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# Douglas-fir genotypic response to seed stratification

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## Summary

Douglas-fir responses to stratification duration were studied using wind-pollinated seeds from 15 seed-orchard clones. Germinative parameters (germination capacity, peak value, germination value, and germination rate and speed) were evaluated in response to four stratification periods (0, 3, 5, and 7 weeks). Significant differences among germinative parameters were observed indicating that the five-week stratification period represents the most appropriate treatment in minimizing variation caused by genetic differences. The results indicate that the International Seed Testing Association (ISTA) rules, which focus only on germination capacity, do not provide an adequate expression of seedlot dormancy, and since the rules are aimed at bulked seedlots, genetic differences, which can be large in heterogenous forest tree seeds, are hidden. The results also demonstrate that extended stratification not only reduces the time in which seedlings become established, but also reduces seedling-emergence variation among parental lines.

## Introduction

Various studies have shown that germination parameters of seeds within a species vary according to seed source, parentage, parental nutritional status, seed pretreatment, seed maturity, environmental preconditioning during seed development, and seed size (see El-Kassaby *et al.*, 1992; Chaisurisri *et al.*, 1992 for review). Conifer seed germination involves three genomes, the seed coat (2n), megagametophyte (1n), and embryo (2n), the maternal contribution to which is four times that of the male (El-Kassaby *et al.*, 1992, 1993). The variation in germination controlled by the dominant maternal genome, often expressed as different degrees of dormancy among the parental lines from which individual seeds are obtained, can be regarded as an evolutionary mechanism for survival (Jain, 1982). This variation maximizes the species' fitness by optimizing the time of germination (Levins, 1969).

Tree seed germination can be considerably improved in many species by treating the seeds prior to sowing by stratification, a process of moist chilling that breaks dormancy (Edwards, 1980). Stratification operates by permitting some individuals in the seed population to germinate more completely, more quickly, and in a more synchronous

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fashion. In most cases, all seedlots within a species are stratified for the same prescribed periods; for example, all Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlots are routinely stratified for three weeks for laboratory testing (International Seed Testing Association, 1985), which is also the period prescribed for sowing in container nurseries in British Columbia (Leadem *et al.*, 1990).

Such standardized pretreatment, whether for nursery sowing or laboratory testing, does not take into account variations in dormancy among the parental lines represented in a bulked seedlot. It has been shown that germination capacities among stratified seeds from a Douglas-fir seed orchard can vary by a factor of almost four, from highest to lowest (El-Kassaby *et al.*, 1992). This study pointed out that stratifying seeds in a uniform manner (mimicking nursery practices) was inadequate for removing dormancy from seeds representing more than one third of the parental lines. Thus, for maximal germination capacity and germination rate to have occurred in seeds from all parent trees, seeds from individual trees would have required stratification treatments customized to their specific requirements (El-Kassaby *et al.*, 1992).

In this study, the effects of genetics (clones) and seed pretreatment (stratification) on germination parameters of seed orchard-produced Douglas-fir seeds are reported.

## Materials and methods

In the fall of 1990, wind-pollinated seeds were collected from individual ramets representing 15 Douglas-fir clones from the Pacific Forest Products' low elevation Douglas-fir seed orchard located in Saanichton, British Columbia (latitude 48°35'N, longitude 123°24'W). The clonal identities of the seeds were maintained during seed extraction and seeds were kept at 2 °C until used.

Using filled seeds only (determined by X-rays), a total of 16 random samples of 100 seeds of each clone were subjected to a standard germination test. Four of the 16 samples were tested without any stratification (i.e., no pretreatment), while the remaining 12 samples were imbibed in water for 24 h and drained in preparation for seed pretreatment. Seed pretreatment consisted of three stratification periods for each set of four samples: 21 (3 weeks), 35 (5 weeks), and 49 (7 weeks) days at 2 °C. Stratified and unstratified samples were germinated simultaneously (i.e., the stratification treatments were applied in such a way that allowed all samples to be placed in the germinator at the same time).

For germination, seed samples were spread in clear plastic germination boxes lined with moistened cellulose wadding (Kimpak®) and filter paper, then placed in a germinator set at an alternating temperature of 30 °C for 8 h followed by 20 °C for 16 h. Light, at approximately 1,000 lux, was provided during the high-temperature period by means of cool-white fluorescent tubes. Germinants were counted every day for 21 days and classified as normal or abnormal according to the ISTA (International Seed Testing Association, 1985) rules.

Results were expressed as: 1) germination capacity (GC), the percentage of seeds that had germinated at the end of the test; 2) peak value (PV), the maximum quotient de-

rived by dividing daily the accumulated number of germinants by the corresponding number of days, which is the mean daily germination of the most vigorous components of a seedlot (Czabator, 1962); 3) germination value (GV), the combination of speed and completeness of germination into a single index (Czabator, 1962); 4) germination rate ($R_{50}$), the number of days required for 50% of the seeds to germinate; and 5) germination speed ($R_{50}'$), the number of days required for 50% of the germinating seeds to germinate (Thomson and El-Kassaby, 1993). It should be mentioned while $R_{50}$ and $R_{50}'$ are used here as germination rate indices, they represent, in fact, times with units of days (see Naylor, 1981, for an evaluation of other indices related to germination time). They are used in this study due to their familiarity to forest nursery growers and tree seed analysts in general.

Data were transformed to normalize the calculated response variables and achieve homogeneity of variances (see Table 1). The germination parameters (GC, PV, GV, $R_{50}$, and $R_{50}'$) were then analyzed using analysis of variance. The following factorial additive linear model was used.

$$Y_{ijk} = \mu + C_i + T_j + CT_{ij} + \varepsilon_{(ij)k}$$

where $\mu$ = overall means,
$C_i$ = clone effect (random effect), i = 1–15,
$T_j$ = pretreatment effect (fixed effect), j = 1–4,
$CT_{ij}$ = effect of interaction between pretreatment and clone, and
$\varepsilon_{(ij)k}$ = residual term.

Source of variation, degrees of freedom, and the expected mean squares are given in Tables 1 and 2. Where significant effects were observed, means were compared using the Student-Newman-Keuls range test.

Table 1. Variation in germination parameters due to genetics (clones) and seed pretreatment (unstratified and 3 stratification times).

| S.O.V. | d.f. | E.M.S.[1] | Germination Parameters (SS%) | | | | |
|---|---|---|---|---|---|---|---|
| | | | GC[2] | PV | GV | $R_{50}$ | $R_{50}'$ |
| Clone (C) | c-1 = 14 | $\sigma^2_e + 16\,\sigma^2_c$ | 22.22** | 7.47** | 11.14** | 4.36** | 3.70** |
| Treatment (T) | t-1 = 3 | $\sigma^2_e + 4\,\sigma^2_{ct} + 60\,\phi_t$ | 51.65** | 88.02** | 83.09** | 92.56** | 93.72** |
| CxT | (c-1)(t-1) = 42 | $\sigma^2_e + 4\,\sigma^2_{ct}$ | 15.15** | 3.07** | 3.53** | 2.44** | 1.84** |
| Residual | ct(r-1) = 180 | $\sigma^2_e$ | 10.98 | 1.44 | 2.24 | 0.64 | 0.74 |

[1] $\sigma^2_c$ = variance among clones; $\phi_t$ = variance among pretreatments; $\sigma^2_{ct}$ = variance due to interaction between pretreatment and clone; $\sigma^2_e$ = variance within treatment within clones.

[2] GC = Germination Capacity, the percentage of seeds that had germinated at the end of the test (Arcsin).
PV = Peak Value, a mathematical expression of the break of a sigmoid curve representing a typical course of germination (square root (0.5 x)).
GV = Germination Value (Czabator 1962), (no transformation).
$R_{50}$ = Germination rate, the number of days required for 50% of the seeds to germinate (1-(1/(x + 1))).
$R_{50}'$ = Germination speed (Thomson and El-Kassaby 1993) (1-(1/(x + 1))).

** Significant at $P \leq 0.01$.

Table 2. Variation in germination parameters due to genetics (clones) and seed pretreatment (3 stratification times).

| S.O.V. | d.f. | E.M.S.[1] | Germination Parameters (SS%) | | | | |
|---|---|---|---|---|---|---|---|
| | | | GC[1] | PV | GV | $R_{50}$ | $R_{50}'$ |
| Clone (C) | c-1 = 14 | $\sigma^2_e + 12\ \sigma^2_c$ | 53.17** | 65.81** | 69.99** | 61.41** | 56.86** |
| Treatment (T) | t-1 = 2 | $\sigma^2_e + 3\ \sigma^2_{ct} + 45\ \phi_t$ | 0.17[ns] | 15.29** | 11.46** | 27.45** | 31.38** |
| CxT | (c-1)(t-1) = 28 | $\sigma^2_e + 3\ \sigma^2_{ct}$ | 7.75[ns] | 5.03* | 4.65* | 3.71** | 3.77** |
| Residual | ct(r-1) = 135 | $\sigma^2_e$ | 38.91 | 13.87 | 13.90 | 7.43 | 7.99 |

[1] See Table 1 for explanation.

## Results

Variation in germination parameters were highly significant ($P \leq 0.01$) for clone, pretreatment, and their interaction (Table 1). Seed pretreatment accounted for the largest proportion of variation (52–94%), while clonal variation and the interaction between seed pretreatment and clone accounted for 4–22% and 2–15% of total variation, respectively (Table 1). Unstratified seeds formed an independent group when the means of the germination parameters were compared among the seeds pretreatments studied (Table 3). Unstratified seeds produced the lowest GC (80%), PV (3), and GV (10) and the highest $R_{50}$ (17 days) and $R_{50}'$ (16 days) values (slowest germination rates) when compared to the three stratification treatments (Table 3, Figure 1, a–d). This polarization made it difficult to determine the differences among the three stratification times used.

To make seed pretreatment more discernible, a complementary analysis was conducted for pretreated seeds (Table 2). Clonal variation now accounted for the majority of the variation (53–70%) in all germination parameters and was highly significant ($P \leq 0.01$), while seed pretreatment and the interaction between seed pretreatment and clone accounted for 0–31% and 4–8%, respectively (Table 2). Pretreated seeds reached a GC of 96% and were not significantly different from each other (Table 3). Thus, extended

Table 3. Student-Newman-Keuls multiple-range tests for germination parameters among the seed pretreatments.

| GC[1] | | PV | | GV | | $R_{50}$ | | $R_{50}'$ | |
|---|---|---|---|---|---|---|---|---|---|
| Time[2] | Ave. | Time | Ave. | Time | Ave. | Time | Ave. | Time | Ave. |
| 5 | 96.47 a[3] | 5 | 10.65 a | 5 | 36.80 a | 0 | 17.37 a | 0 | 15.31 a |
| 7 | 96.43 a | 7 | 9.81 b | 7 | 33.88 b | 3 | 7.37 b | 7 | 7.31 b |
| 3 | 96.20 a | 3 | 9.42 c | 3 | 32.48 c | 7 | 7.37 b | 3 | 7.30 b |
| 0 | 79.88 b | 0 | 3.32 d | 0 | 9.83 d | 5 | 6.70 c | 5 | 6.64 c |

[1] See Table 1 for explanation.
[2] Seed pretreatment in weeks.
[3] Means, within columns, followed by the same letter are not significantly different, $P \leq 0.05$.

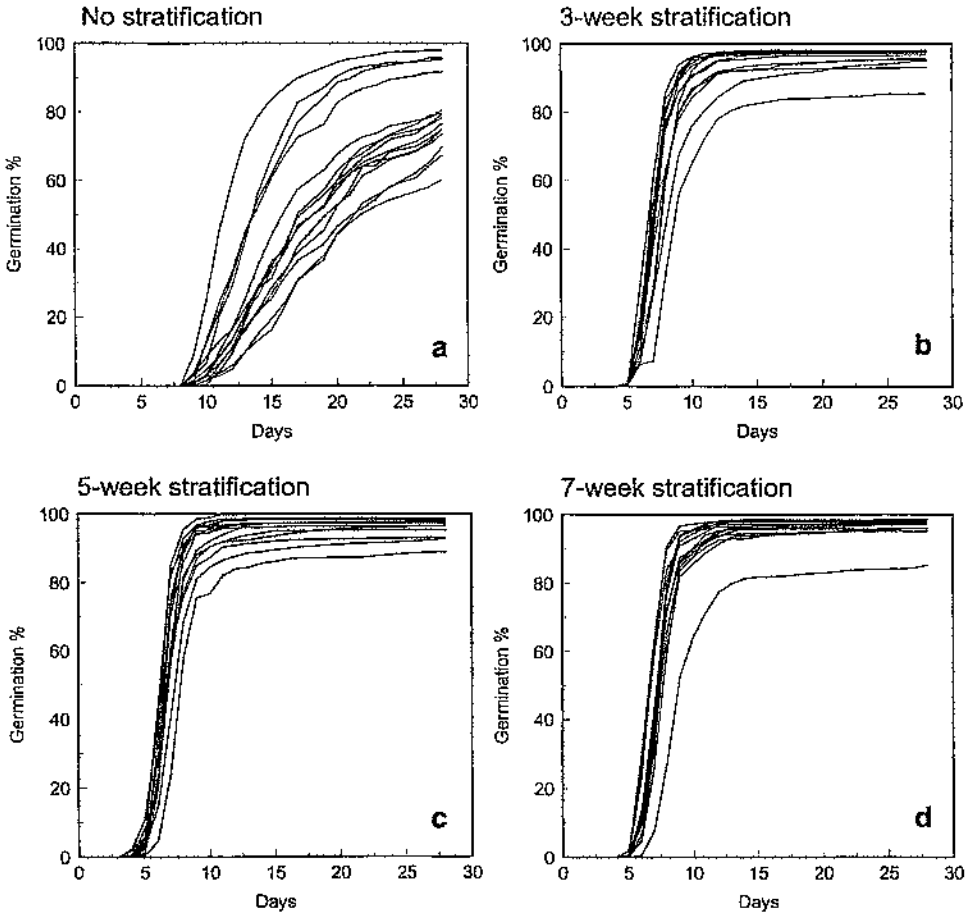


Figure 1. Germination course of 15 Douglas-fir clones. (a, unstratified seeds, and following stratification: b, 3 weeks; c, 5 weeks; d, 7 weeks).

stratification did not produce any improvement in GC and in the case of Douglas-fir, it seems a minimum three-week stratification period is adequate to reach maximum germination. The remaining germination parameters for seed pretreatment, however, all produced highly significant differences ($P \leq 0.01$) (Table 3). Five-week pretreatment formed an independent group when the means of the remaining germination parameters were compared and produced the highest PV (11) and GV (37) and the lowest $R_{50}$ (7 days) and $R_{50}'$ (7 days) values (fastest germination rates) (Table 3). It is noteworthy that while extended stratification did not result in any improvement in GC, the uniformity of germination was further enhanced by the 5-week treatment; germination started on the third day for the 5-week treatment, while the 3- and 7-week treatments started on the fifth day (Figure 1, b–c).

Clonal differences were highly significant ($P < 0.01$) and accounted for a major component of the variation (range: 53–70%) (Table 2). These differences could be summarized by grouping the clones in several statistically different sets based on the Student-Newman-Keuls range test (Table 4). Germination capacity (GC) was the only germination parameter that produced the lowest number of statistically significant sets (4 sets of clones), while PV, GV, $R_{50}$ and $R_{50}'$ were grouped into at least 7 sets, indicating that genetic differences exerted a major influence on germination parameters. For example, clone # 14 had the highest germination value (41) while clone # 2 had the lowest (22), while their respective $R_{50}'$ values were 8 vs. 6 days, respectively (Table 4).

## Discussion and conclusion

The ramifications from these results are threefold. First, the ISTA (International Seed Testing Association, 1985) rules focus on the single parameter of germination capacity (GC) determined after a test period of 21 days for this species; this limits the information that can be gained on lot dormancy because it looks only at completeness of germination. As reported here, increasing stratification from three to seven weeks produced no significant changes in GCs, so on this single parameter basis it would appear that the ISTA stratification prescription (3 weeks) was adequate. But other parameters, $R_{50}$ and PV, monitoring rate of germination, showed that considerable variation in dormancy existed among the 15 clones. Only by increasing seed pretreatment to five weeks were these clonal differences minimized.

Table 4. Student-Newman-Keuls multiple-range tests for germination parameters among the 15 Douglas-fir clones.

| GC[1] | | PV | | GV | | $R_{50}$ | | $R_{50}'$ | |
|---|---|---|---|---|---|---|---|---|---|
| Clone | Ave. | Clone | Ave. | Clone | Ave. | Clone | Ave. | Clone | Ave. |
| 14 | 98.83 a[2] | 14 | 11.48 a | 14 | 40.54 a | 2 | 8.48 a | 2 | 8.21 a |
| 9 | 98.17 a | 13 | 11.18 ab | 13 | 38.74 ab | 7 | 7.66 b | 7 | 7.56 b |
| 4 | 98.00 ab | 9 | 10.65 bc | 9 | 37.35 ab | 1 | 7.43 c | 1 | 7.38 bc |
| 10 | 98.00 ab | 5 | 10.64 bc | 15 | 37.21 bc | 3 | 7.35 cd | 3 | 7.28 cd |
| 15 | 98.00 ab | 15 | 10.62 bc | 5 | 37.19 bc | 11 | 7.32 cd | 8 | 7.23 cde |
| 5 | 97.92 ab | 4 | 10.59 bc | 4 | 37.04 bc | 8 | 7.29 cd | 11 | 7.21 cde |
| 12 | 97.67 ab | 12 | 10.40 cd | 10 | 36.31 bc | 6 | 7.14 de | 6 | 7.10 def |
| 6 | 97.50 ab | 10 | 10.37 cd | 12 | 36.27 bc | 4 | 7.05 ef | 4 | 7.02 efg |
| 13 | 97.08 ab | 1 | 9.89 cde | 1 | 34.22 cd | 12 | 7.00 ef | 12 | 6.97 fg |
| 1 | 96.92 ab | 6 | 9.81 def | 6 | 34.15 cd | 10 | 6.97 ef | 10 | 6.94 fg |
| 8 | 96.25 abc | 3 | 9.50 ef | 3 | 32.60 de | 9 | 6.92 ef | 9 | 6.89 fg |
| 3 | 96.00 abc | 8 | 9.43 ef | 8 | 32.42 de | 5 | 6.83 fg | 5 | 6.80 gh |
| 7 | 94.75 bc | 11 | 9.12 fg | 11 | 30.57 ef | 14 | 6.62 gh | 14 | 6.61 hi |
| 11 | 93.83 c | 7 | 8.61 g | 7 | 29.12 f | 15 | 6.62 gh | 15 | 6.59 hi |
| 2 | 86.58 d | 2 | 7.12 h | 2 | 22.10 g | 13 | 6.50 h | 13 | 6.46 i |

[1] See Table 1 for explanation.
[2] Means, within columns, followed by the same letter are not significantly different, $P \leq 0.05$.

With such pretreatment, the data suggest that the test period usefully could be reduced to 14 days since not only had germination culminated in almost all clones, but also dormancy was more completely removed and all clones germinated more uniformly as determined by $R_{50}$ and PV. Similary, Hoff (1987) recommended extended stratification (up to 15 weeks) to achieve maximum germination in *Pinus monticola* (Dougl.). For Douglas-fir and probably the seeds of many other forest trees, the ISTA rules appear to fall short in establishing seedlot dormancy in focussing only on GC differences. In addition, extended stratification has been shown to significantly improve bare-root nursery emergence in *Picea engelmannii* (Parry ex Engelm.), *Picea sitchensis* (Bong.), *Pinus contorta* (Dougl.) as well as in Douglas-fir when soil temperatures are low (Tanaka *et al.*, 1986; Sorensen, 1991; Jones and Gosling, 1994).

Second, the ISTA rules were devised to test bulked seedlots, but by separating the materials into individual clones the genetic effect becomes more conspicuous. Broad experience has shown that bulked forest tree seed collections from wild stands are not homogenous, in contrast to the seed crops of many highly-bred crop species. Forest seed orchard crops are likely to be even more heterogenous because the matings that occur are between parents brought together from outside their normal crossing range. Thus, seed orchard crops tend to increase genetic diversity, not to reduce it (Chaisurisri and El-Kassaby, 1994; El-Kassaby and Ritland, 1995). Seed orchard seeds, therefore, are likely to require modified pretreatment such as increased stratification, which can only be determined from studying individual clonal requirements.

Third, stratification reduces the time-window within which the seedling crop can be expected to become established. In these tests, countable germinants were first recorded after eight days in unstratified seeds (Figure 1, a), after four days following three and seven weeks' stratification (Figure 1, b and d), but after only three days following five weeks' stratification (Figure 1, c). Half the seeds sown without stratification were countable ($R_{50}$) between 12 days (fastest germinating clone) and 22 days (slowest germinating clone) (Figure 1, a). This was reduced to between 6 and 8 days following three weeks' stratification (Figure 1, b), between 7 and 9 days following seven weeks' stratification (Figure 1, d), and between 6 and 8 days following five weeks' stratification (Figure 1, c). These differences may seem small, but when means were compared, the $R_{50}$ for seeds stratified for five weeks was significantly less than that for seeds stratified for zero, three and seven weeks (Table 3).

Thus, by using the optimal stratification duration, five weeks, in a growth-house a higher percentage of the seedlings will become established more quickly and with less variation among the parental lines, reducing growth-house energy consumption and costs, and permitting the switch from managing sown seeds to a seedling crop to be made sooner. By reducing germination variability among the clones, the seedling crop is more likely to represent the genetic base from which it was derived since there will be less likelihood of eliminating slow-germinating clones by manual thinning (of multiple-seeded cavities) in containerised nursery systems, or by the need to cull sub-optimally-sized plants in traditional nursery systems.

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