Effect of Seed Condition, Stratification, and Germination Temperature on the Laboratory Germination of Loblolly Pine Seed

Earl W. Belcher

Director (retired), USDA Forest Service, National Tree Seed Laboratory
Dry Branch, Georgia

Stratified and nonstratified loblolly pine (Pinus taeda *L.*) *seed of dormant, nondormant, and weak lots were germinated at 6 temperatures for 28 days. Germination percentages and germination values were evaluated. Constant 22 or 25 ºC is best for laboratory germination. An alternating 20-30 ºC and 30-20 ºC sometimes acted as a mean temperature, a low temperature and a heat sum equivalent depending on the seed condition.* Tree Planters' Notes 46(4):139142; 1995.

Several experiments have been conducted to identify the optimum temperature for germinating loblolly pine *(Pinus taeda* L.) seed. Belcher and Jones (1966) reported that a constant 22 /C and an alternating 20-30 ºC provided equal germination on unstratified seed with light, but that 22 ºC provided faster germination. Dunlap and Barnett (1982) found that 13-22 ºC provided even faster germination. McLemore (1966) found optimum germination of loblolly seed in the dark to occur between 17.5 and 27.5 ºC. Barnett (1979) found a peak between 19 and 24 ºC with stratified loblolly seed in container culture. Bonner (1984) found 30-20 ºC to provide the fastest germination on a thermogradient table. His data also suggested that 22 ºC may be too low for optimum laboratory germination of loblolly pine. Dunlap and Barnett (1983) achieved 50% faster germination of loblolly with 35-22 ºC and reported seed source to effect speed of germination.

None of the published data considered the effect of seed condition. Loblolly pine appears in 3 conditions: (1) dormant seed, which will provide greater germination following stratification; (2) nondormant seed, which will provide the same germination either with or without stratification; and (3) weak seed, which has lower germination after stratification. The nondormant lots can also differ in germination rate, so careful selection is required for investigations. This study evaluated germination percent and germination value over a range of temperatures for both stratified and unstratified seed of each of the 3 condition classes.

Materials and Methods

Four lots of each of the three seed condition classes were selected from test samples received at the USDA Forest Service's National Tree Seed Laboratory in 1984. All nondormant lots evaluated had the same rate of germination to minimize confounding. Twelve 100-seed sublots from each of the 12 seed lots were planted on crepe cellulose paper in clear plastic boxes. Six sublots from each seed lot were stratified for 5 weeks at 3 to 5 ºC. One stratified and one unstratified sublot from each lot were germinated at each of 6 temperatures: 20, 22, 25, 30, 20-30, and 30-20 ºC.

Temperatures were controlled within ± 1 ºC. Eight hours of light from cool white flourescent lamps was provided to all tests during one 24-hour period. The 20-30 ºC cycle was 16 hours at 20 ºC and 8 hours at 30 ºC with light. The 30-20 ºC was 16 hours at 30 ºC and 8 hours at 20 ºC with light.

Germination was counted each Monday, Wednesday, and Friday. A seed was considered germinated when the hypocotyl was at least 1 cm long. This allowed for the detection of some abnormalities. Seedlings from one lot were saved on the ninth-day count for evaluation of temperature effects on seedling development. The root length, hypocotyl length, hypocotyl diameter, and hypocotyl color were evaluated. Germination was closed at 28 days.

Germination percentages were adjusted for empty seed and transformed to arc sine for analysis. Germination percent at 28 days and germination value (Czabator 1962) were analyzed by a split-plot ANOV Treatment means were evaluated with the Duncan's multiple range test at the 1% level.

Results and Discussion

Approximately 40% of the variation could not be accounted for in this study. Variation between seed lots (replication) was not significant and accounted for only 4% of the variation. Seed condition and the interaction of seed condition with stratification accounted for 18 and 10%, respectively, in both germination and germination value. Temperature and stratification effects accounted respectively for 12 and 19% of the variation in germination percent whereas for germination value they accounted for none and 29% of the variation.

Temperature had no significant effect on root length (table 1) or hypocotyl diameter. Hypocotyl color darkened as the prevailing temperature decreased. Seed germinated at 20 ºC produced seedlings that developed slowly and, therefore, were reduced in size. At 20-30 ºC, seedlings elongated rapidly and were significantly taller than at all other temperatures.

Week seed produced more than half of all abnormal germinants (table 2). The number of abnormal seedlings was reduced 29% by stratification. The number of abnormals was not significantly different among replications.

The seed lots fell into the three seed conditions that the service test data indicated that they should (figure 1). Stratification improved the total germination for dormant lots, had no significant effect with the nondormant lots, and decreased the total germination on the weak lots. Therefore, the assumption that the study was conducted on seed in 3 physiological conditions is probably correct.

Germination at 22 ºC was significantly higher than at 30, 20-30, and 30-20 ºC, and equal to that at 20 and 25 ºC (figure 2). Dormant lots germinated best at the lower

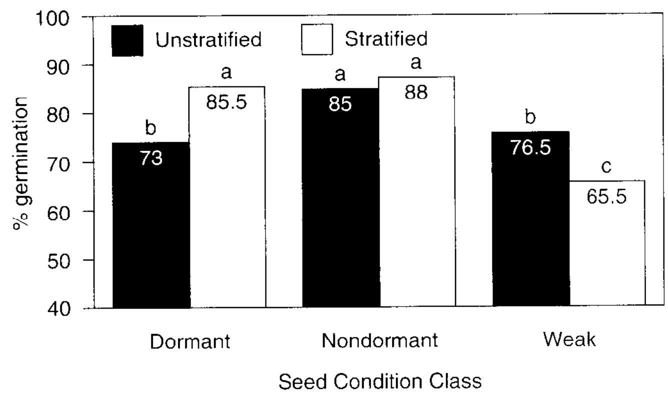

Figure 1—*Mean germination of unstratified and stratified seed of each seed condition class. Bars with different letters are significantly different at the 1% level of probablility.*

Table 1-*Summary of data on seedlings sampled at ninth day from each temperature with a single seed lot*

Temperature (/C)	Top length (cm)	Root length (cm)	Hypocotyl Thickness (cm)	Hypocotyl Color
20	1.6 ± 0.2	1.8 ± 0.5	0.12	Red black
22	3.6 ± 0.3	2.2 ± 0.3	0.12	Med. red
25	3.0 ± 0.5	1.8 t 0.5	0.12	Med. red
30	2.6 ± 0.2	1.5 ± 0.9	0.12	Pale red
20-30	4.4 ± 0.2	1.6 ± 0.4	0.12	Dark red
30-20	3.7 ± 0.4	1.4 ± 0.1	0.12	Dark red

Values are mean ± 1 standard deviation.

Table 2-*Summary of abnormal seedling counts for the 3 seed conditions at each germination temperature*

	No. of abnormal seedlings												
	20 /C		22 /C		25 /C		30 /C		20-30 /C		30-20 /C		
Seed condition	N	S	N	S	N	S	N	S	N	S	N	S	Sum
Dormant	4	1	6	1	0	4	2	3	0	3	1	3	28 b
Nondormant	2	1	4	1	4	1	1	0	0	3	0	2	19 c
Weak	1	2	10	3	6	2	2	3	7	3	12	8	59 a
Totals	7	4	20	5	10	7	5	6	7	9	13	13	
Sum for each temp.	11 c		25 a		17 b		11 c		16 b		26 a		

Values for sums in rows or columns with different letters are significant at the 1 % level of probability.

Table 3- *Summary of germination and difference between paired tests for the 3 seed conditions at each germination temperature*

	Germination percent											
	20 /C		22 /C		25 /C		30 /		20-30 /C		30-20 /C	
Seed condition	N	S	N	S	N	S	N	S	N	S	N	S
Dormant	85	97	87	94	85	92	47	76	7 8	76	52	68
Nondormant	93	92	94	93	92	90	63	81	91	86	71	85
Weak	74	71	88	69	81	58	60	51	79	59	69	68

	Difference between paired tests					
	20 /C	22 /C	25 /C	30 /	20-30 /C	30-20 /C
Dormant	+12	+7	+7	+29	-2	+16
Nondormant	-1	-1	-2	+18	-5	+14
Weak	-3	-19	-23	-9	-20	-1

temperatures of 20, 22, and 25 °C (table 3). Stratification promoted germination of dormant lots at all temperatures except 20-30 °C. Stratification did not promote the germination of nondormant lots at 20, 22, 25 and 20-30 °C but did promote germination at 30-20 and 30 °C. Weak lots gave substantially lower germination with stratification at all temperatures except 20 and 30-20°C. Results at these last two temperatures were similar to the nondormant seed class. The depression of germination with weak lots increased with increasing constant temperature from 20 to 25 °C, and 20-30 °C provided results similar to the mean equivalent of 25 °C.

Loblolly pine is adapted to the moderate temperatures of early spring in the southern United States and, therefore, might be expected to germinate better at moderate temperatures. With increasing constant temperatures, physiological processes are increased until at some point weak seeds are unable to synchronize their biochemical processes correctly and die instead of germinating (Gulliver and Heydecker 1973). This is a likely explanation for the decreasing germination of weak seed lots at higher constant temperatures that was observed in this study.

The germination temperature used can also change the seed condition class assigned to the seed lot. The weak seed lots perform in this study as nondormant when germinated at 20 and 30-20 °C, the nondormant seed perform as very dormant when germinated at 30 or 30-20 °C, and the dormant seed as nondormant at 20-30°C.

Stratification increased the rate of germination for dormant and nondormant lots while weak lots showed no benefit from stratification and germinated as slowly as the unstratified dormant lots (figure 3). Significantly greater germination values were obtained at 22 and 25 °C than at 30 or 30-20 °C, but not at 20-30 °C nor 20 °C (figure 4).

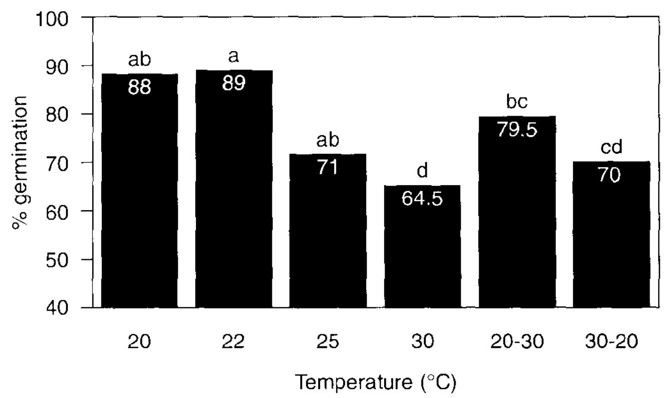

Figure 2—*Mean germination for each germination temperature. Bars with different letters are significantly different at the 1% level of probability.*

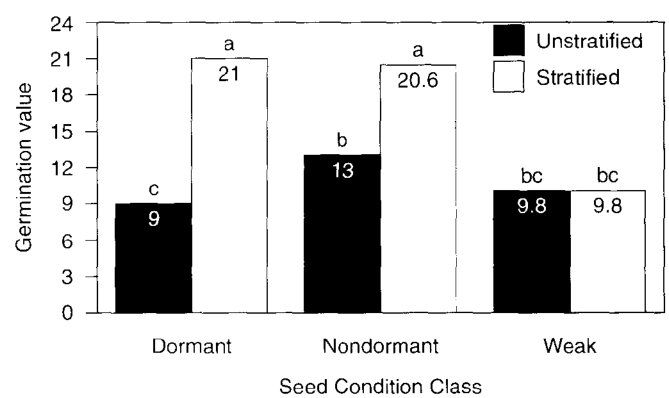

Figure 3— *Mean germination value of unstratified and stratified seed of each seed condition class. Bars with different letters are significantly different at the 1% level of probability.*

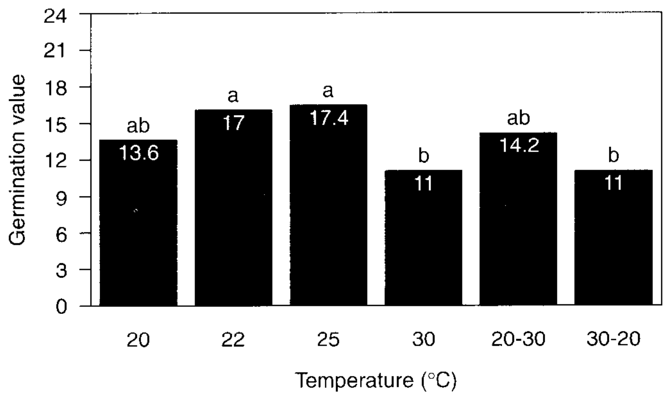

Figure 4—*Mean germination value for each temperature used in the study. Bars with different letters are significantly different at the 1% level of probability.*

Germination values increased with stratification of nondormant lots and the effect increased with increasing constant temperature until 25 °C. When the seed was not stratified, germination value was significantly lower at 30 and 30-20 °C than at 22, 25, and 20-30 °C .

The results of the weak seed were less uniform than the results of the other seed condition classes. At 20, 30, and 30-20 °C the stratified results exceeded the nonstratified. The highest germination values were obtained with nonstratified seed at 22 °C and stratified seed at 30-20 °C. The alternating 20-30 °C results were those expected of its constant heat sum counterpart of 23 °C, but the 30-20 °C did not respond as would be predicted for a constant 27 °C.

Temperature had less effect on determining seed condition class by germination value than it did by germination percent (table 4). The dormant seed lots showed a positive increase at all temperatures when stratified. The increase was less pronounced at the alternating temperatures. Therefore, the classification of a seed lot as dormant was affected by the germination temperature. The nondormant seed lots gave a large positive increase from stratification at 30 and at 30-20 °C, which would classify the lots as dormant. Although the weak seed gave positive and negative responses to stratification, the germination values are all low which is typical of weak seed.

These results do not support Bonner's findings (1984) that loblolly pine seed germinates twice as fast at 30-20 °C as they do at 20 °C, nor that 25 and 30 °C provide the same rate of germination (figure 4). These findings do support the earlier findings of Belcher and Jones (1966) that 22 °C is a reasonable germination temperature for loblolly pine. This study was originally designed with a dark treatment, but that was eliminated from the analysis because all the results in the dark were so much less than in the light. Thus, light is recommended for laboratory germination of loblolly pine seed.

Container nursery managers have sometimes the option to closely control germination temperatures and could use these data to manipulate seed lots. For example, germination of weak seed lots could be maximized by keeping temperatures low, or dormant lots could in a emergency be more successfully germinated without stratification at the alternating temperature of 20-30 °C.

Conclusions

The optimum temperature may be taken to be that temperature at which the highest percentage of germination is attained in the shortest time. The data presented suggest the following optimum temperatures:

Table 4— *Summary of germination value and the difference between paired tests for the 3 seed conditions at each germination temperature*

	Germination percent											
	20 °C		22 °C		25 °C		30 °		20–30 °C		30–20 °C	
Seed condition	N	S	N	S	N	S	N	S	N	S	N	S
Dormant	11.27	24.31	11.23	27.43	13.43	27.08	4.28	17.72	11.75	15.08	4.22	10.22
Nondormant	12.06	17.41	16.45	22.07	16.53	24.65	8.59	21.04	16.73	21.04	9.61	19.98
Weak	7.42	8.81	13.86	10.50	11.35	9.43	6.84	7.75	11.40	9.25	8.32	13.47
	Difference between paired tests											
Dormant	+13.04		+16.20		+13.65		+13.44		-3.33		+6.00	
Nondormant	+5.35		+5.62		+8.12		+12.45		+4.31		+10.37	
Weak	+1.39		-3.36		-1.92		+0.91		-2.15		5.15	

Summary of germination value and the difference between paired tests for the 3 seed conditions at each germination temperature.

Dormant lots— 22 °C or 25 °C
Nondormant lots— 22 °C, 25 °C, or 20-30 °C
Weak lots— 22 °C, 25 °C, or 20-30 °C

Because the condition of a seed lot is not known until after the test is completed, the poorer results of very dormant seed at 20-30 °C suggest that loblolly seed should be germinated at 22 °C or 25 °C in laboratory tests.

Address correspondence to: Earl W. Belcher, PO Box 282, Cave City AR 72521.

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