

Family composition of Douglas-fir nursery stock as influenced by seed characters, mortality, and culling practices

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Application. Large changes in family composition during nursery production can lead to reductions in genetic diversity and unpredictable genetic gains from tree improvement programs. Douglas-fir families were shown to differ in their ability to produce plantable seedlings under operational nursery conditions. The resulting changes in family composition, however, were not great enough to justify the increased costs of segregating families within the nursery in order to better control the composition of outplanting stock.

Abstract. Changes in family composition during nursery production were evaluated by following individual seeds and seedlings of 36 wind-pollinated Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco) families sown in mixture in two operational nurseries in western Washington and Oregon. Families differed significantly in emergence and in percent of seedlings culled for being too small. However, differences were small enough that family composition was largely unaffected. The observed changes in family composition did not markedly reduce genetic diversity and did not affect the genetic gain that may be expected from an improved population. The plantable nursery stock was, for the most part, representative of the composition of families originally sown.

Introduction

Deployment of genetically improved nursery stock of Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco) usually follows the bulking of seed collected from orchard clones or families, and the raising of seedlings from the bulked lots in operational nurseries. Implicit in this approach is the assumption that the genetic composition of outplanted nursery stock represents the genetic composition of the parents of the bulked mixture, which may often include all flowering clones in the orchard. The genetic composition of improved nursery stock, however,

may differ considerably from that of the parents because of pollen contamination or because of differences among parents in male and female flower productivity and phenology, cross-compatibility, self-fertility, seed and cone abortion, and embryo viability (Eriksson et al. 1973; Woessner and Franklin 1973; Friedman and Adams 1981; Griffin 1982; El-Kassaby et al. 1986; Erickson and Adams 1989). In addition, directional selection may occur among families in the nursery as the result of differences in germination, emergence, and mortality (Campbell and Sorensen 1984). Genetic composition may be further altered following standard culling of small seedlings if seedling size differs greatly among families. Differences in seedling size may, in part, be related to differences in seed weight, rate of emergence, and competitive ability.

Reduction in representation or loss of families within the mixture is of particular concern for two reasons: (1) genetic diversity of production forests will be reduced, and (2) realized gains from tree improvement programs will differ from expected. Loss of genetic diversity may lead to a greater risk of destruction from pests and environmental extremes, and to decreased phenotypic stability across variable environments (Trenbath 1974; Hühn 1985). One way to limit loss of families is to collect and process seed separately by parent-tree clone or family, and to grow family seedlots separately in the nursery (Gladstone 1981; Talbert 1982; Johnson and Kellison 1984). Families can then be mixed prior to outplanting, or can be planted separately in a mosaic of family blocks. Although this approach directly controls the seedling composition, it also increases seed orchard and nursery management costs. Thus, the degree to which individual family representation actually is diminished or lost in bulked seedlots is crucial in evaluating the merit of segregating — rather than bulking — families in nursery production.

In this study, we investigated changes from the original composition of 36 open-pollinated families of Douglas-fir sown in equal proportions in mixture into two bare-root nurseries in Washington and Oregon. Factors influencing the final composition were assessed by measuring differences among families in percent emergence, mortality over 2 years, and percent

Tree Improvement Program (Silen and Wheat 1979). The trees were located in second-growth stands in the Coast Range of west-central Oregon, within an area approximately 60 by 15 km in size and between 150 and 430 m in elevation. Mature wind-pollinated cones were collected in fall 1985 and dried for 3 days at 38 °C to allow extraction of seeds. Seeds were dewinged, processed through a blower to remove empty seed, and stored at -10 °C. Seed weights (g/1000 seed) were recorded for each family. Seeds were stratified by soaking in distilled water for 24 h at room temperature and stored at 3–4 °C for approximately 9 weeks (Sorensen 1980).

Study establishment

The experimental design at each nursery was a randomized complete block with 18 blocks, with families assumed to be a random effect. A block consisted of 10 subplots, each containing 36 planting spots. Families were intermixed by sowing a single seed per family in each of the 36 planting spots per subplot. Families were randomly assigned to planting spots once for each of the 18 blocks, and each of the 10 seeds per family per block was sown at the same position in adjacent subplots. The experimental unit (plot) was the 10 seeds per family present in a replication. Each family was represented by 180 seeds per nursery.

Seeds were sown in spring 1987. The seeds were sown in 6 rows spaced 6 inches apart across the nursery bed, with 1 inch between planting spots within a row. This is within the range of densities reported for operational nurseries in the Pacific Northwest (Thompson 1984). Care was taken to try to sow all seeds to the same depth (6 mm). Strings were put across the bed every 6 planting spots along the rows to separate subplots (6 rows × 6 seeds within a row), and individual planting spots were marked with a toothpick in order to accurately track each position. Fine gravel was put on top of each spot after sowing to prevent seeds from being washed away. The cultural regimes, including watering, root wrenching, undercutting, and application of fertilizer and pesticides, were those

poor roots (j-rooted, large tap root, stiff lateral roots, or roots damaged at lifting), or top problems (malformed, forked or multiple stems, or partially dead shoot). At Nursery 2, emergence and survival were poor; thus, seedling heights were not measured, and seedlings were not lifted. Seedlings that normally would have been culled after lifting because of small size or top problems were identified while in the ground.

Germination test

A test was done to provide estimates of each individual family's germination capacity and rate. Seeds were germinated on moist filter paper in covered petri dishes in a controlled environment chamber set at 24 °C with a 24 h photoperiod. Each family was represented by 3 replications (petri dishes) of 50 seeds each. A randomized complete block design was used with 1 petri dish of each family located on a separate shelf in the controlled environment chamber. Numbers of germinants were recorded at varying intervals (more frequently during the period of peak germination) for 32 days. At the end of the test, ungerminated seeds were cut open to determine the percentage of unfilled seeds. Germination capacity was measured as the percent of filled seeds that had germinated during the test. Germination rate was estimated by the slope of the linear regression of the probit transformation of cumulative proportion of germination to each measurement time on the inverse of the measurement time (1/days) (Campbell and Sorensen 1979). Mean germination rate was approximately equal to the inverse of time to 50% germination. Germination capacity and rate were measured separately for each plot (petri dish).

Analyses

Traits considered in analyses included the percentages of total seed sown that emerged (referred to as emergence), produced 2-yr-old seedlings (survival), and produced plantable seedlings after culling (plantable); the percentage of emergents that died by age two (mortality); and the percentage of surviving seedlings culled at the time of lifting because of small size, poor roots, or top problems (culled). Analyses of variance (ANOVAs) were done on family plots (10 planting spots per replication) to test whether families differed significantly in the above traits. Histograms and normal probability plots of residuals indicated that experimental errors were approximately normally distributed with constant variance for all variables, and arcsine and logit transformations had no effect on inferences from the ANOVAs. The relationships of seed weight, percent filled seed, germination capacity, and germination rate to emergence, mortality,

and percent culled were explored by calculating pairwise correlations between family means. In addition, the family mean correlation between 2-yr height and percent culled was estimated.

The effect of changes in family composition on genetic gain associated with improved forests was evaluated with data from family evaluation tests established by the Umpqua Cooperative as part of their tree improvement program. The unweighted mean 15-yr height of all families over all test locations was assumed to be an estimate of the mean height that may be expected from an improved population given equal family representation. The effect of differential representation of families on gain was evaluated by comparing the mean assuming equal representation of families with the weighted mean based on the relative proportions of plantable seedlings per family.

Results and discussion

Emergence and mortality

Mean percentages for both emergence and mortality differed greatly between the two nurseries (Table 1). Emergence and mortality at Nursery 1 were more typical of what can be expected at bare-root nurseries in the Pacific Northwest (emergence of 91.9%, mortality of 2.2%). The poor emergence (58.5%) and increased mortality (28.6%) at Nursery 2 may have been a result of increased incidence of damping-off disease brought on by a cool, wet spring. Although results from Nursery 1 should be given greater emphasis, results from Nursery 2 allow consideration of the effect of poor emergence and increased mortality on family composition. Nearly all mortality at both nurseries occurred during the first growing season.

Differences in emergence among families were significant ($p < 0.05$) at both nurseries, but differences in mortality were significant only at Nursery 2 (Table 1). Mortality at Nursery 1, however, was low in all families. Family means for percent emergence were significantly correlated with percent filled seed at Nursery 1 ($r = 0.44$), but were uncorrelated with all other seed characters (Table 2). The significant (albeit weak) correlation between emergence and percent filled seed was unexpected because differences in filled seed among families were small (91 to 100%). In contrast, emergence was not correlated with percent filled seed in Nursery 2, but was significantly correlated with seed weight. Perhaps, families with smaller seeds were less likely to emerge in Nursery 2 because they were more susceptible to disease, while in the more favorable conditions in Nursery 1, small seeds conferred no disadvantage in emergence.

Table 1. Family variation in percentage of total seed sown that emerged (emergence), produced 2-yr-old seedlings (survival), and produced plantable seedlings after culling (plantable), percentage of emergents that died by age two (mortality), and percentage of seedlings culled at the time of lifting (culled) for various reasons.

Nursery and trait	Mean	Range among families	F ¹	Probability ¹
Nursery 1				
Emergence	91.9	80.0–97.8	2.73	0.0001
Mortality	2.2	0–5.5	1.16	0.25
Survival	89.9	77.8–95.6	2.70	0.0001
Culled, too small	7.2	1.8–18.0	3.66	0.0001
Culled, other reasons ²	5.6	0.7–9.9	0.87	0.68
Plantable	78.3	64.4–87.8	2.53	0.0001
Nursery 2				
Emergence	58.5	41.1–73.3	3.99	0.0001
Mortality	28.6	18.8–41.8	1.48	0.04
Survival	42.1	26.1–58.9	3.86	0.0001
Culled, too small	6.8	1.4–20.7	1.71	0.0001
Culled, other reasons ²	3.4	0–9.5	1.06	0.38
Plantable	37.9	22.8–56.1	4.21	0.0001

¹ F-ratio to test family effect in analysis of variance and probability that family means do not differ.

² Other reasons for culling included poor roots (j-rooted, large tap root, stiff lateral roots, or roots damaged at lifting) and top problems (malformed, forked or multiple stems, or partially dead shoot).

Table 2. Correlations between family means for seed characters and percent emergence, mortality, and culled for being too small.

Seed character	Emergence	Mortality	Culled
Nursery 1			
Seed weight	-0.02	-0.24	-0.66*
Percent filled seed	0.44*	0.18	0.02
Percent germination	-0.07	-0.15	-0.15
Germination rate	0.01	0.05	-0.09
Nursery 2			
Seed weight	0.50*	-0.28	-0.54*
Percent filled seed	0.08	-0.04	-0.20
Percent germination	-0.23	0.09	-0.09
Germination rate	-0.22	-0.11	-0.12

* Significant at $p < 0.05$.

Percent germination of families (filled seed) in the laboratory was not significantly correlated with percent emergence at either nursery (Table 2), but laboratory germination was high (mean 99.6%) and differed little among families (range 95.7 to 100%). For these materials, germination capacity in the laboratory would not be useful for adjusting sowing mixtures to achieve uniformity among families in numbers of emergents. The percent germination of both filled and unfilled seed was no better a predictor of emergence in the nursery than the percent germination of filled seed alone; the family mean correlation between percent germination (filled and unfilled seed) and percent emergence was 0.41 at Nursery 1 and only 0.01 at Nursery 2. In another study of Douglas-fir, we found a correlation of $r = 0.79$ between percent germination (filled seed) and percent emergence (St. Clair and Adams 1991). However, family differences in germination were larger in that study (range 42.5 to 98.5%).

Culling

Numbers of seedlings culled were analyzed separately for each reason for culling. Percentages of seedlings culled for reasons other than size were low and did not differ significantly among families; hence, those culling records were combined into a single class. After combining into a single class, numbers of seedlings culled for reasons other than size still did not differ significantly among families (Table 1). At both nurseries, however, the percent of seedlings culled for being too small did differ significantly among families.

The percentage of each family culled for being too small was negatively correlated with mean family seed weight (Table 2). At Nursery 1, 2-yr height was negatively correlated with percent culled ($r = -0.77$), as expected, and seed weight was positively correlated with 2-yr height ($r = 0.60$). The correlation between seed weight and seedling size in this study was much stronger than that in previous studies of Douglas-fir (Bell et al. 1979; Mangold 1987; Loopstra and Adams 1989; St. Clair and Adams 1991). The strong correlation between seed weight and height in this study may be a result of seedlings from families with heavier seed emerging earlier and consequently being in a better competitive position. However, seed weight does not appear to be related to rate of emergence. Although rate of emergence was not assessed in this study, families did not differ greatly in rate of germination in the germination tests (time to 50% germination ranged from 3.6 to 8.4 days), and rate of germination was unrelated to seed weight ($r = 0.14$) or 2-yr height ($r = 0.15$). Furthermore, St. Clair and Adams (1991) found only a weak relationship between rate of emergence and seed weight ($r = 0.37$).

Implications for genetic diversity

Family differences in emergence, mortality (Nursery 2 only), and percent culled resulted in significant family differences in the percentage of seedlings in each family that survived to the time of lifting and were plantable (Table 1). Assuming equal representation in the final crop, each family would be expected to contribute 2.8% of the plantable seedlings. In Nursery 1, the contributions of each family to the final crop were very close to the mean (range 2.3 to 3.1%). The cumulative contribution curve (Fig. 1) shows that half the families contributed about half (52%) the plantable seedlings. In Nursery 2, the range of family contributions to the final crop was greater (1.7 to 4.1%). Nevertheless, while the most productive family in Nursery 2 produced over twice the number of plantable seedlings as the least productive family, most families produced close to the mean, and half the families still contributed close to half (58%) of the plantable seedlings. Thus, despite statistically significant differences among families in emergence, mortality, and percent culled, changes in family composition were small enough that genetic diversity was nearly unaffected. The seeds used in this study, however, were collected only after cones were mature, and were stratified for a long period. Differences

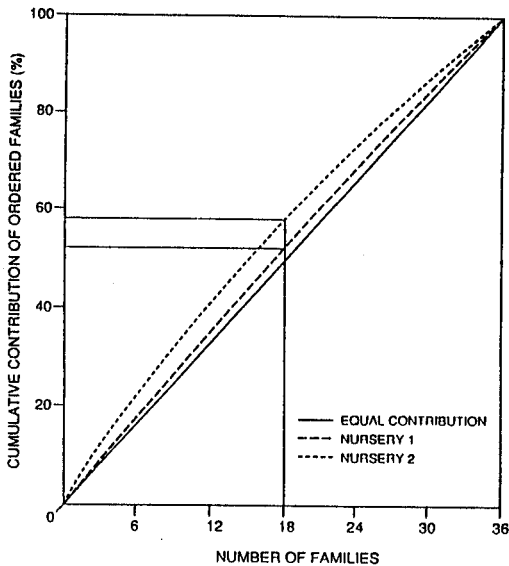


Fig. 1. Cumulative percent contribution of families to final crop of plantable seedlings when families are ordered from the greatest to least contributor.

among families in seed quality and chilling requirements (when not stratified for an adequate time) could lead to larger family differences in emergence than those observed here.

Implications for genetic gain

Assuming equal representation of families in the final crop, the expected genetic gain in 15-yr height would be 9.05 m, based on the observed mean of the 36 families in operational field tests. Expectations given the observed family compositions in Nursery 1 and Nursery 2, as determined by the field test means weighted by family representation in the nurseries, were 9.04 and 9.06 m, respectively. Thus, genetic gain expected from planting the 36 families in this study would be little altered by the changes in family composition observed in the nursery.

Conclusions

Families differed significantly in their ability to survive and produce plantable seedlings under operational seed handling and nursery procedures. Differences in the composition of the final mixture were primarily a result of family differences in emergence and percent culled for being too small. However, differences were small enough that each family's contribution to the final crop of plantable seedlings was nearly equal. Thus, genetic diversity and expected genetic gains were largely unaffected by observed changes in family composition.

Sowing families in pure blocks in the nursery has been suggested to maintain genetic diversity and allow more accurate prediction of genetic gains (Gladstone 1981; Johnson and Kellison 1984). Our results indicate, however, that individual family representation is not altered during nursery production of bulked seedlots. Thus, managing individual families separately in the nursery is unnecessary for purposes of maintaining genetic diversity and ensuring expected genetic gains.

Other advantages that have been cited for segregating families in the nursery include increased crop uniformity, the opportunity to gather incidental information on each family's response to specific nursery practices, and the opportunity to enhance genetic gains through preferential planting of better families, particularly on better sites (Gladstone 1981; Talbert 1982). Enhancing genetic gains through preferential planting of better families does not require pure family blocks in the nursery. Better families may be favoured simply by adjusting the proportions of families in the mixture of seeds to be sown. Controlling the composition of the mixture

of seeds does, however, require that families be handled separately during seed collection. The advantages of pure family blocks for increased crop uniformity and opportunities for incidental information may be real, but the potential benefits must be considered against the extra costs involved in increased handling and record-keeping.

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