Genetic Variation in Germination Parameters among Populations of Pacific Silver Fir[1])

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(Received 28th March 1996)

Abstract

Strong genetic control was detected for germination capacity and germination speed in 6 populations of Pacific silver fir (*Abies amabilis* [Dougl.] Forbes) from northern, central and southern Vancouver Island, British Columbia. Intra-class correlation estimates were high and varied between 0.6 and 0.7 and 0.7 and 0.8 for stratified and unstratified seeds, respectively. Stratified seeds showed marked differences in germination capacity and speed among populations when compared to unstratified seeds. Family differences in germination determined by intra-class correlation, irrespective of seed pretreatment, were the most pronounced factor when compared to regions and/or populations within regions. The implications of these differences to nursery seedling production was discussed. The inadvertent selection for more-rapidly germinating families in bulked seedlots may represent the most important factor affecting the genetic diversity of seedling crops.

Key words: Abies amabilis, germination, stratification, intra-class correlation.

FDC: 165.53; 161.41; 174.7 *Abies amabilis;* (711).

Introduction

Seed quality at the physiological level includes seed viability, germinability and vigour. The viability of a seed is simply its capacity for growth and development (Bewley and Black, 1978). Germinability is a measure of the ability of a population of seeds to germinate, or as Bewley and Black (1978) describe it, "the maximum percentage of seeds that will germinate under favourable conditions." Vigour is more problematic in its definition because of its complexity (Heydecker, 1969). The Association of Official Seed Analysts (AOSA) stated that vigour is operating on at least 2 levels – at the biochemical level, as the coordination of several metabolic events and at the macroscopic level, in the speed and completeness of germination over a range of environmental conditions (Anon., 1976). Vigour is controlled by 2 major factors – one being genetic and the other consisting of various environmental conditions which may occur during seed development, maturation, processing and storage (Heydecker, 1969; Maguire, 1977). Seed vigour is then the sum of all "those properties which determine the potential for rapid, uniform emergence and development of normal seedlings under a wide range of field conditions" (Bonner, 1984).

Germination in coniferous seeds is the culmination of a complex of metabolic activity involving 3 distinct genomes – the diploid embryo surrounded by the nutritional haploid megagametophyte and the diploid maternal seed coat (El-Kassaby *et al.,* 1992) – and specific environmental triggers. In addition, germination responses are likely conditioned by environments encountered by seeds throughout their development (Rowe, 1964).

Stratification, consisting of a moist chilling for a few to several weeks either in some medium (Allen, 1941) or "naked" (Allen and Bientjes, 1954), is a commonly-used dormancy-breaking treatment in temperate zone conifer species (Edwards, 1981 a and b; Wang *et al.,* 1982). Conditions of stratification (or prechilling) are set to approximate the environments that autumn-ripening seeds might find themselves exposed to upon dissemination (Krugman *et al.,* 1974). The degree of dormancy may be expected to show some variation related to climate of origin (Levins, 1969; Thompson, 1981). Campbell and Ritland (1982) found populations of western hemlock (*Tsuga heterophylla* [Raf.] Sarg.) at higher latitudes to exhibit earlier and more rapid germination, a pattern which was detected in other forest tree species also inhabiting climates where cold temperatures limit the growing season (see citations in Campbell and Ritland, 1982).

Stratification has been shown to improve the germination (in terms of capacity and/or speed) of several *Abies* species, which is also taken as evidence that dormancy exists in these seeds (Edwards, 1962, 1981 a and b, 1982). The extent of genetic control over germination in coniferous seeds has been reported to be high due to the high proportion of the maternal genotype in their seed structure (El-Kassaby *et al.,* 1992). However, the effects of population and individual tree genotype on germination of Pacific silver fir (*Abies amabilis* [Dougl.] Forbes) are not known.

In the present study, the impacts of stratification, population, and individual-tree genotype were investigated using single-tree seed collections from 6 populations (grouped by latitude) located in Vancouver Island, British Columbia.

Materials and Methods

Populations sampled and cone collections

Cones were collected from 6 populations of Pacific silver fir representing three collection regions (northern, middle, and southern latitude) of Vancouver Island, British Columbia. Each region was represented by 2 populations (*Table 1*). Within each population, cone samples were collected from 7 randomly selected trees using a cone rake suspended from a helicopter. Cones were collected from individual trees level and the parent-tree identity of cones and seeds was maintained throughout the study. Given the fact that Pacific silver fir was present in these populations as a codominant species with western hemlock, and that the nature of individual tree cone collection was by helicopter, it is likely that sufficient distance (i.e., no co-ancestry) between sampled trees was obtained. The cone collections were made over a 4 week period following

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[1]) This paper represents a portion of Robin Davidson's Ph. D. Dissertation. Robin lost a heroic battle with cancer and prematurely departed the scientific community. Her passing is a great loss of a friend, colleague, and a very bright young scientist.

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cone/seed ripeness check by field personnel. Cones were kept in mesh bags at 4 °C until all collections were made, then air-dried at 12 °C to 20 °C for 2 weeks. Seeds were extracted in a commercial cone-processing facility following standard methods used for the species. Seeds were hand-dewinged and filled seed samples for weight determination and germination test were obtained using a vacuum separation apparatus (Edwards, 1979). Care was exercised at all stages of seed handling to avoid damage to the seed coats, as bursting of resin vesicles has been implicated in reducing germinability of *Abies species* (Bouvarel and Lemoine, 1958; Gunia and Simak, 1970; Kitzmiller *et al.,* 1975).

Table 1. – Sources of populations of Pacific silver fir cones (populations are listed by latitude).

Collection site	Code	Latitude	Longitude	Average elevation (m)
Fleet River	F	48° 39'	124° 06'	710
Mystery Creek	W	48° 48'	128° 09'	40
Sebalhall Creek	B	49° 57'	126° 25'	300
Maquilla Creek	C	50° 04'	126° 21'	500
Hathaway Creek	H	50° 43'	124° 26'	212
Ronning Creek	R	50° 44'	128° 00'	275

Germination test

Double germination tests, modified from the International Seed Testing Association's (ISTA) (1985) rules for the testing of *A. amabilis* seeds, was conducted on 6 random samples each of 50 filled seeds (obtained by X-ray methods) from all 42 trees. One half of the seeds were subjected to a 28-day stratification (prechill) period prior to incubation of all seeds. Stratification entailed placing dry seed samples in clear plastic, closed germination boxes (12 cm x 12 cm x 3 cm) on 3 layers of Whatman #1 filter paper over "Kimpak" cellulose towelling wetted with 43 ml distilled water. Dishes were immediately placed in darkness at 1 °C to 4 °C for 28 days.

Unstratified seeds were set into germination boxes in a similar manner, then placed into 2 incubators concurrently with stratified seeds, so that all tests began at the same time. Prior to the tests, unstratified samples were stored dry at 1 °C. The experiment was too large to be carried out in one cabinet, so it was structured such that 3 samples of both prechilled and untreated seeds from each tree were placed at random on trays in each cabinet. Temperatures were maintained alternately at 30 °C for 8 hours and 20 °C for 16 hours, with light at approximately 1000 lux, using cool-white fluorescent tubes, being provided during the higher temperature period. Germinants were counted 8 times during the 28-day test period with 2 observations during the first week to assess onset of germination. The test period was extended and additional counts were made at 35 days and 42 days to reduce the truncation effect on seeds from later-germinating trees. Germinants were removed when the radicle had reached the length of the seed coat (Edwards, 1982). Germination counts were summarized as 2 response variables: germination capacity (GC), the number of germinants expressed as a percentage of filled seeds, at the end of the test; and germination value (GV), computed according to Czabator (1962). This index of germination is the product of 2 quantities: the mean daily germination (MDG), obtained by dividing the total number of germinants by the length of the test period (in days), and the peak value (PV) which is determined by calculating a cumulative germination percentage for each successive count and dividing by the number of elapsed days. The maximum quotient corresponds to PV, and GV = MDG x PV. The higher the value of GV, the more complete and/or the more rapid the germination process.

Statistical analyses

Previous work on prechilling treatments on Pacific silver fir (Davidson *et al.,* 1984) suggested that the stratification process would alter germination patterns to the extent that it would be very unlikely that homogeneity of variances between treatment groups would be achieved. Given this probability, preliminary analysis of the germination data was carried out using a multiway contingency table approach (Fienberg, 1970) in order to get some estimate of the relationships among the hypothesized sources of variation without invoking the analysis of variance (ANOVA) assumptions of homoscedasticity and normality. The method also accommodates censored data sets. The analysis is based on fitting a log-linear model to individual cell frequencies, in this case, the number of germinants per day. The relative importance of a given factor in the model is determined by obtaining an approximate χ^2 value for the fit of a particular model containing the factor of interest and then refitting the model without that factor and observing the change in χ^2. The magnitude of the difference reflects the relative importance of the term of interest (Schoener, 1970). Not all factors were testable by this method, however, which is not uncommon where models contain both fixed and random effects (M. Grieg, UBC Computing Center, pers. comm.). The analysis did clearly reveal that the stratification treatment was the greatest single factor affecting germination patterns in sampled Pacific silver fir. Further analyses were conducted on stratified and unstratified seeds separately.

An ad-hoc procedure for finding suitable transformations to normalize the calculated response variables and achieve homogeneity of variances was utilized for GC and GV of stratified seed and for GC of unstratified seed. Box's (1949) test for equality of variances was used in conjunction with an appropriate power transformation (all performed using MIDAS statistical software, Fox and Guire, 1976) to obtain variables suitable to ANOVA. Germination value for unstratified seeds fulfilled ANOVA assumptions without transformation. Where transformation was of benefit, results using untransformed variables are included for comparison. A nested-factorial analysis was based on the following model:

$$Y_{ijklm} = \mu + R_i + P_{j(i)} + T_{k(ij)} + C_l + CR_{il} + CP_{jl(i)} + CT_{kl(ij)} + \varepsilon_{m(ijkl)}$$

where:

Y_{ijklm} = the performance of the mth replication from kth tree in the jth population in the ith region in the lth cabinet,

μ = overall mean germination response,

R_i = climatic region (i = 1,2,3),

$P_{j(i)}$ = population within climatic region (j = 1,2),

$T_{k(ij)}$ = tree within population (k = 1,...,7),

C_l = cabinet (l = 1,2),

CR_{il} = cabinet X region interaction,

$CP_{jl(i)}$ = cabinet X population within region interaction,

$CT_{kl(ij)}$ = cabinet X tree within population interaction, and

$\varepsilon_{m(ijkl)}$ = error (m = 1,2,3).

All effects in the model were considered random except R (region) and C (cabinet) which were deemed to be fixed. Expected mean squares were included to indicate appropriate terms for significance testing and to enable estimation of variance components.

The relative magnitudes of variation which may be ascribed to factors in the model are presented as ratios of the appropriate variance components to their sum (CV, expressed as a percentage). The coefficient of intra-class correlation (r, Sokal and ROHLF, 1981), which in these analysis measures the proportion of variation among maternal trees, was also computed. This value is referred to by FALCONER (1981, p. 126) as repeatability and may be viewed as an upper limit of heritability in the broad sense. In addition, the apportionment of variation based on a percentage of the total sums of squares (%SS or eta^2, after FISHER, 1932; LITTLE, 1981; HICKS, 1982) is presented. This method of apportioning variability is appealing because there is no chance of obtaining negative variance components when source contributions are very small (HUEHN *et al.,* 1987). Numerically, the similarity between %SS and respective %CV was demonstrated by MAZE *et al.* (1989). However, when compared with the equivalent variance components, residual variation is usually underestimated using %SS, so that the proportion of the total variation in the data accounted for by other terms should be considered maximal (HICKS, 1982, p. 135).

Results and Discussion

From the results of ANOVA for germination capacity (transformed) of stratified seeds, it was evident that the cabinet effect and its interactions were negligible (*Tables 2a* and *b*). Similarly, no statistical significance was found using response variables for unstratified seeds, prompting the decision to remove the cabinet terms from the model, thus improving the error degrees of freedom. The region of collection (R) was retained in the model despite its small contribution because of its implication for seed crop management. Populations within regions, and trees within populations, accounted for a substantial portion of total variance, as revealed by either %SS or %CV (*Table 2b*).

Table 2a. – Sources of variation, associated degrees of freedom, sum of squares, mean squares, *F* values and associated probabilities for a mixed effects ANOVA model of germination capacity for stratified seeds (transformed)[1]) of Pacific silver fir on Vancouver Island.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	*F*	Probability
Region (R)	2	0.515	0.257	0.155	0.86
Population (P(R))	3	4.991	1.664	11.228	<0.00
Tree (T(PR))	36	5.335	0.148	12.268	<0.00
Cabinet (C)	1	0.003	0.003	0.085	0.78
C X R	2	0.053	0.027	0.695	0.57
C X P(R)	3	0.115	0.038	2.456	0.08
C X T(PR)	36	0.560	0.016	1.288	0.15
Residual	168	2.029	0.012		

[1]) Arcsin transformation

A comparison of germinated vs. ungerminated seeds showed that there was very little difference in the number of germinants of either unstratified or stratified seed between the 2 germination cabinets (less than 2% of the total number of germinants in the test) (*Table 3*). There was, however, a substantial difference in the number of seeds left ungerminated at the end of the test with over 20% of the seeds remaining ungerminating when not stratified, compared to just over 11% for stratified seeds. This result indicates that these samples of Pacific silver fir were dormant. Overall means for germination capacity were 79.6 ± 1.8% (95% confidence intervals, CI) for unstratified, and 89.9 ± 1.8% for stratified seeds (calculated from untransformed data). The influence of stratification on both the total amount and rapidity of germination is reflected more dramatically in the overall increase in germination value, from an average of 4.42 ± 0.22 to 11.37 ± 0.44, being a substantial increase in germination speed.

Stratification has been found to improve total germination in several seedlots of Pacific silver fir (EDWARDS, 1980, 1981 b, 1982; LEADEM, 1986). The present results showed that the variances associated with average GC in unstratified and stratified seeds are equivalent (as expressed by 95% CI), and perhaps more revealing, the variance in GV for prechilled seeds is twice that of unstratified seeds. This is not the typical pattern one would expect from stratification. The usual effect of stratification in conifers is a hastening of germination with a concomitant reduction in variability (ALLEN and BIENTJES, 1954; EDWARDS, 1969, 1982). Examination of the results of hierarchical ANOVA for both unstratified and stratified seeds revealed that the largest source of variability in both GC and GV was associated with differences among trees (*Table 4a* and *b*). Consistently high values of intra-class correlations, obtained for both unstratified and stratified seeds, further emphasize the large inter-tree variation. In most of the analyses, regions

Table 2b. – Sources of variation, percentage of total variation based on sums of squares (%SS), components of variance (%CV) and expected mean squares based on a mixed effects ANOVA model of germination in Pacific silver fir on Vancouver Island. Calculations based on ANOVA described in *table 2a*.

Source of Variation	%SS	%CV	Expected Mean Squares
Region (R)	3.8	3.3	$\sigma^2_e + 6\,\sigma^2_{TPR} + 42\,\sigma^2_{PR} + 84\,\theta_R$
Population (P(R))	36.7	48.8	$\sigma^2_e + 6\,\sigma^2_{TPR} + 42\,\sigma^2_{PR}$
Tree (T(PR))	39.2	28.4	$\sigma^2_e + 6\,\sigma^2_{TPR}$
Cabinet (C)	<0.0	< 0.0	$\sigma^2_e + 3\,\sigma^2_{CTPR} + 21\,\sigma^2_{CPR} + 126\,\theta_C$
C X R	0.4	0.7	$\sigma^2_e + 3\,\sigma^2_{CTPR} + 21\,\sigma^2_{CPR} + 42\,\sigma^2_{PR}$
C X P(R)	0.9	2.2	$\sigma^2_e + 3\,\sigma^2_{CTPR} + 21\,\sigma^2_{CPR}$
C X T(PR)	4.1	1.5	$\sigma^2_e + 3\,\sigma^2_{CTPR}$
Residual	14.9	15.1	σ^2_e

Table 3. – Total number of germinants and non-germinants in all 42 trees involved in a paired germination test of 6 populations of Pacific silver fir. One half of the seeds were subjected to a 28-day stratification prior to testing; seeds from each pre-treatment were further divided between 2 germination cabinets.

Pretreatment	Cabinet	No. Germinated	No. Ungerminated	Total
Unstratified	A	4718	1260	59787
	B	4890	1213	6103
	Total	9608	2473	12081
Stratified	A	5429	607	6096
	B	5317	765	6022
	Total	10746	1372	12188

of collection were relatively unimportant, and the major effect of stratification on the apportionment of variation was the shifting of nearly half of the relative variance associated with individual trees to the population level. Intra-individual variation remains approximately the same (less than 25% of total SS). It was apparent that seed pretreatment by stratification for 28 days has varying effects on populations within the same latitudinal band. SORENSEN and WEBER (1994) observed a comparable shifting of relative variances. They reported that all the family variance in seedling emergence of ponderosa pine (*Pinus ponderosa* DOUGL. ex LAWS.) was within populations for a specific year (1984), but approximately 80% of family variance was shifted to the among populations component for the following year (1985).

There does not seem to be an immediate biological explanation for the apparent change in the relative importance of individual and population effects resulting from seed pretreatment. Seedlot variation in the degree of dormancy in *Abies* is well known (EDWARDS, 1962, 1969) and this might be attributed to the timing of collection, as EDWARDS (1969) observed increased dormancy from early to late collections of noble fir (*Abies procera* REHD.). Cones from both populations within any one collection region in the present study were collected at most one day apart. However, EDWARDS (1980, 1982) reported that maturity differences can exist among cones within the same tree and even among seeds within any one cone. Cones were monitored for embryo maturity via cone-cutting tests but the logistics of collection required that cones be picked from all trees in a given stand on the same day. Thus it is unlikely that the sampled trees represent the same degree of seed maturity within each population. When GCs were considered, no geographic trend in dormancy was evident, since germination in all 6 populations was consistently high. However, this was not the case with germination speed, as will be discussed below.

In response to the stratification regime applied in this study, total germination (GC) was significantly ($P < 0.05$) increased and variability reduced in all populations except *W* (Mystery Creek). Fewer germinants were obtained and germination was more variable for stratified seeds than unstratified seeds in this population. The speed of germination (inferred from GV) improved the least in population *W* (*Table 5*). Its latitudinal counterpart, *F* (Fleet River), on the other hand, already possessing a reasonably high capacity for germination after being exposed only to the conditions of collection and processing (i.e., relatively non-dormant), showed higher, more uniform germination with a marked increase in GV after prechilling 28 days. However, as already noted, the variance of GV increased for stratified seeds in all 6 populations, although this increase was least in population *F* (*Table 5*). This unexpected result merits further examination.

Table 4a. – The percentages of total sums of squares and equivalent variance components from ANOVA associated with each source of variation for germination response variables in unstratified seeds of Pacific silver fir.

Source of Variation	Degrees of Freedom	% Sum of Squares			% Variance Component		
		GC	GC^{1}	GV	GC	GC^{1}	GV
Region	2	1.1	1.0	9.7*	0.00	0.00	11.7
Population	3	5.7	6.6	1.2	0.00	0.00	0.00
Tree	36	74.7*	69.3*	71.5*	79.1*	66.2*	69.8*
Residual	210	18.5	23.1	17.6	20.9	33.8	18.5
r^2		---	---	---	0.79	0.66	0.79

1) Arcsin transformation
2) intra-class correlation coefficient $= \sigma^2_{TPR} / \sigma^2_{TPR} + \sigma^2_{e}$
*) significant at $P < 0.05$

Table 4b. – The percentages of total sums of squares and equivalent variance components from ANOVA associated with each source of variation for germination response variables in stratified seeds of Pacific silver fir.

Source of Variation	Degrees of Freedom	% Sum of Squares				% Variance Component			
		GC	GC^{1}	GV	GV^{1}	GC	GC^{1}	GV	GV^{1}
Region	2	7.5	3.8	4.8	4.4	0.00	0.00	0.00	0.00
Population	3	33.8*	36.7*	29.7*	28.6*	47.9*	50.2*	41.0*	39.4*
Tree	36	42.4*	39.2*	48.5*	50.1*	36.6*	31.4*	42.7*	44.3*
Residual	210	16.3	20.3	17.0	16.9	15.5	18.4	16.3	16.3
r^2		---	---	---	---	0.70	0.63	0.72	0.73

1) Transformation: Arcsin for GC and ($\sqrt{x} + 0.5$) for GV
2) intra-class correlation coefficient $= \sigma^2_{TPR} / \sigma^2_{TPR} + \sigma^2_{e}$
*) significant at $P < 0.05$

Table 5. – Average germination capacity and value (±95% confidence intervals) for 6 populations of Pacific silver fir for both unstratified and stratified seeds.

Region	Population	Germination Capacity		Germination Value	
		Unstratified	Stratified	Unstratified	Stratified
North	R	82.2 (3.7)	92.5 (2.9)	4.55 (0.51)	11.06 (0.94)
	H	76.2 (4.9)	91.0 (2.9)	3.93 (0.51)	11.63 (0.96)
Middle	B	76.2 (5.5)	89.2 (3.5)	4.36 (0.63)	11.82 (1.10)
	C	79.7 (3.7)	94.7 (2.4)	4.16 (0.43)	12.83 (0.69)
South	F	86.6 (3.9)	97.8 (1.4)	5.48 (0.49)	13.70 (0.59)
	W	76.8 (3.7)	68.9 (5.9)	5.34 (0.45)	7.15 (0.90)

Populations *R* (Ronning Creek) and *H* (Hathaway Creek), from northern Vancouver Island, exhibited similar germination values for both unstratified and stratified seeds and both populations had virtually the same levels of variability among trees (*Table 6*). In the mid-latitude collection region (*B*, Sebalhall Creek and *C*, Maquilla Creek), germination behaviour parallels that in the north, although population *C* appeared to respond more rapidly and uniformly to stratification. Germination values were highest in the southernmost collection region without stratification, suggesting that germination is more rapid at lower latitudes. In ponderosa pine, WEBER and SORENSEN (1992) found germination speed to be related to severity of summer drought, being greatest in populations whose seeds developed in short, drought-limiting growing seasons. They concluded that geographic variation in germination speed was highest in populations from regions having the steepest

Table 6. – Mean germination capacity and germination value for individual trees of population *W* (each mean based on 6 50-seed replications) (95% confidence intervals), and population mean values for both germination responses.

Tree No.	Germination Capacity		Germination Value	
	Unstratified	Stratified	Unstratified	Stratified
1	75.1 (2.6)	58.7 (4.6)*	5.23 (0.25)	5.07 (0.69)
2	77.7 (3.6)	64.5 (8.9)	5.86 (0.82)	6.99 (1.73)
3	85.7 (4.2)	85.2 (4.5)	6.95 (0.89)	9.49 (0.54)*
4	54.5 (8.1)	38.2 (7.1)*	2.54 (0.83)	2.36 (0.81)
5	77.0 (4.3)	64.7 (7.0)*	5.54 (0.65)	7.15 (1.62)
6	92.0 (3.2)	95.6 (1.9)	6.23 (0.39)	10.67 (0.51)*
7	75.5 (3.9)	75.7 (9.9)	5.03 (0.48)	8.29 (1.52)*
Mean	76.8 (3.7)	68.9 (5.9)	5.34 (0.45)	7.15 (0.90)

*) significant treatment response, $P < 0.05$

precipitation gradient. However, most of the variation occurred within populations, which supports the findings reported here.

In this study, the average GV for stratified seeds of population *W* was considerably less than that of *F*. This result, when considered along with the substantial individual tree component of variation in germination response (*Table 4a* and *b*) prompted a closer look at germination behavior within population *W*. The average GC and GV of 6 50-seed replications for each parent tree representing population *W* are listed in *table 6*; GCs ranged from 55% to 92% for unstratified seeds and 38% to 96% for stratified seed. The stratification treatment appeared to be detrimental to seeds from 4 of the 7 trees in the sample (mean GC significantly reduced). GV was significantly improved only in the 3 trees in which germination capacity did not diminish in response to stratification (*Table 6*).

Several studies on tree seed maturity reviewed by Edwards (1980) have revealed that immature seeds tend to: a) be lighter in weight; b) germinate slowly if at all; c) show reduced germination as a result of prechilling; and d) be more susceptible to disease. Populations *F* and *W* were the last to be collected (September 30, vs September 8/9 for *B* and *C* and September 28 for *R* and *H*) and also produced the heaviest seeds (42.8 ± 5.3 g and 53.1 ± 12.5 g are the mean values and their standard deviations for 1000-seed weights of populations *F* and *W*, respectively), and the overall average of all 6 populations was 33.3 ± 13.1 g.

Ackerman and Gorman (1969) found that lighter weight seeds had lower germination percentages in lodgepole pine (*Pinus contorta* var. *latifolia* Englem.). Seed weights of ponderosa pine were also correlated with germination capacity and among stand differences were found to be significant in a study by Wang and Patel (1974). In loblolly pine (*Pinus taeda* L.), heavier seeds were shown to have better germination with significant family differences in seed size (Hodgson, 1980, cited in Wang *et al.,* 1982). Correlation of seed weight with germination capacity was also high in wide-ranging samples of white spruce (*Picea glauca* (Moench) Voss) and seed weight was also found to correlate significantly with population latitude in this species (Khalil, 1986). The 1000-seed weight of provenances of Douglas fir (*Pseudotsuga menzesii* [Mirb.] Franco) was found to correlate with altitude of origin (Birot, 1972) and in jack pine (*Pinus banksiana* Lamb.), Chalupa and Durzan (1972) found seed sizes highly correlated with climate of seed origin.

Simple linear correlations among population mean seed weight and germination response variables and also latitude for Pacific silver fir (*Table 7*) indicated that population *W*, with its large seeds and more southerly latitude, was anomalous in its germination behavior. Without *W*, germination speed (inferred from GV, *Table 7*) is closely related to seed weight and given the negative correlation of weight with latitude, faster germination appears characteristic of more southern populations, the reverse of that found by Campbell and Ritland (1982) in western hemlock.

Table 7. – Product-moment-correlations between average seed weight per population of Pacific silver fir and germination response variables and latitude of populations, calculated with and without values for populations *W*.
The critical values of r for 3 and 4 degrees of freedom are 0.878 and 0.811, respectively.

	Germination Capacity		Germination Value		Latitude
	Unstratified	Stratified	Unstratified	Stratified	
Without **W**	0.872	0.763	0.959*	0.731	-0.872
With **W**	0.238	-0.624	0.921*	-0.537	-0.882

*) significant treatment response, $P < 0.05$

In both unstratified and stratified seeds, differences in germination responses are most strongly associated with intertree (family) variation (*Tables 2* and *4*). Farmer and Rienholt (1986) observed large family differences in germination responses of tamarack (*Larix laricina* (Du Roi) K. Koch.) seeds exposed to different temperature and light combinations, and stand and provenance differences were in all cases non-significant, prompting them to speculate a high degree of genetic control over seed quality and germination characteristics, since environments were presumed to be relatively uniform within stands. Significant family variation was also found in yellow poplar (*Liriodendron tulipifera* L.) (Barnett and Farmer, 1978). Both germination capacity and germinative energy were related to parental genotype in controlled crosses of Virginia pine (*Pinus virginiana* Mill.) (Bramlett *et al.,* 1983). The majority of variation in germination percentage was attributed to differences among 19 black spruce (*Picea mariana* Mill.) clones by Stoehr and Farmer (1986), who also observed that a few clones with ”weak, decay-prone“ seeds contributed heavily to this variance. Leadem (1986) found variation among seedlots of Pacific silver fir in response to stratification and suggested differences may be the result of vigour differences among seeds. This high degree of family variation in germination has operational implications for growers of seedlings of Pacific silver fir (and other coniferous species), since rapidly-germinating families within a seedlot maybe selected inadvertently during nursery thinning (El-Kassaby and Thomson, 1995). This can seriously affect the genetic diversity of the seedling stock.

An additional finding of Leadem (1986) was that the ISTA (1985) prescribed incubation temperature (30 °C/20 °C) was unfavorable for Pacific silver fir seeds, which germinated better at alternating temperatures of 15 °C/10 °C. This finding reinforces results from a seedlot of Pacific silver fir germinated on a thermogradient plate at 5 constant temperatures (Davidson *et al.,* 1984) in which it was determined that greater germination occurred at and below 21°C due in part to the prevalence of seed coat fungi at 24 °C and 27 °C, which infected emerging radicles. Mold growth is a frequent problem in germination studies of *Abies* species (Edwards, 1969, 1982; Kitzmiller *et al.,* 1973; Adkins, 1983; Blazich and Hinesley, 1984) and high temperatures increase susceptibility to fungal attack (Leadem, 1986).

During extraction of the seeds in this study, every effort was made to avoid damage to the resin vesicles. The actual function of the resin is unknown in Pacific silver fir, but it has been found to inhibit germination in seeds of white (*Abies concolor* [Gord. and Glend.] Lindl.) and red firs (*Abies magnifica* A. Murr.)(Kitzmiller *et al.,* 1975) and to be a good medium for fungal development (Kitzmiller *et al.,* 1973). When the cones collected from population *W* arrived at the laboratory, molds were visible on cones of 2 of the 17 trees sampled. While seeds of these 2 trees were excluded from the germination test, molds were observed in every germination box, without exception, for all trees in population *W,* by the 4th week of the germination test. Molds occurred in other populations as well, but none were infected to the level seen in population *W*. Stratified seeds displayed more fungal growth than unstratified seeds, suggesting that the stratification conditions may not have been optimal for population *W,* permitting molds to flourish while inhibiting germination.

Although the germination patterns revealed by this study may have resulted in part from seed preconditioning prior to harvest, as suggested by Campbell and Ritland (1982) for western hemlock, the large individual tree effect would argue for a significant genetic component in germination characteristics. Intra-class correlations (i.e., broad sense heritability) estimates were high and ranged between 0.63 and 0.79 indicating that all germination parameters studied are under strong genetic control. These estimates are similar to that reported for several conifers (Chaisurisri *et al.,* 1992; El-Kassaby, 1992; El-Kassaby *et al.,* 1992).

Conclusions

This study did not reveal any strong geographic trend in dormancy when germination capacities were considered, but a trend was evident for germination speed, and the high degree of family variation has important implications for nursery germination. In addition, stratification is likely to affect seedlots differently and its effectiveness may be a complex function of the environments of developing seed, handling conditions as well as inter- and intra-tree variation in dormancy and germination responses.

The large familial component of variation in germination detected in this study suggests that seedling production of Pacific silver fir by individual-tree sowing is essential if the genetic diversity present in the seedlot is to be maintained. The apparent clinal trend in seed size and its relationship to germination performance could also have implications for nursery practice.

Acknowledgements

The authors thank the generous support of Canadian Forest Products Ltd., MacMillan Bloedel Ltd., Pacific Forest Products Ltd., Western Forest Products Ltd., the British Columbia Ministry of Forests, and Reid, Collins nursery in the collection and processing of the research material. Thanks are also extended to L. Charleson, D. Taylor, F. Portlock, J. Emmanuel, and D. McCarthy for technical assistant.

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Variance Structure in *Eucalyptus* Hybrid Populations

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(Received 29th April 1996)

Summary

This article presents the analyses of the first mating designs established in the reciprocal recurrent selection scheme of *Eucalyptus* in Congo. Two selection schemes were involved, the first one concerning *E. urophylla* • *grandis*, the second one concerning *E. urophylla* • *pellita*. For each of the hybrid species, height, circumference and volume were measured until 3 of 4 years (half of the rotation age).

Male additive variance was lower than female additive variance (30% of the additive variance) for the 2 hybrids. It was demonstrated by studying correlation between parent phenotype and parent crossbreeding value in *E. urophylla* that the higher additive variance for females was probably due to selection of males for their phenotypic value. This result implies a weaker selection intensity for the males in order not to havily reduce the variability.

Variance structure was different according to considered hybrids. For circumference, additive variance accounted for 80% of total genetic variance in *E. urophylla* • *grandis* and only 40% in *E. urophylla* • *pellita*. Sampling of parents and/or genetic distance between species could be an explanatory factor of this difference.

Key words: reciprocal recurrent selection, factorial mating design, variance components, *Eucalyptus,* hybrids.

FDC: 165.72; 165,5; 176,1 *Eucalyptus urophylla* x *grandis*; 176.1 *Eucalyptus urophylla* x *pellita;* (672.4).

Résumé

Cet article présente les analyses des premiers plans de croisements réalisés dans le cadre du schéma de sélection récurrente réciproque de l'*Eucalyptus* au Congo. Deux schémas de sélection sont impliqués, le premier concerne *E. urophylla* • *grandis*, le second *E. urophylla* • *pellita*. Pour chacune des espèces hybrides, les mesures de la hauteur, de la circonférence et du volume ont été réalisées jusqu'à la moitié de la révolution (4 ans).

La variance additive des pères, chez les 2 espèces *E. grandis* et *E. pellita* est inférieure à la variance additive des mères; elle ne représente que 30 % de la variance additive totale. Compte tenu de la corrélation entre phénotype du parent et valeur en croisement mise en évidence dans la population des mères *E. urophylla*, ceci peut s'expliquer par une sélection phénotypique des géniteurs mâles avant croisement. Ce résultat implique une intensité de sélection plus faible chez les espèces qui ont fourni les pères, afin de ne pas épuiser trop rapidement la variablité.

La structuration de la variance est différente selon l'hybride: pour la circonférence, la variance additive représente 80 % de la variance génétique chez *E. urophylla* • *grandis* et 40 % chez *E. urophylla* • *pellita.*

Les effets d'échantillonnage dans les populations parentales et/ou la distance génétique entre les espèces semblent être les facteurs les plus explicatifs.

Introduction

In order to improve productivity of clonal plantations of *Eucalyptus* in Congo, a program based on creation of interspecific hybrids was implemented in the 80's. This program has allowed selection of 2 hybrids with high growing potential under the edaphic and climatic conditions of southern Congo: *E. urophylla* • *grandis* and *E. urophylla* • *pellita*. Subsequently, to increase genetic gains in the long term and to optimize clonal varietal output, a reciprocal recurrent selection scheme based on full-sib families was implemented for each hybrid (Vigneron, 1991).

The theoretical interest of such a strategy when 2 species are complementary, not much improved and when varieties are clones or full-sib hybrid families, has been widely discussed (Gallais, 1990). Within this framework, analysis of the first

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