

# Patterns of Variation in First-Year Seedling Traits within and among Douglas-fir Breeding Zones in Southwest Oregon<sup>1)</sup>

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## Summary

To determine the extent of genetic variation within and among Douglas-fir breeding zones in southwest Oregon, we studied six seed and seedling traits in families originating from 14 breeding zones. Families from 25 wind-pollinated parents from each zone were grown in a common garden in Corvallis, Oregon. Seedling traits were measured at the end of the first growing season. On average, 53.9% of phenotypic variation among families was attributable to variation between zones; the remainder reflected differences among families within zones. Patterns of variation among breeding zones were strongly associated with location variables (distance from the ocean, latitude, and elevation) and seemed to reflect adaptation to moisture and temperature gradients in the region. Families from the drier, southern-inland zones had heavier seeds and produced seedlings that grew slower and had earlier budset, less frost damage, and more second flushing than families from breeding zones with cooler, moister climates. Elevation clines were most evident for traits related to phenology; seedlings from higher elevation breeding zones had earliest budset and least frost damage. Regression of family means on location variables accounted for only a small proportion of genetic variation within breeding zones. Redefining current breeding-zone boundaries or reducing zone size probably would do little to reduce the effect of adaptive differentiation associated with geography in southwest Oregon.

**Key words:** Geographic variation, breeding zones, adaptation, Douglas-fir, *Pseudotsuga menziesii*, southwest Oregon.

## Introduction

Breeding programs to improve growth and survival of commercial forests of Douglas-fir [*Pseudotsuga menziesii* (MIRB.) FRANCO] have been established in southwest Oregon. The climate of the region ranges from cool and moist in the coastal areas to hot and dry in the interior valleys (FRANKLIN and DYRNESS, 1973). Superimposed on the broader climatic gradients is extensive microgeographical variation associated with differences in aspect, soils, slope, altitude, and disturbance history. Douglas-fir, the most prevalent and commercially important species in the area, occurs in an extensive range of habitats, from sea level to over 1500 m. The broad genetic diversity (SILEN, 1978; ADAMS and CAMPBELL, 1981) of the species contributes to its extensive ecological range, and significant correlations between seedling characteristics in common-garden studies and topographic or climatic variables of seed sources have been reported in this region (HERMANN and LAVENDER, 1968; GRIF-

FIN and CHING, 1977; CAMPBELL and SORENSEN, 1978; WHITE *et al.*, 1981; SORENSEN, 1983; CAMPBELL, 1986).

Because of its extreme environmental heterogeneity, southwest Oregon has been subdivided into 38 Douglas-fir breeding zones within 13 breeding units (Figure 1). Breeding zones are ecologically similar areas, generally less than 60,000 ha in size, with an elevation range of 150 M to 450 M. Within breeding units, breeding zones correspond to elevation bands. There are 2 to 6 breeding zones within each breeding unit in southwest Oregon (WHEAT and SILEN, 1984). To take advantage of local adaptation in tree improvement programs, each zone is served by a single breeding population of trees from that zone alone. Although the zones were intended to reflect geographically related adaptive variation, little or no information on geographic patterns of genetic variation was available when the zones were designated. The boundaries of both breeding and seed collection zones were instead based primarily on local physiography, meteorological data, and conifer species composition (RUDOLF, 1974). The accuracy with which current breeding zones represent geographic patterns of adaptive variation, therefore, needs to be determined. If adaptively significant variation is common within the current zones, shifts in zone boundaries, reduction in zone size, or both may be necessary to ensure adaptability of genetically improved planting stock. If, on the other hand, breeding zones could be combined with

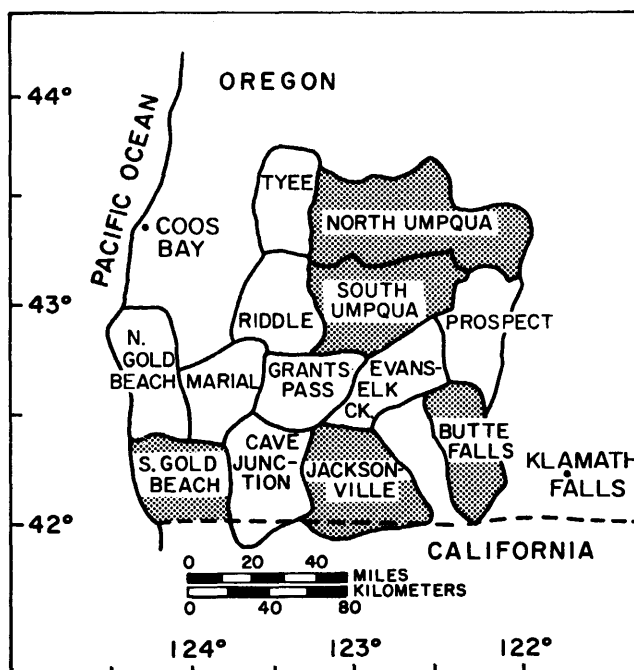


Figure 1. — Douglas-fir breeding units in southwest Oregon. Shaded units were included in this study.

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Table 1. — Southwest Oregon breeding zones included in this study and their elevation ranges.

Breeding unit	Breeding zone	Elevation	
		Designation <sup>a</sup>	Range (m)
North Umpqua	1	L	150-610
	3	M	760-915
	5	H	1065-1220
South Umpqua	1	L	300-610
	3	M	760-915
	5	H	1065-1375
Jacksonville	1	L	450-760
	2	M	760-1065
	3	H	1065-1375
Butte Falls	1	L	760-1065
	2	M	1065-1375
	3	H	1375-1680
South Gold Beach	2	L	0-450
	3	M	450-760

<sup>a</sup>) L = low, M = medium, H = high elevation.

minimal loss of fitness, tree-improvement costs would decrease considerably. Preliminary evidence suggests that present breeding and seed zones take much adaptive variation into account, but considerable adaptive variation may remain within zones (ADAMS and CAMPBELL, 1981; SORENSEN, 1983).

This study was carried out to further describe patterns of genetic variation of Douglas-fir in southwest Oregon and to determine the degree to which breeding zones account for geographically related adaptive differentiation among sources. Previous investigations of geographically related variation in this region involved families derived from very restricted areas (HERMANN and LAVENDER, 1968; SORENSEN, 1983) or from a limited number (70 to 135) of parent trees scattered over a broad area. This paper reports on variation in traits from first-year seedlings grown in a common garden from wind-pollinated seeds from 350 parent trees (25 from each of 14 breeding zones, covering much of the region).

### Materials and Methods

#### Seed Origins

The parent trees were selected originally for inclusion in tree-improvement programs. Two to four hundred parent trees in wild stands made up the initial breeding population in each zone (SILEN and WHEAT, 1979). Only low-intensity selection was applied in choosing these trees, since they were to be evaluated in progeny tests. The 25 trees from each breeding zone in this study were selected at random from this larger collection of trees.

The breeding units (Figure 1) include the major environmental gradients in the region. Mean annual precipitation decreases rapidly with distance from the ocean (from 160 cm in South Gold Beach to 40 cm in Jacksonville and Butte Falls) and increases slightly with latitude (60 cm in the North Umpqua) (FROEHLICH *et al.*, 1982). January mean minimum temperatures decrease with distance from the ocean (25° C to -5° C) and with increasing elevation (FRANK-

LIN and DYRNESS, 1973). Mean July maximum temperatures are lowest in the coastal unit (23° C), followed by the northern inland units (28° C); the southern inland units are the hottest (30° C) (FRANKLIN and DYRNESS, 1973).

The 14 breeding zones included three [low (L), medium (M), and high (H)] from the North Umpqua, South Umpqua, Jacksonville, and Butte Falls breeding units (inland units) and two (L and M) from the South Gold Beach unit (coastal unit) (Table 1). These zones were selected so patterns of genetic variation on elevational transects could be investigated within each unit, as well as along a west-east transect (South Gold Beach → Jacksonville → Butte Falls) and a north-south transect (North Umpqua → South Umpqua → Jacksonville).

#### Experimental Methods

Twenty to 24 seeds from each parent tree (family) were cold-stratified for 31 days and germinated in petri dishes. When the radicles were 2 cm long (late April and early May), 12 germinated seeds per family were planted in two raised nursery beds (cold frames) at the Oregon State University Forest Research Laboratory at Corvallis (latitude 44°30' N, longitude 123°40' W, elevation 90 m). Each frame was filled with Willamette Valley soil covered with 8 cm of forest soil. Seedlings were watered as needed until August 15, when watering was stopped. LOOPSTRA (1984) gives details of germination treatment and seedling culture.

Ungerminated seeds from the same families were x-rayed, and hollow seeds or those with insect larvae in them were removed. Seed weights were then determined on three samples of ten filled seeds per family.

The following characteristics of each seedling were measured at the end of the first growing season:

1) Budset — Terminal buds were scored once each week, beginning July 1. The date when the developing bud was first seen was recorded.

2) Occurrence of second flushing. — Seedlings that set buds temporarily and then reflushed were noted. In these

Table 2. — Random and mixed model analyses of variance for seedling traits.

Source	df	Expected mean squares
<b>Main plot</b>		
Blocks	2	$\sigma_e^2 + 25\sigma_{BUE}^2 + (250)300\sigma_B^2$
Units (U)	3(4)	$\sigma_e^2 + 3\sigma_F^2 + 25\sigma_{BUE}^2 + 75\sigma_{UE}^2 + (150)225\sigma_U^2 \ddagger$
Elevations (E)	2(1)	$\sigma_e^2 + 3\sigma_F^2 + 25\sigma_{BUE}^2 + 75\sigma_{UE}^2 + (375)300\sigma_E^2 \ddagger$
U x E	6(4)	$\sigma_e^2 + 3\sigma_F^2 + 25\sigma_{BUE}^2 + 75\sigma_{UE}^2$
Error "a"	22(18)	$\sigma_e^2 + 25\sigma_{BUE}^2$
<b>Subplot</b>		
Families/zones	288(240)	$\sigma_e^2 + 3\sigma_F^2$
Error "b"	576(480)	$\sigma_e^2$

When degrees of freedom or coefficients of components differ for sets, those for the coastal-inland set are in parentheses.

- $\sigma_e^2$  = Variance from the interaction of replications and family subplot means within main plots.
- $\sigma_F^2$  = Variance from differences among families within breeding zones.
- $\sigma_{BUE}^2$  = Variance from the interaction of blocks and main plot means.
- $\sigma_{UE}^2$  = Variance (fixed effects in mixed model) from the unit-by-elevation interaction.
- $\sigma_E^2$  = Variance (fixed effects in mixed model) from elevations.
- $\sigma_U^2$  = Variance (fixed effects in mixed model) from units.
- $\sigma_B^2$  = Variance from blocks.
- + This term not included in mixed model.
- ‡ In mixed model,  $\sigma_F^2$  estimates fixed effects rather than variances.

seedlings, the final date of budset was used as the budset date.

3) Frost damage — A freeze ( $-2^0$  to  $-3^0$  C) on October 29 damaged from 2% to 74% of the seedlings from each zone. Seedlings with discolored needles one week after the frost were scored as damaged, all other seedlings as undamaged.

4) Seedling height — the distance from the ground to the top of the terminal bud. Heights of severely frost-damaged seedlings that never set a terminal bud were measured to the top of the epicotyl.

5) Epicotyl length — distance from the cotyledons to the top of the terminal bud.

6) Number of branches — total number of branches over 6 mm long.

#### Design and Analyses

Seedlings were planted into blocks according to a split-plot design, replicated three times; breeding zones were the main plots and families nested within zones, the subplots. Main plots were randomized within blocks, and four seedlings per family (subplot) were randomly assigned positions within main plots (i.e., four-tree noncontiguous family subplots). A single row of border trees was planted around the edge of the experimental garden.

Because the number of elevation bands was not the same in all breeding units, two-factorial data sets (inland and coastal-inland) were identified and analyzed separately, with elevation as the first factor and breeding unit as the second. In the inland set, all three elevations (L, M, and H) were analyzed in the inland (North Umpqua, South Umpqua, Jacksonville, and Butte Falls) breeding units only. In the coastal-inland set, the lower two elevation bands (L and M) of all five units were analyzed.

To test the significance of differences among families and breeding zones, both the inland and coastal-inland

sets were analyzed according to a mixed model, with blocks and families within breeding zones assumed to be random effects and elevations and units to be fixed effects (Table 2). If families from different breeding zones are adaptively differentiated to the major environmental gradients (distance from the ocean, latitude and elevation), clinal variation among seed and seedling traits may be observed. To test the significance of these trends, the degrees of freedom (df) for breeding zones were partitioned into single df orthogonal contrasts (Table 3).

The inland data set also was analyzed assuming all effects to be random (Table 2). Although a random model is not strictly correct in this case, it was of interest to obtain at least rough estimates of the distribution of total genetic (family) variation between and within the inland breeding zones that could be attributed to various sources (i.e., units, elevation zones, unit  $\times$  elevation zone interaction and families within breeding zones). The contribution of each source to the total variability was evaluated from estimates of variance components. Three ANOVA's similar to those used for seedling traits were conducted for seed weight, assuming a completely random assignment of family plots (i.e., replicate seed weights) to experimental units.

ANOVA's of total height, number of branches, and date of budset were based on means of the surviving seedlings in each family plot. Analyses of frost damage and the occurrence of second flushing were based on the proportion of surviving seedlings in each family plot that had been damaged by frost or that flushed twice. Only four of the 1050 family plots had no survivors at the end of the first growing season. Missing values for these plots were computed using the equations given in STEEL and TORRIE (1980), and the df for subplot error were reduced appropriately. Unless otherwise indicated, all tests of significance refer-

Table 3. — Analyses of variance (mixed model) for five seedling traits of Douglas-fir from southwest Oregon (inland breeding zones).

Source	df	Date of budset <sup>a</sup>		Frost damage <sup>a</sup>		Frequency of second flushing		Height		Branch number <sup>a</sup>	
		Mean square	F	Mean square	F	Mean square	F	Mean square	F	Mean square	F
<b>Main plots</b>											
Blocks	2	90.45	27.98**	1.150	4.42*	1.475	5.13*	37,697.6	6.08**	19.65	7.97**
Units (U)	(3)	141.98	20.13**	1.333	4.02**	2.075	5.29**	33,420.8	4.89**	25.26	8.60**
North (NU) vs. South Umpqua (SU)	1	30.50	4.54*	0.775	2.42	0.150	0.62	7,759.5	1.18	2.25	0.84
Jacksonville (JV) vs. Butte Falls (BF)	1	128.00	18.18**	1.250	3.78	3.500	8.75**	39,003.8	5.69*	12.00	4.13
North (NU+SU) vs. South (JV+BF) units	1	265.75	37.44**	1.950	5.79*	2.525	6.38*	53,534.5	7.79**	61.25	20.74**
Elevations (E)	(2)	161.01	22.79**	1.925	5.71**	0.013	0.29	7,429.7	1.13	3.76	1.35
linear	1	317.50	44.68**	3.850	11.23**	0.003	0.27	14,296.0	2.12	7.50	2.61
deviation	1	3.50	0.77	0.010	0.23	0.027	0.32	561.5	0.14	0.33	0.19
U x E	6	5.74	1.08	0.192	0.75	0.417	1.27	7,721.1	1.17	2.22	0.83
Error "a"	22	3.23		0.260		0.288		6,204.4		2.46	
<b>Subplots</b>											
Families/zones	288	3.92	1.98**	0.089	1.29*	0.125	1.16	712.5	1.89**	0.50	2.05**
Error "b"	574 <sup>b</sup>	1.98		0.069		0.107		377.8		0.24	

a) These traits were transformed before analysis. See text for details.

b) Degrees of freedom for error "b" were reduced to account for two missing plots.

\* =  $P < 0.05$ ; \*\* =  $P < 0.01$ .

red to in this paper were conducted at the 0.05 level of probability.

Bartlett's test (SNEDECOR and COCHRAN, 1967) for homogeneity of error variances indicated significant ( $p < 0.01$ ) heterogeneity among error variances for three traits: number of branches (NB), frequency of second flushing (FSF), and proportion of seedlings damaged by frost (FD). These traits therefore were transformed to  $\sqrt{NB}$ ,  $\arcsin \sqrt{FSF}$ , and  $\arcsin \sqrt{FD}$  before analysis. However, means in the results are of nontransformed variables.

Since the correlation between seedling height and epicotyl length, based on family means, was nearly one ( $r = 0.99$ ), only data for height are presented. Although significant, correlations between seed weight and the growth traits, height ( $r = 0.34$ ) and branch number ( $r = 0.24$ ), were small. Thus, the growth traits were not adjusted for seed weight differences before analysis.

If environmental variation among locations within zones has resulted in adaptive differentiation associated with elevation ( $X_1$ ), latitude ( $X_2$ ), and distance from the ocean ( $X_3$ ), family means should be at least moderately associated with these location variables, as indicated by a previous study of Douglas-fir seedlings in southwest Oregon (CAMPBELL, 1986). Trait means for the 25 families within each breeding zone therefore were regressed on location variables. Regression equations were fitted to the family means for each of six measured traits by selecting variables from a preliminary model by stepwise multiple regression (NIE *et al.*, 1975). The preliminary model included six variables:  $X_1$ ,  $X_2$ ,  $X_3$ ,  $X_1X_2$ ,  $X_1X_3$ , and  $X_2X_3$ . A total of 84 (6 traits  $\times$  14 breeding zones) equations were fitted.

### Results

Differences among breeding zones (i.e., breeding units (U), elevation (E), and the U  $\times$  E interaction) were significant for all six traits, and differences among families within zones were significant for all traits except fre-

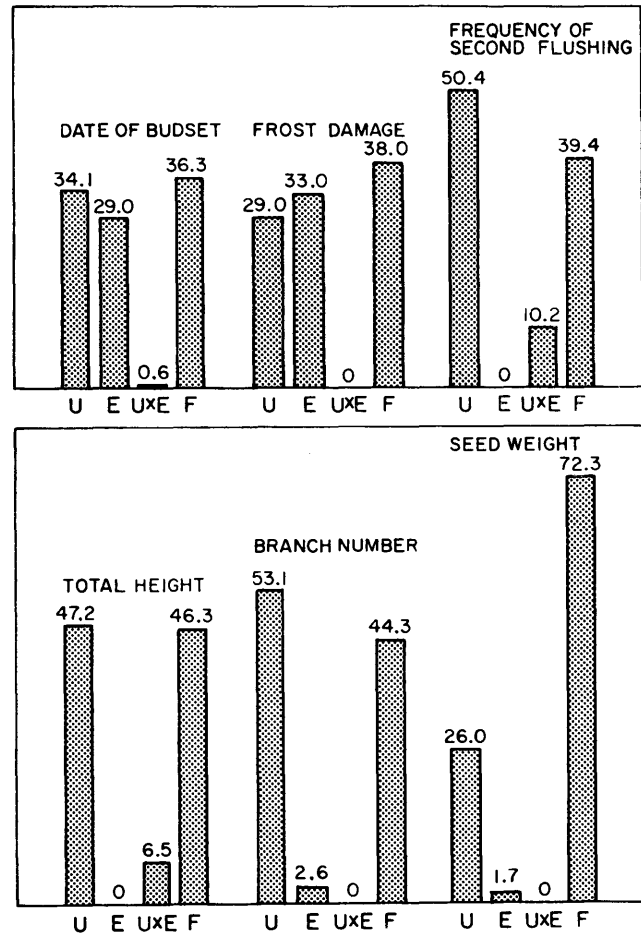


Figure 2. — Percentages of total genetic (family) variation attributable to breeding units (U), elevation zones (E), U  $\times$  E interaction, and families within breeding zones (F) within and among 12 inland breeding zones in southwest Oregon.

Table 4. — Analysis of variance (mixed model) for seed weight (inland breeding zones).

Source	df	Seed weight	
		Mean square	F
Units (U)	(3)	26,454.51	27.05**
North (NU) vs. South Umpqua (SU)	1	3,129.75	3.20
Jacksonville (JV) vs. Butte Falls (BF)	1	662.50	0.68
North (NU+SU) vs. South (JV+BF) units	1	75,565.50	77.26**
Elevations (E)	(2)	3,140.68	3.21
linear (l)	1	4,309.50	4.41*
deviation (d)	1	1,970.75	2.02
U $\times$ E	6	689.52	0.71
Families/zones <sup>a</sup>	288	978.06	29.95**
Error	600	32.66	

<sup>a</sup>) Families/zones mean square was used to test significance of U, E, U  $\times$  E, and the single degree of freedom contrasts.

\*  $p < 0.05$ ; \*\*  $p < 0.01$ .

Table 5. — Breeding unit and breeding zone means for six seed and first-year seedling traits of Douglas-fir from southwest Oregon.

Breeding unit	Breeding zone <sup>a</sup>	Date of budset <sup>b</sup>	Frost damage (frequency)	Second flushing (frequency)	Total height (mm)	Branch number	Seed weight (mg/10)
North Umpqua	L	15.21	0.19	0.133	137.95	4.41	134.44
	M	14.17	0.07	0.123	125.82	3.44	130.55
	H	13.51	0.04	0.163	109.20	2.81	127.80
	Mean	14.30	0.10	0.140	124.32	3.55	130.80
South Umpqua	L	15.54	0.20	0.193	113.39	3.49	140.21
	M	14.62	0.14	0.160	126.96	3.90	134.31
	H	14.29	0.12	0.137	107.67	2.63	133.69
	Mean	14.82	0.15	0.163	116.01	3.34	136.07
Jacksonville	L	14.61	0.15	0.223	112.11	2.25	159.80
	M	14.24	0.13	0.140	116.85	2.43	149.76
	H	13.16	0.04	0.147	113.20	2.32	149.36
	Mean	14.00	0.11	0.170	114.05	2.33	152.97
Butte Falls	L	13.43	0.05	0.217	103.66	1.95	150.84
	M	13.37	0.06	0.310	84.65	1.32	147.46
	H	12.01	0.02	0.323	97.99	1.45	153.40
	Mean	12.94	0.04	0.283	95.43	1.57	150.55
Gold Beach	L	17.00	0.74	0.060	118.07	4.96	144.11
	M	17.00	0.70	0.060	129.41	5.24	130.11
	Mean	17.00 <sup>c</sup>	0.72	0.060	123.74	5.10	137.11

<sup>a</sup>) L = low, M = medium, H = high elevation (see Table 1 for elevation ranges corresponding to designations).

<sup>b</sup>) Weeks after July 1.

<sup>c</sup>) A minimum estimate, because terminals in most Gold Beach trees were destroyed by frost before setting bud.

quency of second flushing (Tables 3 and 4)<sup>3</sup>). In general, breeding zones accounted for much of the total variation among the inland families (Figure 2). This proportion was at least 50% for the five seedling traits and was 27.7% for seed weight (mean over all traits was 53.9%). The relative percentages of variation attributable to breeding units and elevation varied considerably among traits. Elevation zones and breeding units accounted for roughly equal proportions of variation among families in date of budset and frost damage; but for the remaining traits, little or no variation among families could be attributed to elevation differences. For all traits, the proportion of variation resulting from interactions between breeding units and elevations (U × E) was small.

The greatest differences among breeding zones occurred among the coastal (South Gold Beach) and southern inland (Jacksonville and Butte Falls) units. The contrast between South Gold Beach and southern inland units (data not shown) was significant for all traits except budset (which could not be tested; see below); for three traits, the contrast of Jacksonville and Butte Falls was also significant (Tables 3 and 4). Strong clinal patterns were evident; families from the moister, cooler climate of South Gold Beach had lighter seeds and later budset and produced taller seedlings with more branches, than did families from the drier, harsher inland units (Table 5). Particularly strik-

ing was the difference in frost damage between seedlings from the coastal and inland units. Seventy-two percent of the seedlings in the coastal unit were damaged, since most seedlings had not set bud by this date, but damage in seedlings from each of the inland units averaged less than 15%. Because frost often killed the terminals on the South Gold Beach seedlings and budset could not be scored, the coastal-inland ANOVA was not carried out for this trait. Average date of budset, however, must have been at least 17 weeks after July 1, 1.5 weeks later than mean budset data of seedlings from the latest inland zones. Frost damage and weeks to budset were strongly and positively correlated among the 14 breeding zones ( $r = 0.95$ ,  $P < 0.01$ ). Although seedlings from the inland units set final terminal buds earlier, in general, than seedlings from the coast, the propensity to reflush was greater in inland seedlings (Table 5). Average frequency of second flushing was more than four times greater in seedlings from Butte Falls (0.283) than from South Gold Beach (0.060).

Differences in trait means were generally not as great between North Umpqua, South Umpqua, and Jacksonville (north to south) as they were between South Gold Beach, Jacksonville, and Butte Falls (west to east). Nevertheless, the contrast of means between the northern (North and South Umpqua) and southern (Jacksonville and Butte Falls) units was significant for all traits measured (Tables 3 and 4). On average, seedlings from drier southern units had heavier seeds and were smaller (shorter, fewer branches), earlier to set bud, more subject to second flushing, and less damaged by frost than those from the northern

<sup>3</sup>) Since the ANOVA's for the Inland data set are the most relevant to this paper, and the ANOVA's for the Inland and Inland-Coastal data sets were qualitatively similar (LOOPSTRA, 1984), only the Inland ANOVA's for the mixed-model are presented.

units (Table 5). In only one trait, date of budset, was the mean difference between the North and South Umpqua breeding units significant (Table 3).

No significant differences were detected in seedling size (height or branch number) or frequency of second flushing with increasing elevation within breeding units (Table 3). Significant linear trends, however, were found for the other three traits, with families from higher elevations having lighter seeds and producing seedlings with earlier budset and less frost damage, on average, than families from lower elevations (Table 5). Only two of the 36 (6 traits  $\times$  6 contrasts per trait) single-df contrasts involving  $U \times E$  interactions in the inland ANOVA were significant, indicating that trends with elevation were relatively consistent in the different breeding units. Therefore, only the pooled  $U \times E$  mean squares are given in table 3.

In general, variation among families within breeding zones appeared to be associated only weakly with location variables. Fewer than one-third (26) of the 84 regression equations fitted were significant, and significant equations accounted for only about one-quarter (mean  $R^2 = 0.257$ , range = 0.133 to 0.420) of the family variation in a breeding zone. The significant equations were usually complex, involving two or more location variables and interactions between them (LOOPSTRA, 1984). Distance from the ocean was involved in 18 of the 26 equations; elevation and latitude each occurred in 13. Elevation entered six of the eight significant equations involving the two traits related to phenology (frost damage and date of budset). All traits and breeding units were represented among the significant equations.

Because of their complexity, the fitted relationships between location variables and family means for the seedling traits were often hard to interpret. Nevertheless, for 6 of the 10 significant regressions which had a single independent (location) variable, the predicted linear response in family means was in the direction expected from clinal associations between breeding zone means and location variables (Table 6). For example, both date of budset and frost damage decreased with increasing elevation of parent trees in the highest elevation of the South Umpqua breeding unit (South Umpqua-H), the same trend observed among elevations within breeding units (Table 5). No clear association between elevation and branch number or frequency of second flushing was observed among breeding zones, but in two cases (South Umpqua-M and South Umpqua-H) family means of these traits were associated with elevation of parent trees within zones (Table 6). The remaining linear regressions involving a single location variable predicted responses opposite to those expected from breeding zone trends.

### Discussion

Consistent with previous reports on Douglas-fir in southwest Oregon (HERMANN and LAVENDER, 1968; WHITE *et al.*, 1981; SORENSEN, 1983; CAMPBELL, 1986), our results show that genetic variation for seedling and seed traits is extensive in this region. The parent trees in his study were clustered by breeding zone; thus the proportion of total family variation attributed to breeding zones is probably somewhat inflated, relative to that which would be ex-

Table 6. — Regression equations for fitting family means of seedling and seed traits ( $y$ ) to location variables ( $x_1, x_2, x_3$ )<sup>a</sup> of the parent trees within breeding zones when only a single location variable remained in the fitted model.

Unit	Breeding zone		Trait	Equation <sup>c</sup>	$R^2$
	Unit	Elevation <sup>b</sup>			
South Umpqua	M		Frequency of second flushing	$Y = -1.001 + 145(-03)x_1^d$	.142
			Branch number	$Y = -0.206 + 0.367(-01)x_3^e$	.173
	H		Date of budset	$Y = 30.339 - 0.127(02)x_1^d$	.304
			Frost damage	$Y = 2.718 - 0.199(-03)x_1^f$	.370
			Branch number	$Y = 5.035 - 0.304(-03)x_1^d$	.212
Jacksonville	L		Frequency of second flushing	$Y = 1.836 - 0.377(-01)x_3^e$	.208
	M		Seed weight	$Y = 144.243 + 0.891(-01)x_3^f$	.300
Butte Falls	M		Branch number	$Y = -0.931 + 0.341(-01)x_2^f$	.189
South Gold Beach	L		Frost damage	$Y = -1.541 + 0.417(-01)x_2^f$	.305
			Branch number	$Y = -3.176 + 0.809(-01)x_2^f$	.400

a)  $x_1$  = elevation (m),  $x_2$  = latitude (km south of the fifth standard parallel),  $x_3$  = distance from Pacific Ocean (km).

b) L = low, M = medium, H = high elevation (see Table 1 for elevation ranges corresponding to designations).

c) Numbers in parentheses are exponents, base 10.

d) No clear association was observed between breeding zone means for this trait and the fitted location variable.

e) The predicted response is opposite to the direction expected from the association of breeding zone means with the fitted location variable.

f) The predicted response is in the direction expected from the association of breeding zone means with the fitted location variable.

pected if parent trees had been distributed more uniformly. Nevertheless, our results agree with a similar study of family variation within and among seed zones in southwest Oregon, in which the parent trees were uniformly distributed over a broad area (ADAMS and CAMPBELL, 1981). In that study, 43% of family variation (averaged over 13 seedling traits) was attributed to seed zones. (Seed zones are similar in size geographically to breeding zones, but the elevation range (152 m) is narrower.)

Even given the above considerations, breeding zones in southwest Oregon appear to explain a substantial proportion of genetic variation among locations. Furthermore, much of this variation is apparently adaptive, since the patterns of genetic variation among breeding zones are associated with environmental variables. These patterns are consistent with previous common-garden studies of seed sources from southwest Oregon or adjacent regions (HERMANN and LAVENDER, 1968; GRIFFIN and CHING, 1977; CAMPBELL and SORENSEN, 1978; WHITE *et al.*, 1981; SORENSEN 1983; CAMPBELL, 1986) and seem to reflect adaptation primarily to moisture and temperature gradients. For example, earlier budset in seedlings from more southern and interior breeding zones probably results largely from natural selection for early cessation of growth in areas with summer drought. Early budset with increasing elevation of breeding zones similarly is an adaptation to the colder temperatures and greater frequency of early fall frosts at higher altitudes. Strong positive associations between frost damage and weeks to budset among seed sources of coastal Douglas-fir have been reported by CAMPBELL and SORENSEN (1973) and GRIFFIN (1974).

Increase in seed weight with increasing distance from the ocean and with decreasing latitude may also be a selection response to moisture regime. Larger seeds on drier sites may provide more food reserves for initial root penetration, permitting better establishment before onset of summer drought. However, seed weight decreased linearly with elevation (Tables 4 and 5), in contrast to the work of WHITE *et al.* (1981), who found a weak but positive correlation ( $r = 0.35$ ,  $P < 0.05$ ) between seed weight and elevation (from 475 m to 1630 m) of 36 Douglas-fir seed sources in southwest Oregon. The findings of HERMANN and LAVENDER (1968) may explain this difference. They investigated 14 Douglas-fir seed sources, from 457 to 1524 m elevation, in a 16-km  $\times$  24-km area north of the Butte Falls breeding unit. Seed weight decreased with increasing elevation of seed sources up to around 1100 m and then increased again slightly at higher elevations. The overall trend was a weak negative correlation between seed weight and elevation. Thus, increased seed weight appears to be an adaptation to harsh site conditions — either drought (at lower elevations) or cold (at higher elevations). Linear correlations between seed weight and elevation should be negative or positive, depending on whether sampling emphasizes seed sources at lower or higher elevations, respectively.

The higher frequency of second flushing in seedlings from the southern inland zones may be an adaptation to intermittent moisture availability during the growing season (IRGENS-MOLLER, 1967). Thus, seedlings from the inland breeding zones can cease active growth temporarily when moisture is limiting, but reflush to continue growth during favorable periods. Presumably, because moisture is rarely limiting throughout the growing season, the need

for temporary budset has not evolved in coastal seed sources. This hypothesis is supported by the results of KAYA (1987), who grew seedlings from coastal and inland sources in southwest Oregon in two common-garden environments. One was watered every other day during the growing season; in the other, watering was interrupted by a brief (one- or two-month) drought. The frequency of second flushing in the first growing season was very low in seedlings from coastal families, whether watering was continuous (0.001) or intermittent (0.02), and was also low (0.08) in seedlings from inland families under the continuous watering regime. The latter seedlings, however, respond strongly to intermittent moisture availability, with the frequency of second flushing increasing to 0.24.

Trends in breeding zone means with increasing distance from the ocean were based on the southern breeding units (i.e., South Gold Beach-Jacksonville-Butte Falls), where mean annual rainfall decreases rapidly from west to east. Within both South Umpqua-M and Jacksonville-L, however, the annual rainfall pattern is opposite to the overall regional trend, with the highest rainfall occurring in the easternmost portion of the zone. Thus, while associations of trait means with location variables were different at the breeding zone and within breeding zone levels, they could result from adaptation to similar environmental gradients.

Although breeding zones seem to correspond in large part to patterns of geographic differentiation in southwest Oregon, considerable genetic variation remains within zones. Regression of family means on the same location variables strongly associated with patterns of genetic variation among breeding zones, however, accounted for only a small proportion of within-zone families. These regressions should be considered a crude attempt to account for adaptive differentiation among locations within zones, for two reasons. First, each location within a zone was represented by one parent tree. Thus, genetic differences among locations may have been confounded by differences within locations. Second, other variables associated with microgeographical variation in coastal Douglas-fir [e.g., slope aspect (HERMANN and LAVENDER, 1968; CAMPBELL, 1979), sun exposure (CAMPBELL, 1986), and soil type] were not included in the regression equations. Nevertheless, little adaptive differentiation probably occurs along latitudinal, longitudinal or elevation gradients remaining within zones, and redesigning boundaries or reducing breeding-zone size probably would not increase the correspondence between breeding zones and morphological differences associated with geography.

Families from the North and South Umpqua breeding units did not differ significantly in nearly all traits (Tables 3 and 4); consolidating these units or enlarging breeding units in this area might be feasible. Patterns of genetic variation based on seed and seedling traits must be interpreted with caution, however, since the relevance of these patterns to long-term growth and survival in plantations is unknown and can be determined only by field testing (ADAMS and CAMPBELL, 1981). Nevertheless, the adaptive significance of geographic patterns of genetic variation and the importance of breeding zones in lessening risk of maladaptation are well illustrated by the patterns of frost damage (Table 5).

Patterns of genetic variation in seed and seedling traits within and between southwest Oregon breeding zones bear little relationships to those observed for allozyme



loci in the companion study (MERKLE and ADAMS, 1987). Although Douglas-fir is highly variable at the single-gene level, less than 1% of the genetic diversity in 22 breeding zones could be attributed to differences among zones. Furthermore, the small amount of variation in allele frequency among zones was not related to geography. At least in this species, the common-garden approach and allozyme studies appear to give different information when used for identifying geographic patterns of adaptive differentiation.

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#### Literature Cited

ADAMS, W. T. and CAMPBELL, R. K.: Genetic adaptation and seed source specificity. In: HOBBS, S. D. and HELGERSON, O. T. (eds.): Reforestation of skeletal soils. For. Res. Lab., Oregon State Univ., Corvallis. p. 78-85 (1981). — CAMPBELL, R. K.: Genecology of Douglas-fir in a watershed in the Oregon Cascades. *Ecology* 60, 1036-1050 (1979). — CAMPBELL, R. K.: Mapped genetic variation of Douglas-fir to guide seed transfer in southwest Oregon. *Silvae Genetica* 35, 85-96 (1986). — CAMPBELL, R. K. and SORENSEN, F. C.: Cold-acclimation in seedling Douglas-fir related to phenology and provenance. *Ecology* 54, 1148-1152 (1973). — CAMPBELL, R. K. and SORENSEN, F. C.: Effect of test environment on expression of clines and on delimitation of seed zones in Douglas-fir. *Theor. Appl. Genet.* 51, 233-246 (1978). — FRANKLIN, J. F. and DYRNESS, C. T.: Natural vegetation of Oregon and Washington. USDA For. Serv. Gen. Tech. Rep. PNW-8. 417 pp. (1973). — FROELICH, H.

A., McNABB, D. H. and GAWEDA, F.: Average annual precipitation, 1960-1980, in southwest Oregon. Ext. Serv. EM 82:20. Oregon State Univ., Corvallis. (1982). — GRIFFIN, A. R.: Geographic variation in juvenile growth characteristics of Douglas-fir [*Pseudotsuga menziesii* (MIRB.) FRANCO] from the coastal ranges of California. Ph. D. thesis, Oregon State Univ., Corvallis. 153 pp. (1974). — GRIFFIN, A. R. and CHING, K. K.: Geographic variation in Douglas-fir from the coastal ranges of California. *Silvae Genetica* 26: 149-157 (1977). — HERMANN, R. K. and LAVENDER, D. P.: Early growth of Douglas-fir from various altitudes and aspects in southern Oregon. *Silvae Genetica* 17: 143-151 (1968). — IRGENS-MOLLER, H.: Geographical variation in growth patterns of Douglas-fir. *Silvae Genetica* 17, 106-110 (1967). — KAYA, Z.: Genetic variation in shoot-growth patterns of Douglas-fir populations from southwest Oregon. Ph. D. thesis, Oregon State Univ., Corvallis. 123 pp. (1987). — LOOPSTRA, C. A.: Patterns of genetic variation within and among breeding zones of Douglas-fir in southwest Oregon. M. S. thesis, Oregon State Univ., Corvallis. 57 pp. (1984). — MERKLE, S. A. and ADAMS, W. T.: Patterns of allozyme variation within and among Douglas-fir breeding zones in southwest Oregon. *Can. J. For. Res.* 17, 402-407 (1987). — NIE, N. H., HALL, C. H., JENKINS, J. G., STEINBRENNER, K. and BENT, D. H.: Statistical package for the social sciences. (2nd Edition). McGraw-Hill Book Co., New York. 674 pp. (1975). — RUDOLF, P. O.: Tree-seed marketing controls. In: SCHOPMEYER, C. S. (coord.): Seeds of woody plants in the United States. USDA Agric. Handb. 450, United States Government Printing Office, Washington, D. C. P. 153-166 (1974). — SILEN, R. R.: Genetics of Douglas-fir. USDA For. Serv. Res. Pap. WO-35. 34 pp. (1978). — SILEN, R. R. and WHEAT, J. G.: Progressive tree improvement program in coastal Douglas-fir. *J. For.* 77, 78-83 (1979). — SNEDECOR, G. W. and COCHRAN, W. G.: Statistical methods. (6th Edition). The Iowa State Univ. Press. Ames, Iowa 593 pp. (1967). — SORENSEN, F. C. Geographic variation in seedling Douglas-fir [*Pseudotsuga menziesii* (MIRB.) FRANCO] from the western Siskiyou Mountains of Oregon. *Ecology* 64, 695-702 (1983). — STEEL, R. G. D. TORRIE, J. H.: Principles and procedures of statistics, A biometrical approach. (2nd Edition). McGraw-Hill Book Co., New York. 633 pp. (1980). — WHEAT, J. and SILEN, R.: Progress report for the IFA-PNW cooperative tree improvement programs. Industrial Forestry Assoc., Portland, Oregon. 51 pp. (1984). — WHITE, T. L., LAVENDER, D. P., CHING, K. K. and HINZ, P.: First-year height growth of southwestern Oregon Douglas-fir in three test environments. *Silvae Genetica* 30, 173-178 (1981).

## The Incorporation of Early Testing Procedures into an Operational Tree Improvement Program

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#### Abstract

Seedling shoot dry weight can be used as an early testing trait for loblolly pine. Seedlots that have both good germination and a small shoot dry weight in greenhouse tests tend to have average or below average volume growth in field tests. This relationship allows the use of shoot dry weight in the greenhouse to be used as a trait for culling some progenies prior to field testing in a tree improvement program. Two-step testing procedures combine independent culling at the end of the first test (greenhouse) with assortative mating for the second test (field) to both reduce progeny testing costs and increase genetic gain. These procedures increase the efficiency of an operational tree improvement program by reducing the fund-

ing required for field testing to obtain the same or slightly greater genetic gain. The implementation of shoot dry weight as an early testing trait in the first and second generation loblolly pine breeding program for the Western Gulf Forest Tree Improvement Program is discussed.

**Key words:** Loblolly Pine, genetic gain, breeding, progeny testing, assortative mating.

#### Introduction

Tree improvement is widely accepted as a valuable silvicultural tool in the southeastern United States. To meet regeneration demands, approximately 4,000 hectares of seed orchard have been established. About 83 percent of the planting programs in this region utilize improved planting stock produced by these seed orchards. The resulting improvements in growth, form, and disease resistance represent significant economic benefits to forest landowners. Progeny testing is the backbone of any tree improvement program and intensive testing programs

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