

NEAR NEIGHBOR POLLINATION AND PLANT VIGOR IN COASTAL DOUGLAS-FIR

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ABSTRACT

Nineteen seed parents in a young, patchy, second-growth stand, were control pollinated by near (NN) and by far neighbors (FN) and by wind (W) in a heavy flowering year. The progenies were compared in the nursery and for 10 additional years at close spacing in a field test. In a separate test, the aftereffect of isolation-bag environment was evaluated using plants of 10 of the same seed parents. Progeny from W and NN pollinations were slightly, but nonsignificantly smaller than progeny from FN pollination; seed parent \times pollen type interactions were very highly significant. Bagging effect was significant for nursery seedling size but could be eliminated by using seed weight as a covariate, disappeared in the field test, and did not interact with seed parent at any age. Estimated rates of biparental inbreeding in Douglas-fir, based on this test and values from the literature, range from zero to about 20%. Such variation seems compatible with the variation in natural regeneration habits of the species. If the biparental inbreeding is considered as resulting from half-sib mating, the associated inbreeding depression in height is estimated as zero to 2.3%. At the close spacing used in this study, depression in biomass appeared to be 4–5 times as great as depression in height at age 12.

Key words: *Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii*, biparental inbreeding, sib-mating, mixed mating, seed size, cone-environment aftereffect

INTRODUCTION

Individuals whose parents are related by descent usually show depressed vigor. In the mixed mating system of most conifers, relatedness of parents can be as strong as selfing (inbreeding coefficient, $F = 0.5$), or weaker in the form of nonself inbreeding where the parental relationship is usually at the level of half-siblings ($F = 0.125$), full-siblings ($F = 0.25$), or parent-offspring ($F = 0.25$). Given the presence of inbreeding depression, biparental inbreeding may be particularly deleterious because seed set is comparatively good (GRIFFIN & LINDGREN 1985, WOODS & HEAMAN 1989, SORENSEN & CRESS 1994) and inbreeding depression in vigor, though significant, is small enough that it cannot be controlled through early culling or other management procedures (SQUILLACE & KRAUS 1962, DUREL *et al.* 1996, SORENSEN 1997). This paper presents observations on the effect of near-neighbor pollination and presumed nonself inbreeding in a natural second-growth stand of coastal Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii*).

Several factors may influence the rate of nonself inbreeding as measured at the seed or young seedling stage. These include components of stand structure such as density (FARRIS & MITTON 1981, MORGANTE *et al.* 1991, SORENSEN & ADAMS 1993), clustering of

relatives (ELLSTRAND & FOSTER 1983, PARK *et al.* 1984, BRUNEL & RODOLPHE 1986), correlation in flowering phenology (EL-KASSABY *et al.* 1988, ERICKSON & ADAMS 1989, 1990), and genetic control of flowering precocity, intensity and relative maleness vs. femaleness (VARNELL *et al.* 1967, GRIFFITH 1968, EL-KASSABY *et al.* 1989, XIE & KNOWLES 1992). Embryonic genetic load, which varies greatly among individuals in Douglas-fir (SORENSEN 1971) can affect the relationship between rate of inbreeding at the time of fertilization and measured rate at the viable seed stage (LEVIN 1984, KARKKÄINEN & SAVOLAINEN 1993, SORENSEN & ADAMS 1993).

Coastal Douglas-fir is a widespread seral species that often regenerates after natural disasters, particularly fire, and forms pure to nearly pure stands. Seed source after these disasters can be individual trees, tree islands, and stand edges (ISAAC & MEAGHER 1936), which means that population substructuring might differ considerably from place to place and from generation to generation at any one location.

Several isozyme estimates of outcrossing rates, t , have been reported for natural stands of Douglas-fir (SHAW & ALLARD 1982, NEALE & ADAMS 1985, YEH & MORGAN 1987). They indicate a mixed mating system with considerable variation in t . In addition, SORENSEN & WHITE (1988) reported significantly less

16-year-old wind-pollination progenies than for control-cross progenies from the same maternal parents. Significant difference between the two groups remained after deletion of the shortest, presumably self-pollination, seedlings indicating that nonself inbreeding might be contributing to the vigor depression of the wind-pollination progenies.

Purposes of the present test were threefold. First was to verify the SORENSEN & WHITE (1988) results. The original comparison included progenies from only 6 seed parents and pollinations were made in a year of moderate flowering intensity. The present test involved more parent trees and wind pollinations were made in a year of prolific pollen production.

Second was to measure growth of progenies from wind (W), near-neighbor (NN), and far-neighbor (FN) pollinations, and to compare FN-W and FN-NN growth differences with that expected from published estimates of nonself inbreeding in Douglas-fir. In addition, we wished to estimate inbreeding depression in vigor expected from a range of rates of nonself inbreeding.

The third purpose was to get more and longer term data on the aftereffect of cone bagging (PARK *et al.* 1984) on growth in Douglas-fir. Control-cross cones were bagged throughout development, first for pollen isolation and then for insect protection. W cones were not bagged. Bagging of cones affects the environment in which they develop (ROHMEDER & EISENHUT 1959) and increases both cone and seed size in coastal Douglas-fir (SORENSEN & CAMPBELL 1985). Although seed size effect is expected to dissipate with time (SORENSEN & CAMPBELL 1993), it may not do so if the environment that caused the cone and seed size difference includes parental-environment aftereffects similar to those reported for conifer seed produced in nonnative environments (JOHNSEN & ØSTRENG 1994, SKRØPPA, *et al.* 1994), and for perennial herbs when the parental plants were raised under varied environments (LACEY 1996).

MATERIALS AND METHODS

Definitions

We refer to two **types of inbreeding**, self and nonself. The most frequently expected forms of nonself inbreeding in coastal Douglas-fir populations would be half-sib, parent-offspring, and full-sib. Nonself inbreeding is sometimes called **biparental inbreeding** (UYENOYAMA 1986), and we will use that term. **Outcrossing** in the present case implies mating between trees that are not related by descent. Two **rates of inbreeding** are used. The **primary rate** is that occurring at the time of fertilization; the **secondary rate** is that usually

measured at the viable seed or germinant seedling stage (LANDE *et al.* 1994). Inbreeding depression in seed set and height are assumed to be 18% and 6%, respectively, per 0.1 change in F (the inbreeding coefficient), and to be linear between $F = 0$ and $F = 0.5$ (SORENSEN 1971, SORENSEN & MILES 1982, WOODS & HEAMAN 1989, WOODS & STOEHR 1993, SORENSEN & CRESS 1994, SORENSEN 1997).

Materials

The study population was about 80 ha in extent and situated in the lower foothills of the west side of the Cascade Range in Oregon (44°35' N, 122°42' W, 260–300 m). Trees were 20 to 30 years old and an estimated 15 to 20 m tall when pollinations were made for the original study (SORENSEN & WHITE 1988) and 6 years older and 5–6 m taller when pollinated for this test. The stand included a mix of fully stocked areas (trees with approximately 2/3 green crown), less densely stocked areas (trees with 4/5 or almost fully green crowns), and open areas or areas with open-grown individuals. The stand was bordered on the west by land cleared for agriculture and in other directions by older Douglas-fir forest.

Pollinations for the present test were made in 1971, a very good flowering year in the area. Each seed parent was crossed with the tree nearest to it that had pollen (the near neighbor, NN). Distance between NN and seed parent stems ranged from 2 to 24 m (mean 12 m). In 7 pairs, branches of NN and seed parent overlapped. In no case was any tree between the NN and the seed parent. The contrasting far neighbor (FN), a different one for each seed parent, was 100 to 500 m from the seed parent and always with intervening trees between the two. Wind-pollinated (W) cones were collected from the upper crowns of the same trees.

The previous year of pollination was 1965. No numerical records of male and female cone bud production were kept either year, but 1965 was noted as a moderate flowering year, 1971 as a year of abundant flowering. Mean dates of female receptivity extended over 22 days among 6 seed trees in 1965, and over 17 days among 19 seed trees in 1971. Notes on pollination of seed trees indicated individual trees were receptive over 7–10 day periods in 1965; no records were kept in 1971. Two seed parents were common to both years.

The area includes rolling foothills bordering a small stream. Trees lower in elevation near the stream were phenologically later than trees higher on the slope indicating an environmental as well as the expected genetic effect on flowering phenology.

On 10 of the seed trees pollinated in 1971, some branches with wind-pollinated cones were enclosed in bags immediately after pollen flight. Wind-pollinated cones from both bagged and unbagged branches were collected from those trees. The effect of conelet bagging on seed weight and nursery growth was reported earlier (SORENSEN & CAMPBELL 1985); here the effect on longer term growth at close spacing is given.

Seeds were \times rayed; only filled, undamaged seeds were retained. Filled seeds were weighed, stratified, and germinated in an incubator by family plot. Pregerminated seeds were sown so that all seedlings had essentially the same starting date. Analyses were run on plot means. Seed weight was used as a covariate in analyses of nursery size measurements, but not in analyses of the field test measurements.

Experimental design and statistical procedures

Crossing types (NN, FN, W) and the bagging-nonbagging comparisons were evaluated in different tests. Both tests used split-plot designs, the former with 4, the latter with 3 replications. Main plots (seed trees) were randomized within replications; subplots (crossing types or bagging vs. nonbagging) were randomized within main plots. In the nursery, families were represented by 18-tree row plots that extended across the nursery bed. After two years in the nursery, survival ranged from 6 to 18 (mean 14.4) seedlings per family row plot.

After two years in the nursery, seedlings were moved to a field site (44°48' N, 123°20' W, 100 m) and planted at 0.75- by 0.75-m spacing. The experimental design was the same as that used in the nursery except that row plots were 8 plants long. Survival in the field plots at age 12 years ranged from 1 to 8 (mean 6.9) plants.

First-year epicotyl length and second-year total height were measured in the nursery. After 10 additional years in the field, plants were cut off at ground level, and total height, diameter 25 cm above ground level, and fresh weight of top were determined for each plant. Fresh weight of top was obtained by using a sling (for holding the plant) attached to a spring scale suspended from a large tripod. Trees were cut and directly measured and weighed. Harvesting was between August 2 and 24 of the 10th growing season in the field. No precipitation or other interruptions occurred during the harvest period.

The model for analysis of variance of crossing types was,

$$X_{ijk} = \mu + R_i + S_j + \varepsilon_{ij} + M_k + (SM)_{jk} + \varepsilon_{ijk},$$

where: μ – test mean, R_i – replication, $i = 1 \dots 4$, S_j – random seed parent, $j = 1 \dots 19$, ε_{ij} – main plot error, M_k – fixed crossing type (NN, FN, W), $k = 1, 2, 3$, $(SM)_{jk}$ – interaction between seed parent and crossing type, ε_{ijk} = subplot error.

The interaction, $(SM)_{jk}$, was used as the denominator for testing M_k . A similar model was used for analysis of the effect of bagging with the changes, $i = 1, 2, 3$, $j = 1 \dots 10$, and $k = 1, 2$.

Because the NN pollen trees were at various distances from the seed parents, differences in 12-year height and in 12-year top weight (FN value minus NN value) were regressed against the distance between the seed parent and the NN pollen parent. If closer NNs have a higher probability of being related to the seed parent, the FN-NN difference should tend to be larger for closer NNs, and the linear regression coefficient should be negative. The relation should be weak, because male parents will differ in combining abilities and different related matings will vary in the amount by which they depress growth (SORENSEN 1997).

RESULTS

Neighbor and wind pollination

Mortality between outplanting and harvest at age 12 was 13.4%, 13.9% and 15.0% for FN, NN and W progenies, respectively. The differences were very small and did not approach significance but were in the expected direction, assuming NN and W groups included a larger number of weak inbred plants than the FN group. On average, NN progeny were 1.2% shorter and W progeny 0.9% shorter than FN progeny; neither difference was significant. The FN-W difference is much less than the outcross-W difference reported earlier (SORENSEN & WHITE 1988). The difference in this test is in line with inbreeding depression expected based on published isozyme estimates of mating system in coastal Douglas-fir (see Discussion).

Results of both nursery and field-test analyses of size traits for the FN-NN contrast are summarized in short form in Table 1. Five points can be made with regard to NN mating: (1) NN vs. FN contrast was significant only for first year epicotyl length, and in that case NN progeny had longer epicotyls than did FN progeny, the opposite of expectation. In subsequent measurements, FN progeny were larger on average than NN progeny, but not significantly so. (2) In terms of mean values after 10 years in the field, the FN mean was always larger than the NN mean and the wind mean was intermediate. (3) Differences in weight were considerably larger than differences in height.

Table 1. F-test values for analyses of variance of nursery and 10-year field-test measurements of near neighbor (NN), far neighbor (FN) and wind (W) pollination treatments.

| Source of variation | Degrees of freedom | Seed weight | Nursery | | | | Field test | | |
|--------------------------|--------------------|----------------------------|--------------------|-------------------------|-----------------------|------------------------|------------|----------|------------|
| | | | Epi-1 ^a | Epi-1(adj) ^b | Height-2 ^c | Ht-2(adj) ^b | Height | Diameter | Top weight |
| Seed parents (S) | 18 | 1491.54* * ^d | 2.67** | 1.71 | 5.40** | 5.29** | 3.97** | 3.81** | 2.12* |
| Male treatments (M) | 2 | | | | | | | | |
| (NN + FN) vs W | 1 | | 5.17* | 0.22 | 1.79 | 1.09 | 0.10 | 0.01 | 0.12 |
| NN vs FN | 1 | 184.53** 0.12 | 4.45* | 4.25* | 0.01 | 0.23 | 0.83 | 0.90 | 1.18 |
| S x M | 36 | | | | | | | | |
| S x [(NN + FN) vs W] | 18 | | 1.14 | 1.44 | 1.79* | 1.72* | 2.54** | 2.48** | 1.47 |
| S x (NN vs FN) | 18 | 33.08** 20.77** | 2.43** | 2.35** | 4.89** | 4.22** | 3.52** | 3.63** | 3.23** |
| Coefficient of variation | | | 9.6% | | 7.0% | | 4.6% | 7.9% | 20.8% |
| Mean values | | | | | | | | | |
| Far neighbor | | 12.4mg | 9.47 cm | | 23.5 cm | | 7.77 m | 6.86 cm | 15.5 kg |
| Near neighbor | | 12.5 | 9.93 | | 23.3 | | 7.68 | 6.72 | 14.6 |
| Wind pollination | | 10.9 | 9.24 | | 22.9 | | 7.70 | 6.79 | 14.8 |

^a Epi-1 = first year epicotyl length or distance from the cotyledons to the base of the terminal bud.

^b Adjusted (adj) nursery size columns give the results when seed weight is used as a covariate

^c Height-2 = seedling height at end of nursery test

^d Statistical significances are, **, $p < 0.01$; *, $p < 0.05$

(4) The interaction, seed parent \times (NN vs FN), was highly significant ($p < 0.0001$) and large for all size traits. (5) Linear regression slopes for FN-NN difference and distance between NN and seed parent were negative ($r = -0.099$ for top weight, $r = -0.012$ for height), but nonsignificant ($p \gg 0.05$). Regression of top weight difference against distance is given in Figure 1. The 7 dots on the left side of the Figure represent male NNs whose branches overlapped the branches of the seed parent. Scatter around the line was large, no trend other than linear was indicated.

Bagging

Seeds from bagged cones were heavier than seeds from unbagged cones. The seed weight difference persisted in nursery seedling size, but if seed weight was used as a covariate, the bagging effect on seedling height was eliminated. After 10 years in the field, the bagging effect was no longer significant, and the progenies of unbagged cones were very slightly taller and had slightly heavier tops than the progenies from bagged cones (Table 2). Response to bagging in the nursery and the lack of response in the field were consistent across seed parents; i.e., interaction between seed

parent and bagging was nonsignificant (Table 2). Significant genotype \times parental environment interaction effect on seed weight has been reported in cases where parental-environment aftereffects were present (LACEY 1996).

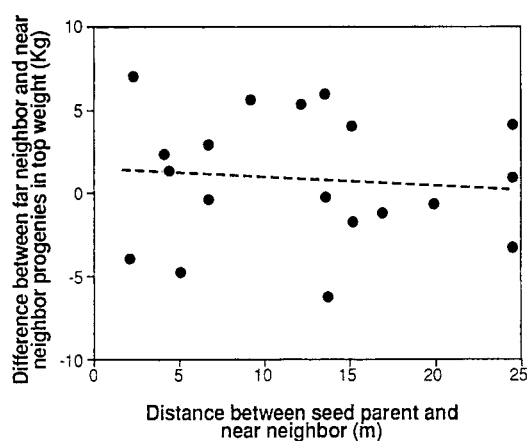


Figure 1. Difference between far neighbor and near neighbor progenies in top dry weight as related to distance between seed parent and near neighbor pollen parent for 19 coastal Douglas-fir seed trees. Equation for line, $Y = 1.392 - 0.054X$ and $r = -0.099$.

Table 2. F-values for analyses of nursery and 10-year field-test measurements of cone bagging study.

| Source of variation | Degrees of freedom | Seed weight | Nursery | | | | Field test | | |
|-------------------------------|--------------------|------------------------|--------------------|-------------------------|--|--------|------------|------------|---------|
| | | | Epi-1 ^a | Epi-1(adj) ^b | Height-2 ^c Ht-2(adj) ^b | Height | Diameter | Top weight | |
| Seed parents (S) | 9 | 522.81*** ^d | 2.34 | 1.74 | 4.84** | 4.36** | 3.07* | 3.50* | 1.69 |
| Cone bagging (B) ^c | 1 | 62.55** | 6.53* | 0.70 | 9.88* | 0.36 | 0.01 | 0.09 | 0.15 |
| S × B | 9 | 1.84 | 1.67 | 1.37 | 0.63 | 0.62 | 1.35 | 0.47 | 0.75 |
| Coefficient of variation | | 10.4% | 9.1% | | 4.1% | | 4.6% | 10.8% | 24.1% |
| Mean values | | | | | | | | | |
| Unbagged cones | | 10.7mg | 6.03 cm | | 29.8 cm | | 7.78 m | 6.71 cm | 15.5 kg |
| Bagged cones | | 11.9 | 6.58 | | 31.1 | | 7.79 | 6.75 | 15.2 |

^a Epi - 1 = first year epicotyl length or distance from the cotyledons to the base of the terminal bud.

^b Adjusted (adj) nursery size columns give the results when seed weight is used as a covariate.

^c Height - 2 = seedling height at end of nursery test.

^d Statistical significance are, **, $p < 0.01$; *, $p < 0.05$.

^e All cones were wind pollinated, bagged cones were bagged from immediately after pollen flight until cone collection, unbagged cones were not bagged at any time.

DISCUSSION

Relation to previous study

This test was installed partly because results in an earlier study (height depression of W compared to control-cross progenies, SORENSEN & WHITE 1988) indicated the possibility of an unusually high rate of biparental inbreeding. Other contributors to the slower growth of the W progeny were also possible: persistent aftereffects of cone bagging and seed size, and chance selection of control-cross male parents with above average combining ability.

In the present repetition of the test, we returned to the same stand but used more seed parents, and made pollinations in a year of more abundant flowering and with a slightly more compressed flowering season. Progenies in the second trial were grown at high density in order to promote inbreeding depression through competitive stress (SCHMITT & EBERHARDT 1990, WOLFE 1993). Under these contrasting conditions, W progeny were a nonsignificant 0.9% shorter than the FN progeny in the current test versus a significant 4.2% shorter than control-cross progeny in the 1965 test. The W progeny were 3.2% shorter in the 1965 test, even after the shortest (presumably self trees) had been deleted (SORENSEN & WHITE 1988).

As in previous studies, seed-weight effect on seedling height was present early, decreased with age in the nursery (ST. CLAIR & ADAMS 1991, SORENSEN &

CAMPBELL 1993), and vanished by age 12 in the field (Table 2). The result was consistent across seed parents, and seemed to eliminate a seed weight effect on W performance in the earlier trial. It also indicated that there was no long-term bag-environment aftereffect on embryo and plant development in coastal Douglas-fir analogous to the parental-environment aftereffects observed in Norway spruce (JOHNSEN & ØSTRENG 1994, SKRØPPA *et al.* 1994).

Thus, the poor performance of the W progeny in the 1965 test must have been genetic, either a high level of natural inbreeding or poor combining ability of the W males compared to a chance group of control-cross males. Given the high genetic control of flowering propensity and phenology (see Introduction), it seems possible that mating in a year (1965) of moderate flowering with a wide spread of flowering phenology in a young stand might be analogous to mating in a stand of low density, and such stands are sometimes characterized by increased inbreeding (FARRIS & MITTON 1981, MORGANTE, *et al.* 1991, SORENSEN & ADAMS 1993).

The two seed parents that were common to both years of pollination gave very inconsistent results: height reductions (W vs. FN-type progenies) of 3.2% and 9.5% at age 16 in SORENSEN & WHITE (1988) vs. 7.8% and -4.5%, respectively, (negative signifying that W progenies were taller than FN progenies) at age 12 in the present test. It is clear that the year × seed parent, or year effect, can be large, but it is not clear if

Table 3. Effect of different levels of half-sibbing at the fertilization stage on half-sibbing at the seedling stage and on inbreeding depression in height due to half-sibbing. Rate of self-pollination is assumed to be 50%^a, rate of background outcrossing 10%^b. The 4 levels of half-sibbing are based on estimates of biparental inbreeding in the literature of coastal Douglas-fir (see text). Relation between inbreeding depression and inbreeding coefficient, F, are considered to be linear for both seed set and height, and assumed to be 18% per 10% increase in F for seed set and 6% per 10% increase in F for height^c (references for these relations are in the text).

| Mating type | Mating at fertilization stage (%) ^d | Mating at seedling stage | | Inbreeding depression in height due to half-sibbing (%) | Inbreeding depression in height with selfs included (%) |
|------------------------|--|--------------------------|------------------|---|---|
| | | (Number) ^e | (%) ^f | | |
| A^g | | | | | |
| Selfing | 50 | 5 | 9.1 | | |
| Near neighbor | | | | | |
| Half sibbing | 0 | 0 | 0 | 0 | 2.73 |
| Outcrossing | 40 | 40 | 90.9 | | |
| Background outcrossing | 10 | 10 | | | |
| B^h | | | | | |
| Selfing | 50 | 5 | 9.3 | | |
| Near neighbor | | | | | |
| Half sibbing | 5 | 3.9 | 7.2 | 0.54 | 3.33 |
| Outcrossing | 35 | 35 | 83.5 | | |
| Background outcrossing | 10 | 10 | | | |
| Cⁱ | | | | | |
| Selfing | 50 | 5 | 9.5 | | |
| Near neighbor | | | | | |
| Half sibbing | 10 | 7.8 | 14.5 | 1.11 | 3.86 |
| Outcrossing | 30 | 30 | 75.8 | | |
| Background outcrossing | 10 | 10 | | | |
| D^j | | | | | |
| Selfing | 50 | 5 | 9.9 | | |
| Near neighbor | | | | | |
| Half sibbing | 20 | 15.5 | 30.7 | 2.30 | 5.27 |
| Outcrossing | 20 | 20 | 59.4 | | |
| Background outcrossing | 10 | 10 | | | |

^a SORENSEN (1982)

^b SILEN (1962), and this paper

^c WOODS & HEAMAN (1989), WOODS & STOEHR (1993), SORENSEN & CRESS (1994)

^d Rates of primary sibbing (LANDE *et al.* 1994)

^e Number of seedling produced from the fertilizations assuming 90% inbreeding depression after selfing (SOSENSEN 1971), 22.5% inbreeding depression after half-sibbing (WOODS & HEAMAN 1989, SORENSEN & CRESS 1994), and 0% inbreeding depression after outcrossing.

^f Rates of secondary sibbing (LANDE *et al.* 1994).

^g Based on estimate of nonself inbreeding in SHAW & ALLARD (1982).

^h Based on estimate of nonself inbreeding in NEALE & ADAMS (1985). Estimates in the study reported are intermediate between levels B) and C).

ⁱ Based on estimates of nonself inbreeding in YEH & MORGAN (1987) and in this paper.

^j Based on estimate of nonself inbreeding in SORENSEN & WHITE (1988)

the cause is due to different levels of relatedness in the W pollen cloud, or to combining abilities of the control-cross males.

Effect of NN pollination in current trial

Overall, NN progenies were 1.2% shorter than FN progenies at age 12. The difference was not significant. This was not surprising. The FN-NN difference was small, but within the range expected based on published isozyme estimates of natural inbreeding in Douglas-fir. The interaction against which it was tested (seed parent \times male type) was large and highly significant. The component of variance for seed parent \times male (S \times M) interaction was nearly as large as the component for S (for example, 629 vs. 847 for S \times M and S, respectively, for height at age 12). Much of the interaction is probably due to variation in breeding values of the males or in specific S \times M combining abilities, but also contributing could be NN matings that involved half- or full-sibs. Although Figure 1 seems to indicate an approximate continuum of FN-NN differences, it is possible that one or more of the largest positive values shown in the Figure are due to matings between relatives.

Effect of biparental inbreeding on growth

Because published estimates of natural inbreeding, particularly biparental inbreeding, have not been related to their impact on tree size, we have used the results from this test and others to illustrate that relationship for Douglas-fir. We use height for illustration, because published estimates of inbreeding depression in vigor usually are given for height.

Assuming no inbreeding in the FN progenies, the 1.2% reduction in height of NN progenies (7.68 m for NN families vs 7.77 m for FN families, Table 1) could result from about 16% half-sib seedlings (i.e., 3 NN males being half-sibs to the seed parents with which they were mated), or from about 8% full-sib seedlings. Plants from half-sib mating ($F = 0.125$) would have an average inbreeding depression in height of about 7.5% (SORENSEN 1997, and unpublished data from older tests). If 16% of the NN progeny were the product of half-sib mating, height reduction due to half-sibbing would be 1.2% ($[\text{frequency of inbreds}] \times [\text{inbreeding depression}] = 0.16 \times 0.075 = 0.012$ or 1.2%).

W and FN families averaged 7.70 m and 7.77 m, respectively (Table 1) to give a height depression of 0.9%. Assuming the height depression was all due to related NN matings (weak self plants eliminated by competitive suppression at the tight spacing), the rate of secondary biparental inbreeding was about 12% if the

parents were related as half-sibs or 6% if they were related as full-sibs. Because sibbing also results in seed loss, these seedling values are equivalent to primary biparental inbreeding rates of about 15% half-sib mating or 10% full-sib or parent-offspring mating (seed loss effect from SORENSEN & CRESS [1994]).

Relationship to isozyme estimates of mixed mating

Isozyme estimators of outcrossing can be derived in two ways, either averaged from single loci or based on multiple marker loci (SHAW *et al.* 1981). The multi-locus model distinguishes between selfs and outcrosses; the single-locus model between all inbreeding and outcrosses. When there is inbreeding other than selfing, the multilocus estimate of inbreeding is expected to be lower than the mean of the single-locus model. For a discussion of assumptions to the models and the effects of violations of the assumptions, see GODT & HAMRICK (1991). In using the following examples, we do not mean to overemphasize the precision with which biparental and self inbreeding are distinguished. Rather we use the published values as a range of estimates that are available for Douglas-fir.

SHAW & ALLARD (1982) report multi- and single-locus estimates for seed produced under open pollination in 8 natural stands in western Oregon and Washington. Differences between estimators (multilocus minus mean of single locus) ranged from 0.04 to -0.04 among stands. Averaged over all stands, the mean difference between multilocus and mean single locus estimates was about 0.01. Little or no inbreeding other than selfing was indicated.

YEH & MORGAN (1987) report results from a Douglas-fir stand in British Columbia. The stand regenerated after fire and was about 30 years old. In this case, they found a large difference between the multilocus estimate (0.11) and the mean single locus estimate (0.26).

NEALE & ADAMS (1985) compared mating system estimates between adjacent shelterwood and uncut natural, mostly old-growth, Douglas-fir in the Oregon Cascade Range. Outcrossing estimates were not significantly different between the two stand types, but the multilocus estimate was higher than the mean single-locus estimates by 0.08 in the uncut stand and nearly the same in the shelterwood (difference = 0.01). The authors argue for nonself inbreeding as the reason for difference between estimates in the uncut stand; that is, family structure and NN mating were present in the unmanaged stand, but the shelterwood cut broke up the family structure.

In Table 3 we have listed 4 combinations of mixed mating that give secondary (i.e., at the seed stage)

biparental inbreeding rates in Douglas-fir comparable to those reported in the literature. In each combination, percentage of primary selfing (selfing rate at the fertilization stage) was assumed to be 50% (SOERSEN 1982) and background pollination 10% (from SILEN 1962 after discounting self pollination). The 40% NN pollination was varied between biparental inbreeding and outcrossing to reflect estimated rates of secondary biparental inbreeding that have been reported in the literature: (A) zero percent (SHAW & ALLARD 1982), (B) 8% (NEALE & ADAMS 1985), and (C) 15% (YEH & MORGAN 1987) with the present test between (B) and (C). All biparental inbreeding in Table 3 is assumed to be between half-siblings. Full-sib and parent-offspring inbreeding, at the same levels of primary inbreeding as used for half-sibbing in Table 3, would yield about 25% fewer inbred seedlings because of more inbreeding depression in seed set after full- than half-sibbing, but about 50% greater inbreeding depression in height of surviving seedlings.

The effects of low to moderate levels of biparental inbreeding on height growth are small, probably too small to be detected in field tests even at rather high rates of biparental inbreeding (Table 3, second from right column, 0.54% inbreeding depression in height at 5% primary half-sibbing to 2.30% inbreeding depression in height at 20% primary half-sibbing).

CONCLUSIONS

Estimated rates of secondary biparental inbreeding equivalent to near zero to about 20% half-sib pollination have been reported for Douglas-fir. Such a range is compatible with intraspecific variation in patterns of natural regeneration.

Contrary to our expectation, estimated inbreeding depression in height caused by biparental inbreeding did not exceed that expected from natural selfing, even at comparatively high rates of biparental inbreeding (Table 3, bottom line). The caveat to this is that biparental inbreds, being more vigorous than selfs, will persist in occupying space and, compared to selfs, have a greater long-term effect on stand productivity.

As the stand develops and stand density increases, height growth is normally favored at the expense of increment in diameter and biomass (BORMAN 1965, DIPPEL 1982, LANNER 1985). In this test which was run for 10 years in the field at close spacing, the difference between FN and NN progenies in biomass was 4–5 times as large as the difference in height (Table 1, bottom, comparison of mean values). That is, the effect of inbreeding, particularly biparental inbreeding, on productivity is likely greater than indicated by height measurements alone.

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