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Aerated Water Soaks Stimulate Germination of Southern Pine Seeds

J. P. Barnett[1]

Soaking loblolly, slash, and shortleaf pine seeds in continuously aerated water at 41° F. speeded germination as much as stratification in polyethylene bags. Soaking at 60° stimulated germination as much as colder soaks and in less time. Although dormant loblolly seeds can be soaked at low temperatures for nearly 5 months without harm, periods up to 60 days are usually sufficient. With less dormant seeds and higher soaking temperatures, periods as short as 2 or 3 weeks may be necessary to prevent germination in water and induction of secondary dormancy. The water should be aerated continuously to keep the oxygen content near saturation.

Stratification has been the standard treatment for overcoming dormancy in southern pine seeds since 1928 when Barton (*3*) reported that it hastened germination. Although in recent years moistened seeds have usually been stratified in polyethylene bags rather than between alternating layers of peat moss (*9, 11*), stratification still requires considerable labor and care. No more than 25 pounds of seeds should be placed in individual bags, and the bags should be opened periodically for aeration.

Many nurserymen may find soaking seeds in cold, aerated water more convenient. Large lots can be treated with little danger of molding or overheating, and with little labor. And research reported here shows that this treatment is as effective as stratification for overcoming dormancy of slash (*Pinus elliottii* Engelm.), loblolly (*P. taeda* L.), and shortleaf (*P. echinata* Mill.) pine seeds.

METHODS AND MATERIALS

The recommendations presented are based on a series of studies into the individual variables affecting treatment success—species, water temperature, duration of treatment, and method and quantity of aeration. In studies in which species was not an experimental variable, loblolly pine seeds were treated because they are the most dormant. In all but a few instances, seeds were from fresh lots collected in central Louisiana.

In most studies treatments were applied to about 3/4 pound sublots in polyethylene bottles that had their tops cut off to make containers 3½ inches in

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diameter and 6 inches deep. Seeds were placed in the containers to a depth of about 4 inches, and water was added until it covered them by about 1 inch. Controls were sublots of the same size stratified in polyethylene bags.

Oxygen content of the soaking water was monitored with a galvanic cell oxygen analyzer. Readings were corrected to concentration of dissolved oxygen, by the Winkler method (*15*). In some studies the airflow, usually furnished by aquarium air pumps, was measured with a flowmeter.

Germination of each treatment replication was tested on duplicate 100-seed samples. Tests were run for 28 days at 72° F. under standard laboratory conditions (*14*). Germination percents and germination values (*5*), which take into acount both speed and completeness of germination, were computed and analyzed. Differences due to treatment were tested for statistical significance at the 0.05 level in all studies.

EFFECTIVENESS

The dependability of aerated soaks was demonstrated many times in the studies. Fowler (*7*) first tried aerated soaks with eastern white pine (*P. strobus* L.). Later, preliminary tests with loblolly pine seeds (*2*) proved encouraging, and led to the studies described.

The first test showed that, in terms of germination values, soaking in continuously aerated or continuously circulated water was about as effective as stratification (table 1). Intermittent aeration, changing water twice weekly, aeration with oxygen, and soaking in unchanged still water were less effective. In this test, treatments were continued for 63 days, and the water temperature was 41° F. The purpose of subsequent studies was to determine optimum soaking conditions.

CONTINUOUS AERATION

The soaking water should be continuously aerated, either by bubbling air through it or by splashing it during a continuous recirculation process. The purpose

Table 1. – *Germination values of loblolly pine seeds soaked for 63 days at 41° F. and oxygen contents of water for each treatment.*

Pregermination treatment	Germination value[1]	Oxygen content[2]
		Mg./ℓ.
Stratification in polyethylene bags	41.2 a	....
Continuous water circulation	38.8 ab	9.1
Continuous aeration	37.0 ab	11.5
Continuous oxygenation	33.7 bc	41.6
Twice-weekly water changes	29.7 c	5.7
Intermittent aeration	28.6 c	4.1
Intermittent oxygenation	18.8 d	4.5
Unaerated water soak	23.7	3.7

[1] Values followed by the same letter do not differ significantly at the 0.05 level. The data for unaerated water soaks were not included in the analyses.

[2] Averages from periodic measurements. Contents for intermittent treatments were measured just prior to water resaturation. The oxygen saturation level for water at 41° F. is 11.9 mg./ℓ.

is to keep the oxygen content of the water near the saturation level achievable with aeration, but bubbling pure oxygen through the water is not recommended. It is very expenisve, results in very high oxygen contents, and is highly damaging to some seed lots. Logically, high oxygen levels would seem advantageous, but Barton (*4*) found that oxygen supplied during the soaking process was deleterious to germination of *Phaseolus* seeds. This effect was attributed to increased water absorption beyond the amount needed for germination. Jones (*10*) later reported that higher-than-average moisture contents in lots of loblolly and slash pine seeds during stratification coincided with lower viability.

Intermittent treatments–twice weekly water changes and periodic bubbling of the soaks with air and oxygen–resulted in significantly lower germination values than stratification and continuous aeration and water circulation (table 1). The poorer results were probably caused by the failure to keep water in these intermittent soaks saturated with oxygen.

Of the most efficient soaking treatments, continuous aeration seems the most practical. Circulation alone is ineffective unless the recirculated water is splashed onto the surface of the soaking solution. It is this action which recharges the water with oxygen. Continuous oxygenation is expensive and seems less effective than aeration. Although water changes were fairly effective in one study, further evaluation indicates that this technique is inferior to continuous bubbling of air through the water.

Amounts of aeration needed were estimated for temperatures of 41°, 59°, and 77° F. Twelve ounces and 6 ounces of seed were placed in containers with 1 pint of water. Soaks of three lots of loblolly seeds were maintained for 28 days.

After the minimal amounts of airflow needed to keep oxygen content near saturation were determined with an oxygen analyzer, the rates of flow were monitored periodically with a flowmeter. Tests run at the end of the soaks showed no marked changes in total viability at any temperatures, but germination values of seeds soaked at 77° were significantly lower than for those soaked at cooler temperatures (table 2). This decrease seems to have resulted from both a slight reduction in viability and slower germination. Thus, the lower germination values may have been caused by the high temperature inducing secondary dormancy in afterripened seeds (*1,6,13*). Alternatively, viability may have been reduced through

Table 2. – *Germination values of loblolly pine seeds in aerated soaks at different temperatures, and minimal airflow necessary to maintain oxygen saturation*

Temperature (° F.)	Germination value[1]		Minimal airflow[2]	
	Initially	After treatment	For 3/4 lb.	For 150 lbs.
			Ml./min.	Cu. in./min.
41	20.5 a	37.0 a	8	96
59	21.2 a	34.7 a	14	168
77	22.1 a	27.0 b	27	324

[1] Within columns, means followed by the same letter do not differ significantly at the 0.05 level

[2] Flows for the 3/4-lb. lots were measured; those for the 150-lb. lots were estimated.

collection between the seedcoat and the megagametophyte of excess water from which the oxygen was depleted (*8*).

The minimal amounts of airflow required to supply the oxygen needs of 3/4-pound seed lots and the computed needs for 150-pound lots of loblolly seed are given in table 2. About 1/8 gallon of water was used with each 3/4-pound lot, and 25-30 gallons would be needed for 150 pounds. Although the minimum airflow necessary for a 150-pound lot of loblolly seed is computed, aeration can easily be provided at much higher levels and probably should be to maintain a margin of safety.

Adequate aeration can be assured by measuring the oxygen content of the water and adjusting airflow to achieve saturation. If this procedure is impractical, a general rule to follow is to provide enough aeration to result in vigorous bubble agitation over about three-fourths of the water surface. Air pumps are adequate; the high pressure developed by compressors is not needed.

DURATION

Dormant loblolly pine seeds require treatment for 2 to 3 months to obtain optimum speed of germination if the water temperature is low. Slash and shortleaf pine seeds, which are usually less dormant, need not be soaked longer than 30 days.

Six lots of loblolly pine seeds were given four pregermination treatments at 41° F. and seed germination was tested after 0, 21, 42, 63, 84, 105, 126, and 147 days. Germination percents in the mid-90's were retained in all treatments through 105 days. Beyond that time, they declined up to 10 percentage points when seeds were soaked in unaerated water or in water changed periodically. Germination values were consistently highest for stratified seeds and those soaked in continuously aerated water. At the end of 147 days they averaged 65.0 after stratification and 62.0 after continuous aeration (table 3). The water change and unaerated soaks had peak germination values of 34.3 and 31.9, which were attained after 63 and 42 days, respectively. None of the six seed lots were adversely affected by either aerated soaking or stratification.

Table 3. – *Germination values of loblolly pine seeds soaked or stratified at 41° F. for up to 147 days*

Length of treatment (days)	Stratification	Aerated soak	Water change	Unaerated soak
0	17.4	17.6	16.7	17.0
21	30.0	30.6	21.5	19.0
42	47.0	45.4	30.2	31.9
63	47.0	47.8	34.3	31.5
84	63.2	59.7	33.6	29.6
105	58.0	55.6	31.9	30.0
126	65.4	62.1	26.6	23.3
147	65.0	62.0	26.8	18.0
Average[1]	49.1 a	47.6 a	27.7 b	21.3 b

[1] Averages followed by the same letter do not differ significantly at the 0.05 level.

Six lots of slash and five of shortleaf were treated in the same way as the loblolly pine seeds in the study above. Since seeds of these species were not nearly as dormant as those of loblolly pine, responses to treatments were not as great. Stratification and aerated soaks were again superior to the other two treatments (table 4).

Table 4. – *Germination values of slash and shortleaf pine seeds subjected to various pregermination treatments at 41° F.*

Length of treatment (days)	Stratification	Aerated soak	Water change	Unaerated soak
		SLASH PINE		
0	26.8	25.7	26.2	25.7
21	34.4	38.2	34.9	33.2
42	34.6	28.3	8.1	18.5
63	39.8	41.0	24.7	25.2
Average[1]	33.9 a	33.3 a	23.5 b	25.6 b
		SHORTLEAF PINE		
0	14.7	15.8	17.3	14.8
21	21.2	19.2	12.3	13.0
42	27.0	19.7	9.9	7.5
63	29.1	20.4	7.8	5.0
Average[1]	23.0 a	18.8 a	11.8 b	10.1 b

[1] Species averages followed by the same letter do not differ significantly at the 0.05 level.

Slash pine seeds stratified and soaked in aerated water germinated 86 and 88 percent after 63 days, and germination values averaged 39.8 and 41.0 respectively. There was no marked improvement in either rate or amount of germination beyond 21 days of treatment. Changing water and unaerated soaking were significantly poorer in promoting and maintaining viability. Dissolved oxygen contents of the water in the aerated soaks were maintained at about 11 mg./1.(near saturation). Oxygen contents of the water change and unaerated treatments were lower and there were no differences–both averaged 1.5 mg./1.–when oxygen measurements were made just prior to the twice-weekly water changes.

The shortleaf seeds were 1 or 2 years old when the study began. The results obtained were essentially the same as with slash seeds (table 4). Germination averaged 74 and 79 percent and germination values averaged 20.4 and 29.1 for aerated water soaking and stratification, respectively, after 63 days. The water-change and unaerated soak treatments resulted in a steady decline in viability and germination values.

TEMPERATURE

Temperatures of 34° to 42° F. are recommended for aerated soaks if there is no need to shorten the period of treatment. Higher temperatures, 60° to 70°, will overcome dormancy faster, but soaks must not be prolonged beyond 2 or 3 weeks or germination will start in the water.

The effects of 41°, 59°, 77° F., and ambient outdoor temperatures on seeds in aerated soaks and stratification were evaluated on three lots of loblolly pine seeds. Ambient air and water temperatures averaged 65° and 61°, respectively, over the duration of the study. Germination was tested initially and after 7, 14, 21, and 28 days of treatment.

The results again confirmed earlier ones in that aerated soaks promoted germination as well as stratification. Viability of all seeds, except those at 77° F., remained high throughout the study. Seeds either stratified or aerated at 41°, 59°, and ambient temperatures germinated 90 percent or more and there were no differences between pregermination methods. Germination was markedly lower at 77°, and at this temperature, stratification was superior. Only at 77° were differences due to length of treatment of sufficient magnitude to be important; viability decreased with each increase in length of treatment beyond 7 days. Germination values followed the same trend (fig. 1).

The loss of germinability of seeds soaked at high temperatures and after extended periods cannot be attributed to the temperatures alone. Measurements of oxygen contents in the soaks revealed that aeration was not being provided at rates sufficient to maintain oxygen saturation. Although oxygen contents of the 41° and 59° F. soaks remained near saturation, aeration at 77° was supplying only about half of the saturation level. This deficiency is probably the primary reason for the rapid deterioration at this temperature.

Seeds stratified in polyethylene bags began to mold heavily after 21 days at 59°, 77° F., and ambient temperatures. Even if other problems were overcome, long periods of treatment at warm temperatures would not be practical because germination begins to occur in both stratification and water soaks after about 21 days of treatment.

In soaks, warm temperatures for short periods may be as effective as low ones for long periods. This trend has been noted before (*3, 12, 13*), but until now the danger of heating and molding was too great to use warm temperatures for stratification.

The effect of temperature on rate of oxygen use by seeds was determined for two replications of loblolly seeds placed in oxygen-saturated water at 41°, 59°, and 77° F. Oxygen depletion was measured, the water resaturated, and the cycle repeated. As expected, the water held more dissolved oxygen at cold than at warm temperatures and seeds depleted oxygen faster at warm temperatures. After the seeds were fully imbibed, they depleted available oxygen to an equilibrium level of about 0.9 mg./l. in 4 to 8 hours. The average difference between the high and low temperature in time needed for this depletion was about 3 hours.

The equilibrium level after oxygen depletion is too low to maintain seeds for long periods. It is clear that recharging every few days is insufficient to maintain dissolved oxygen at necessary levels.

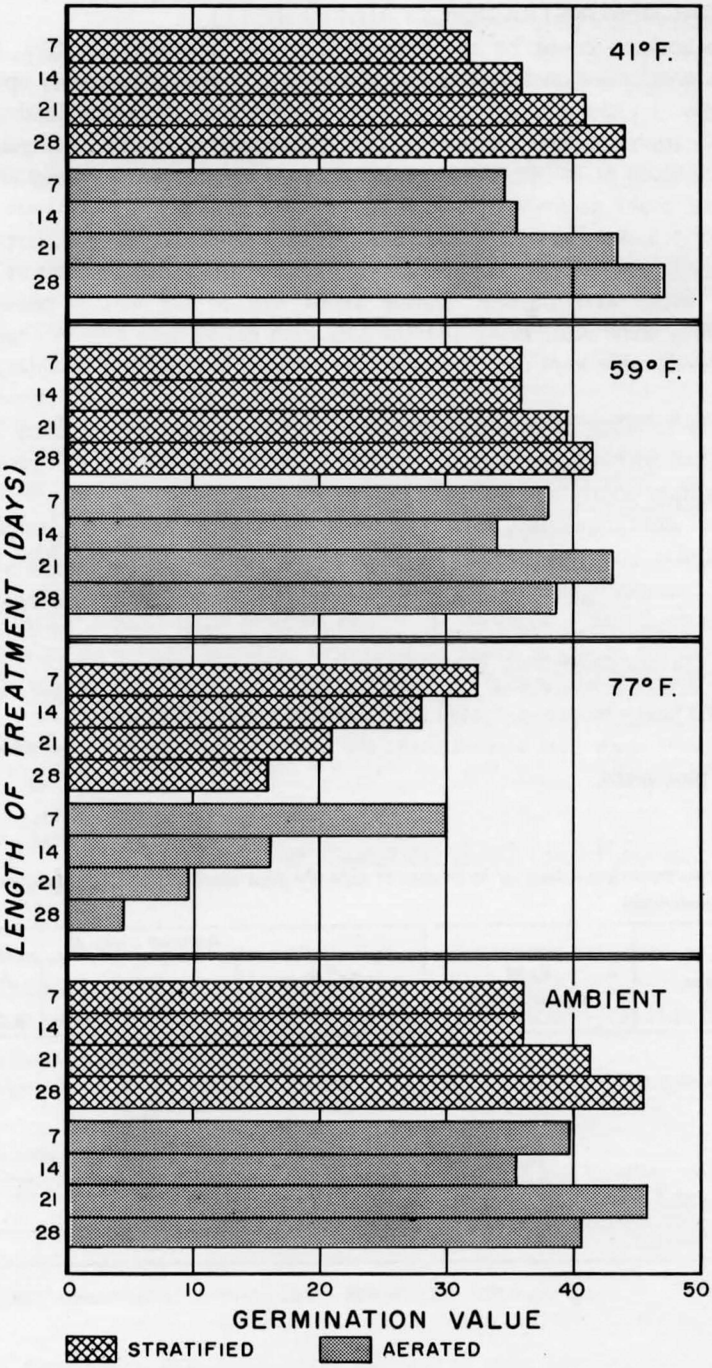

Figure 1. – *Germination values of loblolly pine seeds after several lengths of pregermination treatment at various temperatures. The average germination value of untreated seeds was 24.6.*

PRACTICAL DEMONSTRATION

Before soaking could be recommended, it was necessary to show that the treatment is effective on seed lots near the size for commercial nursery operations. Accordingly, a 150-pound lot of 1-year-old loblolly pine seeds was divided to test three replications of four treatments: (1) cold stratification in polyethylene bags; (2) aerated soaks at 34° F., (3) aerated soaks at 34° with seeds in burlap sacks; and (4) aerated soaks at ambient temperatures. Five pounds of seeds were used in treatments 1 and 4, and 25 and 15 pounds in treatments 2 and 3, respectively. Average air and water temperatures of the ambient soaks were 55° and 51° for the time the soaks were outside. During about half of the 42-day period, night temperatures were subfreezing and the lots were moved into cold storage at 34°. Oxygen levels were kept near saturation by measuring them and adjusting airflow rates.

Viability of seeds of all treatments averaged 90 percent or more after 42 days. Seeds soaked within burlap bags germinated least, but the difference in germination of 4 percentage points is of little practical importance.

The ambient-temperature soak resulted in the highest germination values, while seeds soaked in burlap sacks again responded least (table 5). An interaction between length of treatments and pregermination methods indicates that germination values of treatments 2 and 4, soaks at 34° F. and ambient temperatures, increased more rapidly than the others as length of treatment increased. Burlap sacks, which were tested as a means of facilitating handling, resulted in slower response, probably because the heavy burlap restricted circulation of well-aerated water.

The results show that aerated soaks are practical for promoting germination of southern pine seeds.

Table 5. – *Germination values of large lots of loblolly pine seeds after various pregermination treatments*

Length of treatment (days)	Cold stratification	Aerated soaks at		
		34° F. Loose seeds	34° F. In bags	Ambient temps. Loose seeds
0	14.6	13.0	12.6	11.9
7	18.5	20.3	20.0	18.8
14	22.9	23.7	21.6	25.6
21	26.0	30.7	21.5	34.6
28	26.5	30.1	26.8	39.0
35	23.9	26.4	22.4	29.2
42	29.5	33.9	28.4	37.5
Average	23.1	25.4	21.9	28.1

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