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Stratification Period and Germination of Douglas-Fir Seed From Oregon Seed Orchards: Two Case Studies

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Abstract

Effect of stratification period (S) and incubation temperature (T) on germination behavior were tested by using two groups of Douglas-fir orchard seedlots that had given low germination percentages in standard tests. One group of seedlots that had experienced different cone-drying regimes, but otherwise were treated comparably, were germinated at T = 15 and 25 °C after S of 21, 42, and 84 days. Cone-drying conditions affected germination behavior. Particularly, low-humidity, high-temperature drying decreased the rate and percentage of germination, but if seeds were given 84-day S, then germination was good, even at 15 °C. A second group of five seed lots represented three elevational blocks from one orchard, two blocks sampled in 2 years. Seedlots were tested as above, except S of 21 days was omitted. Year of collection and, to a lesser extent, orchard block affected germination behavior, but in all cases germination was 90 percent or more, and rate and uniformity were good if seeds received 84 days of S. At 15 °C incubation, only 84 days of S gave satisfac tory germination. At 25 °C, 21 days of S gave satisfactory percentage of germination, but rate and uniformity were much improved by longer stratification. Preliminary recommendations for stratification are given and discussed for different germination temperatures.

Keywords: Germination percent, germination rate, standard germination test, chilling, dormancy.

Introduction

Although pregermination treatments and germination tests for Douglas-fir *(Pseudotsuga menziesii* (Mirb.) Franco) seeds are standardized (for references, see Schopmeyer 1974: 678-679), some apparently good seedlots germinate slowly and poorly under these conditions. In several instances, poor germination has involved seeds from Douglas-fir orchards in Oregon. A common mix of Douglas-fir seeds from the State of Oregon J.E. Schroeder seedling orchard at St. Paul, which averaged 97 percent filled, ranged from 69 to 91 percent germination in the standard test. Variation in germination percentage was associated with several drying treatments that the common lot of cones received after harvesting.[1] Seeds from five lots collected during 2 years at the David T. Mason clonal orchard, Sweet Home, Oregon,

[1] Kanaskie, Alan; Cook, Bill; Jaeger, Richard; Hamm, Phil. 1987. Effect of cone storage conditions on Douglas-fir seed yield and viability, cone mold, and seed-borne pathogens. General File 2-2-1-300. 10. Unpublished material available from Department of Forestry, Salem, OR.

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averaged 88 percent firm but only 48 percent germination after 21 days in the standard test used at Oregon State University Seed Laboratory, Corvallis. Finally, Liang Hsin[3] reported that Douglas-fir seeds collected in the Bureau of Land Management Orchard, Colton, Oregon, germinated more slowly and less completely in their containerized nursery than did seeds from collections in natural stands.

Previous experience with a large number of seed lots over many years had suggested that slow and poor germination of healthy-appearing Douglas-fir seeds is often due to inadequate stratification. In this note, I report on additional germination tests of seeds from the Schroeder and Mason Orchards (described above). Long chilling periods were included in these tests and, because chilling is reported to broaden the range of temperatures seeds will germinate under (Allen 1967, Vegis 1963), two incubation temperatures were used. The lower temperature approximated the situation seeds would face in spring nursery sowings; the higher temperature approximated the mean temperature of the standard germination test. Germination percentage and rate were satisfactory at both temperatures if the seeds were adequately chilled. The results are preliminary, but they indicate a potential germination problem and a possible solution.

Because the seeds came from operational seed orchard collections in which orchard personnel evaluated cone and seed maturity and prescribed methods of cone and seed processing, I refer to the tests as case studies.

Case Study I. Schroeder Orchard-Effect of Postharvest Drying Conditions

Materials and Methods

Open-pollinated, whole-crown cone collections were made in autumn 1986 from trees in each of 10 full-sib families in the Vernonia block (northwest Oregon Coast Range). Trees in this block were 15 years from seed at the time. Because of the large spread in relative cone and seed maturity among trees within the blocks, seed maturity of each cone-bearing tree was evaluated by color of seed wing and ease of separation of seed and wing from the cone scale. Cones were sampled twice weekly, and trees with the most mature seeds were harvested first. Cones were collected when seeds in the sampled cones had brown wings and the filled seeds separated readily from scales. A bulk cone rrro was made up that consisted of 30 bushels of cones from each of the 10 families, but represented different numbers of trees per family. **AIS** cones were thoroughly mixed on a large plastic tarpaulin, then placed into sterile ny= ion mesh laundry bags (1 bushel of closed cones—about 700 cones—in a 2-busheJ bag) (see footnote 1). Five bags were transported to each of seven drying facilities. In my tests, I used seed samples from cones dried at four locations and sampled from three bags at each location. All seed samples were subsequently kept separate according to drying location and bag within location. The four locations were:

1. Colton Seed Orchard—closed shed with screened opening at the bottom and louvered opening at the top of the walls. Ambient temperature and relative humidity were not monitored but often had wide diurnal fluctuations during Sate summer and early autumn (when the cones were in the shed).

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[2] Personal communication, Howard Dew. 11987. Barringer and Associates, Inc., Sweats Home, OR 97386.

[3] Personal communication, Liang Hsin. 1983, Forest geneticist, Bureau of Land Management,' 825 NE. Mulnomah Street, Portland, OR 97208

2. Corvallis Forestry Sciences Laboratory—drying room with dehumidifier, about 30 °C and 10 percent relative humidity.

3. Dorena Tree Improvement Center—drying room, 16-22 °C, 50-60 percent relative humidity.

4. Elkton Nursery—growth chamber, 10 °C, 30-50 percent relative humidity. Because of space limitations in the growth chamber, smaller cone bags were used. About 85 cones were placed in 28- by 53-centimeter (flat dimensions) mesh bags. Cone-to-bag volume ratio was the same as for the larger bags.

Locations 2 (drying room) and 4 (growth chamber) had the most controlled environ ments but differed greatly in temperature. Location 2 also had much lower relative humidity than did 4. Location 1 had the most variable conditions and the closest to ambient.

After 6 weeks of drying, cones were returned to the Schroeder Seed Orchard for processing. Because this experiment was established to monitor cone molds and seed-borne pathogens (see footnote 1), each bag of cones was processed separ ately. Between extractions all equipment was surface sterilized with a 0.1-percent solution of sodium hypochlorite to prevent cross-contamination by fungi. Tumblers and cleaners were cleaned with compressed air between extractions. Seeds were extracted in drum tumblers (cones tumbled 4 minutes), dewinged, cleaned in a Clipper cleaner[4] and stored about 12 months at 0 °C initially, then at -15 °C until the stratification treatments started.

Four 60-seed samples representing each cone bag were placed in fine-mesh cloth packets (made from commercial veiling fabric with about 9 holes per centimeter), soaked 24 hours in aerated water in the laboratory (21-22 °C), shaken to remove excess water, put in a zip-lock plastic bag along with damp paper towels and strati fied at 2-4 °C in a walk-in cooler. Three stratification periods were used: 21,42, and 84 days. Soaking and chilling were arranged so that all stratifications were concluded at the same time.

Seeds were sown on two circles of Whatman no. 2 filter paper in 8.7 centimeters in diameter by 1.3 centimeters deep plastic petri dishes with covers. A layer of vermiculite was placed under the filter papers and moistened with 23 milliliters of distilled water. During germination, distilled water was added as needed to maintain the filter papers at a visually constant moisture content (papers wet, but without surface water on the seeds or a water meniscus between paper and seed). The first water was added about day 8 at 25 °C incubation temperature and about day 20 at 15 °C.

Seeds were incubated at two constant temperatures, 15 °C and 25 °C. Germination was counted 23 times at gradually increasing intervals (4.75 hours between the first two counts, 14 days between the last two) starting when the first germination was observed (Campbell and Sorensen 1979). A seed was considered as germinated

[4] The use of trade name is for information and convenience of reader, and does not constitute official endorsement or approval by the U.S. Department of Agriculture of any product to the exclusion of others that may be suitable.

when a white root tip could be seen clearly—a root tip about 2 millimeters long. Cracked and swollen seeds and abnormal germinants (reverse, twins) were tallied but were excluded from the counts used in the analyses.

Petri dishes for each combination of drying treatment and stratification period were assigned to random positions in each of two blocks in each of the two incubation temperatures (chambers). Blocks were separate shelves within a single chamber. In the low-temperature chamber, germination rate could not be estimated for seeds that had received short stratification, because too few seeds germinated. Observations from each chamber therefore were analyzed separately. The statistical model was,

$$X_{ijkl} = M + R_i + D_j + B(D)_{jk} + S_l + (DS)_{jl} + [B(D)S_{jkl}] + e_{ijkl},$$

where X_{ijkl} = observation for one dish,
M = grand mean,
R_i = random blocks,
D_j = fixed drying locations,
$B(D)_{jk}$ = random bags within drying location,
S_l = fixed stratification periods,
$(DS)_{jl}$ and $[B(D)S_{jkl}]$ = fixed interactions,
e_{ijkl} = experimental error,
i = 1,2 blocks,
j = 1, 2, 3, 4 drying locations,
k = 1, 2, 3 bags in each drying location, and
l = 1, 2 stratification periods at low incubation temperature, and
= 1, 2, 3 stratification periods at high incubation temperature.

Mean squares for drying facilities were tested against bags within locations. The interaction mean square, drying location by stratification period, was tested against bags within location by stratification period. Other main effects and interactions were tested against experimental error.

The test ended after 48 days, and three germination traits were analyzed:

1. **Ungerminated seeds.** Seeds were x rayed and proportions of filled seeds that had not germinated were determined for each petri dish. Because of large variation among treatments, values were transformed to an arc sine $\sqrt{\text{percent}}$ before analysis to obtain homogeneous variances (Snedecor and Cochran 1967: 327). Plotting of residuals (Box and others 1978: 221) indicated that the transformation was appropriate.

1. **Mean germination rate** was calculated for the germinated seeds on each pefri dish. The rate expresses daily development toward germination after entering incuba tion; for example, if a seed requires 8 days to germinate, the embryo development rate for that seed is 1/8 or 0.125 units per day (Campbell and Sorenseo 1979, Hegarty 1973). Higher rates mean fewer days to germinate. When incubation was at 25 °C (but not 15 ^{0}C), variances of rates were heterogeneous (Snedecor and Cochran 1967: 296) and positively related to the mean. Transformation to logarithms removed the heterogeneity.

3. Spread (or uniformity) of germination for the sample of seeds within a petri dish was expressed as **standard deviation of rates.** It was readily calculated from the rates (Campbell and Sorensen 1979: table 1).

With only 21-day stratification, too few seeds germinated (five or fewer seeds in 15 of the petri dishes) at 15 °C incubation to reliably estimate mean rate and standard deviation of rate within dishes. Therefore, only 42- and 84-day stratifications were included in the analyses of variance of rate and standard deviation at 15 °C.

Results

Percentage of germination was strongly affected by stratification period and, to a lesser but still significant extent, by location where the cones had been dried. F-values (table 1) and differences in percentage of germination (table 2) for all treatments were much larger when incubation was at 15 °C rather than 25 °C. The effect of bags within drying location was not significant, so only location means, based on the three bags per location, are presented.

At 25 °C incubation, all stratification periods were sufficient for essentially complete germination (table 2). At 15 °C incubation, 84 days of stratification gave 95 percent germination or more, 42 days marginally satisfied stratification requirements, and 21 days was insufficient for all seedlots. Seeds from cones dried at locations 1 and 3 generally had higher germination percentages than seeds from cones dried at locations 2 and 4, but only at the intermediate stratification (42 days) was germination percentage strongly affected by drying location.

Table 1—Analyses of variance of percentage of germination (transformed to arc sine √percent) of Douglas-fir seeds from cones dried at 4 locations, stratified for 3 durations, and incubated at 2 temperatures

Sources of variation	Degrees of freedom		Incubation temperature 15 °C F-value	15 °C P	25 °C F-value	25 °C P
Blocks	1		0.83	0.3690	2.03	0.1627
Stratification periods (S):[a]	2		1621.36	.0000	15.02	.0000
Linear		1	3182.48	.0000	23.40	.0003
Nonlinear		1	60.25	.0000	6.64	.0148
Drying treatments (T):	11					
Locations[b]		3	70.22	.0000	4.15	.0477
Bags/locations		8	.33	.9468	1.38	.2401
S x T:	22					
S x locations		6	9.31	.0002	2.97	.0382
S x bags/locations		16	.53	.9092	.81	.6672
Error	35					
Error coefficients of variation			10.8%		37.2%	

[a] Stratification periods were 21, 42, and 84 days and were treated as linear on the log scale.

[b] Cone drying locations were Colton Seed Orchard, Forestry Sciences Laboratory at Corvallis, Dorena Tree Improvement Center, and Elkton Nursery.

Table 2—Percent germination (calculated from back-transformed values of arc sine √percent ungerminated) after 48 days of filled seeds from a bulk lot of Douglas-fir cones dried at 4 locations, stratified for 3 durations, and germinated at 2 temperatures

	Drying location[a]			
Stratification period	1	2	3	4
15 °C incubation:				
21 days	8.8	3.0	6.1	5.6
42 days	82.7	56.9	79.0	56.4
84 days	99.9	95.9	99.8	98.8
25 °C incubation:				
21 days	99.9	96.9	99.5	98.0
42 days	100.0	99.8	100.0	100.0
84 days	100.0	99.9	99.9	100.0
Standard test[b]	90.0	78.8	69.0	90.8

[a] The four drying locations were, respectively, Colton Seed Orchard, Corvallis Forestry Sciences Laboratory, Dorena Tree Improvement Center, and Elkton Nursery.

[b] Germination percentages obtained at the Seed Testing Laboratory, Oregon State University, Corvallis, in a test lasting 21 days.

Mean rates of germination at both incubation temperatures were affected by stratification period and drying location (table 3). Doubling the stratification period (for example, from 21 to 42 days) consistently reduced by 30 to 40 percent the mean days to 50 percent germination (table 4).

Seeds from cones dried at location 2, which generally had the lowest percentage of germination (table 2), were also the slowest to germinate (table 4).

Spread in time when germination occurred was analyzed as standard deviation) of rates of germination for seeds within petri dishes. Results of the analyses of variance at the two incubation temperatures are shown in table 5.

Spread of germination is presented as the number of days required for the central 80 percent of the seeds to germinate (table 6). Increasing stratification period beyond 21 days greatly increased the uniformity of germination; in general, each doubling of the stratification period reduced the spread in days by about 50 to 60 percent. As a result, if the stratification period was 84 days, germination was very uniform, even at an incubation temperature of 15 °C.

Again, seeds from cones dried at location 2, which had .relatively stow and incomplete germination (tables 2 and 4), stand out (particularly if given shorter stratification) as having a relatively long period during] which seeds germinated.

Table 3—Analysis of variance of mean germination rates (1/days) (transformed to logarithms) of Douglas-fir seeds from cones dried at 4 locations, stratified for 3 durations, and Incubated at 2 temperatures

	Incubation temperature							
	15 °C				25 °C			
Sources of variation	D.F.[a]		F-value	P	D.F.[a]		F-value	P
Blocks	1		18.21	0.0001	1		9.49	0.0042
Stratifications (S):[b]	1		2007.11	.0000	2		795.33	.0000
Linear		—[c]	—	—		1	1590.59	.0000
Nonlinear		—	—	—		1	.06	.8079
Drying treatments (T):	11				11			
Locations[d]		3	17.67	.0007		3	53.13	.0000
Bags/locations		8	1.59	.1824		8	.24	.9802
S x T:	11				22			
S x location		3	.70	.5780		6	1.96	.1321
S x bags/location		8	1.05	.1824		16	.46	.9504
Error	23				35			
Error coefficients of variation			3.5%				7.0%	

[a] Degrees of freedom.
[b] Stratification periods were 21, 42, and 84 days and were treated as linear on the log scale.
[c] Germination rates at 15 °C after 21 days of stratification were not included in the analysis because too few seeds germinated.
[d] The four drying locations were Cotton Seed Orchard, Corvallis Forestry Sciences Laboratory, Dorena Tree Improvement Center, and Elkton Nursery.

Table 4—Days to 50 percent germination (backtransformed from logarithms of mean rates [1/days] of germination) for seeds from a bulk lot of Douglas-fir cones dried at 4 locations, stratified for 3 durations, and Incubated at 2 temperatures

	Drying location[a]			
Stratification period	1	2	3	4
15 °C incubation:				
21 days	—[b]	—	—	—
42 days	10.6	12.2	10.8	11.3
84 days	6.8	7.6	7.0	7.1
25 °C incubation:				
21 days	9.9	11.5	10.2	11.2
42 days	6.1	7.1	6.0	6.4
84 days	4.5	4.9	4.4	4.5

[a] The 4 drying locations were, respectively, Colton Seed Orchard, Corvallis Forestry Sciences Laboratory, Dorena Tree Improvement Center, and Elkton Nursery.
[b] Too few seeds germinated at 15 °C after 21 days of stratification to estimate rate.

Table 5—Analyses of variance of spread of germination (standard deviation of germination rates [1/days] of seeds within petrl dishes) of Douglas-fir seeds from cones dried at 4 locations, stratified for 3 durations, and Incubated at 2 temperatures

	Incubation temperature					
	15 °C			25 °C		
Sources of variation	D.F.[a]	F-value	P	D.F.[a]	F-value	P
Blocks	1	6.64	0.0132	1	3.97	0.0548
Stratifications (S):[b]	1	.26	.6125	2	120.72	.0000
Linear	—[c]	—	—	1	230.75	.0000
Nonlinear	—	—	—	1	10.69	.0026
Drying treatments (T):	11			11		
Locations[d]	3	9.57	.0050	3	12.04	.0024
Bags/locations	8	.59	.7726	8	1.23	.3124
S x T:	11			22		
S x locations	3	.70	.5780	6	1.96	.1321
S x bags/locations	8	1.05	.1824	16	.46	.9504
Error	23			35		
Error coefficient of variation		2.9%			2.6%	

[a] Degrees of freedom.
[b] Stratification periods were 21, 42, and 84 days and were treated as linear on the log scale.
[c] Standard deviations at 15 °C after 21 days of stratification were not included in the analysis because too few seeds germinated.

[d] The 4 drying locations were Cotton Seed Orchard, Corvallis Forestry Sciences Laboratory, Dorena Tree Improvement Center, and Elkton Nursery.

Table 6—Number of days needed for the central 80 percent of the seeds to germinate (days between 10 percent and 90 percent germination) for seeds dried at 4 locations, stratified for 3 durations, and Incubated at 2 temperatures

	Drying location[a]			
Stratification period	1	2	3	4
15 °C incubation:				
21 days	—[b]	—	—	—
42 days	6.9[c]	12.0	7.2	8.2
84 days	2.7	4.3	3.0	3.0
25 °C incubation:				
21 days	11.3	22.7	13.1	16.8
42 days	5.3	10.1	5.5	5.8
84 days	3.3	4.6	2.8	3.1

[a] The *4* drying locations were Colton Seed Orchard, Corvallis Forestry Sciences Laboratory, Dorena Tree Improvement Center, and Elkton Nursery.
[b] Too .few seeds, germinated, to calculate values.
[c] Values were calculated from means and standard daviations backtransformed from logarithms of germination rates (l/days) for seeds within patri dishes.

Discussion

The test evaluated four groups of seeds that came from a common mix of cones. The seedlots differed only in how the cones were dried before seed extraction. Seed samples from these lots were given standard germination tests[5] and had 69 to 91 percent germination (table 2, bottom line) at the end of the 21-day test. The relatively low standard-test germination percentages associated with locations 2 and 3 (table 2, bottom line) suggested that method of cone drying had either affected germination behavior or damaged some seedlots.

Standard test conditions included 21 days of stratification and incubation at 30 °C in the day and 20 °C at night with 8-hour photo and thermoperiod (mean temperature about 23 °C). Previous work shows that stratification influences the width of the temperature range giving full germination (Thompson 1970, Vegis 1963), and transition from temperatures giving full germination to those giving greatly reduced germination can be quite abrupt (Roberts and Smith 1977, Simon and others 1976). This indi cated that 21 days of stratification under standard conditions may have been insufficient for full germination of some of these seedlots. The results of my tests indicated that this was the case. With long stratification, germination was rapid, uniform, and nearly complete for all seedlots at both incubation temperatures (tables 2, 4, and 6).

Seeds from cones dried at location 2 gave the poorest germination with short stratification periods. At this location, cones were dried at the highest temperature and lowest relative humidity. Final drying of these cones probably was more rapid and exposure to warm, low-humidity conditions was longer than for cones dried at the other locations. Others have observed that length of cone storage (Bloomberg 1969, Walkenhorst 1984) and rate of seed drying (Belcher 1986) modify stratification requirements. Belcher (1986) reports that rapid seed drying can deepen dormancy.

Finally, a comment on the interaction, S by location, in table 1. Occasionally, seedlots appearing fully viable give quite variable germination or emergence in some sowings but not in others. The germination percentages listed in table 2 indicate that one cause might be the influence of stratification period on the width of the temperature range giving full germination (Vegis 1963). With one combination of stratification period and incubation temperature (42-day, 15 °C), germination percentages in table 2 differed much more than with the other combinations. If cone and seed handling, genetic variation, or other influences have resulted in large variation in dormancy among seedlots, a stratification period satisfying the requirements of only an intermediate proportion of seeds can cause large variation in the germination behavior. Lengthening stratification will decrease the proportion of dormant seeds. Consequently, another benefit of longer stratification is that it increases the probability of getting good seedling yields from all or nearly all seedlots.

Amount and type of cone mold differed among storage facilities, but there was no relation among cone mold and seed yield, viability, or disease (see footnote 1).

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[5] **Seed Laboratory, Oregon State University, Corvallis 97331.**

Case Study II. Mason Orchard-Effect of Orchard Block and Year of Collection

in standard germination tests (see footnote 5), five seedlots from a clonal orchard begun in 1960 (David T. Mason Seed Orchard, managed by Barringer and Associates, Inc., Sweet Home, Oregon) gave much slower and poorer germination than expected[6] (table 7). Further testing of these seedlots is described in the following sections.

Materials and Methods

The orchard consists of selections from the west slopes of the Cascade Range in Oregon. It is divided into low- (<600 meters, 25 clones), mid- (600-900 meters, 21 clones), and high-elevation (>900 meters, 65 clones) blocks. The orchard itself is located at about 400 meters elevation. Cones for this study came from 1982 and 1985 collections.

[6] Personal communication, Howard Dew. 1988. Barringer and Associates, Inc., Sweet Home, OR 97386.

Table 7—Percentage of germination[a] after 21 and 28 days incubation for 5 seedlots from 2 years of collection and 3 elevational blocks In the D.T. Mason Douglas-fir Seed Orchard

Seedlot	Incubation period	
	21 days	28 days
	- - - - *Percent* - - - -	
1982:[b]		
Low elevation[c]	45	57
Midelevation	31	51
High elevation	56	71
Mean	44	60
1985:		
Midelevation	44	70
High elevation	64	74
Mean	54	72
Overall mean	48	65

[a] Tests were conducted at the Seed Laboratory, Oregon State University, Corvallis, with 21 days of stratification and incubation at 30 °C/20 °C day/night temperature with 8-hour photo and thermo-period. Seedlots averaged 88 percent filled seeds.

[b] Year of collection.

[c] Elevational blocks were low, <600 meters; mid, 600-900 meters; high, >900 meters.

Cones were collected and processed operationally. Emphasis was put on collecting close to cone maturity; that is, embryos filling embryo cavities, female gametophytes firm and not milky, seed wings turning brown.[7] Individual trees were harvested approximately in the order that their seeds reached maturity. In both years, all clones contributed to the seed mix, but some clones contributed more seeds than others and not the same clones were most productive in both years.

Nylon mesh bags (bushel size) were half filled with cones. Bags were brought daily into a storage facility at the orchard where they were placed on racks with free air circulation around each bag. The storage building had doors on one side, which were left open. Ambient air (temperature usually 10- to 20 °C) was circulated with a large fan.

At the end of cone collection, bags were sorted by clone and shipped to the seed plant.[8] At the cone and seed plant, bags were again placed on racks in a building with open eaves and circulating fans.[9] Final drying, done just before cone tumbling, lasted 2-3 days in a closed room (temperature 30-37 °C) with a moisture pump dehumidifier. (Relative humidity in the room was not recorded for this drying, but it usually decreases from about 40-50 percent to about 25 percent during drying [see footnote 9].) Dried cones were tumbled, and seeds were dewinged and cleaned. Processing of a cone sample from the 1985 collection indicated incomplete extraction due to poor flaring. These cones therefore were moistened by placing the sacks of cones outdoors in the rain, then re drying as above and tumbling. Incomplete extraction, before moistening, was general for the Mason cones in 1985. The cause of poor flaring and initial extraction is unknown, but appeared to be due to cone insects and pitch. Condition of embryo and female gametophyte before cone collection indicated it was not due to seed immaturity. Cleaned seeds were bulked by orchard block and stored in porous containers on open shelves at 20-25 °C until moisture content of seeds reached about 6-8 percent, dry weight basis.

Three random seed probes were taken from each of the five Mason Orchard seedlots. Initially, one sample of each seedlot was sent to the Seed Laboratory (see footnote 5) for standard germination tests. Because of poor germination (table 7), a second sample of seeds from each seedlot was sent to the same facility for viability tests using the tetrazolium staining reaction, and a third sample of about 900 seeds per seedlot was set aside for further evaluation of seed quality and stratification requirements.

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[7] Personal communication, Howard Dew. 1989. Barringer and Associates, Inc., Sweet Home, OR 97386.

[8] G. Barnes Enterprises, 1210 Vincent Place, Cottage Grove, OR 97424.

[9] Personal communication, Gerald Barnes. 1989. G. Barnes Enterprises, 1210 Vincent Place, Cottage Grove, OR 97424.

The 900-seed samples were x rayed and the number of empty, partially filled, and insect-damaged seeds counted. Ten filled seeds were taken from each seedlot. Five seeds from each seedlot were weighed, dried 24 hours at 80 °C in a forced-air oven, reweighed, and moisture content determined. The other five seeds from each seedlot were soaked 24 hours in aerated distilled water at room temperature (ca. 22 °C), surface dried, weighed, oven dried as above and reweighed. Before reweighing, oven-dried seeds were brought to room temperatures over CaCO3 in a sealed jar. Tetrazolium tests, x rays, and moisture evaluations were to determine if the comparatively low germination percentages in the standard tests were due to poor seed quality.

The percentage of ungerminable seeds identified on the x rays averaged 5.6 percent (range, 4.0 to 7.8 percent) for the five seedlots. An additional 4.0 percent (range 2.0 to 5.3 percent) was identified as ungerminable (cracked seedcoats, rotted or swollen) or abnormal germinants (reverse, twins) at the end of the germination test. Estimated maximum germination thus averaged about 90 percent. Seedlots averaged 90 per cent (range, 87 to 93 percent) normal staining reaction in the tetrazolium test. Moisture percentages, fresh-weight basis, averaged 4.9 percent (range, 4.6 to 5.2 per cent) for seeds directly from storage and 27.3 percent (range, 26.8 to 27.8 percent) after 24-hour imbibition at room temperature. The dry-seed values are within the normal range for temperate conifers (Ching 1966, Huss 1954, Ryynanen 1984, von Schonborn 1964) but 1-2 percent lower than I usually have found.[10] Values for soaked seeds are also within the normal range (Ching 1966; also see footnote 10). These preliminary tests did not indicate any reason that filled seeds should not germinate well.

Seeds were prepared for germination testing as in case study I. Percentage of germination (transformed to arc sine $\sqrt{\text{percent}}$), mean rate (transformed to logarithms), and standard deviation of rate were determined for the five orchard seedlots by using two stratification periods (42 and 84 days) and two constant incubation temperatures (15 °C and 25 °C). Tests at both temperatures were run concurrently in separate incubators. Petri dishes representing each combination of seedlot and stratification period were randomly assigned to positions on each of two shelves within each incubation chamber to serve as replications within temperatures. All stratification period-incubation temperature combinations gave sufficient germination to be analyzed for all traits, and were analyzed as a combined experiment (LeClerg and others 1962: 215).

The statistical model was,

$$X_{ijkl} = M + T_i + C_i + B(T)_{ij} + S_k + (TS)_{ik} + U + (TL)_{il}$$
$$+ (SL)_{kl} + (TSL)_{ikl} + e_{ijkl},$$

where X_{ijkl} = observation for one dish,
M = grand mean,
T_i = fixed incubation temperatures,
G_i = random chamber effects, which-were-assumed = 0,
$B(T)_{ij}$ = random replications within incubation temperatures,
S_k = fixed stratification periods,

[10] Unpublished observations, author.

Table 8—Analysis of variance of percent germination (transformed to arc sine √percent) and mean rate (1/days) of germination (transformed to logarithms) of Douglas-fir seedlots from 3 Mason Orchard elevational blocks collected in 1982 and 2 of the same blocks collected in 1985

Sources of variation	Degrees of freedom		Percent		Rate	
			F-value	P	F-value	P
Temperature (T)[a]	1		297.33	0.0033	1572.76	0.0006
Replication/T (error a)	2					
Stratification (S)[b]	1		83.51	.0000	321.33	.0000
S x T	1		83.81	.0000	1.56	.2278
Seedlots (L):	4		3.80	.0208	23.95	.0000
1982 vs. 1985		1	13.45	.0018	35.47	.0000
Among blocks/82		2	.06	.9383	11.63	.0006
Between blocks/85		1	1.61	.2205	37.07	.0000
L x T:	4		1.94	.1476	2.73	.0617
(82 vs. 85) x T		1	7.17	.0154	5.51	.0306
Among blocks/82 x T		2	.07	.9298	2.02	.1619
Between blocks/85 x T		1	.44	.5171	1.37	.2568
L x S:	4		1.78	.1766	3.06	.0433
(82 vs. 85) x S		1	2.60	.1241	3.60	.0740
Among blocks/82 x S		2	2.18	.1415	1.79	.1959
Between blocks/85 x S		1	.15	.7000	5.09	.0368
L x S x T:	4		.89	.4923	1.37	.2834
Remainder (error b)	18					
(Error b) coefficients of variation			9.1%		2.8%	

[a] Incubation temperatures were 15 °C and 25 °C.

[b] Stratification periods were 42 and 84 days.

L_l = fixed seedlots,
$(TS)_{ik}$, $(TL)_{il}$, $(SL)_{kl}$, $(TSL)_{ikl}$ = fixed interaction effects,
e_{ijkl} = experimental error,
i = 1, 2 incubation temperatures,
j = 1, 2 replications within temperatures,
k = 1, 2 stratification periods, and
l = 1, 2, 3, 4, 5 seedlots.

Mean square for incubation temperatures was tested against blocks within temperatures; stratification periods, seedlots, and interactions were tested against experimental error (table 8).

Results

Given adequate stratification (42 days or longer at 25 °C incubation, 84 days at 15 °C incubation), germination of filled, sound seeds was high (table 9).

Significant effects were associated with incubation temperature, stratification period, seedlot, and several interactions among them (table 8). Analysis of standard deviation of rate is not listed because, as in case study I, results were similar to those based on mean rates.

Collections in 1982 and 1985 differed in germination percentage and mean rate (table 8). Seeds collected in 1985 generally germinated more completely (table 9), slightly faster (table 10), and more uniformly (table 11) than seeds collected in 1982. Because the low-elevation orchard block was not included in 1985, the two years are not equivalent. The difference among blocks within years for germination percentage was not significant (table 8), however, indicating the year effect was not due to the blocks sampled. Restricting the year comparison to midelevation and high-elevation blocks still indicated a difference between years (tables 9,10,11). (See also the results from the standard test in table 7.) Clonal composition of seedlots differed between years. Because collections in both years included seeds from all clones within blocks (see footnote 7), it is suggested that the year effect probably was not genetic but due to annual environmental influences or to cone and seed handling effects; for example, the remoistening of the 1985 cones.

Mean germination rate differed significantly among blocks within years in both years (table 8).

There was large interaction between incubation temperature and stratification period in their effect on germination percentage. At 25 °C, most seeds germinated regardless of stratification period (table 9). At 15 °C, 42 days of stratification was inadequate, particularly for seeds collected in 1982 (table 9).

Table 9—Germination percent (calculated from backtransformed values of arc sine √percent) of Douglas-fir seeds from 3 elevational blocks at the Mason Seed Orchard collected in 2 years, stratified 42 and 84 days, and incubated at constant 15 °C and 25 °C

	Incubation temperature and stratification period			
	15 °C		25 °C	
Seedlot	42 days	84 days	42 days	84 days
1982:[a]				
Low elevation[b]	42	91	99	99
Midelevation	30	96	100	99
High elevation	25	98	98	100
Mean	32	95	99	99
1985:				
Midelevation	53	99	100	99
High elevation	75	99	100	100
Mean	64	99	100	100
Overall mean	45	97	99	99

a Year of collection.
b Elevational blocks were low, <600 meters; mid, 600-800 meters; high, >900 meters.

Table 10—Mean days to 50 percent germination (calculated from backtransformed values of logarithms of mean germina tion rates [1/days]) of Douglas-fir seedlots from 3 elevatlonal blocks at the Mason Seed Orchard collected in 2 years, stratified 42 and 84 days, and incubated at 15 °C and 25 °C

Seedlot	Incubation temperature and stratification period			
	15 °C		25 °C	
	42 days	84 days	42 days	84 days
1982:[a]				
Low elevation[b]	14.2	11.3	6.5	5.1
Midelevation	16.4	11.8	7.7	5.6
High elevation	15.2	10.8	6.5	4.5
Mean	15.3	11.3	6.9	5.1
1985:				
Midelevation	15.6	10.6	6.8	4.9
High elevation	15.0	8.8	5.3	3.8
Mean	15.3	9.7	6.0	4.4
Overall mean	15.3	10.7	6.6	4.8

[a] Year of collection.
[b] Elevational blocks were low, <600 meters; mid, 600-900 meters; high, >900 meters.

Table 11—Number of days needed for the central 80 percent of the seeds to germinate (days between 10 percent and 90 percent germination) for seedlots from 3 elevational blocks at the Mason Seed Orchard collected in 2 years, stratified 42 and 84 days, and Incubated at constant 15 °C and 25 °C

Seedlot	Incubation temperature and stratification period			
	15 °C		25 °C	
	42 days	84 days	42 days	84 days
1982:[a]				
Low elevation[b]	11.5[c]	7.0	10.4	4.2
Midelevation	14.2	8.2	7.8	6.2
High elevation	15.2	5.8	7.4	2.8
Mean	13.6	7.0	8.5	4.4

Footnotes on next page.

Table 11—continued

	Incubation temperature and stratification period			
	15 °C		25 °C	
Seedlot	42 days	84 days	42 days	84 days
1985:				
Midelevation	13.5	6.4	8.8	4.2
High elevation	8.8	3.8	3.9	2.3
Mean	11.2	5.1	6.4	3.2
Overall mean	12.6	6.2	7.7	3.9

[a] Year of collection.

[b] Elevational blocks were low, <600 meters; mid, 600 to 900 meters; high, >900 meters.

[c] Values calculated from backtransformation of logarithms of mean germination rates (1/days) and the standard deviations of seeds within a petri dish.

Discussion

Like the results in case study I, germination of filled, healthy seeds from the Mason Orchard was essentially complete when they were adequately stratified. But adequate stratification was important. At 15 °C, which was chosen as being roughly equivalent to spring nursery soil temperature, 42-day stratification gave only 45-percent mean germination at the end of the 39-day test (table 9).

Doubling the stratification period reduced the spread of germination (days required for the central 80 percent of seeds to germinate) by about half (table 11). As a result, seeds incubated at 15 °C, if stratified for 84 days, had more uniform germination on the average than did seeds incubated at 25 °C and stratified for 42 days; that is, germination was uniform even at cool temperatures if seeds were adequately stratified. Conversely, germination at high temperatures, even if complete, was unnecessarily spread out if stratification was short.

Variation among seedlots in germination traits was present for blocks within years (table 8, rate columns only) and between years (table 8, percentage and rate). Difference among elevational blocks within years is presumably a seed source, or genetic, effect and has been observed in other investigations (Campbell and Ritland 1982, Levins 1969, Tanaka 1976, Weber 1988). The year effect could be due to differences in annual environmental preconditioning of the seed while on the tree (Akpan and Bean 1977, Alexander and Wulff 1985, Jensen and Noll 1959, Mohamed and others 1985, Sawhney and others 1985, VonAbrams and Hand 1956, Wang 1976). It could also be due to aftereffects of cone-handling differences in the two years. Such effects are well known (Bonner 1987, Jensen and Noll 1959, McLemore and Bamett 1966, Silen 1958, Woods and Blake 1981) and were associated with drying location in case study I of this report.

General Discussion and Conclusions

In the introduction, I said that some presumably viable seedlots from Oregon Douglas-fir orchards germinated poorly in standard tests and in some nursery sowings. In presenting these case studies, it has been my intent to use some of these seedlots to determine if the seeds were indeed of poor quality or if standard testing conditions had not adequately evaluated viability. The indication from the case studies was that the standard germination test did not indicate true germinability. The cause was inferred to be insufficient stratification. I suggest that consideration be given to re-evaluating (expanding or modifying) the standard tests used for Douglas-fir; particularly, use of stratification longer than 21 days should be considered. Even if only a minority of seedlots are affected, they involve material of high commercial and reforestation value. Perhaps an expanded test need be used only with those seedlots presumed good but that appear poor in initial testing.

Three observations from the case studies have consequences for nursery use of seeds from Oregon orchards. First, stratification requirements were strongly dependent on germination temperature (table 2). For complete germination in cool substrates (15 °C was the cool temperature in my tests), long stratification may be essential. This implies that nursery workers should have information about temperature of the soil or substrate the seeds will be germinated in. In cases where substrate temperatures are low, they may wish to consider methods of modifying temperature (Guarigla and Thompson 1985). Second, seed source, cone handling, and perhaps annual environmental effects caused variation in germination traits (tables 1, 3, 5, and 8). Long stratification, compared to short, decreased this variation, sometimes considerably (for example, table 6). Third, even when short stratification gave complete germination, longer stratification increased rate and improved uniformity (tables 4, 6,10, and 11). Quicker emergence in the nursery has several advantages. It decreases length of the danger period between sowing and emergence (Heydecker 1973/1974), lengthens the growing season and, other things being equal, results in larger (Barnett and McLemore 1984, Boyer and others 1985, Schell 1960) and stockier (Sorensen 1978) seedlings. In addition, lower variance of germination rate may decrease variation in seedling size (Waller 1985).

From the results of the case studies, the following preliminary recommendations are made for stratifying of Douglas-fir seeds from seed orchards of western Oregon. Because these recommendations are based on only two case studies, additional work is needed with seeds from other orchards and additional years. Recommendations are based on getting full germination percentage.

Substrate temperature	Stratification period
°C	*Weeks*
>23	3-5
18-22	7-9
<17	11-13

Some Pacific Northwest seed plants and nurseries already stratify Douglas-fir seeds up to 13 weeks,[11] so the recommendations are within the range of current practices. Because substrate temperature is such an important factor, spring sowing in most outdoor nurseries probably should include stratifying for at least 8 weeks to meet the above recommendations.

Stratification, particularly if longer than 2 months, needs to be done with care (Bonner and others 1974). Long stratification may reduce germination percentage slightly if seed quality is poor (although rate and uniformity are still increased). Reduced germination percentages were observed if seeds were immature when harvested (Allen 1958a, Sorensen 1980), if cones or seeds received prolonged processing (Allen 1958b), and after very long seed storage (Wakeley and Barnett 1968). Metabolic activity and heat release occur in stratification. Seed must be packaged so that heat buildup will not occur within the package (Bonner and others 1974). Insertion of an aeration tube into the stratification bag should aid exchange of gases and heat (see footnote 11). Seed molds and fungi grow during stratification. Their growth during long stratification has been reduced by massaging the stratification bags weekly (see footnote 11). Finally, seeds can germinate in stratification if they have low stratification requirements (Tanaka 1976) or if unfavorable weather for nursery sowing lengthens an already long stratification period. This potential problem can be overcome by surface drying the seeds and holding them at 2 °C at a reduced moisture content (Danielson and Tanaka 1978, Leadem 1986, Tanaka and Edwards 1986).

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