

Effects of sib mating and wind pollination on nursery seedling size, growth components, and phenology of Douglas-fir seed-orchard progenies

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Abstract: Polymix outcross (X), full-sib (FS), and wind-pollination (WP) families were produced on 25 seed trees and 10 half-sib families on 10 of the same trees in a *Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii* seedling seed orchard. Seedlings were raised at two sowing densities for 2 years in the nursery, and inbreeding depression in seedling size related to inbreeding effect on growing season length and growth rate. Seedling mortality was light and not affected by inbreeding. Mean inbreeding depression (ID) for 2-year size traits was 6% (height) and about 8% (diameter) per 10% increase in F , the inbreeding coefficient, and was linear with the increase in F over the range of F used. Both amount of ID and its fit to linearity differed greatly among seed trees. Elongation season was significantly and slightly shorter for FS than for X families; second-year relative elongation rate was nonsignificantly larger for FS than for X families. Inbred families had nonsignificantly larger within-plot variance and significantly larger coefficients of within-plot variance than X families. Sowing density was not a significant factor except in diameter and height/diameter ratio. Results are discussed in terms of plant growth habit and possible gene action. Wind-pollination, compared with X, families were significantly shorter by 3.8% and significantly smaller in diameter by 4.6%, with much variation among family groups. About half of the height difference could be explained by seed weight; the remainder could have been due to pollen contamination or natural inbreeding. Progenies of the two pollen types did not differ for phenological traits, even though the seed orchard was in a drier, more inland location than the parent-tree locations. Progenies of WP had nonsignificantly larger within-plot variance than X progenies.

Résumé : À partir d'un verger à graines de semis de *Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii*, des descendance originant de pollinisation libre (WP), de croisements biparentaux (FS) et de croisements dirigés à l'aide d'un pollen composé (X) ont été obtenues à partir de 25 arbres semenciers, en plus de 10 demi-fratries produites par 10 de ces arbres. Les semis ont été cultivés pendant 2 ans en serre selon deux intensités d'ensemencement. L'effet de la dépression consanguine sur la taille des semis était relié à l'effet de la consanguinité sur la longueur de saison de croissance et le taux de croissance. La mortalité chez les semis était faible et sans lien avec la consanguinité. La dépression consanguine moyenne (ID) chez les caractères de taille des semis à 2 ans était de 6% pour la hauteur et d'environ 8% pour le diamètre, pour chaque tranche d'augmentation de 10% du coefficient de consanguinité F . Cette dépression consanguine affichait une relation linéaire avec l'augmentation de F pour l'étendue des valeurs de F étudiées. Cette valeur de ID et son adéquation à une relation linéaire différaient beaucoup d'un arbre semencier à l'autre. La période d'élongation de la pousse était légèrement plus courte pour les descendance découlant de FS comparativement à celles découlant de X et ce, de façon significative. De plus, le taux relatif d'élongation pour la seconde saison de croissance n'était pas significativement plus grand pour les descendance découlant de FS, comparativement à celles découlant de X. Les descendance consanguines affichaient une variance intra-parcelle plus élevée mais de façon non significative, ainsi que des coefficients de variance intra-parcelle significativement plus grands, comparativement aux descendance découlant de X. L'intensité d'ensemencement n'avait pas d'effet significatif sur les caractères mesurés à l'exception du diamètre et du rapport hauteur/diamètre. L'auteur discute des résultat en termes d'habitude de croissance des semis et de possibles effets des gènes. Les descendance découlant de la pollinisation libre affichaient une hauteur et un diamètre significativement plus petits, respectivement de l'ordre de 3,8 et 4,6%, comparativement aux descendance découlant de X. De plus, il y avait beaucoup de variation quant à ces caractères parmi les groupes de descendance. Environ la moitié de la différence en hauteur pouvait être expliquée par le poids des semences. Le reste de la différence pouvait être due à la contamination pollinique ou à la consanguinité naturelle. Malgré la localisation du verger à graines sur un site situé plus à l'intérieur des terres et affichant des conditions climatiques plus sèches que celles des localités d'origine des arbres parents, les descendance originant des deux types de pollen ne démontraient pas de différences quant aux caractères phénologiques. Les descendance découlant de WP affichaient de façon non significative une variance intra-parcelle plus grande que celle des descendance découlant de X.

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Introduction

Wind-pollinated species are mixed mating in the sense that the pollen pool for any individual usually contains a mixture of unrelated outcross pollen, self pollen, and pollen with lower degrees of relatedness such as from full and half-siblings. The inbreeding component of mixed mating usually has a severely deleterious effect on survival at the embryo stage and on survival and vigor at the plant stage (Charlesworth and Charlesworth 1987). Generally, for both survival and vigor, inbreeding depression is reported at certain times or ages without regard to ontogenetic changes in its expression. When observations have been made over time, it has been observed that magnitude of inbreeding depression can differ between life stages (Husband and Schemske 1995) and can differ among components contributing to growth (Voldeng and Blackman 1973). Voldeng and Blackman (1973), Donaldson and Blackman (1973, 1974), and others studying growth of maize (*Zea mays* L.) hybrids and inbreds have reported that inbreeding depression seems to be greater in early postemergence growth rate than in later growth rate, and greater in net assimilation rate than in leaf-area ratio. Analogous to the lesser inbreeding depression in later growth of maize, Sorensen and Miles (1982) found in a 10-year-old field test of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii*), in which height of self and outcross plants had been measured annually, that inbreeding depression in elongation rate at ages 5–9 years was less than inbreeding depression in total height at age 9 years. This indicated that inbreeding depression in relative elongation rate might be greater in the early postemergence phase than in the young sapling stage in Douglas-fir, at least in the absence of severe competition. The present investigation looks at the early ontogeny of inbreeding depression in more detail by observing in a nursery test the effect of low to moderate levels of inbreeding on a number of traits, including elongation-season length and relative elongation rate in year 2. Additionally, the effect of inbreeding on within-plot variance and the linearity of inbreeding depression across inbreeding coefficients (F) of 0, 0.125, and 0.25 were determined. Because matings were made in a seedling seed orchard, a second objective was to include wind-pollination families in the nursery test in order to contrast the performance of families from wind and orchard polymix pollinations of the same orchard seed parents.

This paper is a sequel to Sorensen and Cress (1994), which reported the effect of inbreeding on cone and seed traits for these same families.

Materials and methods

Crosses were made in 1989 in the Vernonia block of the J.E. Schroeder Cooperative Seed Orchard managed by the Oregon State Department of Forestry. The orchard contains full-sib families from biparental matings of selected trees, plus some half-sib relationships, i.e., two families with one common parent. The original source of the parent material was in the northwest Oregon Coast Range; the Schroeder Orchard is located in the Willamette Valley of western Oregon. Description of the material is given in detail in Sorensen and Cress (1994).

Orchard flowering and pollen production was very heavy in 1989 because of stimulation treatments the preceding spring (Ebell 1971, 1972). Flowering period was compressed because of unusually warm weather at the time of pollen shed and female conelet receptivity; all pollinations were made within a 9-day period.

Twenty-five seed trees were pollinated with polymix outcross and full-sib pollens. Fourteen of the same seed trees also were pollinated with half-sib pollen, of which four families were deleted because of possible misidentification (Sorensen and Cress 1994). Self-pollinations were not included because low self-fertility of the orchard trees made it very difficult to get enough filled seeds to include in a seedling test (Woods and Heaman 1989, Sorensen and Cress 1994). To approximately match reproductive phenology of pollen donors with phenology of seed trees, four different polymixes were used successively during the pollination season. Each polymix included 3–5 donors; thus within each family, outcrossed progeny were both full and half-siblings. Wind-pollinated cones were collected from the same seed trees. Wind-pollination families potentially differ from outcross families in (1) relative proportions of full and half-sibs within maternal families, (2) rate of natural inbreeding (excluded from outcrosses, not from wind pollinations), (3) pollen parents with different breeding values, and (4) "bagging" environmental effects. Outcrossed and sib-pollinated cones were bagged throughout cone development, first to prevent natural pollination and later to protect from cone and seed insects. Wind-pollinated cones were not bagged. Isolation bags change the microclimate around developing conelets and cones (Rohmeder and Eisenhut 1959). In Douglas-fir, bagging results in larger cones and heavier seeds inside the bags than outside (Sorensen and Campbell 1985).

Cones were harvested just before seed shed, seeds were extracted and cleaned, and empty seeds were removed by blower, or from x-rayed plates if the sample was small, and stored at -10°C . In February, seeds were removed from storage, soaked for 24 h in aerated water at ca. 20°C , stratified for 2 months at $2\text{--}3^{\circ}\text{C}$, surface dried, and sown in a raised nursery bed. Seeds were sown in small depressions in the soil surface, covered with ca. 0.5 cm granite grit, and the surface of bed was kept moist with periodic hand watering until emergence was complete. Two seeds per spot were sown and thinned to one at the end of the first season. Thinning was systematic, i.e., east seedling removed or north seedling removed if east-west separation could not be made.

Several size and phenology traits were measured or recorded (Table 1). There were several types of minor damage (damping off, frost, *Lygus* bug, etc.), but they occurred individually at a low frequency (<3% of seedlings affected), and the effects were not analyzed.

Experimental design was split split plot with three replications. Two sowing densities were used because of the possibility of inbreeding depression in competitive ability (Schmitt and Ehrhardt 1990; Latter and Mulley 1995). Whole-plot densities, 7.6×6.9 cm (190 seedlings/ m^2), and 9.9×9.5 cm (106 seedlings/ m^2) were randomized within replications; seed-tree groups (seedlings from outcross, full-sib, etc., pollinations of one seed tree) were randomly assigned to subplots within densities; and pollen type sub-subplots were completely randomized as 12-seedling noncontiguous plots within each seed-tree group. Two rows of border seedlings were sown along each edge of the test; six buffer rows were used between density plots; and six border rows were sown on the ends of the test. The three replications were sown April 1 and 2, 4 and 5, and 8 and 9, 1991, with one density plot in one replication being sown on each of the paired dates.

Because only 10 seed-tree groups included half-sib families, the data were analyzed in two sets. Set 89A included 3 replications, 2 densities, 25 females, and 3 pollen types (outcross, full sib, and wind pollination). Replications and females were random; densities and pollen types were fixed effects. Set 89B, which was used to test linearity between inbreeding depression and F , had 10 females and 3 pollen types (outcross, half sib, and full-sib).

The format for analysis of variance included single degree of freedom contrasts for pollen types and comparable contrasts for interactions involving pollen types. In 89A, contrasts were (1) outcross + wind versus full sib and (2) outcross versus wind.

Table 1. Traits analyzed in trial 89.

Trait and symbol	Description (units)
Size traits	
CN	Cotyledons per seedling (no.)
HYPO	Hypocotyl length, distance from ground surface to cotyledons (mm)
Ht1	Final height to base of terminal bud, year 1 (mm)
Ht2	Final height to base of terminal bud, year 2 (mm)
HtInc2	Height increment, year 2 (Ht2 - Ht1)
Vht2	Within-plot variance in Ht2
CVHt2	Coefficient of within-plot variance in Ht2
Dia2	Diameter, year 2, measured below cotyledons (hundredths of mm)
Ht/Dia2	Height/diameter ratio, year 2 (mm/mm)
ER2	Elongation rate, year 2 (HtInc2/ESL2 in weeks) (mm/week)
RER2	Relative elongation rate, year 2 (ln Ht2 - ln Ht1)/ESL2 in weeks(mm·mm ⁻¹ ·week ⁻¹)
Phenology traits	
EMERG	Emergence date, recorded when seed coat or hypocotyl visible above ground surface, observed daily (days from sowing)
BS1	Date of bud set, year 1, recorded when bud scales first visible on terminal shoot, observed twice weekly (Julian calendar date); if seedling second flushed, final bud set date was analyzed
BF2	Date of bud flush, year 2, recorded when green needles first visible within terminal bud, observed Monday, Wednesday, Friday (Julian calendar date)
BS2	As BS1, except observed weekly
ESL1	Elongation season length, year 1 (BS1 - EMERG) (days)
ESL2	Elongation season length, year 2 (BS2 - BF2) (days)

The null hypothesis in the latter contrast was that controlled outcross and wind pollination give equivalent vigor and phenology. In 89B, where pollen types were outcross ($F = 0$), half-sib ($F = 0.125$), and full sib ($F = 0.25$), the contrasts tested linearity and nonlinearity of the relation between inbreeding depression and F .

Inbreeding depression, in percent, was calculated as $I_i = [1 - (W_i/W_x)]100$ (Lynch 1988), where subscript i can be full sib (fs) or half-sib (hs) and W is the performance of the inbred (i) and outcross polymix (x). Values were calculated for each seed tree individually, and the mean for a trait was averaged from the seed-tree values (Lynch 1988). A negative I means that the inbred had a larger value for the trait than did the outcross. Difference between outcross and wind pollination was calculated in the same way, $E_{wp} = [1 - (W_{wp}/W_x)]100$. A positive value means that the wind treatment has depressed performance relative to outcross.

Two measures of within-plot variability or inequality were analyzed: variance and coefficient of variation. Variance was analyzed, because within-plot variance is used in heritability estimates. Coefficient of variation, a standard measure of relative variation, is highly correlated with other measures of inequality (Weiner and Thomas 1986) and was used to adjust for the effect of inbreeding depression on mean values. Pollen \times seed tree interactions were always highly significant. The relative contribution of the three pollen types to the pollen type \times seed tree interaction was partitioned using Wricke's (1962) ecovalence procedure. This was done for Ht2 only.

Elongation rate was evaluated in two forms, absolute and relative. Absolute elongation rate (ER) is the elongation per time period, independent of the initial size of the plant. Relative elongation rate (RER) is expressed as mm·mm⁻¹·time period⁻¹ and takes into account or adjusts for the initial size of the plant. Both measures were used on HtInc2 to evaluate the extent to which inbreeding depression in height (the common measure of size for young trees) occurs early in development versus is a multiplicative effect over growth periods.

Inspection of residuals and lack of correlation between sample means and variances indicated that transformations were not needed for analysis of phenological and size traits.

Results

Trial 89A (outcross, full sib, and wind) provided the strongest test of inbreeding because it was based on the most seed parents (25). Results from this trial will be presented first; results from 89B will be included only to test the linearity of the relation between inbreeding depression and F .

Results from the analysis of variance of 89A are given in Table 2. The test includes two pollen-type contrasts. The first (P1) is the contrast of outcross [(outcross + wind pollination) pooled] families versus full-sib families. Results for P1 will be presented first followed by the contrast, outcross versus wind-pollination families (P2 in Table 2).

Effect of inbreeding

Mortality

Mortality was very low, only 1.0%. Equality of percent mortality among pollen treatments was tested with χ^2 analysis; differences were small but significant ($\chi^2 = 16.085$, $df = 3$, $p = 0.0011$). Percent mortality was 0.4, 1.4, 1.7, and 0.7 for outcross, half-sib, full-sib, and open-pollination treatments, respectively.

Growth and phenology

P1 contrast (outcross + open pollination versus full sib) was significant for all but three traits (Table 2); that is, almost all traits showed a response to inbreeding. The amount and direction of the inbreeding effect is given in the left half of Table 3. The few negative signs in the "Mean" column show that full-sib families on average had a larger value for that trait than did the pooled outcross + wind-pollination families; negative signs in the left value in the "Range" column mean that some

Table 2. Effect of pollen-type contrast (P1 = outcross + wind pollination versus full sib; P2 = outcross versus wind pollination),^a seed tree (S), density (D), and their interactions^b on several phenological, growth, and size traits in the nursery in trial 89A.

Trait ^c	P1	P2	S	S×P1	S×P2	D×P1	D×P2	D×S×P1	D×S×P2
EMERG	0.9****	ns	21.9****	1.6****	0.8****	ns	ns	ns	ns
CN	4.5***	ns	54.5****	10.0****	3.7**	ns	ns	ns	ns
HYPO	0.5*	1.1**	51.3****	3.8****	1.2**	ns	ns	ns	ns
BS1	ns	ns	34.9****	3.8****	1.4***	ns	ns	ns	ns
BF2	2.2****	ns	50.3****	4.9****	1.2***	ns	ns	1.0**	ns
BS2	2.6***	ns	44.2****	5.6****	ns	ns	ns	ns	ns
ESL1	0.5**	ns	22.4****	2.1****	0.9***	ns	ns	ns	ns
ESL2	1.7***	ns	48.3****	4.6****	1.4***	ns	ns	1.0*	ns
Ht1	8.6****	1.4**	22.0****	2.4****	0.9****	1.0**	ns	ns	ns
Ht2	22.4****	1.4**	15.5****	4.4****	1.4**	ns	ns	ns	ns
HtInc2	19.6****	0.8*	8.7***	4.2****	1.5**	ns	ns	ns	ns
ER2	15.1****	ns	18.7****	4.3****	ns	ns	ns	ns	ns
RER2	ns	0.7**	22.1****	2.2****	1.3*	ns	ns	ns	ns
Dia2	26.5****	1.2***	11.4****	3.5****	1.2****	0.4****	ns	ns	ns
Ht/Dia2	10.5****	ns	18.6****	6.9****	1.6**	0.2*	ns	ns	ns
Vht2	ns	ns	14.0****	5.8*	7.6****	ns	ns	6.2*	ns
CVHt2	6.0****	ns	12.8****	9.2****	6.6**	ns	ns	6.7**	ns

Note: Results are given as the percents of total sums of squares for significant effects and are based on progenies grown at two densities. Further explanation is given in the text. ns, nonsignificant, $p > 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

^aThis trial includes 25 seed trees.

^bDensity and seed tree × density are omitted from the table, because they were not significant for any trait.

^cDescription of traits is in Materials and methods section.

Table 3. Inbreeding (I_{fs})^a and wind-pollination (E_{wp})^b effects in percents in trial 89A (1989 pollinations: outcross, full sib, and wind pollinations).

Trait ^c	I_{fs}			E_{wp}		
	Mean	SE	Range	Mean	SE	Range
EMERG	-0.5	0.1	-1.9 to 0.5	ns	—	—
CN	-2.3	0.9	-9.9 to 8.0	ns	—	—
HYPO	3.6	1.0	-11.5 to 11.5	3.0	0.6	-4.5 to 5.9
BS1	ns	—	—	ns	—	—
BF2	-2.0	0.5	-7.4 to 2.1	ns	—	—
BS2	-0.2	0.05	-0.7 to 0.3	ns	—	—
ESL1	0.6	0.2	-0.6 to 2.7	ns	—	—
ESL2	2.3	0.6	-2.9 to 7.9	ns	—	—
Ht1	13.5	1.4	0.3 to 24.6	5.2	0.8	-1.6 to 14.3
Ht2	15.0	1.2	3.5 to 23.6	3.8	0.8	-2.8 to 11.5
HtInc2	15.6	1.3	3.3 to 25.6	3.2	0.9	-4.7 to 12.0
ER2	13.3	1.4	-3.1 to 24.1	ns	—	—
RER2	-0.9 ^d	0.7	-6.9 to 4.0	-2.1	0.6	-9.0 to 3.8
Dia2-W	19.9	1.7	5.8 to 35.1	4.6	0.9	-1.8 to 12.4
Dia2-N	21.4	1.3	4.7 to 29.9	^e	—	—
Ht/Dia2-W	-7.1	1.2	-20.4 to 6.1	-1.1	0.7	-10.1 to 4.4
Ht/Dia2-N	-8.6	1.5	-22.4 to 3.5	^e	—	—
Vht2	ns	—	—	ns	—	—
CVHt2	-22.5	4.7	-73.3 to 11.4	ns	—	—

Note: Inbreeding depression of full sibs is at $F = 0.25$. Values are based on 25 seed trees. All values are in percentages.

Negative I_{fs} or E_{wp} means that the full-sib or wind-pollination value is larger than the outcross value; ns means effect is not significant. If pollen treatment interacted with density, values are given for both densities. SE, standard error.

^a $I_{fs} = [1 - (W_{fs}/W_x)]100$, where I is inbreeding depression in percent at $F = 0.25$ and the subscripts fs and x stand for full-sib and polymix outcross pollination, respectively.

^b $E_{wp} = [1 - (W_{wp}/W_x)]100$, where E is depression due to wind pollination, the subscript wp stands for wind pollination, and the other symbols are as above.

^cSee Materials and methods for description of traits. Wide (W) and narrow (N) refer to the two densities, which significantly affected only the two traits involving Dia2 and only for I_{fs} .

^d I_{fs} not significant for RER2, but included in the table, because it is discussed in the text.

^eInteraction (outcross versus wind pollination) × density was not significant for E_{wp} ; therefore, values are the same for both densities.

Table 4. Mean values for age 2 heights (Ht2) and diameters (Dia2) for each seed parent and pollen type.

Seed tree	Ht2 (cm)				Dia2 (mm)			
	WP	X	HS	FS	WP	X	HS	FS
3	53.2	58.7	47.8	47.0	6.35	7.03	5.46	5.26
4	58.2	58.5		48.7	7.23	7.17		6.23
5	54.9	57.0		49.5	6.11	6.09		4.89
6	55.5	60.8		53.2	6.09	6.89		5.88
8	53.7	56.9		53.6	5.77	6.44		5.44
10	52.1	54.0		45.2	5.83	6.37		4.71
12	50.5	54.4	49.6	42.3	6.08	6.72	5.86	4.79
13	52.2	56.9		47.7	6.08	5.13		5.13
21	56.7	55.6		47.9	6.58	6.36		5.08
25	60.2	64.2	61.8	53.1	6.77	6.98	6.48	5.01
27	55.5	59.1		47.3	6.19	6.59		4.60
29	60.9	62.1		58.4	6.38	7.15		6.27
31	59.6	60.0		55.8	6.42	6.50		5.56
33	58.7	61.4		52.4	6.72	7.42		5.18
34	65.0	65.3	64.8	53.4	7.09	7.28	6.72	6.04
35	57.4	64.9		49.3	6.46	7.37		5.42
36	61.2	65.9		50.0	6.93	7.36		5.39
37	57.9	56.3		48.2	6.51	6.58		5.33
38	59.4	59.4	57.7	57.1	7.36	7.37	6.31	6.06
43	63.0	64.5	60.9	52.4	7.20	7.37	7.08	5.22
45	59.6	59.9	53.9	57.8	7.15	7.02	6.31	6.66
63	56.7	57.1	55.5	51.1	6.66	6.63	6.35	5.71
65	59.0	60.8		46.4	6.70	7.01		5.03
66	58.5	63.1	57.9	55.6	6.82	7.24	6.42	6.25
69	59.8	60.5	56.8	48.0	7.23	7.42	6.90	5.65
Mean(25) ^a	57.6	59.9		50.0	6.59	6.91		5.47
Mean(10) ^b	58.6	60.7	56.7	51.8	6.87	7.11	6.39	5.66

Note: WP, wind pollination; X, polymix outcross; HS, half sib; FS, full sib.

^aBased on all 25 seed trees.

^bBased on the 10 seed trees that received half-sib pollen.

full-sib families had larger values than pooled outcross + wind-pollination families from the same seed tree.

Compared with outcrosses, seedlings from full-sib matings on the average emerged later, had more cotyledons and shorter hypocotyls, flushed later in year 2, and had significantly shorter elongation seasons in both years. Differences in phenology, though significant, were small in magnitude (0.6 and 2.3% in ESL1 and ESL2, respectively, Table 3). With regard to size, full sibbing reduced Ht2 by 15.0% (6.0% per 10% increase in *F*) and Dia2 by 19.9% (wide spacing, 8.0% per 10% increase in *F*) and 21.4% (narrow spacing, 8.6% per 10% increase in *F*). Family means for Ht2 and Dia2, averaged across densities, are given in Table 4. Ht/Dia2 was increased by inbreeding, more in the narrow (-8.6%) than in wide (-7.1%) spacing. The interaction between pollen type and density was significant for both Dia2 and Ht/Dia2 (Table 2, $D \times P1$).

For all traits, the interaction, seed tree (*S*) \times P1, was highly significant, mostly very highly significant ($p < 0.0001$) (Table 2). In other words, seed parents differed greatly in their response to inbreeding, even for traits in which there was not, on the average, a significant inbreeding effect. For all size traits except HYPO, I_{fs} values for all families were positive (Table 3, no negative values in the "Range" column); that is, all full-sib families showed inbreeding depression in size traits, but the

range in depression was large, from 3.5 to 23.6% in Ht2, for example.

Inbreeding increased within-plot variance (VHt2) by 6.8% compared with the outcross groups, but the difference was not significant (Table 2). When within-plot variance was expressed as coefficient of variation (CVHt2), full-sib families had significantly ($p < 0.0001$, Table 2) greater variation than the outcross progenies by 22.5% (Table 3).

Most unexpected was the inbreeding effect on elongation rate in the second growing season. Absolute rate (ER2), as expected, showed inbreeding depression (Table 2), but relative rate (RER2) was greater for full-sib progenies than for outcross progenies by 0.9%. Even though the difference was nonsignificant, it was surprising that in the second year the inbreds were elongating at a faster rate than were the outcrosses. Eckert and Barrett (1994) report similar results for inbreeding depression in height in *Decodon verticillatus* L. between the second and sixth weeks after emergence.

Linearity of inbreeding

Trial 89B evaluated the linearity of inbreeding depression across $F = 0, 0.125$, and 0.25 for families from 10 of the 25 seed trees. Results for all traits and including interactions among inbreeding level, seed tree, and density of seedlings

Table 5. Linear (I_{lin}) and nonlinear (I_{nlin}) effects of inbreeding at $F = 0$ (outcross), $F = 0.125$ (half-sib), and $F = 0.25$ (full sib) for several phenological, growth, and size traits in the nursery in trial 89B.

Trait ^a	I_{lin}	I_{nlin}	S	$S \times I_{lin}$	$S \times I_{nlin}$	$D \times I_{lin}$	$D \times I_{nlin}$	$D \times S \times I_{lin}$	$D \times S \times I_{nlin}$
EMERG	ns	ns	21.7****	0.6*	1.7****	ns	ns	ns	ns
CN	5.1*	ns	33.3****	8.6***	9.3***	ns	ns	ns	ns
HYPO	2.3*	ns	49.9****	1.3**	5.2****	ns	ns	ns	ns
BS1	ns	ns	26.4****	2.3****	4.4****	ns	ns	ns	ns
BF2	ns	ns	33.4****	4.5****	10.1****	ns	ns	ns	ns
BS2	ns	ns	34.2****	ns	8.3***	ns	ns	ns	ns
ESL1	0.9*	ns	24.8****	1.2****	2.5****	ns	ns	ns	ns
ESL2	ns	ns	29.8****	4.3****	11.5****	ns	ns	ns	ns
Ht1	9.1****	ns	22.3****	2.3****	2.7****	ns	ns	ns	ns
Ht2	20.3****	ns	21.7****	4.0****	3.6****	ns	ns	ns	ns
HtInc2	18.6****	ns	15.0****	3.6****	3.6****	ns	ns	ns	ns
ER2	12.6***	ns	24.5****	4.4****	5.5****	ns	ns	ns	ns
RER2	ns	ns	21.9****	2.6**	3.5****	ns	ns	ns	ns
Dia2	27.5****	ns	8.1****	3.9****	3.6****	0.4*	ns	ns	ns
Ht/Dia2	8.6**	ns	21.9****	6.7****	5.0****	0.5*	ns	ns	ns
Vht2	ns	ns	18.9**	5.7*	9.7***	ns	ns	ns	ns
CVHt2	ns	ns	13.0*	10.0**	9.5**	ns	ns	ns	ns

Note: Results are given as the percentages of total sums of squares for significant effects and are based on progenies from 10 seed trees grown at 2 densities. Further explanation is given in the text. I , effect of inbreeding, linear and nonlinear; S , seed tree; D , density. ns, nonsignificant; $p > 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

^aSee Materials and methods for description of traits.

in the nursery bed are given in Table 5. Linear (I_{lin}) and nonlinear (I_{nlin}) effects are compared by using the percent of total sums of squares (%SS) contributed by the sources of variation (Hicks 1982; Hühn et al. 1987). Percent of variation expressed in this way should be considered maximal, but %SS is suitable for comparing importance of I_{lin} and I_{nlin} because they always have the same degrees of freedom and the same error term.

For all traits, if the effects of inbreeding were significant, only the linear term was significant. This is well illustrated for Ht2 and Dia2 in Figs. 1 and 2. For Ht2, %SS for $I_{lin} = 20.3\%$, %SS for $I_{nlin} = 0.1\%$; for Dia2, %SS for I_{lin} and I_{nlin} were 27.5% and 0%, respectively. The tests were only moderately sensitive, coefficients of variation for the error term used in testing linearity were 12.4% for Ht2 and 14.0% for Dia2. However, the small %SS for I_{nlin} and Figs. 1 and 2 all show that deviations from linearity for the pollen-type means were very small, at least at low to moderate levels of inbreeding.

With one exception, $S \times I$ interactions were significant for all traits. For size traits, %SS was about equivalent for $S \times I_{lin}$ and $S \times I_{nlin}$; for phenological traits, $S \times I_{nlin}$ was 2 or more times larger than $S \times I_{lin}$. The only significant interactions with density involved Dia2 and Ht/Dia2. Surprisingly, for this group of families, high sowing density compared with low sowing density reduced Dia2 for outcrosses more than for inbreds (the slope of the line in Fig. 2 is steeper in low than in high density). This was true in both absolute (Fig. 2) and relative terms (not shown).

Polymix outcross versus wind pollination

On the average, wind- and cross-pollination families did not differ for any phenological trait, but wind-pollination families were slightly, but significantly, smaller (Table 2). Presumably,

and Cress 1994) and the difference was already present in hypocotyl length. The interaction, seed tree \times (outcross versus wind pollination), was highly significant for all size and phenology traits ($0.0019 < p < 0.0001$ for various traits). The range in E_{wp} for size traits is shown in Table 3. The range in E_{wp} is always considerably less than the range in I_{fs} for the same trait, but the range in E_{wp} values is still quite large and always includes positive and negative values, i.e., for some of the females the outcross progeny were more vigorous, for others the wind-pollination progeny were more vigorous.

Discussion

Mortality

Mortality was very low and even though inbreeding increased mortality, the increase was slight (from 0.4% for outcrosses to 1.7% for full-sib families). In an earlier Douglas-fir nursery trial (Sorensen and Miles 1974), selfed seedlings had 21% mortality versus 3% for outcross seedlings, and a small additional excess mortality for 8 years after establishment in a spaced field test (Sorensen and Miles 1982). All these results, but particularly those involving lower levels of inbreeding, indicate that inbred seedlings, once established, have high survival in the nursery and for at least 8 years after outplanting (Sorensen and Miles 1982). The low mortality of inbreds is surprising because it is in contrast with isozyme studies that found that the excess homozygosity in embryos of wind-pollination families is absent or much reduced in seedling or young pole-sized stands (reviewed in Bush and Smouse 1992). Most isozyme surveys have been made in naturally regenerated populations, and sampling has been done at the seed and then older seedling stages. Perhaps a much greater loss of more

Fig. 1. Nursery heights of 2-year-old polymix outcross, half-sib, full-sib, and open-pollination progenies from 10 seed trees, and sown at two densities, 106 and 190 plants/m². Inclined lines connect mean values; vertical lines represent 1 SD; squares and circles are individual family means.

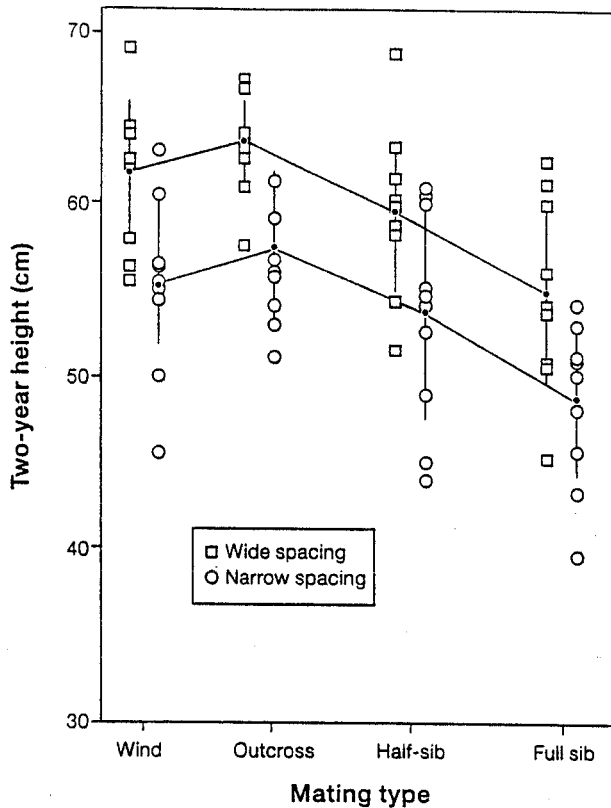
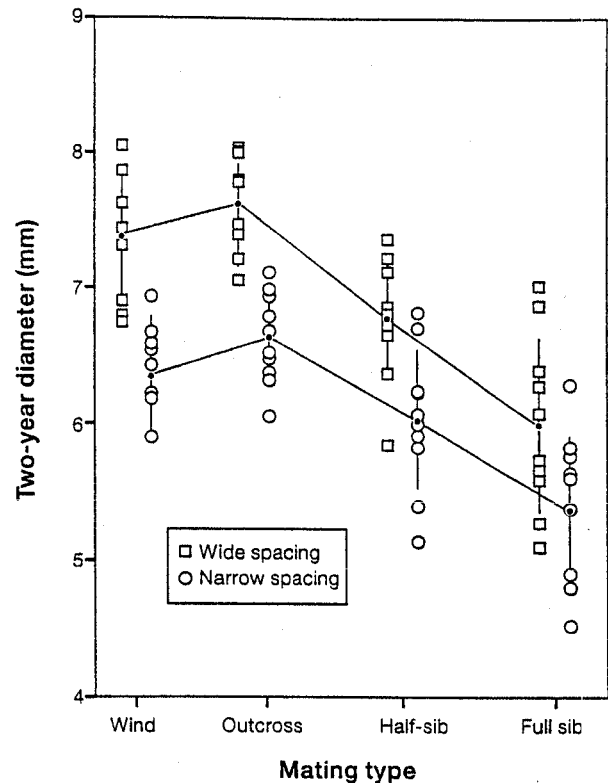


Fig. 2. Nursery diameters below cotyledons of 2-year-old polymix outcross, half-sib, full-sib, and open-pollination progenies from 10 seed trees, and sown at two densities, 106 and 190 plants/m². Inclined lines connect mean values; vertical lines represent 1 SD; squares and circles are individual family means.



benign conditions of the nursery (Harper 1977, p. 579). The results of the present test and Sorensen and Miles (1982) indicate, however, that these more homozygous individuals, if once established, may have the ability to persist in the population except in cases of extreme environmental or competitive stress (see also next paragraph). In the embryo, most inbreeding mortality seems to occur very shortly after fertilization (Orr-Ewing 1956; Hadorn 1961). Perhaps it is an analogous population response that under natural regeneration there is much loss of the remaining more homozygous individuals during or shortly after emergence.

Development of inbreeding depression in size traits over time

A large effect of inbreeding or genetic homozygosity on early development has been pointed out previously (Buchholz 1945; Voldeng and Blackman 1973; Donaldson and Blackman 1973, 1974). In the present test, emergence of full-sib seedlings compared with outcross seedlings was delayed slightly and hypocotyl lengths were shorter. Both were likely due to inbreeding depression in embryo size because seeds from full-sib seedlings weighed slightly less than seeds from outcrosses (Sorensen and Cress 1994), and to less vigor of the inbred embryos (Sarkissian et al. 1963). Date of first-year bud set did not differ between pollen types, but the extension season was slightly shorter for inbreds because of their slower (i.e., later) emergence. In the second year, relative extension rate did not differ between inbreds and outcrosses, and increase in percent in-

breeding depression for height was slight (Table 3, Ht2 vs. Ht1). A longer term field test of Douglas-fir showed only slight inbreeding depression of the height-growth trajectory prior to crown competition (Sorensen and Miles 1982). Wiener et al. (1988, 1992), in a comparison of inbred and outbred sheep, reported inbreeding depression in early size and skeletal size but not in growth rate.

In summary, it seems that in Douglas-fir, differential photosynthetic capital associated with inbreeding must be established early, and thereafter, in the absence of competitive or environmental stress, relative height-growth rate at least is little affected by inbreeding. Likely contributors to the equalization of RER2 are two allometric mechanisms. In young plants, growth rates tend to be inversely related to plant size (van den Driessche 1992 and references therein). Compared with open-grown plants, plants undergoing competitive stress will direct relatively more of their total assimilate to height than to diameter increment (Bormann 1965; Dippel 1982; Lanner 1985). Because inbred plants are short when competition starts in the nursery bed, it follows that they should have more depression in diameter than in height. This has occurred in the present test and is illustrated in two ways: Ht/Dia2 shows negative inbreeding depression, i.e., is larger for inbreds than for non-inbreds (Table 3), and the depression is significantly more negative at narrow than at wide spacing (Table 3, also Table 2, which shows that the interaction, P1 × density, for Ht/Dia2 is significant, $p = 0.0168$).

Inbreeding effects on nonsize traits

Even though inbred seeds and presumably embryos were smaller than outcross seeds and embryos (Sorensen and Cress 1994), cotyledon number of inbreds was significantly greater than cotyledon number of noninbreds (Table 3), as also has been observed in other conifers (Fowler 1965; Sorensen and Miles 1974; Sorensen et al. 1976). Number of cotyledons presumably is determined by initiation of growing points in the very young embryo; later size traits are determined by cell division and expansion. I can only speculate that it is a case of altered regulation of differentiation, perhaps analogous to the effect in animals where inbreeding has been found to decrease concentration of hormones that regulate development (Kosowska and Zdrojewicz 1996a, 1996b).

There was a small, but significant, effect of inbreeding on phenological or developmental traits (Table 2). Considering just year 2 phenology, bud flush (BF2) was later, bud set (BS2) was slightly later, and elongation season length (ESL2) was slightly shorter for inbreds than for outcrosses (Table 3). Inbreeding reduction in ESL2 was 2.3% (1.3 days), which should have contributed only slightly to inbreeding depression in size, but makes the negative inbreeding depression in relative elongation rate still more surprising.

Inbreeding depression in elongation season contrasts with most observations on annual plants, where inbreeding typically delays late-season phenological development and maturation (Sing et al. 1967; Borchers 1968; Wolfe 1993). There are constraints associated with flowering in the development of annual plants (Geber 1989) that could make the response of phenology to inbreeding different for annual plants than for the annual cycle of vegetative growth in conifers. Late-season development of annuals includes reproductive maturation, a more or less fixed process possibly prolonged by a lower rate of mitotic activity in inbreds than in outcrosses (Sarkissian et al. 1963). On the other hand, the annual elongation of many woody plants, including most conifers, is largely expansion of the cells of a preformed bud with some intercalary growth (Romberger 1963; Cannell et al. 1976). If terminal buds of inbreds are small compared with terminal buds of crosses, perhaps the former can complete their expansion phase slightly faster than the latter.

Inbreeding depression and within- and among-plot variability

Full-sib progenies were more variable phenotypically than outcross progenies in two measures: they had larger within-plot variances, particularly if expressed as coefficient of variation, and they contributed more to the variance for interaction with seed parent (48.5% versus 28.9% and 22.6% for outcross and wind pollen types, respectively), based on Wricke's (1962) ecovariance partitioning of the interaction. Similarly, standard errors for I_{fs} were always larger than standard errors for E_{wp} (Table 3).

Theoretically, within-family variance should decrease with inbreeding (Falconer 1960), but Snyder (1968), Sniezko and Zobel (1988), and Hardner and Potts (1995) also found larger within-plot variances for inbreds compared with outcrosses in several tree species. Increased within-plot variance can have several potential causes: (1) less stability of the inbreds to within-plot microsite variability (Robertson and Reeve 1952; Haldane 1954), (2) genetic and competitive interactions between neighboring plants in a heterogeneous mixture of inbreds and

outcrosses (Griffing 1989), (3) rare, recessive alleles (Robertson 1952; Heywood 1993), and (4) the effect of initial inbreeding on additive variance (Ruano et al. 1996). This test cannot distinguish between the causes; however, the large variation among families in I_{fs} Vht2 (not shown) and CVht2 (Table 3) suggests that the cause may be genetic rather than environmental. The considerably larger contribution of inbred than outcross and open-pollination families to pollen type \times seed tree interaction and the larger variability in I_{fs} than in E_{wp} may indicate quite large variation among seed parents in distribution of rare recessive alleles or in nonadditive genetic effects.

Linearity of inbreeding depression

Regression between mean performance and decrease in heterozygosity should be linear unless there is interaction between loci due to linkage or epistasis (Wright 1922). In the present material, there was no significant deviation from linearity in any trait (Table 5, column I_{nin}) as an average response. Curvilinearity was also nonsignificant for seed traits (Sorensen and Cress 1994). However, the interaction between I_{nin} and seed tree ($S \times I_{nin}$) was relatively large and significant ($0.01 < p < 0.0001$) for all seedling traits (Table 5) and for some seed traits (Sorensen and Cress 1994). This suggests the presence of linkage and interloci interactions that average out across families. Comparable variability among lines in response to different levels of inbreeding has been reported in maize (Burton et al. 1978) and *Tribolium castaneum* (Pray and Goodnight 1995). The latter citation includes illustrations indicating significant inbreeding quadratic \times line interaction, but no overall curvilinear response to F . See Pray and Goodnight (1995) for detailed discussion of possible causes.

Cross versus wind pollination

The seed orchard in which the pollinations were made is located at low elevation in the Willamette Valley of western Oregon. The seed parents are native to the Coast Range in northwest Oregon, much closer to the Pacific Ocean than is the orchard site. Common garden tests have shown significant difference in phenology associated with distance from the ocean (Campbell and Sorensen 1978; Sorensen 1983). The lack of significant phenological difference between wind and outcross families in this test (0.1, 0.6, and 0.0 day difference in BS1, BF2, and BS2, respectively) indicated minimal effective pollen contamination in the year of this study.

A similar conclusion came from seedling size comparison. Seeds from cross pollination were 5.2% heavier than wind-pollination seeds (Sorensen and Cress 1994). Outcross 2-year family mean heights were 3.8% greater than heights of wind-pollination families (Table 2). In an earlier study (Sorensen and Campbell 1985), a 5.2% difference in seed weight was associated with a 1.9% difference in 2-year height (seed weight effect on diameter was not determined). The remaining difference between outcross and wind-pollination families in Ht2 (1.9%) could be due pollen contamination with parents of lower vigor or to a small amount of natural inbreeding. With regard to the latter, even if wind depression is entirely due to inbreeding (natural selfing and sibbing), it indicates that the amount of sib pollination is low, and is not a serious problem in a well-designed Douglas-fir seedling orchard.

Within-plot variance was larger, but not significantly so, for wind than for outcross families by 7.2% ($F = 0.84$, $df = 1,48$,

$p = 0.3645$); similarly within-plot coefficient of variation was insignificantly larger by 8.5% ($F = 3.24$, $p = 0.0782$). The difference is logical and could have been due to more males in the wind than in the outcross pollen mix or to slight partial inbreeding (Lande and Schemske 1985; Sorensen and White 1988). In either case, larger within-plot variance would cause inflation of the denominator of the heritability equation for wind compared with single crosses or polymix outcrosses.

Practical considerations

Inbreeding depression in vigor is quite severe for Douglas-fir. For seed orchard management, the problem should be most serious if expected coefficient of inbreeding in orchard seed is moderate (e.g., $F \leq 0.25$), because seed set is still quite high at this level of inbreeding (Woods and Hearman 1989; Sorensen and Cress 1994). Allometric partitioning of growth that favors height increment means that plants with $F \leq 0.25$ will be difficult to cull in the nursery, and if established as members of plantations, they likely will persist as suppressed, intermediate, and even codominant trees. Unfortunately, crown position will be maintained at the expense of diameter and volume increment. As stressed in Sorensen and Cress (1994), the goal for management of orchards with family structure is to minimize frequency of within-family matings. To accomplish this, it is recommended that there be few members per family, that distance between family members be maximized, and that cones be collected in years when large pollen crops have been induced in the orchard. That was the situation in the present test, and on the average, wind-pollination progenies compared quite favorably with outcross progenies from the same seed trees.

Finally, I will comment on the potential role of seedling tests in orchard management. Most allozyme evaluations of seed orchard mating patterns (e.g., Adams and Birkes 1989; Pakkenen and Pulkkinen 1991) indicate that pollen contamination is potentially a serious problem. Pollen contamination in the Schroeder Orchard has not been tested with isozymes, but this seedling study indicated that the effect of contamination on phenology and vigor in this particular year was not serious. What this suggests is that seedling tests, and maybe even longer term tests in some cases, would be a valuable adjunct to allozyme investigations. The latter indicate percent contamination; the seedling tests would help to judge the impact of contamination on traits considered to be associated with adaptation and vigor. Seedling and longer term tests are time-consuming and expensive. They would not be appropriate if allozyme evaluation indicates little contamination. However, if contamination is moderate to high and the orchard is sited in an environment different from the parent tree locations, nursery and field tests could be useful in guiding management strategies and investments (Mikola 1982; Stoehr et al. 1994; Pulkkinen et al. 1995). Seedling and longer term tests have been used for these purposes in Finland, but rarely elsewhere.

As well, the seedling comparison could show if there was a small proportion of seed trees whose wind-pollination progeny performed much less vigorously than did outcross progeny from the same seed tree. Identification of these seed trees or clones in a seedling test would allow their deletion from the producing parents in the orchard. This would be analogous to culling low-vigor parents by using a nursery test as the first stage of selection in two-stage selection in family evaluation trials (Adams and Aitken 1991).

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