

Comparison of Seed Stratification Methods for Western White Pine.

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Better seed stratification is needed to break the dormancy in western white pine, to obtain more rapid and uniform germination. Four stratification methods were compared. Warm/cold stratification was superior to cold stratification methods, especially for low germinating lots.

In the past, the efficiency of the blister rust testing program at the Dorena Tree Improvement Center has been limited by the low germination percentages exhibited by some of the seedlots selected for the testing program. Germination percentage varies greatly among seedlots, ranging from 0 to almost 100 percent. Approximately 15 percent of the seedlots sown for testing purposes were in the zero to marginal range. These lots will probably not have enough surviving seedlings to provide a sufficient base for rust testing. Lack of sufficient base due to poor germination has been an ongoing problem at Dorena. Overcoming this problem would increase the efficiency of the rust testing program by an estimated 10 to 15 percent.

The low germination is not due to low viability or poor germination but to dormancy, and is expressed in large numbers of firm seeds, which do not germinate. We are looking for a method that will overcome dormancy. Dorena personnel have performed a number of small studies over the past few years aimed at developing stratification techniques which will result in higher overall germination percentages among seedlots. Such techniques have been worked out fairly successfully for sugar pine, but western white pine has been less tractable. Current research has not attacked this problem to a degree that would benefit the Dorena program. Further study is needed to upgrade stratification techniques.

BACKGROUND

The current method of stratification being used

at Dorena consists of a 48 hour soak in 1 percent hydrogen peroxide (H₂O₂) followed by 120 days chilling at 34-38°F. This does not appear to be sufficient for some western white pine seed lots to overcome germination blocks. This problem is not limited to Dorena. A survey of other nurseries in the northwest indicated poor germination in some lots. Wind River Nursery soaks the seed for 24 hours and then puts it in cold stratification for 90 to 120 days. They have, however, gone to fall sowing for western white pine to improve stratification. J. Herbert Stone Nursery uses a 24-48 hour running water soak, followed by 100 days cold stratification in a nylon mesh bag layered in peat. Coeur d'Alene Nursery soaks the seed in a home laundry bleach (2 parts bleach to 3 parts water) for 10 minutes, then rinses them for 4 days under running water, followed by cold stratification in the refrigerator for 45 to 50 days.

Past Dorena studies indicate that a different combination of H₂O₂ concentration and length of soak may yield better results. Research by Anderson and Wilson (1966) and D.W. Taylor (personal communication) suggests that a combination of warm stratification at room temperature, followed by cold stratification, would be more effective in promoting germination than cold stratification alone.

Other factors that may affect germination are the use of infrared irradiation (Works and Boyd, 1972) and seed moisture content (Edwards, 1981). Recent work indicates that relatively small variations in moisture content of the seed during stratification may lead to significant differences in germination. Edwards (1981) has found that the optimum germination in *Abies grandis* occurs when seed is dried to 35 percent moisture content following the soak treatment. McLemore and Burnett (1968) report that dormancy was least in *Pinus taeda* seeds when stratified at a moisture content greater than 20 percent. However, the optimum moisture content for stratifying western white pine seed is as yet unknown.

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OBJECTIVE OF STUDY

The objective of this study was to compare different stratification methods for western white pine to see which would result in more rapid and uniform germination among families. Improving the uniformity of germination should lead to increased efficiency in Dorena's rust testing program. Moisture uptake was a problem that this study will address. Cold stratification vs. warm/cold stratification would be compared as well as a 1% H₂O₂ soak vs. household bleach soak followed by rinsing for four days in running water.

METHODS AND MATERIALS

Twenty families were used in this study. Five families with less than 50% germination in previous tests and five with greater than 50% germination, along with 10 families of unknown performance. All methods of stratification received a 48 hour soak except those lots which were washed for 4 days in running water. All seed had been stored in the freezer at 0°F. Fresh seed may give entirely different results. Total length of stratification for all lots was 120 days.

The following methods of stratification were compared in this study.

Method 1. Dorena operational stratification.

- A. 48 hour soak in 1% H₂O₂.
- B. Drain.
- C. Cold stratification at 34-38°F. for 120 days.

Method 2. Warm/cold stratification.

- A. 48 hour soak in 1% H₂O₂.
- B. Surface dry.
- C. 30 days warm stratification at 50°F. in growth chamber.
- D. Dry to 30-35% moisture content and then put into cold stratification at 34-38°F. for 90 days.

Method 3. University of Idaho Method.

- A. Soak seed 10 minutes in a solution containing 2 parts sodium hypochlorite (clorox) and 3 parts water.
- B. Rinse seed in flowing water for 4 days.
- C. Drain.
- D. Place seed in nylon mesh bag and layer in peat at 34-38°F. for 120 days.

Method 4. Modified University of Idaho Method.

- A. Soak 48 hours in 1% H₂O₂ solution.
- B. Drain.
- C. Place seed in nylon mesh bag and layer in peat at 34-38° for 120 days.

The seed were sown in large flats (40"X48"X12") filled with "Forestry Mix" growing media. Only sound seed were sown. Two seed were planted per spot and spots were about 4" apart. There were 2 replications of 5 planted spots per family or 20 seed sown per family per treatment method. The seed was sown on March 31, 1985, and germination was recorded periodically throughout the next 48 days.

RESULTS AND CONCLUSIONS

Results from the water uptake portion of the study are presented in Table 1.

Table 1. Water Uptake in Stratification Test

Family Tree Number	Number	Weight of 20 seed (grams)	Moisture content after 48 hr. soak (percent)	Moisture content after warm strar. (percent)
1	119-01167-006	0.452	43.6	44.0
2	119-01167-007	0.358	39.1	41.5
3	119-03124-382	0.329	35.1	42.7
4	119-03124-390	0.361	34.2	32.5
5	119-06023-513	0.302	31.7	35.5
6	119-06024-612	0.393	32.3	32.3
7	119-06025-526	0.422	33.3	34.4
8	119-06124-661	0.387	32.7	36.7
9	119-06124-662	0.371	32.9	41.4
10	119-06125-538	0.246	37.7	42.0
11	119-15043-920	0.434	31.4	38.4
12	119-15045-935	0.352	34.7	39.5
13	119-17113-337	0.324	36.5	47.0
14	119-17113-340	0.312	37.5	40.5
15	119-17115-715	0.395	33.6	36.8
16	119-17115-816	0.287	36.9	46.2
17	119-18035-393	0.437	27.0	36.7
18	119-18034-397	0.412	29.4	35.3
19	119-20046-025	0.466	35.6	45.9
20	119-20046-044	0.543	33.9	34.6

It appears that water uptake was not a problem in this study. The moisture content of the seed in storage was approximately 6%. Moisture content of the seed after the 48 hour soak ranged from 27.0 to 43.6%. The moisture content in all lots appears to be adequate for germination, as the lot with the least moisture content germinated about as well as the lot with the most moisture (85 vs. 90% respectively).

Results of germination from the four stratification methods are presented in Tables 2 - 5.

Table 2. Rate and Proportion of Germination of Seed Following Stratification by Method 1: The Dorena Operational Procedure.

Family Number	Date:	4/19	4/25	5/6
	Day No.	20	30	41
	Number of emergent seedlings per 20 planted seed.			
1	12	13	18	
2	9	10	10	
3	11	14	17	
4	16	16	18	
5	0	0	2	
6	12	13	13	
7	2	3	4	
8	4	5	9	
9	10	11	16	
10	1	2	6	
11	15	16	19	
12	18	19	19	
13	10	13	16	
14	3	3	5	
15	10	12	15	
16	13	15	16	
17	13	14	17	
18	17	17	18	
19	15	15	15	
20	20	20	20	
OVERALL %	53	58	68	

Table 4. Rate and Proportion of Germination of Seed Following Stratification by Method 3: University of Idaho Method.

Family Number	Date:	4/19	4/25	5/6
	Day No.	20	30	41
	Number of emergent seedlings per 20 planted seeds.			
1	11	13	15	
2	11	11	12	
3	9	14	16	
4	15	15	15	
5	0	1	6	
6	12	16	18	
7	5	6	7	
8	8	9	9	
9	9	11	12	
10	7	11	12	
11	11	14	18	
12	19	19	19	
13	12	13	16	
14	5	5	10	
15	11	16	20	
16	14	18	19	
17	13	15	19	
18	17	19	19	
19	20	20	20	
20	20	20	20	
OVERALL %	57	67	76	

Table 3. Rate and Proportion of Germination of Seed Following Stratification by Method 2: Warm/Cold Stratification.

Family Number	Date:	4/19	4/25	5/6
	Day No.	20	30	41
	Number of emergent seedlings per 20 planted seed.			
1	13	14	17	
2	11	14	15	
3	15	17	19	
4	18	19	20	
5	0	1	16	
6	14	15	15	
7	12	17	19	
8	12	17	20	
9	13	16	18	
10	5	8	18	
11	8	16	17	
12	14	16	18	
13	13	17	18	
14	14	17	20	
15	9	12	19	
16	3	10	20	
17	9	15	19	
18	11	17	19	
19	16	17	17	
20	12	18	20	
OVERALL %	56	73	91	

Table 5. Rate and Proportion of Germination of Seed Following Stratification by Method 4: Modified University of Idaho Stratification.

Family Number	Date:	4/19	4/25	5/6
	Day No.	20	30	41
	Number of emergent seedlings per 20 planted seeds.			
1	9	10	15	
2	7	8	10	
3	7	9	14	
4	13	13	15	
5	0	1	2	
6	10	11	13	
7	4	7	8	
8	8	8	8	
9	11	14	15	
10	7	8	11	
11	14	15	17	
12	20	20	20	
13	13	14	17	
14	3	3	6	
15	15	18	18	
16	17	17	20	
17	11	12	17	
18	16	17	19	
19	17	17	18	
20	20	20	20	
OVERALL %	56	61	71	

Germination at 41 days appears to be the best time for comparison. Little change was noted after that time and seed which had germinated by that time would be useful in the rust test. Overall germination for Methods 1-4 are 68, 91, 76, and 71 respectively. The Warm/Cold Stratification Method does stand out above the rest.

The important issue at Dorena is "how well do the poorest lots do with Method 2, and does it have any adverse effect on the best germinating lots". The 5 best germinating lots are compared with the 5 poorest germinating lots. Results are presented in Table 6.

Table 6. Comparison of Germination in Best and Poorest Families.

Five Best Germinating Families

Family Number	Stratification Method (Number of Germinates at 41 Days)			
	1	2	3	4
4	18	20	15	15
11	19	17	18	17
12	19	18	18	20
18	18	19	19	19
20	20	20	20	20
Percent	94	94	90	91

Five Poorest Germinating Families

5	2	16	6	2
7	4	19	7	8
8	9	20	9	8
10	6	18	12	11
14	5	20	10	6
Percent	26	93	44	35

There appears to be very little difference in germination in the best germinating lots, regardless of stratification method used. Method 2 does not appear to have an adverse effect on lots which are easy to germinate. When stratification methods were compared for the 5 poorest germinating lots, Dorena's method had only 26% germination. These lots would probably be lost in rust test, as they would have insufficient base for a valid test. The warm/cold stratification method brought these lots up to the same germination percentage as the best germinating seed lots (93%). The germination using the modified University of Idaho method (35%) was 9% better than the Dorena method (26% germination). The germination by the University of Idaho method (44%) was 9% better than the modified Idaho method (35% germination). The largest improvement in germination was obtained by use of the warm/cold stratification methods on the poorest

germinating lots. This 67% increase in germination over Dorena's operational method is highly significant.

These results indicate that significant improvement can be made in seed germination by using warm/cold stratification method. We now have a good method to use for stratification of western white pine seed, but additional fine tuning may yield further improvements.

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