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Effects of Inbreeding on Coastal Douglas-fir: Nursery Performance

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Abstract

In advanced generation seed orchards, low levels of inbreeding may be inevitable as relatedness among individuals in breeding populations increases with each generation. Unlike selfing, low level inbreeding can produce relatively large number of viable seeds. Following previous study on the effects of inbreeding on coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) filled seed production, the present study investigated inbreeding on nursery performance over various cross-types, including outcrosses (inbreeding coefficient F=0), crosses between half-sibs (F=0.125), between full-sibs (F=0.25), between parents and offspring (F=0.25), and selfing (F=0.5). Significant differences were found among cross-types for germination, seedling mortality, seedling diameter and height, and nursery cull rate. Inbreeding also increased among-family genetic variability. Cumulative losses of seedlings at the nursery stage were 18, 33, 31, 36 and 43%, respectively for the above types of crosses. This result indicates that seeds with low levels of inbreeding may produce relatively large numbers of seedlings that meet nursery culling standards and could be used for reforestation, resulting in negative impacts on the genetic gain realized in field plantations.

Key words: inbreeding depression, nursery performance, cull rate, genetic variability

Introduction

Inbreeding depression is a common phenomenon in forest tree species, particularly in conifers (reviewed by Williams and Savolainen, 1996). Strong inbreeding depression has been observed at different life history stages in many forest trees, including seed development (Griffin and Lindgren, 1985; Sorensen and Cress, 1994), growth performance in nurseries (Sniezko and Zobel, 1988; Sorensen, 1997), and at the early stage of field trials (Durel et al., 1996; Lundkvist et al., 1987; Orr-Ewing, 1976; Sorensen and Miles, 1982).

In first generation seed orchards, self-pollination is typically the only form of inbreeding. It can reduce both seed yields and genetic gain. As self-fertility is low in many species, including Douglas-fir (Sorensen, 1973; Sorenson, 1971), the selfing rate in viable seed produced in first generation seed orchards is low (Burczyk, 1998; Prat and Caquelard, 1995), even lower than that of seed lots collected from stands grown under natural conditions (Prat and Burczyk, 1998). Thus, the negative impact of inbreeding on genetic gain in offspring from first generation seed orchards is expected to be small.

Inbreeding may become more serious as advanced breeding programs generate a large number of trees sharing one or more common ancestors. The best individuals are often from the same families, and selection will result in some degree of relatedness. Thus, in advanced generation seed orchards, in addition to selfing, some low levels of related matings will likely occur. Low levels of inbreeding, such as mating between half-sibs or full-sibs, have much greater capabilities of producing viable seed than selfing (Sorensen and Cress, 1994; Woods and Heaman, 1989). Thus, if the seeds with low levels of inbreeding can produce acceptable seedlings for reforestation, and if these seedlings grow slower than outcross trees as found in some other conifers (Durel et al., 1996), then low levels of inbreeding may have greater impact on the growth and yield of field plantations than selfing.

In order to investigate the effects of various levels of inbreeding on seed production and growth performance, and the rela-

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tionship between inbreeding depression and inbreeding coefficient (F), Woods and Heaman (1989) made a series of crosses covering a wide range of inbreeding coefficients. These included outcrosses (F=0), crosses between full-sibs (F=0.25), between half-sibs (F=0.125), and between parents and offspring (F=0.25), as well as first- (F=0.5) and second-generation selfing (F=0.75). They found that the level of inbreeding had no significant effect on rate of cone abortion, but a strong linear relationship between inbreeding coefficient and the number of filled seeds per cone was observed. Similar results were also reported by Sorensen and Cress (1994).

The present study is sequential to Woods and Heaman's (1989) study. The objective is to investigate the effects of inbreeding on seed germination, seedling mortality, seedling growth performance, and nursery culling rate. The incremental and cumulative loss of seedlings at various levels of inbreeding at the nursery stage is estimated. This information, combined with the effects of inbreeding on filled seed production, will provide knowledge to determine the probability of producing acceptable seedlings when related clones are able to cross in a wind-pollinated seed orchard. Although the effects of inbreeding on seedling traits have been reported in several studies (Sniezko and Zobel, 1988; Sorensen, 1997), there are no published reports on the effects of various levels of inbreeding on nursery culling rate and cumulative seedling loss from an operational perspective. The effects of various levels of inbreeding on genetic variability will also be estimated for germination and seedling growth traits. As pedigree information is known for all crosses, parental effects on outcrossed and inbred seedling performance will be discussed.

Materials and Methods

Founders and mating design

The original parents (founders) and the mating design for producing progenies with various levels of inbreeding, as well as outcrossed controls, are described in Woods and Heaman (1989). Nine unrelated founder clones from parent trees selected in natural stands in south coastal British Columbia were crossed in a 4 x 5 factorial to produce 20 full-sib families to form the outcrossed (OC) population. The same families from previous matings were used to develop the crosses between half-sibs (HS), between full-sibs (FS) and between parents and their offspring (PO). Each of the nine founders was self-pollinated to produce S1 families, from which a number of trees were self-pollinated again to produce second-generation selfs (S2). The mating scheme was designed to create an even contribution of each founder to the various levels of inbreeding. A total of 162 crosses were made (*Table 2* in Woods and Heaman, 1989).

Nursery experiment

Seeds used for this study were from Woods and Heaman's (1989) experiment. In total, 152 genetic entries across the six cross-types (*Table 1*) were included. As only one viable family was generated for S2 due to severe inbreeding, this cross-type was excluded from the data analysis. Seeds were sown in PSB 415A styroblock containers in a greenhouse at the Cowichan Lake Research Station of the BC Ministry of Forests. These containers have a volume of approximately 130 cubic centimeters and a depth of 15 cm, with 80 cavities per block. A single seed was sown in each cavity, and most families had 160 seeds sown.

Each block was divided into four quadrants, and genetic entries were each sown into eight quadrants in eight different blocks such that no styroblock contained more than one quadrant of a genetic entry. Sowing was further arranged so that each genetic entry was presented in four inner and four outer quadrants. The styroblocks were randomly placed throughout the greenhouse and rearranged 8 times during the growing season to minimize the effects of greenhouse location. After one growing season, seedlings were subjected to nursery culling standards used at the time by the British Columbia Ministry of Forests. Seedlings that met the standards were subsequently planted in field tests (manuscript in prep.).

Table 1. – Number of families included in the nursery experiment for each of the six cross-types.

Cross-type	Number of families sown
Outcross (OC)	20
Half-sib (HS)	69
Full-sib (FS)	37
Parent-Offspring (PO)	17
First-generation selfing (S1)	8
Second-generation selfing (S2)	1

Data collection

Measurements were taken on 80 seedlings per genetic entry. To minimize edge effects on seedling growth, only seedlings from the four inner quadrants were used. Families with less than 80 seedlings available from inside quadrants were supplemented with seedlings from the inside rows of the outer quadrants. Germination per quadrant was observed. Seedling mortality, percent of abnormal seedlings, as well as growth traits, including the height to first budset, total height and lammas growth (height growth resulting from second and subsequent flushes), were recorded following the first year of growth.

Seedling culling

Seedling culling carried out by nurseries to eliminate seedlings that do not meet reforestation standards. To examine the effects of inbreeding on seedling cull rate, four culling standards (*Table 2*) were simulated and compared. Among the four culling standards, standard 2 and 3 are close to real practice, while standard 1 and 4 are extreme. Cull rates were also simulated and compared for minimum and maximum acceptable heights, and minimum diameter, both individually and in combination within each culling standard. For culling on maximum height, a seedling was considered culled only if its diameter was also smaller than the target value.

Table 2. – Culling standards used to simulating the effect of inbreeding on cull rate.

Culling Standard	Height (cm)		Diameter (mm)	
	Minimum	Maximum	Minimum	Target
1	12	25	2.5	3.0
2	15	30	3.0	3.5
3	18	40	4.0	4.5
4	21	45	4.5	5.0

Data analysis

ANOVA was performed using GLM procedures in SAS statistical software (SAS Institute Inc., 1989) to detect differences among cross-types and among families within cross-types for germination and seedling growth traits, as well as cull rate. Least-square means of cross-types were compared to detect if differences were significant between each pair of cross-types. The linear models are:

[1] $y_{ijk} = \mu + x_i + f_{ij} + E_{ijk,}$

[2] $y_{ij} = \mu + x_i + E_{ij}$

where

y is each observation;

μ is the general mean;

x_i is the fixed effect of the i^{th} cross-type;

f_{ij} is the random effect of the j^{th} family within the i^{th} cross-type;

E is the random error.

Model [1] was used for the traits observed with replications within families, including germination and seedling growth traits; while model [2] was used for traits observed with no replications within families, including seedling mortality, percent of abnormal seedlings, and cull rate. Arcsine square-root transformation was applied prior analysis to proportional data, including germination, seedling mortality, percent abnormal seedlings, and cull rate.

Since the original mating design balanced the contribution of each parent to each cross-type or inbreeding level, variation in performance of various cross-types due to effects of founders should be minor. Additionally, due to the complicated nature of the mating design, the removal of parental effects across cross-types is difficult (WOODS and HEAMAN, 1989). However, the analysis of parental effects by cross-type allows for comparison of maternal, paternal and grand-parent effects, and improves precision in detecting the differences among cross-types. There was only one observation per family for seedling mortality, the proportion of abnormal seedlings, and cull rate, thus parental effects could not be analyzed for these traits. Parental effects on germination and growth traits were analyzed by performing ANOVA using pedigree information for all cross-types except selfs (S1). Linear models for this analysis were based on the models used by WOODS and HEAMAN (1989) with slight modifications as follows:

[3] OC: $y_{ijk} = \mu + M_i + F_j + MF_{ij} + E_{ijk}$

[4] HS: $y_{ijklm} = \mu + M_i + F_j + CGF_k + CGM_l + E_{ijklm}$

[5] FS: $y_{ijk} = \mu + GF_i + GM_j + GFGM_{ij} + E_{ijk}$

[6] PO: $y_{ijk} = \mu + P_i + F_{j(i)} + E_{ijk}$

where

y is each observation;

μ is the cross-type mean;

M_i is the effect of the ith male parent;

F_j is the effect of the jth female parent;

MF_{ij} is the interaction between jth male parent and jth female parent;

CGF_k is the effect of the kth common grandfather;

CGM_l is the effect of the lth common grandmother;

GF_i is the effect of the ith common grandfather

GM_j is the effect of the jth common grandmother

$GFGM_{ij}$ is the interaction between the ith grandfather and jth grandmother;

P_i is the effect of the ith full-sib family;

$F_{j(i)}$ is the effect of the jth female within ith family; and

E is the random error.

All effects in the models [3] – [6] were treated as fixed. The average seed weight of each family was added to the model as a covariate for seedling growth traits, as it often has significant effects on seedling growth (SORENSEN and CAMPBELL, 1993). In order to remove parental effects listed in the models, residuals from each ANOVA, added to the cross-type mean, were used for further analyses. ANOVA was performed again with the new data set to detect the differences among the cross-types and among families within cross-types using model [1].

Regression analyses were performed at the family mean level to determine the relationships (linear, nonlinear or both) between performances of various cross-types and inbreeding coefficient using the following model:

[7] $y_{ij} = a + b_1F_i + b_2F_i^2 + E_{ij}$

where a is the constant; b_1 and b_2 are regression coefficients; F_i and F_i^2 are the linear and quadratic forms of inbreeding coefficient of the ith cross-type. If the quadratic term was not significant, the model was simplified to:

[8] $y_{ij} = a + b_1F_i + E_{ij}$

Among- and within-family variation was evaluated based on the coefficient of variation (CV). The CV of family means within each cross-type was used to estimate among-family variation, whereas the average of CV of seedlings within a family was used for within-family variation.

Results

Effects of inbreeding and their relationships with inbreeding coefficient

Germination, seedling mortality and the percent of abnormal seedlings

Germination was high, averaging over 94 % across all cross-types. Significant differences were found among cross-types before and after parental effects were removed (*Table 3*). Outcrossed progeny (OC) had 99 % germination, significantly higher than HS (94 %), FS (94 %) and S1 (95 %) ($P<0.05$). Crosses between parents and their offspring (PO) had significantly higher germination (97 %) than crosses between full-sibs (FS) (95%) although these two cross-types had the same inbreeding coefficient. The linear relationship between germination and inbreeding coefficient was very weak, although significant ($r^2=0.052$, $P=0.0049$).

Seedling mortality after one year growth in the nursery was close to zero for all cross-types except S1 (4 %), which was significantly higher than other cross-types ($P<0.05$). The percent of abnormal seedlings was generally low, and differed significantly among cross-types (*Table 3*). PO crosses had a significantly higher percentage of abnormal seedlings (11 %) than OC (2 %), FS (3 %) and HS (5 %). Differences among OC, HS, FS and S1 (5 %) were not statistically significant. Neither seedling mortality nor percent of abnormal seedlings was significantly correlated with inbreeding coefficient.

Growth traits

The height to the first budset showed significant ($P<0.05$) differences among cross-types. These differences became more evident after parental effects were removed (*Table 3*). Total height and diameter differed significantly among cross-types only after parental effects were removed. Lammas growth did not show significant differences either before or after parental effects were removed. Inbred cross-types had significantly smaller seedling diameter (4–5 %), height to first budset (5–7 %) and total seedling height (4–6 %) than outcrosses, but greater (although not significant) lammas growth (13–50 %). However, no significant differences were detected for diameter or total height among inbred cross-types (*Figure 1*). For height to the first budset, HS crosses were significantly smaller than

Table 3. – Results of ANOVA of seed germination, seedling mortality, percent abnormal seedlings, and seedling growth traits before and after parental effects were removed.

Trait	Effect	df	With parental effect		Parental effect removed	
			SS[1]	*F* (*P*)	SS	*F* (*P*)
Seedling mortality	Cross-type	4	0.232	4.86 (0.001)		
	Error	146	1.741			
Abnormal seedlings	Cross-type	4	0.420	3.43 (0.010)		
	Error	146	4.462			
Germination	Cross-type	4	1.377	4.19 (0.003)	1.33	13.63(<0.001)
	Family(cross-type)	146	11.999	3.21 (<0.001)	3.56	0.97 (0.577)
	Error	421	10.572		10.57	
Height to first bud	Cross-type	4	5866.2	2.44 (0.049)	5609.0	11.20 (<0.001)
	Family(cross-type)	146	87671.6	33.79 (<0.001)	18286.6	7.05 (<0.001)
	Error	11615	206393.4		206393.4	
Total height	Cross-type	4	5085.7	1.31 (0.269)	4715.9	6.19 (<0.001)
	Family(cross-type)	146	141966.8	35.29 (<0.001)	27794.9	6.91 (<0.001)
	Error	11615			320072.0	
Diameter	Cross-type	4	6575.8	1.56 (0.187)	6421.1	4.95 (<0.001)
	Family(cross-type)	146	153418.7	16.84 (<0.001)	47305.7	5.19 (<0.001)
	Error	11615	724772.4		724772.4	
Lammas growth	Cross-type	4	165.3	0.22 (0.929)	140.3	1.12 (0.350)
	Family(cross-type)	146	27843.8	23.88 (<0.001)	4581.9	3.93 (<0.001)
	Error	11615	92769.9		92769.9	

[1] Type III sum of squares, which is the sum of square that results when that variable is added last to the model.

FS and S1 (*Figure 1B*). A weak but significant linear negative relationship (r^2=0.16, P<0.0003) was found between diameter and inbreeding coefficient. No significant relationships were detected between height growth traits and inbreeding coefficient.

Cull rate

The average cull rates for minimum (*Figure 2A*) and maximum heights (*Figure 2B*) were generally low, especially for maximum height (less than 0.02). The cull rates on minimum diameter were substantial (*Figure 2C*), and differed considerably among the four standards (*Figure 2*). This is consistent with the distributions of height and diameter of seedlings of different cross-types. The separation among cross-types at the low value end was wider for diameter than for height. Significant differences in cull rate for height only occurred when seedlings were culled for the minimum height of standard 4. At this standard, PO showed the highest cull rate, significantly higher than OC and FS (*Figure 2A*). These culling standards were applied to the growth data, and did not consider seedling

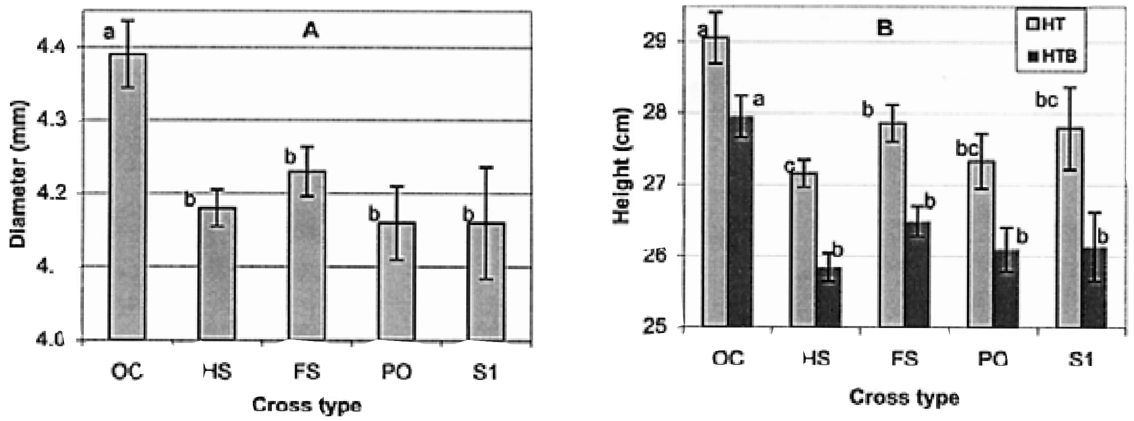

Figure 1. – Least-square means of seedling diameter (A) and height (B) for different cross-types (OC=outcrossed, HS=cross between half-sibs, FS=cross between full-sibs, PO=cross between parent and offspring, and S1=self-pollinated). The means followed with the same letter are not significantly different at the level of P<0.05. HT=total height; HTB=height to first bud set .

health, root development, and seedling form. However, these other factors are typically secondary to size, particularly diameter, in terms of contribution to culling.

Cull rates for minimum diameter showed significant differences among cross-types for all standards simulated (*Figure 2*). Inbred cross-types consistently had significantly higher cull rates than outcrossed seedlings, with S1 showing the highest rate. HS, FS and PO cross-types had intermediate values (*Figure 2C*). However, a significant linear relationship was found between inbreeding coefficient and cull rate only for the standards 3 and 4. The combination of culling for minimum and maximum height, and minimum diameter (*Figure 2D*) demonstrated very similar patterns to culling for minimum diameter, as most culling was attributable to seedlings not meeting the minimum diameter criterion.

Among- and within-family variation

Variation among families within cross-types was significant for all traits analyzed before and after parental effects were removed except germination (*Table 3*), which was significant only before parental effects were removed. Both among- and within-family variation increased considerably with the level of inbreeding for germination (*Figure 3*). Selfing produced significantly larger within-family variation than other types of crosses. For diameter and height growth traits, among-family variation also increased substantially with the level of inbreeding. The changes in within-family variation with inbreeding, however, were relatively small, although significant differences were detected between some inbreeding crosses and outcrosses (*Figure 3*).

Parental effects

Most parental and grandparental effects on germination and growth traits were significant for different cross-types (*Table 4*). Seed weight, used as a covariate, had significant effects on seedling growth in most cases. The amount of total variation accounted for by all types of parental and grandparental effects across all cross-types was 30–40 % for germination, 8–13 % for diameter, 23–25 % for height and approximately 20 % for lammas growth. The effect of mothers was generally greater than the effect of fathers or the interaction between mothers and fathers, indicating some maternal effects persisted even after the removal of seed weight effect. Grandparents also had significant effects, in most cases, on half-sib and full-sib crosses. The effects of grandmothers were greater than that of grandfathers on full-sib crosses for all traits.

Cumulative loss of seedlings at the nursery stage

Cumulative nursery losses due to no germination, mortality, abnormality, and culling was estimated for each cross type. Culling standards 2 and 3 are close to operational standards and were the only standards used for these estimates. As shown in *Figure 4A,* cumulative losses ranged from 19 % for OC to 43 % for S1, and were linearly correlated with inbreeding coefficient (r^2=0.82, P<0.05). Of the four potential types of loss included, culling on height and diameter accounted for the

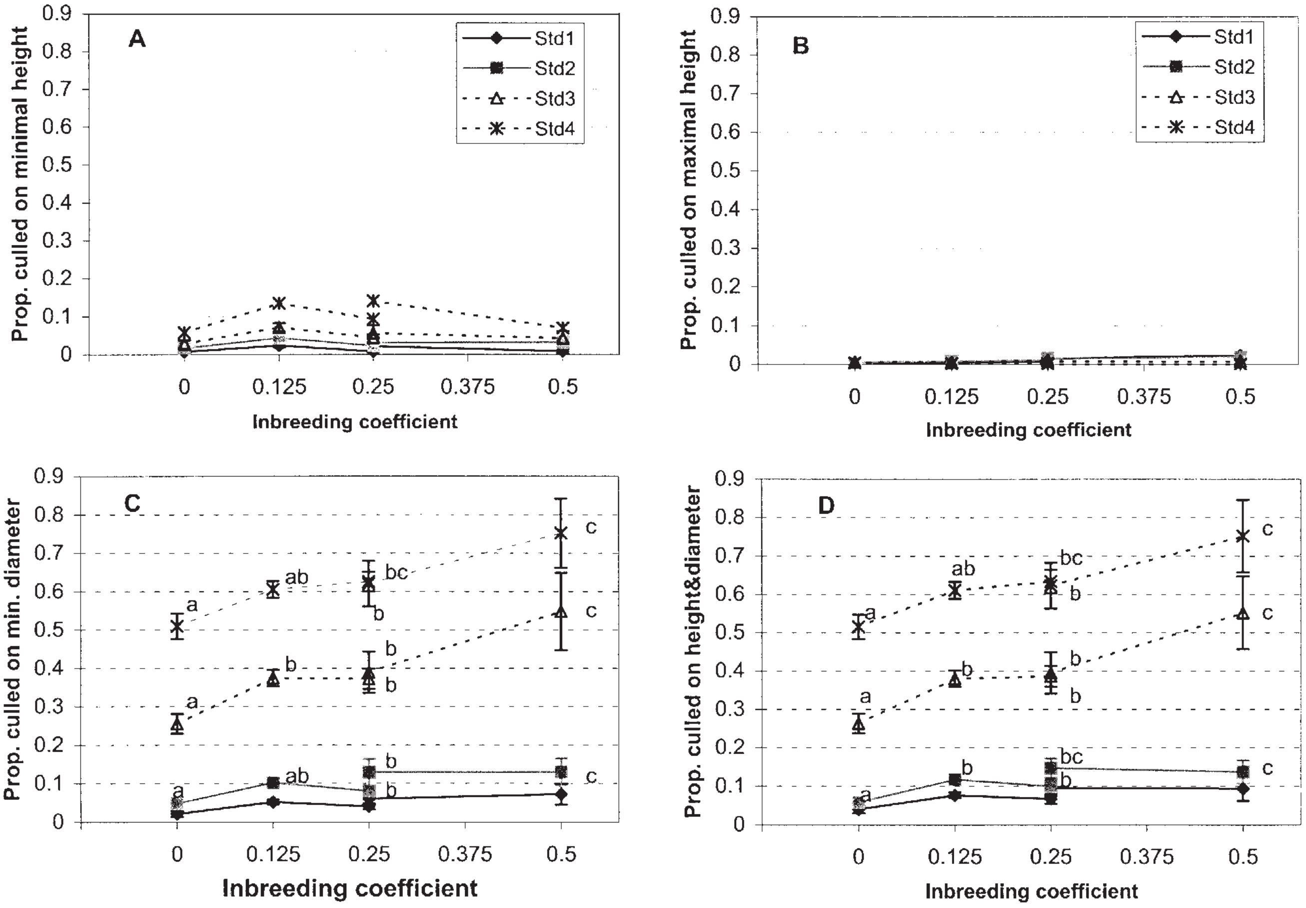

Figure 2. – Nursery cull rates at various levels of inbreeding using the culling standards listed in *Table 2.* The means followed with the same letter are not significantly different at the level of P<0.05. Proportion culled due to seedlings not meeting, minimum height (A), maximum height (B), minimum diameter (C), and the combination of minimum height and diameter (D).

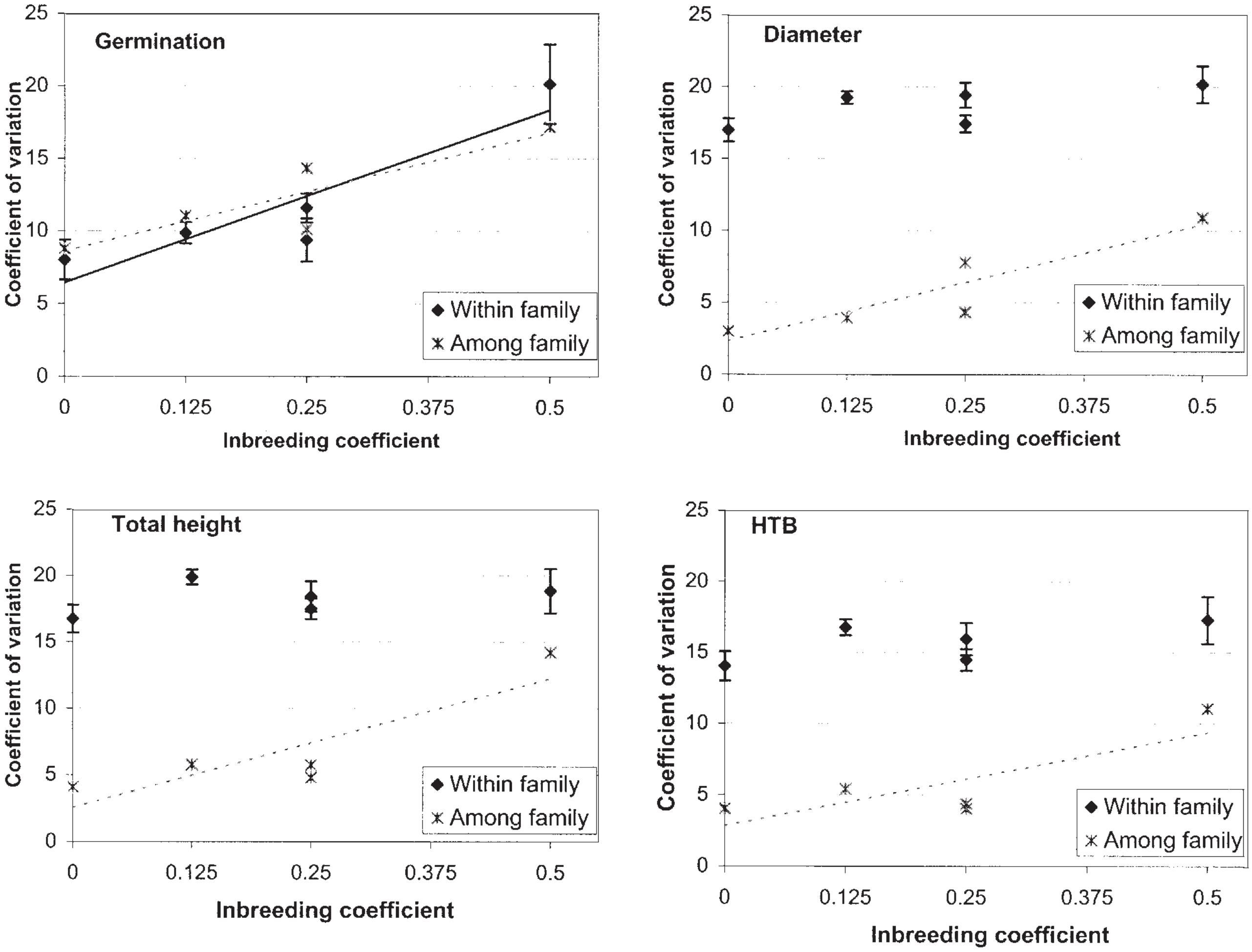

Figure 3. – Among- and within-family variation versus degree of inbreeding for seed germination, seedling diameter, seedling total height and the seedling height to the first bud (HTB).

greatest reduction from the number of seeds sown to the number of acceptable seedlings lifted.

Discussion

As breeding programs advance, relatedness among individuals will likely increase. Top-ranking trees are often from the same families or share common ancestors. Therefore, if advanced generation wind-pollinated seed orchards contain related trees, both selfing and low levels of inbreeding may occur. Selfing impacts on genetic gain are not a serious concern for Douglas-fir, as low self-fertility greatly reduces the number of viable seeds compared to outcrossing (Burczyk, 1998; Prat and Burczyk, 1998; Sorenson, 1971). However, low levels of inbreeding such as mating between full- or half-sibs, can produce a relatively high proportion of viable seeds (Sorensen and Cress, 1994; Woods and Heaman, 1989).

If a large proportion of the seeds obtained from low levels of inbreeding can produce seedlings that meet nursery culling standards, these inbred seedlings will be used for operational reforestation, and may negatively impact realized genetic gain in plantations. In the present study, we found that, relative to outcrossing, seeds with low to intermediate levels of inbreeding had significantly lower rates of germination, and the resulting seedlings had lower average height and diameter growth, and higher cull rates. These factors collectively resulted in higher levels of loss from the seed to the final seedling stage for inbreds (31–37 %) relative to outcrossed families (19 %). Selfed families were found to have the highest level of loss during the nursery stage (43 %).

No significant differences were detected between most inbred crosses and outcrosses for seedling mortality and the percentage of abnormal seedlings. Similar results were reported for the same species by Sorensen (1997). He found significant inbreeding depression from full-sib mating in seedling height and diameter, but not in seedling mortality.

Based on genetic theory, inbreeding is expected to severely reduce the fitness of trees (Falconer, 1989; Lynch and Walsh, 1998). In addition, allozyme studies (Bush and Smouse, 1992) show that inbreeding results in an excess of recessive, alleles in homozygous form, some of which may be deleterious. The low mortality observed at the nursery stage in this study could be due to most of the lethal homozygotes being eliminated during the period of embryo development, as the number of filled seeds per cone substantially differed among various levels of inbreeding (Woods and Heaman, 1989).

The degree of inbreeding depression found for seedling growth traits in the nursery was small compared to other studies in nursery traits with Douglas-fir (Sorensen, 1997) and to field tests for a number of other species (reviewed by Durel et al., 1996; Williams and Savolainen, 1996). One possible explanation is that the optimum greenhouse conditions used in this study may have alleviated the inbreeding depression, as observed by Husband and Schemske (1996) and reviewed by Lynch and Walsh (1998).

Table 4. – Results of the ANOVA of parental effects on seedling traits for different cross-types (*F-statistics* and *Pr>F*). Effects of seed weight on seedling growth traits were removed by including seed weight as covariate in general linear models [3]-[6]. CGF = common grandfather; CGM = common grandmother; GF = grandfather; and GM = grandmother.

				Trait		
Cross-type	Source	Germination	Diameter	Total height	Height to first bud	Lammas growth
OC	Male (M)	5.41(0.002)	18.21(<0.001)	15.23(<0.001)	29.95(<0.001)	16.70(<0.001)
	Female (F)	4.05(0.006)	26.90(<0.001)	91.51(<0.001)	49.09(<0.001)	70.40(<0.001)
	M*F	0.97(0.489)	7.11(<0.001)	10.47(<0.001)	15.02(<0.001)	2.45(0.004)
	Seed weight		39.56(<0.001)	39.00(<0.001)	57.50(<0.001)	0.01(0.912)
HS	Male	1.99(0.013)	18.56(<0.001)	21.61(<0.001)	28.14(<0.001)	5.95(<0.001)
	Female	8.45(<0.001)	16.77(<0.001)	28.77(<0.001)	40.79(<0.001)	17.70(<0.001)
	CGF	3.13(0.027)	0.29(0.835)	11.63(<0.001)	17.22(<0.001)	4.42(0.004)
	CGM	0.82(0.4435)	7.49(<0.001)	7.60(<0.001)	8.52(<0.001)	2.72(0.066)
	Seed weight		3.58(0.059)	6.49(0.011)	6.11(0.013)	1.06(0.302)
FS	GF	1.47(0.225)	28.54(<0.001)	23.77(<0.001)	68.72(<0.001)	46.58(<0.001)
	GM	3.05(0.019)	48.20(<0.001)	85.83(<0.001)	85.34(<0.001)	70.62(<0.001)
	GF*GM	2.97(0.002)	12.55(<0.001)	29.24(<0.001)	32.92(<0.001)	9.48(<0.001)
	Seed weight		12.24(<0.001)	11.86(<0.001)	37.88(<0.001)	5.48(0.019)
PO	Family	3.25(0.018)	52.04(<0.001)	136.44(<0.001)	135.34 (<0.001)	101.88 (<0.001)
	Female(family)	0.89(0.478)	1.98(0.094)	3.84(0.004)	1.43(0.221)	4.52(0.001)
	Seed weight		0.00(0.996)	8.01(0.005)	3.08(0.079)	7.86(0.005)

In the present study, germination and seedling diameter were found to have a weak but significant, negative linear relationship with inbreeding coefficient. No nonlinear relationships were found between any observed trait and inbreeding coefficient. Theoretically, these linear relationships indicate that the inbreeding depression is attributable to additive effects of the genes across loci (FALCONER, 1989). However, these relationships were very weak, and there were no significant differences among different types of inbred crosses for these two traits (*Figure 1*). In addition, germination was generally high (above 94%) and diameter varied only within a narrow range (between 4.15 and 4.4 cm). Thus, no strong inference of gene

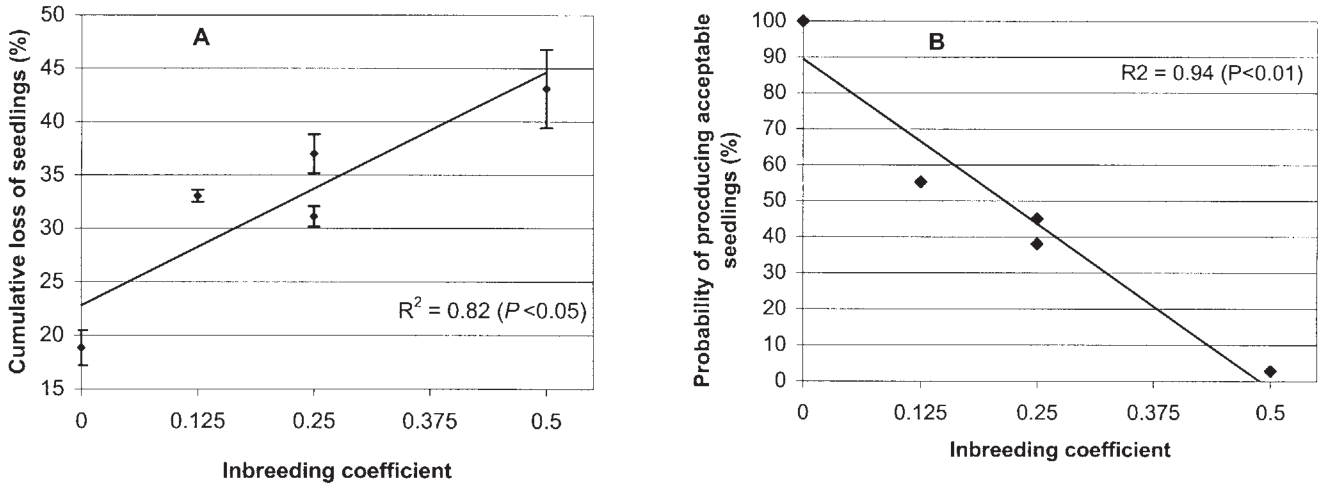

Figure 4. – Cumulative nursery losses from the stage of sowing seed through to lifting acceptable seedling (A), and probabilities of producing acceptable seedlings for reforestation for various levels of inbreeding relative to outcrosses, assuming the same probability of mating. The cumulative nursery losses were from seed germination, seedling mortality, percent abnormal seedlings, and culling caused by various level of inbreeding.

action can be made based on these relationships. Negative linear relationships between growth and inbreeding coefficient have previously been observed for both seedling height and diameter for coastal Douglas-fir (SORENSEN, 1997) and for loblolly pine (SNIEZKO and ZOBEL, 1988).

Theoretically, inbreeding increases among-family variation and reduces within-family variation if the genetic variation is due to genes which act additively (FALCONER, 1989; LYNCH and WALSH, 1998; ROBERTSON, 1952). Substantial increases in among-family variation were found in the present study for germination and seedling growth traits including diameter and height, in agreement with theory. However, within-family variation also increased with level of inbreeding. This trend was stronger for germination than for other traits (for seedling diameter and height). Although the increase of within-family variation contradicts theory, similar results have been observed in many studies for forest tree species (GEBUREK, 1986; HARDNER and POTTS, 1995; SNIEZKO and ZOBEL, 1988; SORENSEN, 1997). Possible explanations have been reviewed by WILLIAMS and SAVOLAINEN (1996) and SORENSEN (1997). Hypotheses include 1) the effects of recessive alleles at low frequency; 2) the conversion of additive x additive epistasis to additive genetic variance; and 3) inbred trees may be more likely to exhibit low phenotypic plasticity to environmental stress.

In comparison with the impacts of inbreeding on trait means, inbreeding effects on genetic variation are much more substantial at the nursery stage, particularly the effects on among-family variation. For example, the significant differences in cull rate among various levels of inbreeding appear to be due to the increase of genetic variation with inbreeding than to differences in means among inbreeding classes.

Between the two cross-types with the same inbreeding coefficient (FS and PO), PO crosses produced more severe inbreeding depression for all traits observed except germination, consequently a higher proportion of seedlings would be lost at the nursery stage than for FS crosses. However, the cause of such differences is unclear.

Parental and grandparental effects on nursery traits were smaller than these effects on filled seed production (about 50 %) in the previous study of (WOODS and HEAMAN, 1989). The removal of seed weight effects (used as a covariate) reduced maternal effects by a limited amount, as seed weight accounted for less than 5 % of the total variation in all seedling traits within cross-types. Strong maternal effects were observed for all nursery traits assessed, despite the removal of seed weight effects. This was consistent with other studies (KAYA and ISIK, 1997; SAMUEL et al., 1972). The strong parental effects found in the present study suggest the possibility of selecting parents that show relatively little inbreeding depression. This may become an important consideration in decisions to include related individuals in advanced generation seed orchards.

Although low levels of inbreeding result in a significantly greater loss of seedlings compared to those from outcrossed seed, a relatively large proportion of seedlings may still meet nursery culling standards. Combining the previous study of seed production (i.e., number of filled seeds per cone) of the same cross-types (WOODS and HEAMAN, 1989) with the results of the present study, we were able to estimate the relative probabilities of producing acceptable seedlings for various levels of inbreeding relative to outcrosses (*Figure 4B*). With the same chance of mating, selfing has a 4 % chance of producing filled seeds and 3 % chance of developing acceptable seedlings relative to outcrosses. In contrast, crosses between full-sibs, between parents and offspring, and between half-sibs have a 50 to 70 % probability of producing filled seeds and 40 to 55 % probability of producing acceptable seedlings for reforestation. Therefore, low levels of inbreeding have a much greater chance of producing seedlings that meet the nursery culling standards and of being used in plantations than selfing. Since trees with low levels of inbreeding have slower growth rate than outcrossed trees (DUREL et al., 1996), they may negatively impact the realized genetic gain of plantations.

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