

Breeding Graft-compatible Douglas-fir Rootstocks (*Pseudotsuga menziesii* (MIRB.) FRANCO)

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Summary

A study encompassing 24 years was conducted to determine if a breeding program could produce highly graft-compatible rootstocks. Twenty-seven trees of apparent high graft compatibility were selected and crossed to produce 226 control-pollinated families. Seedlings were grown, field planted, and grafted with test scions. Graft unions from field tests were evaluated anatomically for internal symptoms of incompatibility. Average compatibility of progeny from the 226 crosses was 90.6%, compared with 65% in native populations. Breeding values were calculated for each parent by the best linear prediction (BLP) procedure. Average compatibility resulting from crossing among the top 10 parents was estimated by breeding values as 95.4%. Field-test results of progeny from 34 crosses among the 10 most compatible parents showed 96% compatibility.

In addition to field-tests for graft compatibility, nursery tests of seedlings from 124 crosses were evaluated for second-year vegetative bud flush and seedling height. It was possible, while maintaining adequately high levels of graft compatibility, to breed both for resistance to spring frost damage and for increased seedling height.

Key words: Incompatibility, grafting, seed orchards, understocks, graft rejection.

Introduction

Graft incompatibility losses ranging from 30% to 60% percent occurred in Douglas-fir (*Pseudotsuga menziesii* (MIRB.) FRANCO) seed orchards during the 1950s and 1960s (SILEN and COPES, 1972). Numerous investigations of the problem were undertaken by the author in 1964. Results of those studies revealed anatomical (COPES, 1970), phenological (COPES, 1969), and isozyme (COPES, 1978) traits that were useful in differentiating compatible grafts from incompatible grafts. With the anatomical testing method, graft compatibility was shown to have a narrow sense heritability of 0.81 (COPES, 1974). Two-stage screening procedures to facilitate the identification of highly compatible trees were evaluated (COPES, 1981). The procedure of intensively testing a smaller number of trees in the first stage was more effective than less intensively testing a larger number of trees in the first stage.

A field study evaluated the compatibility of control and wind-pollinated progenies of 3 highly compatible parents (COPES, 1982). Progenies of the 3 parents were grafted in 6 orchards in Oregon, Washington, California, and British Columbia. The rootstock by orchard interaction effect was not significant, so breeding and testing for graft compatibility could be done safely at one location and selected families used in orchards with similar environments. The average compatibility of unselected parents was 65%.

The study reported in this paper, the second and final stage of research evaluating the potential of breeding highly graft compatible Douglas-fir rootstocks for the coastal region of the Pacific Northwest, was begun in 1974 and continued through

1995. The primary objective was to determine how much gain in rootstock compatibility was possible through breeding highly compatible parent trees, and involved breeding, growing, and graft-testing the progenies of 226 control-pollinated families from 27 parents selected for graft compatibility in stage one. Breeding values were calculated from best linear predictions (BLPs) for graft compatibility, seedling height, and vegetative bud flush. The floral phenology of 19 of the 27 parent trees was determined for female strobili opening.

Materials and Methods

In 1974, parent trees for breeding were selected from orchards and graft tests in western Oregon and Washington. Parent trees were selected on the basis of compatibility as scion-donors when grafted upon unselected rootstocks with a broad genetic base. Only parent trees with $\geq 90\%$ compatibility as scion-donors were selected for breeding. Sixteen selections were from adolescent trees, 11 or 13 years old, that came from a prior test of 2 screening procedures (COPES, 1981). Four adult parents were identified in unpublished grafting trials conducted by the author, and 7 adult parents were identified in seed orchards. The orchard parents were ≥ 50 years old and were evaluated in this study because their survival in grafted orchards revealed them to have the greatest proportion of visually healthy grafts several years after grafting. For further evidence of their compatibility, 50 scions from each parent were subsequently grafted upon 50 unrelated rootstocks and evaluated 2 years later using the anatomical test described by GNOSE and COPES (1975).

The selected parents were grafted in a clone bank near Corvallis, Oregon. Breeding began in 1974 and continued through 1991. The breeding plan was to breed each tree with at least 10 of the 27 parents. The crosses completed each year were dependent on availability of female strobili, reproductive bud phenology, and pollen availability. The mature trees produced abundant and frequent female and male pollen strobili. The pollen from those trees often was used on female strobili of adolescent trees because the younger trees were deficient in pollen production. This crossing produced 226 crosses that were evaluated anatomically for compatibility.

Each year, 50 or more seeds of each cross collected in the previous year were germinated and grown in nursery beds at the Forestry Sciences Laboratory in Corvallis, Oregon. The seedlings were lifted 2 years later in January or February and field planted at an orchard site near Corvallis, Oregon. Fifty seedlings from each family were planted at 1.0-meter by 3.3-meter spacing. The seedlings were grown for 2 years at the field site before grafting. In March or April, 1 scion from each of 50 randomly selected parents was grafted upon seedlings of each cross. Different scion-parents were grafted each year. When a cross had less than 50 vigorous seedlings at the time of grafting, multiple clones were grafted upon some of the larger

and more vigorous rootstocks. Additional grafting of a rootstock family was done the following spring if the family had less than 25 grafts surviving at the end of the first growing season. Seedlings of 47 of the 226 families were intentionally planted in 2 or 3 different years to provide information about graft-test repeatability. In addition, seedlings of 19 reciprocal crosses were planted so maternal and paternal effects could be evaluated.

The graft unions were collected for anatomical investigation in September or October after completing 2 years of growth. Graft collections, micro technique procedures, and anatomical symptoms of incompatibility used to determine compatibility were as described by GNOSE and COPES (1975). Average percent compatibility of each family was determined.

In a supporting investigation to provide information to rank parents for vegetative bud flush and seedling height, 12 crosses were randomly selected and seeds were sown in the nursery beds during one or more of the years, 1989, 1990, or 1991. In the nursery, a randomized, complete-block design was used. Blocks were contiguous areas of one long nursery bed. In each block, every cross sown that year was represented by a row of 20 trees. To provide linkage across years, seeds from 7 crosses were sown in multiple years (2 of the same crosses in 1989 and 1990, and 5 of the same crosses in 1990 and 1991). Tree heights were measured to the nearest 0.5 cm after growth ceased at the end of the second growing seasons. Vegetative bud flush was assessed in 1990, 1991 and 1992. Bud assessments began the start of the second growing season when about 50% of the seedlings exhibited new needles. The percentage of seedlings in each family with visible new needles was used to rank parents for earliness of vegetative bud flush. A similar ranking of female strobili for earliness of bud opening was obtained for 19 of the 27 parents. The female strobili data were extracted from 1985, 1987 and 1988 flowering information published by COPES and SNIĘZKO (1991).

In a few cases, poor seedling survival following field planting or scion survival following grafting reduced the number of unions available for anatomical study to less than 50. Only families with compatibility values based on microscopic examination of ≥ 25 unions are documented in this paper. All documented crosses were weighted equally in statistical analyses. Vegetative bud flush and seedling height data for each of the 3 years were standardized to overall means of 59% and 57 cm, respectively. Compatibility percentages did not require transformation, but vegetative bud flush and seedling height had skewed distributions where the means and standard deviations were related and required log transformation. Analyses of variance for height, reproductive bud flush, and vegetative bud flush were done with SAS's General Linear Model Procedures (SAS, 1989). Level of significance was $\alpha = 0.05$. The BLP analyses were used to estimate breeding values for percent compatibility, vegetative bud flush, and seedling height. The BLP values (WHITE and HODGE, 1989) and parent means were calculated from family means across years. Analyses were done both across and within years for seedling height and vegetative bud flush.

A computer simulation was used to estimate the number of crosses required to accurately determine the compatibility of a parent. Of the 27 original parents, 12 parents had compatibility data from 17 or more crosses and were used in this simulation because the parents had enough crosses to permit extensive sampling. From all crosses of a parent, a sample of 3 crosses was chosen randomly and the average compatibility was calculated. This was repeated for all 12 parents. The 12 parent means were correlated with their true parent means, and the mean of the 12 parents was plotted in figure 1. A true parent

mean was the average compatibility of all crosses of the parent. This simulation was replicated 10 times at the 3-cross level, and the entire process was repeated for each sample size from 4 to 15 crosses. The determination of the number of crosses required to accurately determine the compatibility of a parent was made by visual observation of the plot of correlations versus the number of crosses sampled.

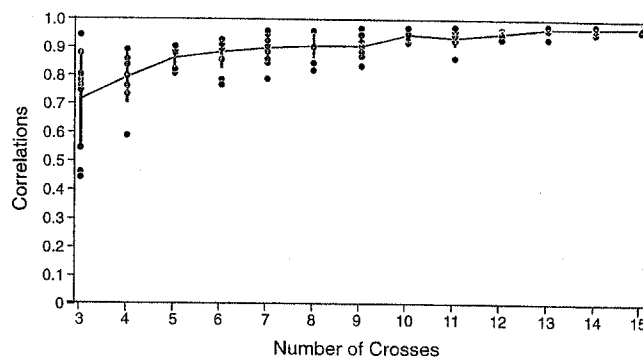


Fig. 1. - Correlation of the true compatibility means of 12 parents with sample means derived from field test of 3 to 15 crosses per parent. Ten composite means of the 12 parents were plotted for each sample size and a graph line connected the 13 sample-level means.

Results

Average compatibility of each parent is shown in table 1. Parents were ranked in order from highest to lowest compatibility. The mean of all 27 parents was 90.7% (std. dev. 5.9). Twenty of the parents averaged $\geq 90\%$ compatibility. The percentage of families of each parent that were $\geq 95\%$ compatible ranged from 0% to 59%. The crosses among the top 10 parents produced families that averaged 93% compatibility. The average number of families tested per parent was 16.7 (range of 4 to 31 crosses). The average difference among the 19 reciprocal crosses was 4.9%, while the between-year difference for the 47 families grown and tested in multiple years was 2.3%. Neither difference was significant.

Estimates of breeding values of the 27 parents were derived from BLP analyses of untransformed data. The estimates were calculated for compatibility, vegetative bud flush, and seedling height (Table 2). Ranking was listed in the order of most to least compatible, tallest to shortest seedlings, and earliest to latest flushing parents. Breeding values for graft compatibility of all 27 parents averaged 90.6%, and 16 parents were $\geq 90\%$ compatible. Breeding value of individual families ranged from 73.6% to 100%.

Considerable variation was found among crosses in dates of vegetative bud flush. Breeding values for vegetative bud flush averaged 56.3% and ranged from 21% to 83% (Table 2). Parental difference among the 3 plantings made in different years was significant for general combining ability ($p \leq 0.0001$), while specific combining ability was significant in 2 of the 3 plantings. Breeding estimates were calculated by BLP values and ordinary means. The correlations between the 2 methods were generally between 0.78 and 0.91.

The average breeding value for seedling height was 59.4 cm and cross means ranged from 48.8 cm to 76.3 cm (Table 2). The tallest family was 56% taller than the shortest family. Reciprocal cross effects were not significant for any of the 3 plantings ($p = 0.31$ to 0.70). Thorough interpretation of analysis of variance results for seedling heights and vegetative bud flush are

Table 1. – Parents are ranked in descending order for average compatibility determined from field tests. The number of families tested, percent compatibility, and percentage of families $\geq 95\%$ compatible are listed.

Parent	Compatibility rank	Number of families	Average compatibility (%)	Standard deviation (%)	Families $\geq 95\%$ compatible (%)
312	1	10	94,5	5,3	50
102	2	16	93,8	7,1	56
23	3	13	93,7	4,9	46
16151	4	27	93,4	5,0	52
20	5	11	92,8	5,5	54
65	6	28	92,8	5,2	43
197	7	17	92,6	6,1	59
165	8	15	92,1	6,8	53
9218	9	18	92,1	7,1	56
241	10	13	92,0	4,0	23
25	11	4	91,8	7,2	25
17	11	20	91,8	5,3	20
O-4	13	6	91,6	5,4	33
3	14	13	91,5	5,7	38
109	15	22	91,4	5,8	41
16131	16	24	91,3	6,1	42
172	17	15	91,2	5,0	40
16161	18	21	90,9	4,9	38
106	19	14	90,4	6,7	21
97	20	18	90,1	7,0	39
14	21	13	89,9	6,6	23
2	22	17	89,6	5,5	23
16162	23	31	89,3	7,5	19
223	24	9	85,5	9,0	11
8211	25	25	85,3	5,3	4
H-4	26	5	84,5	5,5	0
20237	27	27	81,7	5,1	0
Mean		16,7	90,7	5,9	33,6

not presented because of the unbalanced nature of the crosses tested.

Breeding values were not calculated for earliness of opening of reproductive buds, but results were presented in the form most useful to a breeder. The data for each parent were presented simply as the number of days separating the first opening of megastrobili of each parent from the date of first opening of megastrobili of the earliest parent (8211). Parents were ranked from earliest to latest dates of bud opening (Table 2). About 10 days separated flower opening of the earliest and latest parents.

Linear correlations between seedling height, vegetative bud flush, and reproductive bud flush revealed only 2 significant correlations – seedling height by percent vegetative bud opening $r = -0.52$, $p = 0.004$) and vegetative bud opening by floral

bud opening ($r = +0.51$, $p = 0.02$). Vegetative phenology of crosses of parent 241 departed greatly from this relation and had seedlings that opened their vegetative buds much earlier than would be expected from predications based on opening of floral buds. Crosses of parents 20 and 8211 exhibited the opposite response; the vegetative buds flushed much later than indicated by the flushing of their of floral buds. SORENSEN and CAMPBELL (1971) report on the lack of correlation between floral and vegetative bud opening.

Compatibilities of families among the 10 parents with the largest breeding values are shown in table 3. Six of the top 10 parents were used as males and all 10 parents were used as females. No self-pollinated families were made, so there were 54 potential families. Thirty-four of the potential 54 families were field-tested. Estimated family values were calculated for

Table 2. — Estimated breeding values calculated from best linear prediction (BLP) are listed for parent mean compatibility, vegetative bud flush, and second-year seedling height. Parents are ordered from largest to smallest values for graft compatibility. Floral bud data were calculated from simple means. Ranking and number of families measured (N) are listed.

Clone	Best linear prediction breeding values									Floral bud opening ¹	
	Graft compatibility			Vegetative bud flush			Seedling height			Days after earliest clone opened	Rank
	Mean	Rank	N	Mean	Rank	N	Mean	Rank	N		
	(%)	(1-27)		(%)	(1-26)		(cm)	(1-26)		(1-19)	
312	100.0	1	10	— ²	—	—	—	—	—	—	—
16151	97.1	2	27	65.6	9	23	54.3	15	23	5.0	9
102	96.6	3	16	72.0	7	4	51.4	23	4	—	—
65	96.0	4	28	64.5	10	25	52.1	20	25	2.0	2-3
197	95.2	5	17	53.4	16	14	66.7	7	14	9.0	16
17	94.6	6	20	21.2	26	16	56.7	13	16	9.7	18-19
9218	94.1	7-8	18	59.4	13	10	59.6	11	10	3.3	5-6
16131	94.1	7-8	24	72.5	6	16	55.8	14	16	3.3	5-6
20	93.4	9	11	44.6	21	6	75.0	3	6	4.0	7
3	93.2	10	13	47.7	20	6	76.3	1	6	8.0	12-15
23	92.9	11-13	13	25.7	25	8	68.3	5	8	9.7	18-19
165	92.9	11-13	15	75.8	4	15	49.4	24	15	3.0	4
109	92.9	11-13	22	62.5	12	14	53.5	16	14	8.0	12-15
172	92.6	14	15	41.0	22	6	67.9	6	6	6.3	10
25	92.2	15	4	29.3	24	1	72.5	4	1	—	—
16161	90.6	16	21	74.2	5	12	48.8	26	12	5.0	8
2	89.8	17	17	50.8	17	6	52.9	18	6	8.0	12-15
O-4	88.8	18-19	6	70.4	8	4	52.1	21	4	—	—
97	88.8	18-19	18	49.3	19	14	59.1	12	14	8.0	12-15
106	88.2	20	14	50.8	17	2	75.3	2	2	—	—
241	88.1	21	13	83.3	1	7	49.2	25	7	9.3	17
16162	87.7	22	31	80.2	2	18	52.2	19	18	2.0	2-3
H-4	86.2	23	5	31.6	23	1	65.7	8	1	—	—
223	84.7	24	9	59.4	14	4	51.8	22	4	—	—
14	82.8	25	13	78.2	3	6	63.4	9	6	—	—
8211	79.1	26	25	62.9	11	6	53.3	17	6	0	1
20237	73.6	27	27	57.0	15	4	61.4	10	4	7.0	11
Mean	90.6		16.7	56.3		9.1	59.4		9.1	5.8	

¹) Floral bud flush data determined from 1985, 1987, and 1988 breeding seasons (COPES and SNIĘZKO, 1991).

²) — denotes where data were not collected.

all 54 families by averaging the male and female breeding values for each family. The field-test mean of the 34 crosses was 96%, and the breeding value mean for all 54 crosses was 95.4%. The lowest estimated value for a cross among the top 10 parents was 93.6%.

The computer simulation procedure used to determine the number of crosses necessary to estimate compatibility of parents indicated that reasonably accurate estimates were possible from field-test results of 5 to 7 crosses (Figure 1). Highly accurate estimates were obtained with ≥ 10 crosses.

Table 3. -- Percent graft compatibility of crosses among the 10 parents with the largest breeding values. Field-test result (FT) and estimated breeding value (BV) are listed for 34 and 54 crosses respectively.

Female parents	Test type	Male parents						
		16151	102	65	17	9218	16131	Mean
312	FT		100	97				98
	BV	98	98	98	97	97	97	98
16151	FT			92	98			95
	BV		97	96	96	96	96	96
102	FT	100		97		100		99
	BV	97		96	96	95	95	96
65	FT	100						98
	BV	96	96		95	95	95	96
197	FT	96	99	98	92	98	100	97
	BV	96	96	96	95	95	95	95
17	FT	100	94	88				95
	BV	96	96	95		94	94	95
9218	FT	97		95				96
	BV	96	95	95	94		94	95
16131	FT	88			98			93
	BV	96	95	95	94	94		95
20	FT	86		96	92	100	95	94
	BV	95	95	95	94	94	94	94
3	FT	95		95	93	90	95	94
	BV	95	95	95	94	94	94	94
Mean	FT	95	98	95	95	97	96	96
	BV	96	96	96	95	95	95	95

Discussion

Results indicated that it is possible to breed among highly compatible parents and produce rootstocks with adequate compatibility to ensure high levels of graft survival. Crosses among the 10 most compatible parents produced crosses that averaged 95% compatibility, and crossing among the 5 most compatible parents produced progeny that averaged 97% compatibility. Compatibility is great enough that future breeding for compatibility is not planned. The 11 parents with the lowest breeding values can be excluded as sources of future rootstocks because the remaining 16 highly compatible parents contain adequate genetic diversity to ensure the production of healthy and vigorous rootstocks (JOHNSON, 1998).

There may be traits in addition to graft compatibility that should be considered when breeding rootstocks. For example, if seed orchard sites are subject to severe frosts in early spring, a breeder may choose to sacrifice a small amount of compatibility to create a group of special families that open their vegetative buds later in the spring. In this case, parents 3, 17, 20, 23, and

172, which were the 5 latest parents, may be selected for breeding rather than the 5 parents having the highest compatibility ranking. Progeny from breeding among these 5 parents would average 93% compatibility and open their buds 35% later than would progeny from crossing among the 5 most compatible parents.

Situations may exist in which larger seedling rootstocks are desired. Selection and breeding among parents 3, 20, 23, 172, and 197 would produce the tallest rootstocks. Seedling from those families produced families that grew 26% taller than seedlings from crosses among the 5 parents with the greatest compatibility. (Note the possible confounding of vegetative bud flush and seedling height.). Four of the 5 parents selected for seedling height also were the same parents selected for late vegetative bud flush. It is possible that freeze damage to early flushing families reduced their heights at the end of the second year. Freezing temperatures occurred early in the second growing season of 2 of the 3 plantings. Damage to succulent shoots of seedlings from crosses of early parents may have resulted in reduced growth in comparison to seedlings from crosses of later

parents that escaped those early freezes. Crossing the 4 parents found in both selections should produce seedlings that are taller, have increased resistance to damage from spring frosts, and average 2% less compatibility than families from crosses among the 10 most compatible parents.

A slightly different ranking of parents for compatibility occurred when the rankings were based on field-test results versus rankings based on estimated breeding values derived from BLP analysis. Parents 23, 242, and 16131 were ranked 11 and 3, 21 and 10, 7 and 16 based on field tests and breeding values, respectively. Parents 23 and 242 seemed to have lower average compatibility based upon field tests because they had been crossed accidentally with a greater proportion of less compatible parents than normal. The reverse situation occurred with parent 16131; it was crossed only with highly compatible parents. The estimated breeding values are likely more accurate because the BLP procedure adjusts for crossing imbalance.

Phenological ranking of female buds will assist breeders in determining which parents can be easily crossed. The average 10-day difference in opening of buds separating the earliest and latest parents necessitates planning when deciding which parents to isolate for female strobili and which to use as males. Pollen can be collected, applied the same year, or preserved in cold storage (COPES, 1987; WEBBER, 1987) and used in future years. Phenology information was available only for 19 of the 27 parents, but that included 12 of the 14 most compatible parents. Unfortunately, phenology and growth data were not recorded for parent 312, the most compatible parent in this study.

Computer sampling indicated that the compatibility of a parent could be estimated with reasonable or high accuracy if as few as 5 to 7 or ≥ 10 crosses, respectively, were field tested. These results were likely influenced by the truncated nature of the parent population. The initial screening of potential parents by graft-testing before breeding resulted in selection of parents that were more compatible than the native, unselected population (91% versus 65% compatibility, respectively) (COPES, 1982). The ability to determine the compatibility of putative parents without more extensive field-testing should significantly reduce costs associated with developing new rootstocks.

Use of highly compatible families as rootstocks in seed orchards has saved considerable cost and time in orchard establishment as less mortality occurs and less regrafting of incompatibility losses is required. The economic benefit of more complete stocking of healthy trees is obvious. The unseen benefit of obtaining seed with greater genetic gain or diversity due to increased panmixia during pollination exists, but is more difficult to determine.

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