The Effect of Stratification Temperature on the Germination of Two Southern Pines

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For many years most of the pine seed used in the United States has been processed by public forest nurseries. These nursery operators have adequate storage facilities, seed testing equipment and experience to successfully process large volumes of seed for the production of trees for transplant purposes. The problems associated with nursery operation are unique. Large numbers of seed are used on relatively few acres. The trees are raised under intensive-care management. Irrigation is an integral part of nursery management and provides certain guaranteed levels of performance of the planted seed.

Most lots of loblolly pine *(Pinus taeda* L.) *and shortleaf pine (Pinus echinata* Mill.) require some form of pre-germination treatment for breaking natural dormancy and for uniform control of emergence. It has been standard practice to write broad stratification recommendations for seed such as those presented by Wakeley (1954) which suggests periods of from 30 to 60 days at temperatures of between 35° F. and 40° F. for most lots. In most instances operators have worked out schedules that seem optimum for local situations and apply those pre-germination treatments which have proved successful through experience.

During the early 1950's, direct seeding as a means of artificial regeneration was thought to have too many problems for dependable use. High volumes of seed were required to insure success, and loses due to insect, rodent and bird depredations were excessive. After much research,

[1]The following cooperators supplied seed for the study: Herron Lumber Company, Idabel, Oklahoma; The International Paper Company, Camden, Arkansas; Forest Products Division of Olin Mathieson Chemical Corp., Monroe Louisiana; The Oklahoma Division of Forestry, Oklahoma City, Oklahoma.

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by the middle 1960's chemical treatments for seed protection had been developed and many operators were using direct seeding methods as standard procedures. For the most part, users of the direct seeding methods were advised to follow standard nursery practices for pre-germination treatments on dormancy. Most operators continued to use moist-cold stratification at temperatures between 35° F. and 40° F. for periods of 30 to 60 days (Mann and Derr, 1961).

In order for direct seeding to be successful Mann and Derr (1961) state that it is essential for germination to be as rapid as possible, even if the pre-germination treatment results in a reduced total germination. This conviction is predicated upon the idea that bird, rodent and insect losses will be reduced as seed exposure to these factors is reduced.

Rapid germination is dependent upon having seed ready to germinate when delivered to the site. This introduces an administrative management problem which requires flexible alternatives. Most large-scale aerial seeding projects are handled by contract flying agencies. Such operators attempt to set up work schedules several months in advance and, of course, must service a wide variety of users in order to justify the capital investment necessary for adequate performance.

The period of time for optimum direct seeding is rather short in any one area and "hot seed" requires immediate attention. Seeding schedules therefore must be adhered to reasonably well. A user may project a seeding date of March 15 and place large volumes of seed in pre-germination treatment one to two months prior to that date. If, due to weather conditions or unforeseen problems, the contract operator finds himself either behind or ahead of schedule, the user is placed in a predicament. Seed that has been stratified for long periods of time may very well germinate in treatment, if seeding is delayed. On the other hand, if the treatment schedule is shortened the desired field results may not be obtained.

It therefore seems desirable that pre-germination methods be worked out which provide the direct seeder some flexibility in dates for sowing and still insure the rapid, uniform germination required for successful tree establishment.

Research Review

Basic recommendations regarding stratification of the southern pines were made by Wakeley (1954). Essentially they were to stratify Southern pine seed between 35° F. and 40° F. for 30 to 60 days. Swafford and Jones (1960) found that in shortleaf pine 47-day stratification gave either the highest or second highest total germination for all lots tested, but they found that a 90-day period aided the speed of germination more than the shorter periods. Essentially the same results were obtained for loblolly pine. It is presumed that stratification temperatures were held constant in these studies since the objective was to evaluate stratification mediums.

One conclusion that can be drawn from the above cited work is that between lots of seed there is considerable variation in the effect of pre-germinative treatments and either speed of germination or total germination can be improved by some form of treatment to almost all lots of seed.

Workers at the Eastern Tree Seed Laboratory (1964) found that loblolly pine seed responded to a small degree when soaked in hydrogen peroxide in the presence of light. The real prospects for this insight may be in the reduced period necessary for treatment or in improving germination in slow-to-moderate lots. At the present time, however, it seems unwise to write sets of general recommendations using this technique for commercial operations.

Wenger and Trousdell (1958) in their treatise on loblolly pine in the South Atlantic Coastal Plain make the following generalizations:

> **"Germination begins in the spring when temperature becomes favorable, usually at about the same time that established trees begin vegetative growth. Temperatures below 40° F. preclude germination, and the best range is 65° F. to 80° F."**

Perhaps the implication here is that seed must attain a balanced temperature with the soil before germination takes place. Allen (1962) verified the importance of incubation temperatures on speed and completeness of germination. While Allen's work dealt with Douglas fir, the evidence (insofar as the variables under consideration are concerned) seems to focus upon the incubation temperature as being of prime importance. Allen (1962) also stated that: "the longer the period of stratification the faster and more complete the germination at all temperatures". This seems to verify the validity of making general recommendations for all seed lots.

Afanasiev (1944) attempted several pre-germination treatments on redbud *(Cercis canadensis* L.) and considered two different stratification temperatures after various treatments with H_2SO_4. His results indicate the presence of variation in germination speed as a result of these differences, but they were only slight.

Graber (1965), in studying white pine, found that stratification temperature significantly affected only the rate of germination. He stratified seed at three temperatures, 36° F., 40° F. and 50° F. He found that germination increased with each temperature increase, but that premature germination occurred at 50° F. when stratification time was sixty (60) days or longer.

McLemore (1966) undertook a study similar to the one reported here with much broader objectives. He studied the temperature effects on dormancy and germination of loblolly pine using a dark environment throughout the project. He found differences in germination due to stratification temperatures, length of stratification, and germination temperature as well as differences due to the interactions of all combinations of these. McLemore indicated that the most notable differences were due to length of stratification. Germination values and percent germination both increased with lengthened stratification temperatures. He speculated that because his studies were performed in darkness the increases due to temperature were amplified and in the presence of light the results in favor of temperature would have been less. In conclusion McLemore stated that:

> **"Although stratification for 45 to 60 days may be sufficient to obtain fast germination of loblolly seed in light, this study demonstrated that 112 days at 10° C. is necessary to completely after-ripen seed in the absence of light. A somewhat longer period appears necessary at 5° C. The advent of subsurface sowing in direct-seeding operations may require that seeds be stratified for longer periods than were customary".**

Seidel (1965) found in shortleaf pine that stratification for 60 days results in faster germination but that total germination is retarded when compared to 20 days stratification. These results were similar to those reported by McLemore and Czabator (1961) with loblolly pine. Seidel further found that 60° F. storage after 60 days stratification accelerated germination rate over 38° F. storage but depressed total germination. This may well indicate a point where management must make a decision regarding goals of seeding. Trade-offs in benefits from treatments must be decided upon.

There are many reports which indicate the effect of varying degrees of light, oxygen fumigation, exposure to radiation, etc., on various tree seeds, but there does not seem to have been any other attempt to improve speed or total germination through regulation of the pre-germination temperatures.

Czabator (1962) describes an improved index for evaluation of seed performance which combines completeness of germination and speed of germination. The index is called the "germination value". It gives more weight to the speed of germination than to completeness and for direct seeding purposes this is desirable. Seidel (1963), McLemore (1966) and others have made regular use of the germination value as an index to evaluate differences in seed performance.

The germination value is found by using the following relationship:

G.V. = P.V. × MDG

where G.V. = germination value

P.V. = peak value is the percentage of filled seed that germinates to the day of peak seeding emergence ÷ the number of days required to reach the peak rate.

MDG = mean daily germination.

As can be observed, the mean daily germination gives an expression of total germination while the peak value is an index of the most vigorous component of the seed (Czabator 1962).

It has been concluded by these authors that there are many factors which affect the germination of southern pine seed and there are equally as many ways to evaluate seed performance. Germination percent, peak value, germination value, and others have all received some use. It is our contention that a seed tester is free to use any evaluation he chooses, provided he matches it with the objective of the study. If, for example, one wanted to evaluate seed solely for direct seeding purposes, it would perhaps be wise to use the peak value. However, if the interest is in total response to treatment, germination percentage might be a better test.

Much has been written about stratification mediums. Moist sand and moist peat moss have been the traditional favorites of commercial nurseries. In the last few years, however, stratification in polyethylene bags has gained much favor. Hosner, Dickson and Kahler (1959) reported that total germination was not significantly different with stratification in polyethylene bags than that from wet sand stratification. Lehto (1960) reported results of a single test using polyethylene bags for stratification and cites several advantages insofar as seed handling is concerned. It has been the author's experience[3] in stratifying large lots of seed for industrial direct seeding projects that the polyethylene bag procedure produces excellent results.

General Remarks

Once seed scheduled for direct seeding operations has been placed in stratification, the sequence of events is pretty well fixed until the seed is delivered to the site. Seed left in stratification too long will germinate and those seeds with cracked seed coats or extended hypocotyls will not withstand the handling necessary to deliver them to the planting site.

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Seed treated for less time than the scheduled treatment will not attain their fullest germination potential. This, of course, then produces less than optimum results in terms of established trees.

A second factor related to the problem deals with amounts of seed required. Many workers feel that when large supplies of genetically improved seed are available, it will not be possible to afford the high volumes of seed traditionally used in aerial direct seeding trials. On the other hand, if pre-germination treatments could be developed which would allow better performance of fewer seed, it is possible that direct seeding could continue to be used as a large-scale application technique. It is with this background that the project herein described was initiated.

Objectives of the Study

1. To determine whether the speed of germination for loblolly and shortleaf pine can be affected through regulation of stratification temperatures and, if they can,

2. To develop the most appropriate treatment schedules for the species.

Procedures

In the fall of 1963 loblolly pine seed was collected from four geographic sources. One collection was made in southeast Oklahoma, one in northeast Texas, and two from north central Louisiana. The chief difference in the Louisiana collections was that one lot of seed was properly handled in process and the other was not. Each was considered in the study as a separate seed source.

The year 1963 was a very poor seed year for shortleaf pine. The suppliers were unable to obtain any fresh seed. A sample of seed which was collected in 1956 and maintained in storage was obtained from the Oklahoma Division of Forestry.

Moisture content, whole seed counts and untreated germination were determined for each of the five seed sources. Table 1 shows the basic data collected for each of these lots.

Fifteen treatment combinations were selected. By examination of Table 2, it can be seen that the group of treatments 1 through 6 cover the range of general recommendations for stratification of southern pine seed. The group of treatments 7 through 10 were designed to determine the effect of changing temperatures over a basic stratification period of 36 days. It was thought that for optimum results all seed might best be stratified for some basic period. Treatments 11 through 15 were selected

Table 1. Seed Quality Before Treatment

Source	% Moisture Content	% Whole Seeds[1]	Untreated Germination %[2]
A 1956 Oklahoma shortleaf	--	98.25	59
B 1963 Texas loblolly	8.2	87.95	2
C 1963 Oklahoma loblolly	6.3	98.00	2
D 1963 Louisiana loblolly (poor)	7.8	98.60	5
E 1963 Louisiana loblolly (good)	8.5	98.55	31

[1]Averages of 20 random samples of 100 seeds each.
[2]Percent seed germinated after 60 days in germination environment with no pre-germination treatment.

Table 2. Treatments

Treatment Number	
1	Temperature 38° F. for 38 days
2	Temperature 38° F. for 45 days
3	Temperature 38° F. for 52 days
4	Temperature 38° F. for 59 days
5	Temperature 38° F. for 66 days
6	Temperature 38° F. for 36 days
7	Temperature 38° F. for 36 days + 7 days at 45° F.
8	Temperature 38° F. for 36 days + 7 days at 45° F. + 7 days at 52° F.
9	Temperature 38° F. for 36 days + 7 days at 45° F. + 7 days at 52° F. + 7 days at 59° F.
10	Temperature 38° F. for 36 days + 7 days at 45° F. + 7 days at 52° F. + 7 days at 59° F. + 7 days at 66° F.
11	Temperature 38° F. for 7 days
12	Temperature 38° F. for 7 days + 7 days at 45° F.
13	Temperature 38° F. for 7 days + 7 days at 45° F. + 7 days at 52° F.
14	Temperature 38° F. for 7 days + 7 days at 45° F. + 7 days at 52° F. + 7 days at 59° F.
15	Temperature 38° F. for 7 days + 7 days at 45° F. + 7 days at 52° F. + 7 days at 59° F. + 7 days at 66° F.

to test the effect of temperature change without reference to a basic period.

A random sample of seed was selected from each seed lot for each of the 15 treatments and for each of the four replications. Replications were carried out at one-year intervals.

Seed were soaked in tap water for 24 hours in polyethylene bags and drained. The bags were closed at the top, providing ample air supply, and placed in a regular residential refrigerator which was equipped with a thermostatic control that maintained the desired temperature ± 2° F. There was, of course, a temperature adjustment when the door was opened, but this was not considered to be a limiting factor.

The treatments were so designed and scheduled that stratification was in effect going on under similar conditions for several treatments at once. As the particular treatment time and temperature were reached, the seeds were removed and placed in germination tests. One hundred seeds were selected at random from each treatment and placed in flats filled with a combination of sterile vermiculite and sand. Seeds were watered every day and germination counts were made. Germinated seeds were removed once the seed was lifted from the soil medium. Germination status was checked for 60 days past sowing. It was judged that any germination taking place past this point was not useful for direct seeding trials.

Peak values, germination percent, and germination values were determined for all replications of all lots of seed and all treatments and were subjected to analysis in an attempt to select optimum treatments.

Discussion and Results

Table 3 lists the means of each of fifteen treatments for all five seed sources. Means were calculated for germination percent, peak value and germinative capacity. As would be expected after judging trends from these lists, the pooled analysis of variance showed highly significant treatment differences.

Table 3A Mean[1] Germination Percent for Each Treatment in Seed Source

Treatment Number	A	B	C	D	E
1	89.5	65.1	91.0	83.9	70.2
2	58.5	56.0	96.4	80.4	84.4
3	73.2	55.8	76.7	74.5	88.7
4	68.7	55.6	90.3	76.8	78.6
5	80.1	58.5	75.2	77.3	77.1
6	79.1	65.4	58.1	69.0	59.6
7	79.6	49.7	68.4	76.0	74.8
8	90.6	50.8	82.6	87.5	78.1
9	85.5	48.9	87.2	91.5	83.2
10	80.1	46.0	81.1	93.6	81.9
11	61.3	23.8	37.0	34.2	30.2
12	66.9	23.0	46.9	53.0	48.9
13	60.8	36.1	70.6	77.8	68.7
14	68.2	35.0	67.8	84.4	82.9
15	65.6	43.2	74.5	82.6	73.8

[1]Mean of four replications

Table 3B Mean[1] Peak Value for Each Treatment in Seed Source

Treatment Number	A	B	C	D	E
1	5.45	2.24	3.88	3.30	3.06
2	3.31	2.38	4.96	3.52	4.15
3	4.97	2.08	3.10	2.93	4.14
4	4.54	1.78	3.47	3.07	3.74
5	7.16	1.58	2.95	3.27	3.80
6	5.71	1.24	2.31	2.42	2.33
7	5.77	1.61	2.62	3.14	3.34
8	6.43	1.78	4.21	4.94	4.81
9	7.34	2.42	4.51	4.78	4.78
10	14.77	2.61	5.30	5.31	4.75
11	4.64	.55	1.11	1.30	1.46
12	4.30	.63	1.37	4.37	4.73
13	4.50	1.13	2.93	8.83	6.73
14	4.92	1.34	2.88	3.61	3.67
15	11.23	1.55	3.53	7.52	5.74
Value of F for treatment & treatment X replication Tests of Significance[2]	1.78	4.35	3.94	.78	.61

[1]Mean of four replications
[2]F values greater than 2.80 indicate significant differences @ the 99% level with 14 and 28 degrees of freedom.

Table 3C Mean[1] Germination Values for Each Treatment in Seed Source

Treatment Number	A	B	C	D	E
1	8.04	2.18	5.82	4.61	4.20
2	3.69	1.95	7.82	5.24	6.26
3	6.02	1.69	3.92	3.60	6.10
4	5.12	1.23	5.26	3.80	5.34
5	9.37	1.06	4.02	3.95	4.74
6	7.65	1.03	2.88	2.83	2.31
7	7.52	1.54	2.87	4.08	4.33
8	9.54	1.51	5.76	7.31	6.60
9	10.24	1.84	6.53	7.15	6.98
10	20.94	1.85	7.34	8.20	6.80
11	5.33	.32	.93	1.03	1.07
12	5.08	.34	1.08	4.56	3.78
13	4.63	.79	3.37	13.32	8.54
14	5.77	1.03	3.30	5.02	5.45
15	14.98	.98	4.33	9.49	6.96
Value of F for treatment & treatment X replication tests for significance[2]	1.60	3.07	3.17	.78	.82

[1]Mean of four replications
[2]F values greater than 2.80 indicate significant differences @ the 99% level with 14 and 28 degrees of freedom.

In addition, the analysis showed significant differences in replications, sources, years and in their interactions. It was anticipated that there would be a source difference since the A source was shortleaf and the others were loblolly. It was further anticipated that like species would perform differently due to the inherent differences in the seed, together with the environmental differences for the specific locations during seed development.

The analysis of variance by seed source on germination value revealed inconsistent treatment performances. Significant differences were indicated for seed sources B and C. These sources had the lowest base germination of any of the loblolly seed. A similar analysis on peak value indicated significant differences on the same sources. Examination of the means in Table 3 shows that the level of both the germination and the peak values was not as high as the level for other seed sources. It is perhaps in this kind of seed, where germination potential is low, that pre-germination treatments are of most value.

Treatments

Another analysis of variance was carried out on the germination value data to look at the effect of treatments within similar groups of time and temperature treatments. Treatments 1 through 5 were grouped because stratification temperatures were held constant, but time in stratification was increased. Treatments 6 through 10 were grouped since they shared a common 36-day period at 38° F. and then had increasing temperature with increasing days.

The last group of treatments was 11 through 15. This group was identical to the 6 through 10 group except that they did not have the common 36-day period of 38° F. at the beginning. This group was subjected to changed temperature with changed time.

Table 4 gives a summary of treatment ranks of seed sources based on the mean germination values of each treatment. It is interesting to note that while the treatment differences were not statistically significant, there is a tendency for the same treatment to show up in the top four ranks across all seed sources. Furthermore, it seems clear that long stratification without increasing temperature is of little value since treatment five (#5) appears only one time in the rankings.

Table 5 gives similar data for rankings by the peak value. These data indicate exactly the same thing. The peak value does not select the same ranks as germination value, because it measures only the initial germination surge and does not take into consideration total germination. Some lots of seed had different total germination potential and

Table 4 Summary of Treatment Ranks G.V.

A—G.V. Ranks of All Treatments by Seed Source

Seed Source	Best Treatment Ranks 1st	2nd	3rd	4th
A	10	15	9	8
B	1	2	10	9
C	2	10	9	8
D	13	15	10	8
E	13	15	9	10

B—G.V. Ranks by Seed Source and Like Treatment Sets

Seed Source	Best Treatment Ranks: Treatments 1-5		Treatments 6-10		Treatments 11-15	
	1st	2nd	1st	2nd	1st	2nd
A	5	1	10	9	15	14
B	1	2	10	9	14	15
C	2	1	10	9	15	13
D	2	1	10	8	13	15
E	2	1	9	10	13	15

Table 5 Summary of Treatment Ranks by P.V.

A—P.V. Ranks of All Treatments by Seed Source

Seed Source	Best Treatment Ranks 1st	2nd	3rd	4th
A	10	15	9	5
B	10	9	2	1
C	10	2	9	1
D	13	15	10	8
E	13	15	8	12

B—P.V. Ranks by Seed Source and Like Treatment Sets

Seed Source	Treatments 1-5		Treatments 6-10		Treatments 11-15	
	1st	2nd	1st	2nd	1st	2nd
A	5	1	10	9	15	14
B	2	1	10	9	15	14
C	2	1	10	9	15	13
D	2	1	10	8	13	15
E	2	3	8	9	13	15

this tends to modify the peak value performance when used in calculating the value.

It was hoped that the study would provide clear-cut recommendations as to preferable treatment combinations, but it did not. Least significant difference and multiple range tests were conducted on the treatment means in an effort to determine dissimilar treatments. This analysis proved of little value.

The B section of Table 4 and 5 show the rankings of treatments when treatments were grouped into similar sets as indicated above. The only added insight this provides is that the high temperature treatments have a tendency to recur, indicating that both germination value and peak value can be affected by temperature adjustments during stratification.

Conclusions

There are several insights to be gained from the work. The fact that the statistical analysis indicates significant treatment differences in spite of rather large experimental errors, leads one to conclude that germination values can be improved through temperature regulation of the stratification environment. If the experiment had produced clear-cut results, we would have anticipated gradients in germination values and peak values within treatment groups with an associated increase in them where the temperatures were increased. There seems to be a trend in this direction but it is not statistically significant. There are so many variables affecting the rate of germination that the temperature effects are apparently not strong.

It is concluded, however, that for some lots of seed the germination value and the peak value can be raised by increasing stratification temperature during stratification. In most cases the highest germination values were associated with those treatments (9, 10, 14, 15,) which had the highest temperature at the end of the stratification period.

It is therefore recommended to direct seeding operators that a short period of temperatures in excess of 38° F. may well stimulate improved germination in field trials. It would be better for an operator, faced with a decision of sowing three weeks early or three weeks later than planned to choose the earlier time and increase the stratification temperature for a few days before sowing. This would serve to advance the germination value and reduce the time that seed would be exposed to the forces of nature prior to germination.

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