were major differences in response to the various presowing treatments. The HP and benomyl treatments increased germination over that of the control: performance of the lower quality lot increased by 12 percentage points with the HP and 13 percentage points with the benomyl drench treatments. Treatments that included a 16-hour water soak reduced overall aermination about 22 percentage points.

Table Z-Germination of longleaf pine under laboratory and nursery conditions following seed presowing treatments tested in 1998[a]

Treatments	Seed quality	Test 1			Test 2		
		NTSL	Nurs.	STF	NTSL	Nurs.	STF
		Percent					
Control							
	High	91	64	83	82	85	80
	Medium	65	73	52	66	70	60
	Low	58	52	49	56	49	58
	Avg.	71	70	61	69	68	66
Hydrogen peroxide (HP)							
	High	92	92	76	90	92	88
	Medium	80	71	61	72	70	70
	Low	78	75	60	81	72	63
	Avg.	83	79	66	61	78	73
HP + water soak (ws)							
	High	64	86	88	74	85	88
	Medium	21	36	46	45	32	29
	Low	48	21	44	42	30	21
	Avg.	50	48	59	54	49	46
HP + ws + stratification							
	High	89	64	91	90	95	92
	Medium	25	40	33	49	27	52
	Low	24	15	23	46	45	44
	Avg.	48	46	49	62	56	63
WS + stratification							
	High	92	93	89	94	94	67
	Medium	58	50	34	61	57	60
	Low	41	43	39	53	39	40
	Avg.	63	62	54	69	63	62
Benomyl drench							
	High	92	93	91	92	95	92
	Medium	79	80	69	72	80	74
	Low	70	85	71	64	70	64
	Avg.	80	79	77	76	82	77

[a] Data are averages of 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 by presowing treatment and seed quality conditions in 1998

Treatments	Seed quality condition: High	Medium	Low	Avg.
	Percent			
Control	84	64	54	67
Hydrogen peroxide (HP)	88	71	71	77
HP + water soak (ws)	84	35	34	51
HP + ws + stratification	90	36	33	55
WS + stratification	92	53	42	62
Benomyl drench	92	76	67	76

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 at all locations.

The tests in both 1997 and 1998 indicate that a significant problem in longleaf pine seed performance results from pathogens carried on the seedcoats. Fraedrich and Dwinell (1996) recently reported that the pitch-canker fungus (*Fusarium subglutinans* [Wollenw. & Reinking] Nelson, Toussoun & Marasas f. sp. *pini*) is a cause of significant mortality in longleaf pine germinants. Other species of *Fusarium* are often isolated from conifer seeds and may result in poor germination or pre-emergence and post-emergence disease (Littke 1996). Our results show that treatments to 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 tests by a significant amount (14 percentage points). In the 1996 tests, both the HP and benomyl treatments improved performance of lower quality seedlots. Stocking, overtime, at the two test sites was 15 and 23 percent more for those seeds treated with-the HP and benomyl, respectively. The high-quality lot was largely unaffected by preeowing treatments.

CONCLUSIONS

The results of both tests indicate that treatments to reduce seedcoat contamination will provide maximum improvement in longleaf pine seed performance. Both the l-hour soak in 30-percent HP and the 1 O-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 ST increases the speed of germination of many seedlots by about 3 days, total germination of less than high-quality seedlots is usually reduced by ST treatment. The data confirm results of earlier tests showing that overnight soaking of longleaf seeds (as done in operational ST) may reduce total germination (Bamett and Pesacreta 1993). Data from both the 1997 and 1998 tests that determined the effect of presowing treatment&on nursery stocking show that use of treatments that reduce seedcoat contaminants can markedly improve establishment of germinants in the nursery. Therefore, nursery practices that include treatments for controlling seedcoat pathogens common to longleaf pine seeds would be beneficial. The IO-minute benomyl drench was as effective as the 30-percent HP soak; and it offers a less expensive and safer treatment. We should seek additional labelling of this benomyl treatment because it provides an excellent opportunity to improve performance of typical longleaf pine seedlots.

Table 4—Germination of longleaf pine seeds and seedling stocking following presowing treatments; tested two times in 1998 under laboratory and nursery conditions[a]

Treatments	Test 1: STF	NTSL	Nurs.	Stock.	Test 2: STF	NTSL	Nurs.	Stock.
	Percent							
Control	71	70	61	49	69	66	66	55
	83				81	78	73	70
Hydrogen peroxide (HP)		79	59	64	54	49	46	38
HP + water [illegible] stratification	[illegible]	[illegible]	49	[illegible]	62		63	57
			54			56	62	36
[illegible]	[illegible]	[illegible]	77	[illegible]	[illegible]	[illegible]	77	74

[a] Germination was tasted at the Pineville STF, NTSL, and Clarldge Nursery. Stocking was determined at the Clarldge Nursery.

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