*Figure 2.* — Hairy roots on medium $ACM_W$ after 28 days of *culture.*

roots increased their fresh weight 45-fold from 84 mg to 3800 mg during the same period with a maximum daily increase of 120 mg/day between the 14th and 38th day (*Fig. 1*). The *in vitro* grown roots ramified considerably (*Table 1*) until a dense network of side roots had formed (*Fig. 2*).

### Discussion

Root cultures and especially hairy root cultures have been established for a variety of herbaceous plant species but only rarely for tree roots (INGRAM and HELGESON, 1980; MUGNIER, 1988). No such cultures have been reported for trees considered suitable for rapid biomass production. In accordance with earlier reports on different plant species (QUATTROCHIO, 1985; RHODES et al., 1987) it was not possible to obtain cultures of normal roots of *Salix alba* with growth rates suited for routine experimentation although some optimization was demonstrated. Since hairy roots of herbaceous plants generally have much higher growth rates than normal roots, *Salix* plants were infected with *A. rhizogenes* and roots with high growth rates (elongation, ramification, fresh weight) were obtained. These putatively transformed roots (TEPFER, 1990) will serve as an host source for the study of root symbionts and pathogens.


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# Genetic Control of Germination Parameters in Douglas-fir and its Importance for Domestication


By Y. A. EL-KASSABY[1],[2], D. G. W. EDWARDS[3] and D. W. TAYLOR[3]



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[1] Canadian Pacific Forest Products Limited, Tahsis Pacific Region, Saanich Forestry Centre, 8067 East Saanich Road, RR # 1, Saanichton, B. C., V0S 1M0, Canada

[2] Faculty of Forestry, University of British Columbia, Vancouver, B. C., V6T 1W5, Canada

[3] Forestry Canada, Pacific Forestry Centre, 506 West Burnside Road, Victoria, B. C., V8Z 1M5, Canada



### Abstract

The genetic control of germination in Douglas-fir [*Pseudotsuga menziesii* (MIRB.) FRANCO] was studied using wind-pollinated seed collected from 19 seed orchard trees. Seed-donor trees and the seed orchard were carefully selected to minimize any environmental-precondi-

tioning effects. Broad-sense heritabilities of germination parameters ranged from 0.80 for germination rate to 0.93 for germination value. Germination capacities among trees varied by a factor of nearly four, from highest to lowest. It was concluded that Douglas-fir germination, especially germination speed, is under strong maternal control, however, no relationship between seed size, expressed by 1000-seed weight, on either germination capacity or speed was observed. The impact of these genetic differences on the diversity of nursery seedling crops is discussed.

*Key words:* Douglas-fir, germination, heritability, maternal effect, environmental-preconditioning.

## Introduction

Improvements in plant crops, whether for food, ornamental or other aesthetic uses, are associated with reductions in genetic diversity. This is the basis of the agricultural model in which selected, breedable lines of plants or varieties are used to produce crops characterized by maximal homogeneity. The genetic base of new generations of the crop become extremely narrow. In effect, many major agricultural crops have been custom "designed" to meet specific needs. This has led to highly successful, massive, improvements in food production that, along with better crop husbandry, have become the foundation of the economies of many developed nations. For example, wheat yield in Europe increased by 105% between 1967 and 1983, and 47% of this increase is due to the production of new varieties (National Institute of Agricultural Botany, 1989).

Attempts at improving forestry crops follow the agricultural model, but for a variety of reasons that largely relate to the extended life cycle of trees, developments lag far behind. Tree-improvement programms still remain, by and large, attemps at domestication of wild species. Successful domestication of any wild-tree population requires knowledge of ,and respect for, the biological diversity of the species.

Forest tree-improvement programs are structured on three main stages: selection, breeding and testing. After testing, superior genotypes are propagated to establish seed orchards for seed production; seeds collected from the seed orchards are then used for seedling production for reforestation purposes. For effective seedling production in a nursery, germination and growth traits of the young plants have to be uniform. In modern, "high-tech" containerized nurseries, in which seedlings are grown in individual, small containers, production costs require that each and every container hold a growing seedling; to achieve this, growers typically sow more than one seed per cavity, then remove unwanted germinants to achieve uniformity. However, the goal in the seed orchard is to maximize diversity. Thus, a paradox exists: achieving uniformity of emergence in the nursery is in conflict with maintaining diversity of the species if genetic variability in seedlings emergence exists. While some dramatic gains have been achived from tree improvement, knowledge of the components that have genetic control with adaptive significance is still lacking for several species of major economic importance.

The first seed orchard was established in coastal B. C. in 1963, and by 1985, 35 orchards were devoted to the production of improved coastal Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] seeds (Hansen, 1985). Between 1985 and 1989, 59% of all sources of coastal Douglas-fir seeds were collected from seed orchards. During this same period 12.2 million seedlings were grown, of which 7.2 million (59%) were from seed orchard seeds. Sowing requests received by the British Columbia Ministry of Forests for the 1990 growing season will consume almost 273kg of Douglas-fir seeds from coastal sources. Of these, 47% (approximately 129 kg) will be obtained from seed orchards and will be used to grow more than 8.6 millions seedlings (M. Pelchat, B. C. Ministry of Forests, Silviculture Branch, per comm., September 1990).

While these statistics illustrate a major domestication effort, very little ,if any, information has been gathered on the genetic control of germination of Douglas-fir seeds, a species known to exhibit considerable heterogeneity in wild populations. This study was designed to examine some aspects of genetic control of germination, and to determine how the integrity of the diversity of the species is being affected through domestication efforts.

## Materials and Methods

In the fall of 1988, the cone crops of 19 unrelated trees were harvested from Canadian Pacific Forest Products Limited's 6-ha, full-sib Douglas-fir seed orchard located in Saanichton, B. C. (latitude 48° 35', longitude 123° 24'; elevation 50 m). Tree by tree, cones were counted, then placed in burlap sacks and stored in an open-sided, freely ventilated shed for eight weeks before seed extraction. Seeds were extracted and cleaned by hand, and filled and empty seeds (identified by X-ray) were separated using an aspirator separator. The total number of filled seeds produced by each tree was determined from the average weight of ten samples (1,000 seeds each) plus the total weight of seeds per tree.

Using filled seeds only, four replications of 100 seeds each from each tree were prechilled for 21 days at +2°C prior to a standard germination test. Only prechilled seeds were used in this study to mimic the standard Douglas-fir seed treatment for container seedling production in coastal British Columbia. All seed samples were spread in clear plastic germination boxes lined with moistened cellulose wadding (Kimpak) and filter paper, and placed in an incubator set at an alternating temperature of 30°C for 8 hours followed by 20°C for 16 hours. Light, at approximately 1,000 lux, was provided during the high-temperature period by means of cool-white fluorescent tubes. Germinants were counted on alternate days for 28 days and classified as normal or abnormal according to the ISTA (International Seed Testing Association, 1985) rules. Results were expressed in four ways: (1) germination capacity (GC), the percentage of seeds that had germinated at the end of the test, (2) germination rate ($R_{50}$), the number of days required for 50% of the seeds to germinate, (3) peak value (PV) (Czabator, 1962), the maximal quotient derived by dividing daily the accumulated number of germinants by the corresponding number of days (which is the mean daily germination of the most vigorous component of the seedlot, and is a mathematical expression of the tangent drawn through the origin of the sigmoid curve representing a typical course of germination), and (4) germination value (GV), computed by Czabator's (1962) procedure, which combines both speed and completeness of germination into a single value. The higher the GV, the faster and/or the more complete the germination.

Germination parameters (GC, $R_{50}$, PV, and GV) were analysed using a simple one-way ANOVA after appropriate data transformation. Data transformations were

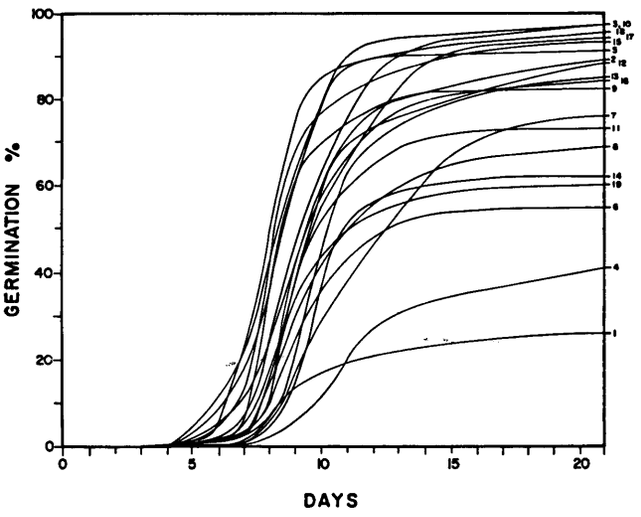


*Figure 1.* — Germination curves for 19 Douglas-fir families.

conducted using an ad-hoc procedure for finding appropriate transformations to normalize the calculated response variables and achieve homogeneity of variances. Source of variation, degrees of freedom, and the expected mean squares are given in *table 1*. Where significant treatment effects were observed, means were compared using the Student-Neuman-Keuls range test. The germination course of every tree was graphically presented to represent visual comparisons (*Figure 1*). Using the multiple measurements concept of animal breeders (FALCONER, 1960, pg. 142), the repeatability of these parameters was determined and used to represent the broad-sense heritabilities (replications were considered as multiple measurements per tree) of the four germination parameters.

The relationships between the 1,000-seed-weight and the four germination parameters were assessed using PEARSON'S product-moment correlation to investigate the presence/absence of seed size-germination parameters associations.

*Table 1.* — Estimates of variance components, significance level, and broad-sense heritabilities ($h^2_b$) for germination parameters of 19 Douglas-fir families.

| Source of Variation | Degrees of[1] Freedom | Expected[2] Mean Square | Germination Parameters[3] GC | $R_{50}$[4] | PV | GV |
|---|---|---|---|---|---|---|
| Among trees | t-1 | $\sigma^2_w + K\sigma^2_B$ | 0.058** | 0.00011813** | 3.602** | 1.681** |
| Residual | t(r-1) | $\sigma^2_w$ | 0.005 | 0.00003039 | 0.348 | 0.134 |
| $h^2_b$ | | | 0.92 | 0.80 | 0.91 | 0.93 |

[1]) t = # of trees (19); r = # of replications (4)

[2]) $\sigma^2_B$ = variance among trees; $\sigma^2_w$ = variance within trees; K = coefficient of variance component (4)

[3]) GC = germination capacity; the percentage of seeds that had germinated at the end of the test (Arcsin)

$R_{50}$ = germination rate; the number of days required for 50% of the seeds to germinate (1—1/x+1)

PV = peak value; a mathematical expression of the break of a sigmoid curve representing a typical course of germination (no transformation)

GV = germination value (CZABATOR, 1962), ($\sqrt{x+0.5}$)

[4]) ANOVA for $R_{50}$ was only conducted on trees with GC >50% for all four replications (t=16)

**) Significant at $P \leq 0.01$

## Results

Highly significant differences ($P<0.01$) were observed among the 19 trees for all four germination parameters (*Table 1, Figure 1*). Estimates of broad-sense heritabilities, determined from the among-tree source of variation, were high and significant. They ranged from 0.80 ($R_{50}$) to 0.93 (GV), indicating the presence of high genetic control of germination in Douglas-fir.

Germination capacity (GC) varied widely, from less than 26% (tree # 1) to over 96% (tree # 10) (*Table 2*). Although there were exceptions, trees that germinated nearly completely also germinated quickly, and vice versa; for example, tree # 18, which had the third highest GC (95.25%), germinated the fastest ($R_{50}$ = 8.1 days). This relationship was more clearly observable between GC and peak value (PV) (*Table 2*). Seeds of trees # 1, 4 and 6 germinated less than 50% for one or more replications, and it was decided to exclude them from the analysis of $R_{50}$. (Statistical manipulation of indeterminant values, such as treating them as missing observations or replacing them by the average of the other replications, tends to overestimate the seeds' real biological capabilities.) Since PVs can be calculated even when germination does not reach 50%, and since this parameter is also a measure of germination speed, there might be a relationship between PV and $R_{50}$. The relationship was demonstrated by an $r^2$ of —0.95. Thus, among the 16 trees for wich $R_{50}$ could be determined, those with low $R_{50}$s (i. e., fast germination) produced high PV's; for example, seeds of tree # 18 required 8.1 days to reach 50% germination, and produced the highest PV (8.83), while seeds of tree # 7 had the highest $R_{50}$ (12.6 days) and the lowest PV (4.53) (*Table 2, Figure 1*). GV is dependent upon completeness of germination (defined by CZABATOR, 1962, as the mean daily germination obtained by dividing the accumulated number of germinants by the total number of days in the test) and speed of germination (PV), so it was not surprising to observe a close agreement between the relative ranking of the 19 trees for GV, PV and $R_{50}$ (*Table 2*). GVs varied from a high of 40.09 (tree # 18) to a low of 2.25 (tree # 1).

None of the correlation analyses between 1,000-seed-weight and the four germination parameters showed any significant association, indicating that neither speed nor

*Table 2.* — STUDENT-NEWMAN-KEULS multiple-range tests for germination parameters among 19 Douglas-fir trees[1]).

| Germination Capacity (GC) | | Germination Rate ($R_{50}$)[2] | | Peak Value (PV) | | Germination Value (GV) | |
|---|---|---|---|---|---|---|---|
| Tree # | x | Tree # | x | Tree # | x | Tree # | x |
| 10 | 96.75 a | 7 | 12.6 a | 18 | 8.83 a | 18 | 40.09 a |
| 3 | 96.50 a | 8 | 11.6 ab | 3 | 8.49 a | 3 | 39.06 a |
| 18 | 95.25 ab | 14 | 11.4 ab | 5 | 8.08 ab | 5 | 35.15 ab |
| 15 | 93.50 ab | 19 | 11.4 ab | 15 | 7.74 ab | 15 | 34.46 ab |
| 17 | 93.50 ab | 13 | 10.3 bc | 10 | 7.17 bc | 10 | 33.01 ab |
| 5 | 91.50 abc | 11 | 10.3 bc | 9 | 7.08 bc | 17 | 28.14 bc |
| 2 | 88.50 bc | 17 | 9.8 c | 2 | 6.59 cd | 9 | 28.14 bc |
| 12 | 88.50 bc | 16 | 9.7 c | 12 | 6.49 cd | 2 | 27.86 bc |
| 13 | 85.00 cd | 12 | 9.6 cd | 17 | 6.31 cd | 12 | 27.32 bc |
| 16 | 83.75 cd | 2 | 9.5 cd | 16 | 6.03 cd | 16 | 24.10 cd |
| 9 | 82.25 cde | 10 | 9.2 cde | 13 | 5.70 de | 13 | 23.07 cd |
| 7 | 76.00 def | 9 | 8.5 def | 11 | 5.36 de | 11 | 18.78 de |
| 11 | 73.25 ef | 3 | 8.5 def | 14 | 4.73 ef | 7 | 16.52 e |
| 8 | 69.25 fg | 15 | 8.3 ef | 8 | 4.62 ef | 8 | 15.52 e |
| 14 | 62.00 gh | 5 | 8.2 ef | 19 | 4.56 ef | 14 | 14.05 e |
| 19 | 60.25 gh | 18 | 8.1 f | 7 | 4.53 ef | 19 | 13.20 e |
| 6 | 55.00 h | | | 6 | 4.11 f | 6 | 10.91 f |
| 4 | 41.00 i | | | 4 | 2.44 g | 4 | 4.91 g |
| 1 | 25.75 j | | | 1 | 1.79 g | 1 | 2.25 h |

[1]) Trees sharing a common letter are not significantly different at $P<0.05$.

[2]) Three trees (#'s 1, 4 and 6) were not included in the test due to the indeterminant value of their $R_{50}$ caused by GC<50% in all or some replications.

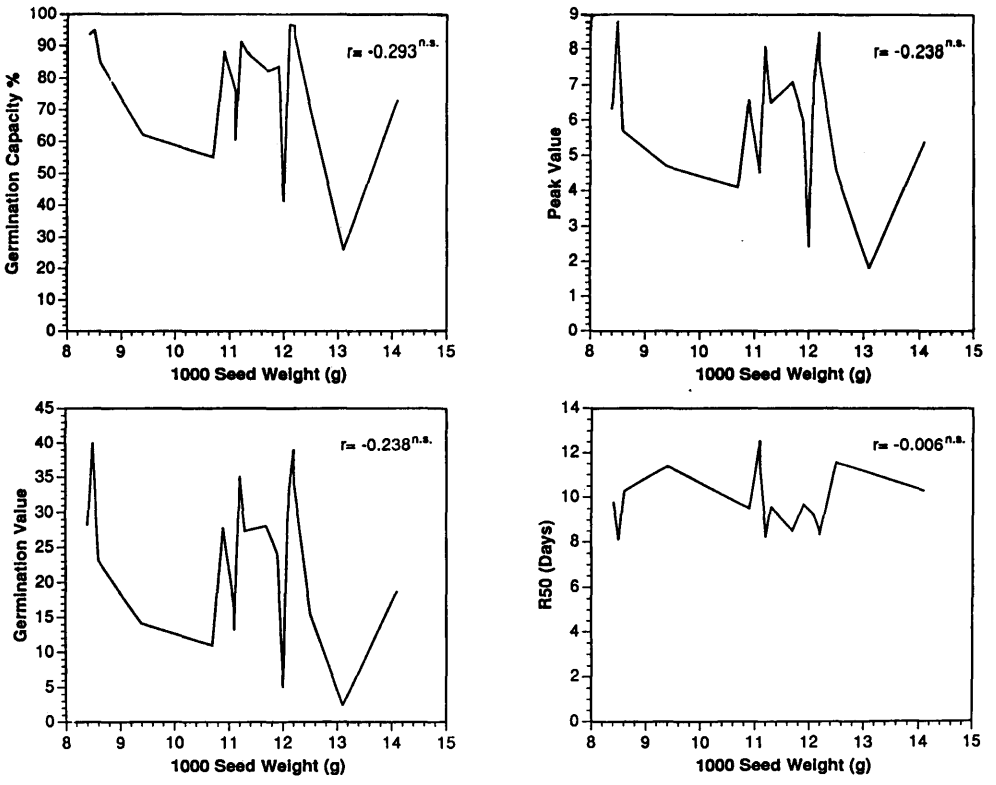


*Figure 2.* — Relations between germination parameters (GC, $R_{50}$, PV and GV) and 1000-seed weight (ns: not significant $P<0.05$).

completeness of germination was associated with mean seed weight ($r = -0.296$, $-0.244$, $-0.240$ for GC, PV and GV, respectively, $n = 19$, $P>0.05$, and $r = -0.008$ for $R_{50}$ $n = 16$, $P>0.05$) (*Figure 2*).

## Discussion

Genetic studies have shown a large maternal effect on the germination of several plants (Parker, 1968; Eagles and Hardacre, 1979; Stanton, 1984; Schmitt and Antonovics, 1986) including coniferous species (Greathouse, 1966; Bramlett et al., 1983; Davidson, 1990). Germination of conifer seeds is the result of much complex metabolic activity involving three distinct genomes: the diploid embryo, the haploid, maternally-derived, nutritional megagametophyte, and the diploid, maternal, seed coat (*Figure 3*) — as well as specific environmental triggers. Furthermore, germination responses are likely conditioned by environments encountered by the seeds throughout their development. Therefore, the apportionment of the total phenotypic variance of any germination parameter to various hypothesized sources (i. e., among and within genetic entities) may be miselading if maternal effects, as well as environmental preconditioning, are not considered.

Maternal effects in plants have been classified into three distinct classes: cytoplasmic genetic, endosperm nuclear aid maternal phenotypic (Roach and Wulff, 1987), all of which are evident in coniferous seeds. Recent molecular-genetics work on organelle (plastids and/or mitochondria) inheritance in conifers has confirmed that the mitochondrial genome is exclusively maternal (Neale and Sederoff, 1989). The maternally-derived megagametophytic tissue in coniferous seeds represents exclusively maternal genes (Foster and Gifford, 1974); fertilization is not necessary for this tissue to form. It has been shown in *Zea mays* L. that the endosperm tissue (consisting of two parts maternal and one part paternal genes) contains enzymes important for germination and it is also the source of nutrients for the developing embryo (Harvey and Oaks, 1974). Because the megagamethophytic tissue in coniferous seeds is exclusively maternal, the female parent has the sole genetic role in determining the characteristics of this nutrient source. Studies by El-Kassaby et al. (1986) and McLean and El-Kassaby (1986) presented evidence of genetic control of mineral profiles in seeds of *Picea sitcensis* (Bong.) Carr. and Douglas-fir that were exclusively maternal.

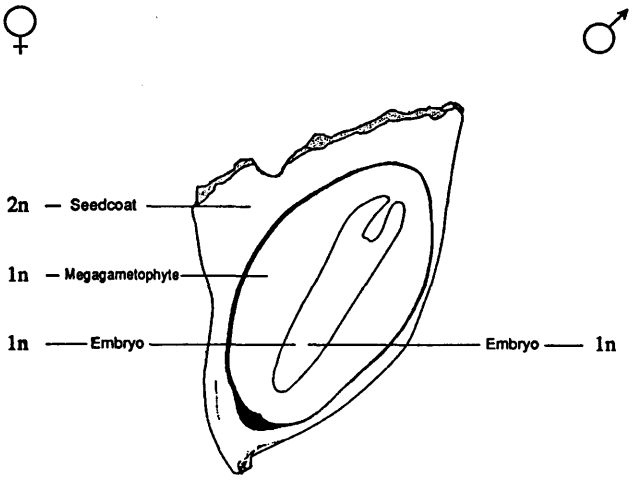


*Figure 3.* — The genomes of a mature Douglas-fir seed. Total gene contributions is 4n:1n (maternal:paternal).

The maternally-derived seed coat that surrounds the embryo and nutritional tissue is an important determinant of seed dormancy and germinations traits. Examples from forest tree seed studies have also implicated the seed coat in dormancy, especially in pine (LARSEN, 1925; PITEL and WANG, 1985; HOFF, 1978; BARNETT, 1976). The papery, inner membrane (between the hard outer seed coat and the megagametophyte) has been described as restricting water absorption in *Pinus lambertiana* DOUGL. (BARON, 1978), oxygen transport in *Pinus jeffreyi* MURR. (STONE, 1957) and *Pinus strobus* L. (KOSLOWSKI and GENTILE, 1959), and as a major site of dormancy in *Pinus monticola* DOUGL. (PITEL and WANG, 1985; HOFF and STEINHOFF, 1986; HOFF, 1987). EDWARDS (1969) presented evidence that implicated the seed coat (and the megagametophyte) as providing mechanical restraint in noble fir (*Abies procera* REHD.) seeds, and KITZMILLER et al. 1975, as well as a number of other researchers (EDWARDS, 1982), have speculated on some form of seedcoat dormancy in several true fir species. In Douglas-fir, DE MATOS MALAVASI et al. (1985) observed that when stratified seeds were dried to 35% or 25% moisture contents, the bulk of the moisture was lost from the seed coat, while the megagametophytic and embryonic tissues remained unchanged; after three months' storage, moisture within dried seeds had not equalized. It was suggested that this rapid loss of moisture from the seed coat of stratified seeds was associated with improved metabolic activites in the embryo and gamethophyte, leading to improved seed germination, that is, in overcoming dormancy (DE MATOS MALAVASI et al., 1985). There is also compelling evidence from other plants that has demonstrated maternal-envelope control over seed dormancy and germination (KOLLER, 1969); HILU and DEWET, 1980; MAYER and POLJAKOFF-MAYBER, 1982; NOODEN et al., 1985). ROACH and WULFF (1987) indicated that conflict between the maternal-genotype (via the seed coat and other structures) and the embryo-genotype may develop to maximize the species' fitness by optimizing the timing of germination (LEVINS, 1969; THOMPSON, 1970). This conflict maximizes fitness through regulating germination to reduce offspring competition (ELLNER, 1986), or to ensure success in a temporally heterogenous environment (WESTOBY, 1981; WESTOBY and RICE, 1982).

Evidence of environmental preconditioning on seed dormancy and germination, as well as on the subsequent performance of the progeny, is well ducumented for several plant species (DURRANT, 1985; HIGHKIN, 1958; KOLLER, 1962; AUSTIN and LONGDEN, 1965; HILL, 1965; EVENARI *et al.*, 1966; KARSSEN, 1970; NELSON et al., 1970; BASKIN and BASKIN, 1973; MOORE and EGINGTON, 1973; GUTTERMAN et al., 1975; HEIDE et al., 1976; MAXON SMITH, 1976; NOODEN et al., 1985). This phenomenon was recommended for investigation in forestry by ROWE (1964), but few studies have been conducted on tree species to adress the impact of environmental preconditioning on eiter seed germination or progeny performance. Studies in Scandinavia were the first to evaluate the impact of seed production of northern clones grown in southern locations (BJORNSTAD, 1981; LINDGREN and WANG, 1986; JOHNSEN, 1989a, b; JOHNSEN et al., 1989). Recently, the impact of cultural practices on seed germination has been studied in *Pinus taeda* L. (STRUVE et al., 1989) and in Douglas-fir (EL-KASSABY et al., 1990b). CAMPBELL and RITLAND (1982) reported, after studying the germination responses of 12 populations of *Tsuga heterophylla* (RAF.) SARG.,that differences observed among populations conceivably could result from either seed preconditioning before harvest, or from the refined adjustment of populations to natural selection, or both. In general, they found populations from higher latitudes to exhibit earlier and more rapid germination, a pattern detected in other forest tree species inhabiting climates where cold temperatures limit the growing season (STEARNS and OLSON, 1958; MERGEN, 1963; ROCHE, 1969).

Persistent environmental-preconditioning effects were minimized in the study reported here by the use of seeds collected in the same corp year from trees of the same age growing on the same site with equal growing space. In addition, the seeds used to produce the parent trees forming the orchard were collected from crosses made on grafted clones growing in the same orchard site. Therefore, it can be expected that any environmental-preconditioning effects that might have been operating on the clonal trees, preconditioning that would reflect the original environmental conditions of the clones' ortets, should have been buffered by going through two generations of reproductive cycles — one generation to produce the seeds used to produce the orchard trees and another to yield the seeds used in this study. Two reproductive cycles have been proposed as a protocol for removing the maternal environmental effect in crop plants (NELSON et al., 1970; BASKIN and BASKIN, 1973; QUINN and COLOSI, 1977; ALEXANDER and WULFF, 1985). Although the seeds used in this study were produced after wind pollination ,pollen-pool homogeneity was confirmed from earlier studies using allozyme markers (EL-KASSABY and DAVIDSON, 1990, 1991 and in preparation); pollen-pool frequencies were shown to be a representative sample of the orchard trees. Additionally, pollen contamination was virtually non-existent, due to the relative isolation of the orchard and the crop cultural practices used (EL-KASSABY and DAVIDSON, 1990, 1991).

It is important during the interpretation of germination results to distinguish variations due to direct and maternal genetic effects from those of persistent environmental influences. In the study reported here, maternal open-pollinated Douglas-fir seeds were used in the estimation of broad-sense heritabilities of germination parameters. Because of the presence of maternal effects, it is believed that these estimates are exaggerated. Furthermore, coniferous seed responses are susceptible to change by other environmental factors, such as different times of seed harvest and ripening (ALLEN, 1958). These pseudo-preconditioning factors cannot be avoided in collections from natural stands and produce effects mimicking or masking genetic differences among populations (CAMPBELL and RITLAND, 1982). Pseudo-preconditioning factors, such as differences in flowering phenology, date of cone collection, and cone-storage treatment, have been proven to have no significant effect on crops collected from the same Douglas-fir seed orchard that provided the seeds for his study (EDWARDS and EL-KASSABY, 1988). Certain cultural practices have also been shown to have no significant effect on seed performance (EL-KASSABY et al., 1990a, b).

Bulked seedlots usually are composed of an unknown number of seed parents with undetermined proportions of viable seeds representing each parent, and have been derived from a heterogenous pollen pool. In the study reported here, the germination tests were conducted only on stratified seeds, since stratification is the standard treatment applied to Douglas-fir seeds prior to sowing in

container nurseries. Stratification, which has been shown to improve germination of several coniferous species (EDWARDS, 1980) including Douglas-fir (ALLEN, 1941), is a treatment that is commonly applied to overcome dormancy; thus, responses to stratification can be taken as evidence that dormancy is present (EDWARDS, 1962, 1980). Studies of coniferous seeds that are based on bulked seed samples (i. e., seedlots composed of a mixture of seeds from several trees )usually suffer from the "experimental material-noise" that produces confunding, and often misleading, results. For example, a germination test on a bulk sample from all 19 trees would not have revealed that some replications from tree # 1, 4 and 6 failed to achieve 50% germination ,so that an $R_{50}$ could not be calculated (*Table 2*). Whereas an average $R_{50}$ for such a bulk lot could have been obtained, this would have failed to represent the very poor germination of certain component trees. However, studying germination traits on an individual-tree (family or clone) level revealed the inadequacy of $R_{50}$ as a germination rate parameter. PV (peak value), on the other hand, can be calculated whatever the level of germination (*Table 2*), so the poor germination speed of seeds from tree # 1, 4 and 6 can be accurately represented; thus an average PV for a bulk lot would be more meaningful.

Since the germination tests in this study were conducted on an individual-tree basis, it was possible to reveal (i) that there was no relationship between seed size (as expressed by 1,000 seed-weight) and any of the germination parameters (*Figure 3*), and (ii) the presence of substantial differences is the numbers of filled seeds left ungerminated at the end of the test (GCs, *Table 2*). While the viability of these ungerminated seeds was not verified (by tetrazolium or other means), most can be assumed to have remained dormant. This differential dormancy was revealed after stratifying all seedlots in a uniform manner that mimicked nursery practice; a uniform treatment was clearly inadequate for removing dormancy in trees # 1, 4, 6, 19, etc., but was sufficient (or nearly so) in trees # 10, 3, 18, 15, etc. (*Table 2*). Such differential dormancy as shown in our data means that for maximal germination to have been realized in all seedlots, seeds from individual trees require stratification treatments customized to their specific requirements.

Alternatively, since seeds from all 19 trees were stratified uniformly, the results (*Table 2*) indicated that for equal numbers of seedlings from all trees to be included in a nursery crop, there would have to be approximately 3.8 times more seeds from tree # 1 than from tree # 10, 2.4 times from tree # 4, 1.8 times from tree # 6, and so on. If the seeds from all the study trees had been processed and extracted in bulk, as is normal operational practice, not only would such equalization have been impossible to achieve, but also the genetic representation in the ensuing seedling crop most likely would have been biased towards those trees, with high or low germinating seeds, that produced the highest quantities of seeds. Thus, as the data reported here demonstrate, bulking the seeds from a seed orchard without knowledge of the contribution that individual trees make to the mixture can be disadvantageous in growing the new forest. Additionally, had the study been conducted on a bulked seedlot from the 19 trees, a weighted germination percentage (based on the total number of filled seeds produced by each tree — see Materials and Methods) of 74.28% would have been obtained. If such a seedlot was used for nursery sowing, sowing rules to ensure the presence of at least one germinant per cavity would require triple sowing (BALMER and SPACE, 1976). Multiple sowing might be justified for individual trees with poor-germinating seeds if their genetic value is high, but multiple sowing of a bulked mixture causes both biological and economic problems. Removal of excesses germinants from multiple sowing amounts to an inadvertent selection yielding reduced genetic diversity and seedling crops with unpredictable allelic frequencies. Such thinning also requires a considerably higher labour cost, and the lost value of the extra seeds sown has also to be considered. Alternatively, single-sowing of seeds for high breeding clones to balance allelic frequencies in the planting stock means increased cost due to wasted nursery space.

The unique structure of conifer seeds clearly represents an adaptive significance. The observed lack of significant correlation between seed size (as expressed by 1,000 seed-weight )and germination in these Douglas-fir seeds further supports this adaptive significance. Therefore, the common practice of sizing seeds during extraction and cleaning should be re-evaluated. SILEN and OSTERHAUS (1979) demonstrated that seed-sizing of bulked Douglas-fir seedlots reducted their genetic diversity. If sizing is carried out, then viable seeds from all size classes must be re-mixed before the seeds are sown if diversity is to be maintained. Our results have demonstrated that germination traits in Douglas-fir are under strong genetic control, a large component of which is maternal. In domestication attempts through seed orchard seed-production programs, as well as nursery seedling production, our data also indicate that a thorough understanding of the biology of seed germination is essential to maintain the integrity of the biological diversity of the species being domesticated.


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