# EFFECTS OF STRATIFICATION AND SIMULATED AGING ON GERMINATION OF DOUGLAS-FIR SEED FROM A CLONAL SEED ORCHARD

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*Received January 16, 2003; accepted April 30, 2003*

## ABSTRACT

Seed from 15 Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) clones were germinated in a factorial design with two pre-treatments (unstratified and stratified) and seven simulated aging periods (0, 2, 4, 7, 10, 12 and 14 days). Simulated aging consisted of high temperature (40 °C) and humidity (100 % RH) exposure, which simulates physiological stresses and consequent deterioration in long-term storage. Seed deteriorated as aging treatments lengthened; no germination occurred after 12 days. Germination parameters (capacity, peak value, speed, completeness) were calculated, and pre-treatment and aging effects evaluated using a mixed model analysis of variance. Germination completeness and speed were higher after two days of aging for stratified seed, whereas only peak value increased for unstratified seed. After four days aging, all parameters decreased. Two days of aging enhanced germination capacity of unstratified seed by 15 %, but stratified seed was still 13 % higher. Douglas-fir seed should be stratified before germination, but unstratified seed can be exposed to 40 °C and 100 % humidity for two days to augment seedling stock during the growing season. *Ex situ* Douglas-fir genetic resource conservation, as well as more adequate representation of planted genotypes across the landscape, can benefit from two days of aging, which would ensure slowly-germinating genotypes are represented in the population.

**Keywords:** *Pseudotsuga menziesii*, germination parameters, stratification, aging.

## INTRODUCTION

Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) is of primary importance in the Pacific northwest coastal forest industry. Its straight bole, rapid growth on productive sites, and high quality wood make it a highly desirable timber and sawlog species (HERMANN & LAVENDER 1990). Stands of massive, ancient Douglas-fir with its deeply grooved bark enhances the aesthetic character of many British Columbia (B.C.) and Washington coastal forests, which are heavily used for recreation and by First Nations people for contemporary and traditional cultural purposes. This species is also important for biodiversity since it provides habitat for many cavity nesting bird and mammal species, bald eagle and spotted owl nest sites (GUTTIEREZ *et al.* 1998; HERSHEY *et al.* 1998), and a plethora of insects, fungi and other microorganisms in its unique contiguous canopy ecosystems (PROGAR & SCHOWALTER 2002).

Nearly all sites from which Douglas-fir is harvested in B.C. are regenerated by planting nursery-grown seedlings (B.C. MIN. FOR. 2002; FOR. GEN. COUNCIL B.C. 2002). Various damaging agents, including frost pockets, vegetative competition and ungulate browsing, have spurred the production of a variety of seedling sizes and ecotypes. Adaptive variation at large and small scales is highly clinal in this species (CAMPBELL 1979; SILEN & MANDEL 1983; REHFELDT 1986), and seed transfer guidelines have been established accordingly (B.C. MIN. FOR. 2002). The efficient production of suitable seedlings can be optimized by understanding physiological and environmental cues to which Douglas-fir responds, and assessing how seed treatments affect the degree of genetic control (EL-KASSABY *et al.* 1993).

While commercial nurseries have been producing large quantities of Douglas-fir seedlings for decades, new research and technologies can always provide additional opportunities for fine-tuning and efficiencies. Stratification is a standard treatment for the species: seeds are left under running water for several days to stimulate metabolic activity, then refrigerated under moist conditions for weeks to months (SORENSEN 1980, 1991; DUMROESE *et al.* 1988). This procedure generally results in more even germination and a higher germination rate. Different provenances and individual trees

have different responses to these treatments; often seed sources with slow initial germination are partially or entirely eliminated (culled), thus reducing their genetic representation in the future stand (ST. CLAIR & ADAMS 1993; EL-KASSABY 2000a,b).

Accelerated, or simulated aging (DELOUCHE & BASKIN 1973; KUENEMAN 1983) is a common technique for agricultural seed viability assessments, but has not yet been operationally incorporated into forest seedling production. It consists of exposing seeds to high temperature and humidity in order to simulate the effects and stresses of long-term storage within a number of days (DELOUCHE & BASKIN 1973). The main application of this technology has been to gauge the effectiveness of storage conditions and the deterioration rate of seeds (CHAISURISRI *et al.* 1993; EDWARDS & EL-KASSABY 1995). In some conifers which have strong physiological dormancy, brief periods of accelerated aging have been found to enhance germination capacity and completeness (CHAISURISRI *et al.* 1993; EDWARDS & EL-KASSABY 1995; WANG & BERJAK 2000). If this treatment is applied correctly, it may reduce the need for time-consuming stratification and achieve more uniform germination, ensuring better proportional representation of various seed sources.

The objective of this study was to quantify the effects and interactions of various degrees of simulated aging on germination of Douglas-fir seed obtained from different genetic backgrounds after subjecting the seed to the typical commercial pre-germination treatment of stratification, and to assess potential *ex situ* conservation applications of simulated aging for Douglas-fir.

## METHODS AND MATERIALS

Five thousand six hundred seeds each of 15 Douglas-fir clones were obtained from a south coastal British Columbia seed orchard. Half of the seeds were pretreated (stratified) in accordance with typical operational guidelines (ISTA 1993), the other half remained as a control (unstratified). They were then subjected to seven levels of simulated aging at 100 % relative humidity (RH) and high temperature (40 °C): 0 (control), 2, 4, 7, 10, 12 and 14 days. Each combination of seed pretreatment and aging level was replicated four times for each clone, with each replicate containing 100 seeds. Seeds were then germinated following the protocol outlined for the species by the International Seed Testing Association (ISTA 1993). Cumulative germination counts were then recorded over the 28-day germination period of the experiment. Treatments were coordinated so that germination began simultaneously for all treatments, and the entire experiment was concluded on the same date. Simulated aging treatments were initiated first for the 14-day aging period, two days later for the 12-day aging period, and so on.

Outlying germination curves, most likely due to damaged germination boxes, were assessed using graphical comparisons; replications deviating from the mean of the other three replications by 25 % or more were excluded from subsequent analysis. Four widely-used germination parameters calculated using the GERM.BAS Quickbasic program (copies of the program are available upon request) were evaluated. These are: R50', germination speed, the number of days required for germination of one half of living (germinable) seed (THOMSON & EL-KASSABY 1993); germination capacity (GC), the percentage of seed germinated by the end of the experiment, in this case, after 28 days; peak value (PV), the mean daily number of germinating seeds of the top-ranked (in terms of germination speed and numbers) seed (EL-KASSABY *et al.* 1992), and germination value (GV), which integrates the former measures by quantifying germination speed and completeness throughout the experiment, calculated by multiplying mean germination throughout the experiment by PV (CZABATOR 1962).

Statistical analysis was conducted on untransformed data using the MIXED procedure in SAS V.8.2© (SAS Institute, Cary, NC, 2001), where clone and replication were random, and pretreatment (stratification) and aging fixed. This analytical procedure was designed to facilitate interpretation of analysis of variance with untransformed data utilizing mixed models. Significance levels were set at $\alpha = 0.05$. Variance components were estimated using the REML (restricted maximum likelihood) iterative procedure. To clarify the effects of stratification on aging, separate analyses were done for each aging duration for stratified and unstratified seed (Equation [1], and for both treatments combined (Equation [2]). Significant differences between fixed effects were assessed using the Tukey test, adjusted for sample size and number of comparisons.

$$y_{cr} = \mu + C_c + \varepsilon_{(c)r} \quad [1]$$

$$y_{tcr} = \mu + T_t + Cc + TC_{tc} + \varepsilon_{(tc)r} \quad [2]$$

where $y_{tcr}$ represents germination parameter $y$, for the $t^{th}$ treatment ($T$), the $c^{th}$ clone ($C$), the $r^{th}$ replicate; m is the grand mean; $TC_{tc}$ is the interaction between the $c^{th}$ clone and $t^{th}$ treatment; brackets indicate nesting within the $r^{th}$ replicate; $\epsilon$ is the residual error term.

## RESULTS

No seeds germinated after 12 days of aging, so the 12 and 14-day aging treatment levels were dropped from the data set, thus analyses were conducted on the remaining five aging levels (0, 2, 4, 7 and 10 days). Sixteen individual outlying germination replicates were also excluded: six unstratified and ten stratified. Stratification had a statistically significant effect on mean values of all germination parameters until the seventh day of simulated aging (i.e., following 0, 2 and 4 days of simulated aging) (Table 1, Figures 1 and 2). For all parameters except R50', stratification caused a statistically significant increase; for R50', the converse occurred, indicative of more rapid germination (a lower R50' reflects a shorter period for 50 % of living seeds to germinate). No significant treatment effects were detectable after seven or ten days of simulated aging. The MIXED procedure only provides Type 3 tests of fixed effects, so it was not possible to determine the statistical significance of the stratification by clone interaction, although standard errors of the mean were small (typically <1–10 % of the mean).

The R50' was consistently highest for the unstratified treatments, and all aging treatments resulted in a lower mean than the control (16.8, Table 1). From two to seven days of aging, R50' ranged from 13 to 14, thus the only rapid decline occurred after ten days of simulated aging (Figure 1a). Germination capacity was stable after two days of aging for stratified clones, but declined steadily after subsequent aging (Figure 1b). Unstratified clones experienced an increase from 65 to 79 % in GC after two days of aging (i.e., a conditioning effect), after which values decreased. By the seventh day of aging there was no difference between the stratified and unstratified treatments. PV for stratified treatments was over 100 % greater than for unstratified treatments in the control and after two days of aging. The difference was less pronounced after four days, and there was no significant difference after further aging (Table 1). Both unstratified and stratified seed experienced higher PV after two days of aging relative to the control, after which values decreased. After two days of aging, both stratified and unstratified seed increased relative to the control: unstratified seed increased from 10 to 16, and stratified seed from 34 to 40, with values decreasing by nearly half after four days of simulated aging, and dropping near zero after further aging (Table 1).

## DISCUSSION

The response of Douglas-fir seed to stratification and simulated aging is clearly an inherent trait found throughout its range. All clones increased their germination capacities when stratified, despite their differing absolute GCs. Germination speed, or R50', tended to show opposite trends to the other three germination parameters. Larger R50' values indicate slower germination since this provides an index of the time for one half the viable seeds to germinate (THOMSON & EL-KASSABY 1993). Stratification resulted in faster germination, but as aging treatments increased in length there was less difference between unstratified and stratified seed. For GC, the overall proportion of seed which germinated, two days of simulated aging caused a physiological effect similar to stratification in unstratified seed, relative to the control (no aging). This effect was also found for Sitka spruce (*Picea sitchensis* (Bong.) Carr.) (CHAISURISRI *et al.* 1993) and mountain hemlock (*Tsuga mertensiana* (Bong.) Carr.) (EL-KASSABY & EDWARDS 1998). Both GV and PV, since PV is a multiplier of GV, had similar trends and values. The increased GC for unstratified seed after two days of simulated aging relative to the control may enable commercial seedling producers to bypass the lengthy stratification period by exposing seed to high temperature and humidity for two days in the event that some seedlots need to be re-sown during the same production

**Table 1. Mean germination parameter values of 15 Douglas-fir clones after simulated aging. Different superscript indicate significant differences between stratification treatments at a given aging level (adjusted $p < 0.05$, Tukey's HSD).**

| Germination parameters[1] | 0 days | | 2 days | | 4 days | | 7 days | | 10 days | |
|---|---|---|---|---|---|---|---|---|---|---|
| | US[2] | S | US | S | US | S | US | S | US | S |
| R50` | 16.80[a] | 8.69[b] | 13.17[a] | 7.38[b] | 13.46[a] | 10.09[b] | 14.10 | 13.29 | 3.91 | 1.74 |
| GC | 64.63[a] | 93.98[b] | 79.34[a] | 93.02[b] | 55.88[a] | 67.48[b] | 8.30 | 8.81 | 1.23 | 0.40 |
| PV | 3.09[a] | 7.48[b] | 4.07[a] | 8.98[b] | 2.93[a] | 4.39[b] | 0.41 | 0.44 | 0.06 | 0.02 |
| GV | 10.16[a] | 33.66[b] | 15.74[a] | 39.95[b] | 8.47[a] | 14.92[b] | 0.30 | 0.38 | 0.02 | 0.01 |

[1] R50`: germination speed, GC: germination capacity, PV: peak value, GV: germination value.
[2] US: unstratified, S: stratified

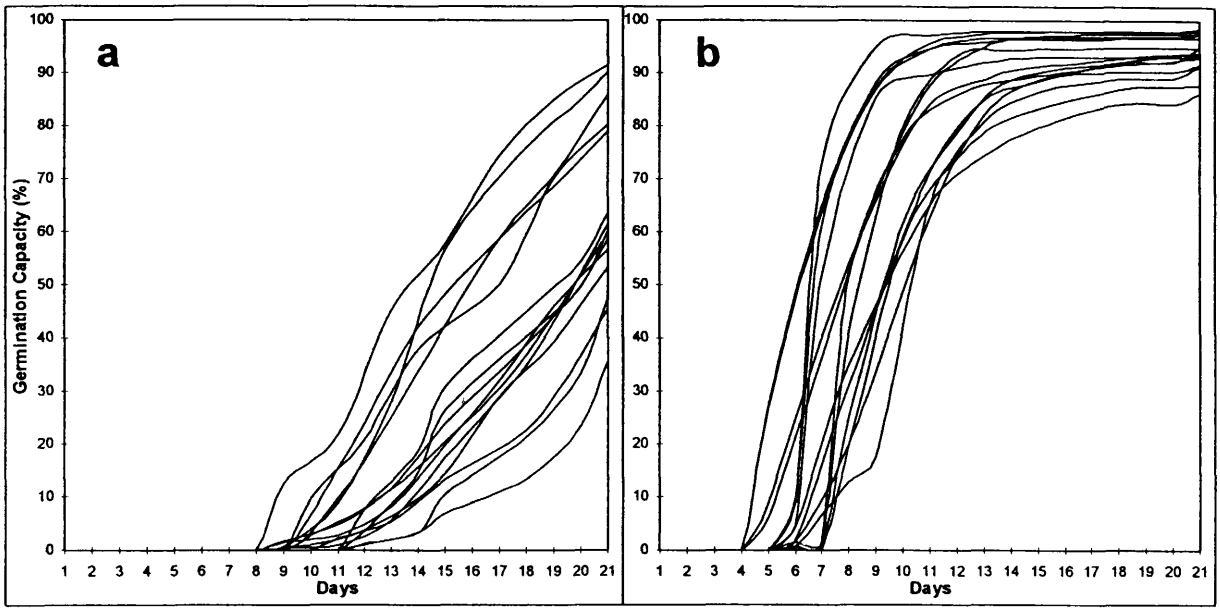


**Figure 1.** Germination courses of 15 Douglas-fir clones with and without stratification pretreatment (a: unstratified; b: stratified).

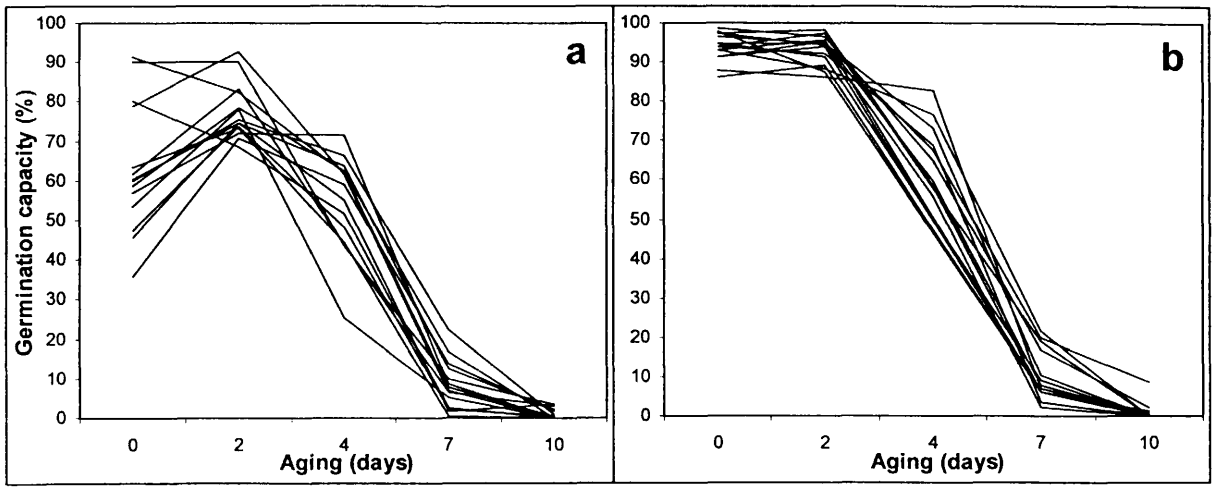


**Figure 2.** Germination capacity of 15 Douglas-fir clones subject to five levels of simulated aging pretreatment (a: unstratified; b: stratified).

season. Given the 13 % higher GC for stratified seeds than for the unstratified after two days of aging, it would still be preferable to pre-stratify seed for commercial production. This treatment is suggested only if unfavorable events occur in the nursery and a repeat sow is considered.

Stratification resulted in an increase in germination speed and increased overall uniformity of germination in terms of proportion and rate of germination across genotypes (Table 1, Figures 1 a, b). With high stress levels (four, seven or ten days of aging), germination was more efficient for stratified seed, although overall germination was far lower than for seed subject to no or two days of aging (Table 1). Stratified seed was thus more robust to prolonged physiological stress.

Naturally regenerated Douglas-fir seed in B.C. is dormant from approximately late August to April (HERMANN & LAVENDER 1990). Seed is frequently contained over winter within partially opened cones, and as little as one third may be released from mature cones in the fall (HERMANN & LAVENDER 1990). This long dormancy period and ability to withstand severe environmental conditions including high humidity and long cold periods is evidence of the adaptive significance of dormancy. Commercial seed producers simulate a shorter version of winter dormancy, and stimulate germination when desired by controlling environmental conditions to meet chilling requirements more rapidly.

Douglas-fir seed still germinates when unstratified, but as apparent from the results of this and other studies, genotypes vary widely in their capabilities and germination is less uniform and complete than in stratified seed (SORENSEN 1980; EL-KASSABY *et al.* 1992; EDWARDS & EL-KASSABY 1995).

Prior studies have revealed that genotypes and allele frequencies found among selected parental trees are often dramatically different in the seedlings produced by typical nursery practices (e.g., EL-KASSABY *et al.* 1993; EDWARDS & EL-KASSABY 1996; SORENSEN 1999; but see ST. CLAIR & ADAMS 1993). These differences arise following culling of genotypes which tend to germinate later or more slowly; such trends are especially pronounced when multiple seeds are sown in one container, where competition among families is exaggerated (EL-KASSABY 2000a). Quantifying the differential responses among clones to stratification and aging will provide managers of production nurseries and seed orchards with requisite information to ensure that the genetic diversity represented in selected parent trees is captured in the next generation of seedlings produced (EL-KASSABY 2000a). Slightly modifying ISTA seed germination standards to accommodate among-family variability may facilitate this (EDWARDS & EL-KASSABY 1995) Taking trees' inherent genetic differences into account when germinating seedlots, which are often bulked for seedling production, will ensure that genotypes with slower initial germination can still be represented in the gene pool (EL-KASSABY & EDWARDS 1998). EL-KASSABY (2000a) noted that performance of extremely young germinants and seedlings is not related to future performance in the field. Sowing a single seed per cavity for these slower germinating seedlots, or keeping individuals within seedlots separate (i.e., not bulking collections) and maintaining the common operational practice of sowing multiple seeds per cavity, can ensure that the maximum genetic diversity is deployed in the field, and conserved in the long term on the land base. Subjecting all, or even only slow-germinating, seedlots to two days of simulated aging may provide a more complete spectrum of genotypes in the population and maintain representation of all families throughout the planting area. Maintaining representative genotypes in seed orchards, clone banks and other *ex situ* conservation applications, specifically including those with variable germination characteristics, can augment current practices which focus primarily on selection for characteristics evident in the mature tree, such as branch angle or bole straightness.

## CONCLUSION

While germination capacity among clones was moderately differentiated, all clones showed consistent responses to stratification, which increased all parameters except R50', where lower values indicate faster germination, and aging, which increased all parameters except R50' after two days. Responses of parameters varied following four days of simulated aging, but mean values of all germination parameters declined sharply after seven days, and very little germination occurred after ten days. Aging past two days caused germination capacity to rapidly decrease. Commercial Douglas-fir seedling producers may benefit in the event of a catastrophe by subjecting unstratified seeds for two days of simulated aging as substitute 13 % less efficacious than stratification in order to maintain the current year's production. Modifying related nursery practices, especially culling more slowly germinating individuals within bulked seedlots, can maintain a more complete spectrum of seedling genotypes, which will be subsequently maintained in the landscape. Managers of *ex situ* Douglas-fir genetic resources, including clone banks and seed orchards, may benefit when more genotypes are represented if families or individuals which germinate more slowly are subjected to two days of simulated aging to enhance germination rapidity, and prevent their loss via culling based on later emergence.

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