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Effect of Test Environment on Expression of Clines and on Delimitation of Seed Zones in Douglas-Fir

R.K. Campbell and F.C. Sorensen
Pacific Northwest Forest and Range Experiment Station, Corvallis, Oregon (USA)

Summary. Clinal models of population structure in an indigenous tree species can be used to delineate seed-collection zones and breeding zones, and to devise transfer rules. Models may be developed by growing populations in test environments; however, a clinal description may be a function of test environments as well as of population genotypes. This possibility was studied by growing seedlings from 40 populations of northwestern U.S. Douglas-fir (*Pseudotsuga menziesii* var. *'menziesii'* [Mirb.] Franco) in eight nursery-bed treatments which contrasted air and soil temperatures and nutrition. Growth traits measured were stem diameter, top height, and dry weight; phenological traits were bud-burst and bud-set dates, extension period, and extension-period midpoint. Population samples interacted significantly with soil temperature for growth traits, and with soil and air temperatures combined for phenological traits. Interactions were at least partly explained by complex clinal associations of seedling performance with elevation, with latitude, and with distance from the ocean of the populations sampled. Both the complexity and the gradient of the clinal pattern depended on the trait and on the specific test environment.

The clinal patterns of greatest complexity were expressed in warm air and soil treatments. Dry top-weights of population samples were associated with latitudes for samples grown in warm soils, but this relationship was not apparent in cool soils. A discrepancy in bud-burst dates between extreme coastal and more inland populations was greatest in warm soil-warm air treatments and was negligible in cool soil-cool air treatments. Populations × temperature interactions were attributed to the differential response of population samples to spring temperature and photoperiod. It is proposed that first attempts at devising a model can be based on nursery or growth-chamber tests, and that test environments should stress contrasting photo- and temperature-regimes.

The estimate of clinal structure in Douglas-fir suggests that there is more risk within northwestern U.S. in moving provenances east-west than north-south, that this risk increases with elevation of provenances, and that north-south transfers are more critical near the coast than inland.

Key words: *Pseudotsuga menziesii* - Genecology - Provenance - Genotype-temperature interactions - Fertilizer interactions

Introduction

Methods for stratifying forest habitats and populations are useful when working with genetic lines (provenances, races, ecotypes, etc.) which sample natural populations in a heterogeneous forest region. In reforestation, this was noted five decades ago by Schotte (1923) who related differences in mortality of planted, non-indigenous provenances of Scots pine to mean annual temperature of their habitats. Subsequently, Eneroth (1926) devised a transfer model for predicting effects of seed transfer and Langlet (1945) proposed rules for stratifying forest regions into "seed collection" or "provenance" zones. More recently, tree breeders have recognized the value of stratification more specifically as a means for reducing genotype-environment interaction components. In field tests of forest species, these are often as large as or larger than the genetic component (King 1965, Morgenstern and Teich 1969). Stratification makes the reference population of environments more homogeneous, thus increasing useful genetic variance by reducing the variance component for genotype-environment interaction (Comstock and Moll 1963).

Based on Langlet's (1934, 1936) demonstration that population diversity is closely correlated with environmental diversity, stratification procedures have usually assumed a clinal response of genotypes to climatic gradients. In concept, at least, most clines are described by fitting regressions of provenance performance to climatic, geographic, or physiographic parameters. Zone dimensions or provenance transfer rules can then be devised, the limits depending on regression line slope in conjunction with a criterion of

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acceptable adaptation (Stern 1964, Morgenstern and Roche 1969, Campbell 1974).

Two factors complicate this procedure. The first occurs because not all traits in populations follow identical clines, a fact documented in tree species (e.g., Holzer 1967, 1969, Hamrick and Libby 1972) and long recognized by genecologists (Langlet 1971). The problem arises in deciding on the trait or traits to use as indexes of growth and adaptation. The second occurs because the pattern of trait response over several levels of an environmental factor (i.e., the response curve) commonly varies among provenances or ecotypes. Curves may differ in intercept, shape, and placement of minima or maxima. Examples of tree species are reported in Jensen and Gatherum (1967), Hermann and Lavender (1968), Lavender and Overton (1972), Fryer and Ledig (1972), Hellmers and Rook (1973), Sorensen and Ferrell (1973), and Campbell and Sugano (1975).

Genotypic values of populations are usually characterized by growing population samples in test environments, e.g., nursery beds or plantation sites. If response curves differ among populations tested at several levels of a single factor, then the ranking of population means in a single environment could vary depending on the test environment (Knight 1970).

Clines are described by fitting estimated genotypic values of populations to regression models. Because ranking may be influenced by test environment, the regression describing a cline also may be affected. Correspondingly, so would the choice of model used in delimiting provenance zones or seed collection zones. The problem is potentially greatest when estimates are derived from the "common garden" experiment, especially with only a single environment, but it may also be of significance when several test plantations are used (Campbell 1974).

In initial efforts to stratify natural populations, a test environment is desirable to the extent that it reveals the adaptive genetic variability within the population. By using environments with less resolving power, we risk devising a model which lacks important variables for describing population structure. Tests in nursery beds, greenhouses, or growth chambers are often used to obtain high resolution but this procedure is criticized because such environments may not be natural--population-sample differences revealed in artificial conditions may have little adaptive significance and, consequently, little relevance to field conditions.

We gain confidence that the population-sample differences exposed by an "artificial" test are adaptively significant if they are associated with environmental differences at population origin. And confidence is enhanced if, for example, genetic variation is clinally structured and if genotype-environment interactions are also clinally structured. In terms of the response curve analogy, such a relationship implies that curve shapes differ among population samples, and, furthermore, that curve shapes change along clinal gradients. Such a complex structure of genetic variation is difficult to account for except as a refined adjustment of populations to natural selection.

The main objective in the present study was to examine the effect of test environment on regressions of two developmental-cycle traits (bud burst, bud set) and three growth traits (height, diameter, dry weight) on location parameters of populations in coastal Douglas-fir seedlings. Subsidiary objectives were:

1) evaluation of environmental factors which we have identified as having potential for exposing genetic variation among population samples and for participating in genotype-environment interactions

2) a preliminary description of topographic and geographic clines for these developmental-cycle and growth traits in Pacific Coast Douglas-fir

3) preliminary recommendations regarding seed-transfer of Douglas-fir

Experimental results came from a sample of 40 populations from western Washington and Oregon, grown in eight different nursery-bed environments.

Materials and Methods

We sampled 40 populations (24 within 0.75 km of a U.S. Weather Bureau Station) of Douglas-fir in its range between the Pacific Ocean and the crest of the Cascade Range between latitudes of 42°N and 48°15'N. Elevations of collection locations are from 6 to 1,432m above sea level (a.s.l) and distances from the ocean are from 5 to 244 km, the mean location being 45.0°N, 112.4 km from the ocean and 602 m a.s.l. Collections sampled the region fairly uniformly with some underrepresentation of high elevations in north coastal areas and of low elevations in the Cascade Range. Simple correlations among locations for latitude and elevation, latitude and distance from the ocean, and elevation and distance from the ocean were, respectively, $r = -.27, .28, .53$. "Growing-season" lengths (mean last spring minimum of 0°C to mean first fall mini-

mum of 0°C based on 22 years of record) at weather station collection-locations ranged from 142 to 278 days.

The experiment was established in nursery beds in Corvallis, Oregon, as a split-plot design using population-sample subplots within environmental-treatment main plots. The eight environmental treatments were replicated in two randomized blocks (Li 1964, p. 515). Environments were created by factorial arrangement of the following treatments: fertilized vs. nonfertilized, cool soil vs. warm soil, "cool air" vs. "warm air". Environmental plots had surrounding rows of non-counting seedlings from a common source. Each population-sample subplot contained five seedlings, one from each of five open-pollinated single-tree collections at a location.

The warm-soil treatment was produced by burying plastic-coated electric heating cables at 15-cm depth and spacing, and heating was applied continuously from time of sowing of seed (June 4, 1969) to harvesting (October 1970). The warm-air treatment, obtained by erecting small polyethylene-covered frames over appropriate plots, was designed to raise air temperature by the greenhouse effect. Treatment started August 15, 1969, and ended June 10, 1970. Cool-soil and cool-air plots were, respectively, unheated and uncovered. Fertilizer plots were treated, before seeding, with a commercial fertilizer (6N, 10P, 4K) at a rate of 37 kg nitrogen per hectare.

Soil-temperature differences between treated and untreated plots varied, depending on measurement depth, time of day, season, and rainfall patterns. During the growing season, at 10-cm depth, differences were rarely greater than 5.5°C or maxima higher than 21°C.

We did not record air temperature continuously, but continuous recording under similar polyethylene frames in a subsequent comparable experiment has shown temperatures in frames to be marginally warmer in the early morning (about 0.5°C) and considerably warmer in early evening (about 5°C), with greatest differences on overcast days. As shown by shade-cloth experiments (Allen 1975), this treatment may alter humidity, irradiance, eddy diffusivity, and CO_2 concentration, as well as temperature. In comparison to natural environments of Douglas-fir in the Pacific Coast region, treatment environments could be classed as ranging from the relatively mild, infertile, mesic site to the very mild, very fertile, humid site.

Total heights, basal stem diameters, and dry weights of stem and needles above the cotyledon scar (dry top weight) were measured at the end of the second growing season. Mean bud-set and bud-burst dates of the terminal bud were determined for the second growing season. Seedlings were scored for bud-burst every 3.5 days - i.e., Monday a.m. and Thursday p.m.; later they were scored every 2 weeks for bud set. From these data, two secondary traits were derived: (1) extension period, which equaled mean bud-set date minus mean bud-burst date, and (2) extension period midpoint, which equaled mean bud-burst date plus one-half the terminal extension period.

In an unbiased experiment with properly scaled data, a significant population-sample × environment interaction implies either that the ranking of population-samples or the relative magnitude of differences between population-samples is not identical in all environments. The interaction is measured by the difference between two differences; that is, by the failure of a difference between population samples to be the same in two environments. In this experiment, these differences are hypothesized as resulting from population-samples responding variously to the two environments, in clinal relationship to population origin. When interactions were statistically significant in analyses of variance, we examined population responses in relation to elevation (X_1), latitude (X_2), and distance from the ocean (X_3) of population origin to determine if the trait responses were clinal or population-specific. This was done for each level of an interacting environmental treatment. If, for example, interactions involved air temperature, population-sample means were averaged over replications in subplots in the cool-air treatment and a regression equation was then fitted to the means. The same process was repeated for means in the warm-air treatment. This provided two regression equations which were solved for a set of selected values for latitudes, elevations, and distances from the ocean (i.e., 44°, 46°, 48°N latitude; 100-, 600-, 1, 100-m elevation; 5, 50, 150, 200 km from ocean). When calculated responses were plotted graphically, the result was a set of curves for describing performance of population-samples in each test environment. Respectively, two and four sets of curves were required to illustrate differences originating from first- and second-order interactions.

Equations were fitted to population-sample means by selecting predicting variables from a preliminary model by stepwise multiple regression. Our preliminary model included 18 "independent" variables made up as an expansion series of polynomial first- and second-order terms of latitude, elevation, and distance from the ocean. Such a series with two independent variables (e.g., elevation and latitude) has the form:

$$Y = B_0 + B_1X_1 + B_2X_2 + B_3X_1X_2 + B_4X_1^2 + B_5X_2^2 + B_6X_1^2X_2^2.$$

From the 18 variables, the stepwise procedure builds an equation variable by variable, by adding in sequence all those variables that contribute significantly ($P<.05$) in reducing sums of squares in the dependent variable. The process is completed when no more variables are admitted to the equation and no more are rejected.

After the equation was chosen, differences between observed and calculated values were examined to ensure that error variances were homogeneous and that there was no visual evidence for lack of fit of data to equations. A final test was to determine if the observed F-ratios (regression mean squares)/(residual mean squares) exceeded the 5-percentage point of the F-distribution point by four times. This is the "four-times rule" (Draper and Smith 1966, p. 64) for rating the fitted equation as a satisfactory predictor, in that the variaton in response values predicted is substantially larger than the standard error of the response.

Results

Phenological traits were influenced mainly by air and soil temperatures, and growth traits by fertilizer (Table 1). Bud burst and, secondarily, extension-period midpoint, came earlier in both warm soil (warm vs. cool soil) and warm air (warm vs. cool air - Table 2); extension-period and bud set were not significantly changed by environmental treatment.

Table 1. Analyses of variance for seven traits: mean squares and significance tests

Variation source	d.f.	Bud burst (half-weeks)	Bud set (2-weeks)	Extension period length (days)	Extension period midpoint (days)	Log_{10} stem diameter (mm×10)	Log_{10} stem height (cm)	Log_{10} dry top weight (g×10)
Air temperature (A)	1	785.13**	12.36	2334.3	5463.2**	.031	1.646**	.527
Soil temperature (S)	1	163.96*	11.55	7.7	2134.6**	.105	.004	.300
Fertilizer (F)	1	34.72	16.91	6114.1	341.3	1.994**	7.280**	17.795**
Blocks (B)	1	51.61	17.47	6999.9	278.5	.013	.212	.001
AS	1	13.47	.22	374.4	10.1	.049	.140	.286
AF	1	17.88	.03	156.6	73.0	.115	.106	.506
SF	1	4.95	4.61	1431.8	124.0	.021	.038	.046
ASF	1	8.23	.01	125.4	19.7	.001	.010	.017
Error a[1]	7	16.94	3.90	1419.4	131.0	.022	.077	.159
Provenance (P)	39	14.16**	1.95**	414.9**	173.7**	.032**	.059**	.194**
PA	39	1.55	.31	84.0	18.7	.004	.004	.017
PS	39	1.86	.25	60.0	21.0	.007*	.006*	.022*
PF	39	1.43	.29	91.5	14.0	.005	.005	.019
PB	39	1.58	.32	93.9*	17.1	.003	.002	.009
PAS	39	2.03*	.42*	119.5**	23.9	.005	.004	.016
PAF	39	1.38	.28	76.6	16.4	.003	.003	.011
PSF	39	1.04	.20	53.9	12.4	.006	.004	.019
PASF	39	1.47	.21	61.0	14.2	.004	.004	.017
Error b[2]	273	1.36	.27	63.5	19.2	.004	.004	.014
Experimental mean		7.75	2.12	116.5	162.4	1.519	1.378	1.546

[1] Pooled treatment × block interactions
[2] Pooled population-sample × block interactions
* Significant at $P < .05$
** Significant at $P < .01$

Table 2. Main plot treatment means averaged over all other treatments and 40 populations

Treatment	Bud burst (half-weeks[1])	Bud set (2-weeks[2])	Extension period (days)	Extension period midpoint (days after Dec. 22)	Log_{10} stem diameter (mm×10)	Log_{10} stem height (cm)	Log_{10} dry top weight (g×10)
Cool air	8.86)**	2.26	115	165)**	1.512	1.328)**	1.518
Warm air	6.65	1.98	118	159	1.526	1.429	1.575
Cool soil	8.26)*	2.25	117	164)**	1.506	1.376	1.525
Warm soil	7.25	1.99	116	161	1.532	1.381	1.568
Fertilizer-low	7.52	2.28	120	163	1.463)**	1.272)**	1.380)**
Fertilizer-high	7.99	1.96	113	162	1.575	1.485	1.713

[1] To convert to days after Dec. 22, use (half-weeks × 3.5 + 77)
[2] To convert to days after Dec. 22, use (2-weeks × 14 + 191)
*, ** Main-effects significance level (Table 1)

Fertilizer treatment increased diameters, heights, and dry top weights by 30, 60, and 115 percent, respectively (retransformed from Table 2).

Population samples differed significantly ($P<.01$) in all traits, but magnitudes of the differences depended partly on environmental treatment as indicat-

Table 3. Regression equations for fitting population performance (Y) in test environments to factors of provenance origin: elevation in meters (X_1), latitude in degrees north (X_2), and distance from Pacific Ocean in kilometers (X_3)

Y in equation pertains to:	Regression equation[1]	R^2	$s_{y.x}$	d.f.	F	Four-times rule[2]
1. $\log_{10}$(dry top weight (g) × 10)						
a. Cool soil	$Y=1.57-3.45(-12)X_1^2X_3^2$	.36	.085	1,38	21.3**	+
b. Warm soil	$Y=1.84-5.29(-02)X_2+1.85(-04)X_2X_3 -9.21(-09)X_1X_3^2$	.75	.065	3,36	26.4**	+
2. $\log_{10}$ height (cm)						
a. Cool soil	$Y=1.41-2.35(-12)X_1^2X_3^2$	.59	.036	1,38	54.5**	+
b. Warm soil	$Y=1.44-7.75(-04)X_2^2-3.21(-12)X_1^2X_3^2$	.73	.037	2,37	51.1**	+
3. $\log_{10}$ diameter (mm × 10)						
a. Cool soil	$Y=1.57-1.65(-02)X_2+4.07(-04)X_3-1.79 (-12)X_1^2X_3^2$	.46	.035	3,36	10.4**	-
b. Warm soil	$Y=1.58-7.65(-04)X_2^2-1.86(-06)X_1X_2^2$	.45	.040	2,37	15.1**	+
4. Extension period (days)						
Cool air						
a. Cool soil	$Y=121.21-6.99(-05)X_1X_3$	.37	7.29	1,38	22.7**	+
b. Warm soil	$Y=132.58-2.87X_2-1.50(-04)X_1X_3 +3.84(-10)X_1^2X_3^2+5.17(-06)X_2^2X_3^2$	.60	4.06	4,35	12.9**	+
Warm air						
c. Cool soil	$Y=119.53-2.22(-08)X_1^2X_3$	.20	4.59	1,38	9.8**	-
d. Warm soil	$Y=123.19-7.44(-02)X_2^2-1.64(-10)X_1^2X_3^2$	.45	3.75	2,37	15.1**	+
5. Bud set (in 2-weeks after July 1)						
Cool air						
a. Cool soil	$Y=2.62+1.60(-08)X_1^2X_2^2-3.15(-11)X_1^2X_3^2$	.49	.41	2,37	17.7**	+
b. Warm soil	$Y=2.34+7.99(-05)X_1X_2-5.55(-06)X_1X_3$	.57	.26	2,37	25.0**	+
Warm air						
c. Cool soil	$Y=2.25-1.88(-09)X_1^2X_3$	.29	.31	1,38	15.6**	-
d. Warm soil	$Y=2.04-1.37(-11)X_1^2X_3^2$	.45	.28	1,39	30.6**	+
6. Bud burst (in half-weeks after March 9)						
Cool air						
a. Cool soil	$Y=4.54+3.74(-03)X_1+7.19(-01)X_2 -4.69(-07)X_1^2X_2$	.55	.86	3,36	14.5**	+
b. Warm soil	$Y=6.18+5.56(-01)X_2-7.92(-07)X_2^2X_3^2$	.28	1.16	2,37	7.2**	-
Warm air						
c. Cool soil	$Y=5.16+5.03(-01)X_2-1.11(-04)X_2^2X_3$	.35	.84	2,37	9.8**	-
d. Warm soil	$Y=3.37+6.64(-01)X_2+9.36(-06)X_2X_3^2 -4.48(-04)X_2^2X_3$	.51	.59	3,36	12.6**	+
7. Extension period midpoint (days after winter solstice; Dec.22)	$Y=158.03+1.06X_2+3.29(-06)X_1^2 -2.12(-10)X_1^2X_3^2$	.62	2.10	3,36	19.8**	+

[1] Numbers in parentheses are exponents, base 10. X_2 is coded as degrees north latitude minus 40

[2] Four-times rule: Equation is an inadequate predictor when calculated F value is not four times greater than tabular $F_{p=0.05}$, equation inadequate if minus (-)

** Equation significant at probability < .01

ed by two types of significant interaction (Table 1):

1) population-sample × soil-temperature interaction - relative seedling sizes among population samples, as measured by stem diameter, stem height, and dry top weight, were influenced by soil temperature;

2) population-sample × soil-temperature × air-

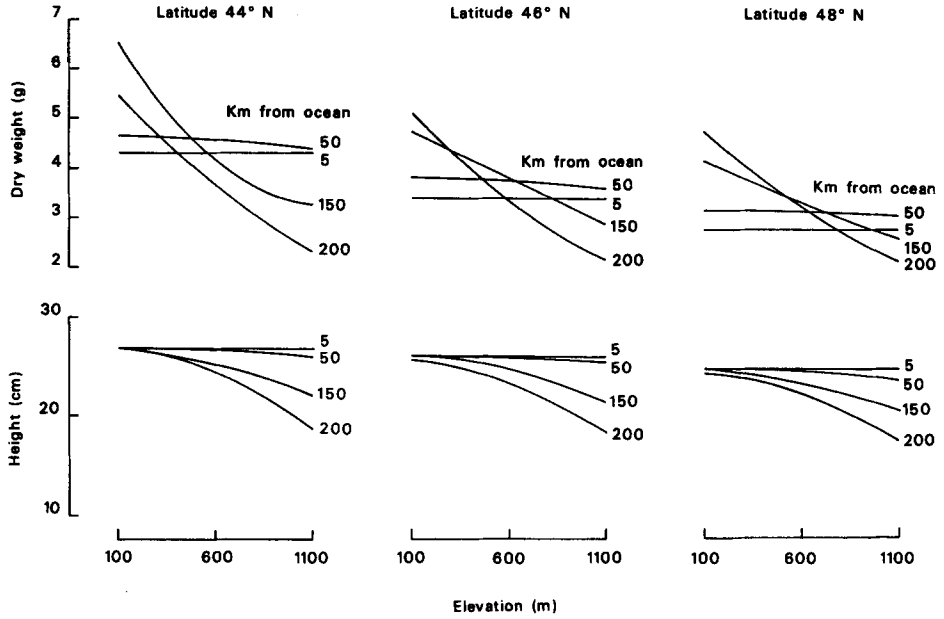

Fig. 1. Clines of dry top-weights and heights retransformed from logarithms (5 and 50 lines are in Coast Ranges, 150 and 200 lines are in the Cascades) - population samples grown in warm soil

temperature interactions - phenological traits of population samples in soil-temperature treatments were additionally affected by air temperature.

When population-sample responses were fitted by stepwise regression to factors of population origin, regression mean squares were highly significant ($P < .01$) for all traits. Several equations were found to be inadequate by the "four-times rule" (Table 3), but even in these cases, response surfaces calculated from such "inadequate" equations generally followed patterns established by the "adequate" regressions. Of equations predicting population-sample growth (height, diameter, dry top weight), those for cool soils were less satisfactory than for warm soils: cool-soil equations either were inadequate predictors (Table 3, equation 3a) or explained smaller proportions of variation in provenance means (Table 3, compare R^2, equation 1a with 1b, etc.). For phenological traits, equations were least satisfactory in the warm-air, cool-soil environment (Table 3, equations 4c, 5c, 6c); better in warm-soil, warm-air; and cool-soil, cool-air combinations (equations 4a, 4d, 5a, 5d, 6a, 6d); and best in the warm-soil, cool-air treatment (equations 4b, 5b), except for bud burst, which was poorly predicted (equation 6b).

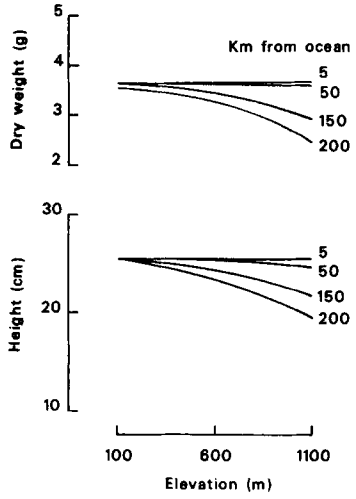

Fig. 2. Clines of dry top-weights and heights retransformed from logarithms - population samples grown in cool soil

Trends for the several traits are presented graphically in Figures 1-9. Rather than describe results in detail, we have chosen those for just four traits, dry top-weight, stem height, extension period, and date of bud set, to illustrate the effect of test environment on clinal patterns.

Dry top-weight and height are used as examples for growth traits. When population-samples were grown in warm soil, their dry weights were significantly related to all three geographic variables - latitude, elevation, and distance from ocean (Table 3,

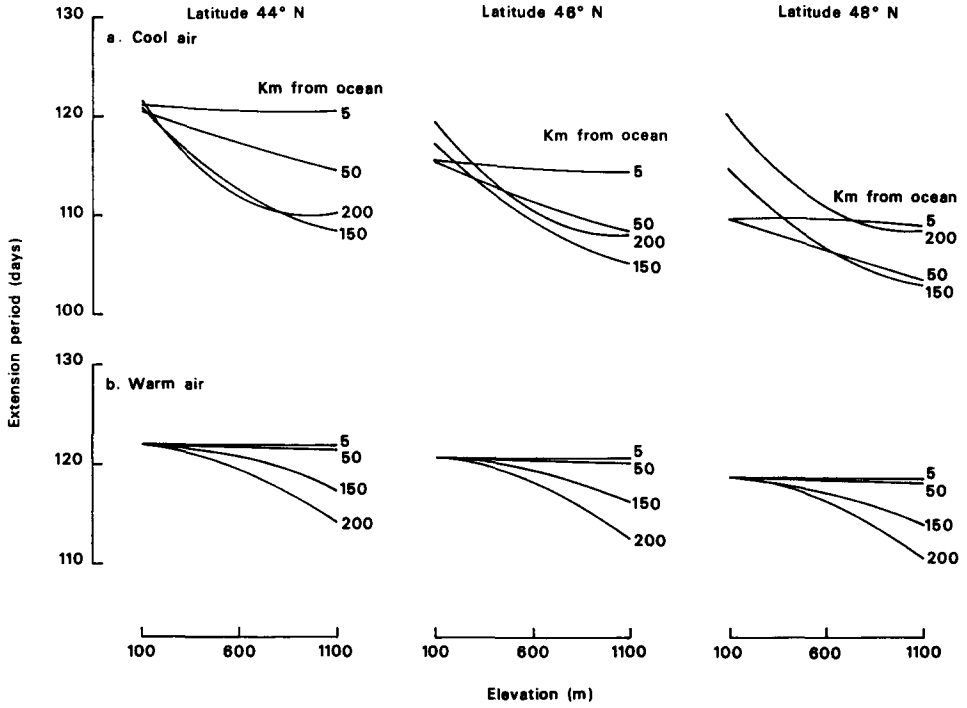

Fig.3. Clines in extension period as established in warm soil: a) as influenced by cool air; b) as influenced by warm air

equation 1b). The relationship is complex, as can be seen when the equation is solved for a set of latitudes, elevations, and distances from the ocean (Fig.1). In comparison, when population-samples were grown in cool soil, population latitude was not a significant predictor of dry top-weight (Table 3, equation 1a). Consequently, trends from the solved equation did not include latitudinal influences, and latitude is not a classification in the trend graph (Fig.2). The difference in pattern among soil temperatures is similar for height (Figs.1 and 2). For both traits, in the warm-soil test, genetic variation among sources is associated with latitude, elevation, and distance from ocean of populations; in the cool-soil test, genetic variation was evident mainly among Cascades sources (150 and 200 km from the ocean) from different elevations.

The relationship of extension period to population origin also varied depending on test environment. The contrast was greatest in warm soil where, in cool air, extension period was strongly related to latitude of populations near the ocean (Fig.3a and Table 3, equation 4b). In comparison, the effect of latitude among Cascades populations was negligible (Fig.3a). In warm soil, warm air, extension period changed only slightly with population latitude, the change being consistent across all combinations of population elevation and distance from the ocean (Fig.3b). In cool soil, genetic variation among populations was not associated with latitude, regardless of population elevation, distance

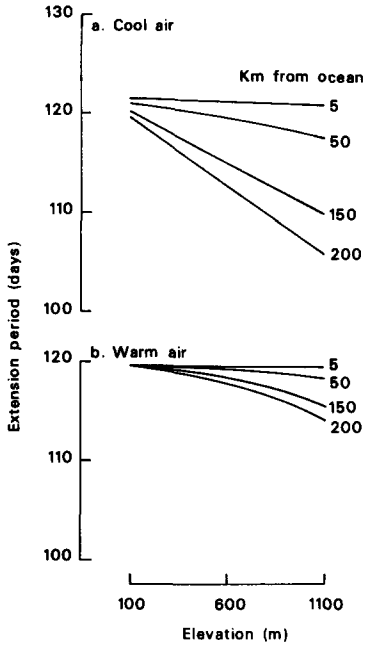

Fig.4. Clines in extension period as established in cool soil: a) as influenced by cool air; b) as influenced by warm air

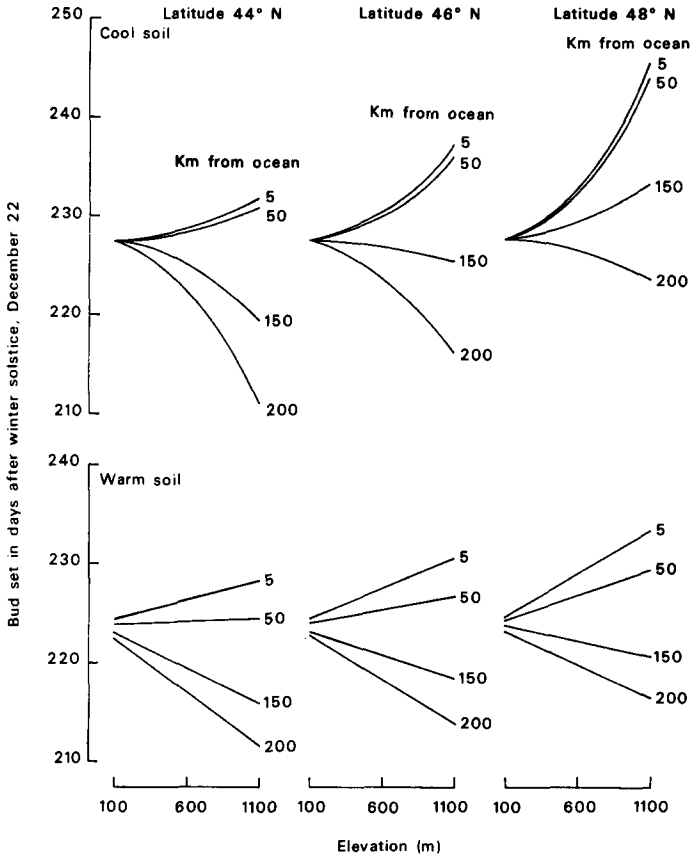

Fig.5. Bud-set trends of population-samples in cool air

from ocean, or the test's air temperature (Fig.4 and Table 3, equations 4a and 4c). Also, regardless of air- and soil-temperature combination, the trend with elevation is steeper and more curvilinear for Cascade population-samples (150 and 22 km from ocean) than for coastal population-samples (5 and 20 km from ocean).

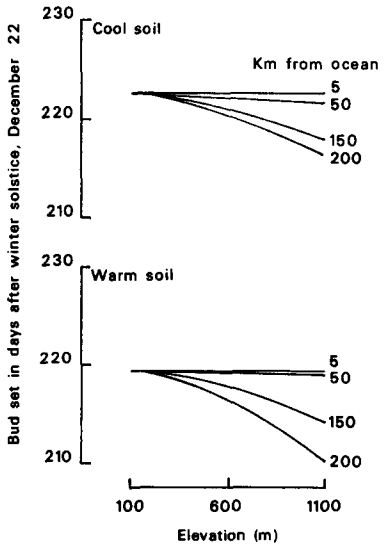

Fig.6. Bud-set performance trends of population-samples tested in warm air

The final example of the four illustrating effects of test environment deals with bud set, the major contrasts in clinal patterns occurring between cool air and warm air (Table 3, equations 5a, 5b, and 5c, 5d). In cool air, provenance latitude in combination with elevation and distance from the ocean is a partial predictor of bud set (Fig.5). In warm air, regardless of soil temperature, there is no relation of population-sample bud-set with latitude (Fig.6).

An exception to the general pattern of interactions occurred with the extension period midpoint (Fig.7). Population-samples differed in midpoint and these differences were related to all three physical factors considered (latitude, elevation, distance from the ocean; Table 3, equation 7), but the samples performed consistently in all test environments; and interactions were not significant (Table 1).

In general, in comparisons between the test environments involved in statistically significant inter-

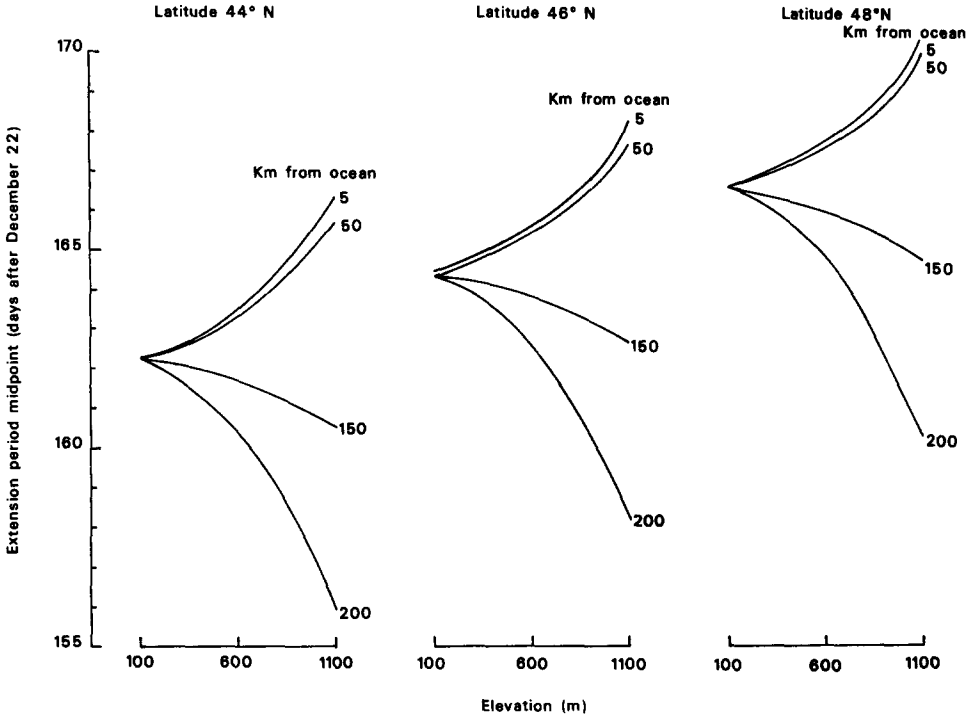

Fig.7. Clines of extension period midpoint

actions (Table 1), regression surfaces differed substantially between environments. This indicates that interaction may be mainly a function of the clinally graded responses of population-samples to test environments. Evidence is provided by compairing expected and observed interaction effects. As noted previously, interaction is failure of a difference between population-samples to be the same in two environments. For this failure to be statistically significant, and further, to be clinally structured, the expected difference in population response to environments, as generated by prediction from regression, should be correlated with the actual difference. To test this, an expected population response was computed for each population location by appropriate equations from Table 3. One set of predictions was made for each interacting environment in a comparison. Then, the expected differential response for each population-sample was calculated by subtraction between environments. In the final step, these differences were compared with observed differences. This was done for dry top-weight and extension period. Dry top-weight largely integrates the separate measurements of stem height and diameter, the two other growth traits; extension period is derived from measurements of bud burst and bud set, the developmental-cycle traits.

The correlation between expected and actual differences in dry top-weight of population-samples grown in cool soil vs. warm soil was $r = .41$. For extension period, interaction measured a differential response between soils in the two air treatments, involving four regression equations (Table 3). In this comparison, the correlation between expected and actual differences was $r = .44$. Both coefficients are highly significant ($P < .01$) but their small sizes indicate that observed interaction-effects were not closely associated with the expected interaction-effects derived from regression equations. However, inherent in the analyses are two sources of error, to be discussed below, and either could reduce the sizes of correlation coefficients considerably below their true values. In spite of this, coefficients were statistically significant. We interpret this as evidence that interaction-effects were strongly related to population-sample origin and were clinally structured.

The first source of imprecision in correlation results because population-sample means were subject to sampling error. Sampling variances contributed

doubly to error in fitting the regressions from which expected values were derived and also in calculating the observed differences. On the average, for extension period, sampling variances were 36 and 74 % of the variances in population-sample means in cool soils and warm soils, respectively. For dry top-weight comparable values were 14 and 17 %. Thus, the true values of correlation coefficients undoubtedly have been substantially underestimated.

The second source of imprecision results because not all relevant independent variables were included in regression analyses. Land form aspect, relief, and exposure, factors which we did not measure at seed-collection points, all influence local climates (Geiger et al 1933, 1934, Utaaker 1963, Baumgartner 1964, McGee 1974) and presumably natural selection. Adaptive plant responses caused by local climatic variation may not have been completely explained by the independent variables we used. Consequently, population-sample means would not have been predicted as accurately as in a complete model. The regressions for cool soils, especially, included this type of error - sampling variances in cool soils were relatively small, yet only about a third of variability in population-sample means was explained by regression (Table 3, equations 1a, 4a, 4c).

Discussion

a. Interactions and Clinal Patterns

Population-sample × environment interactions, when they occurred at a statistically significant level, were consistently connected with temperature rather than fertilizer, even though main effects of fertilizer were larger than main effects of temperature in most traits (Table 2). Soil temperature was involved in all such interactions, but effects of air temperature appeared to be equally as important in significant interactions related to phenology. Thus, Douglas-fir populations from the region we sampled apparently are not genetically differentiated in response to the nutrient levels we used, but are differentiated in their response to soil and air temperatures.

Different test environments exposed different patterns of clinal genetic variation, the patterns also depending on the trait involved. For height, bud burst, and bud set, clinal structures in different test environments were similar. Among low-elevation populations, genetic variability that was related to latitude or distance from the ocean was generally small. Among higher-elevation populations, variability was greater the higher the elevation, and variability among higher-elevation populations was greater in some test environments than in others. Together, these two factors accounted for differences in clinal patterns.

For other traits, clinal patterns in different test environments were so diverse as to seem qualitatively distinct. Dry top-weight provided the most striking example. When population samples were grown in cool soil, genetic variation could not be related to latitude (Table 3, equation 1a, and Fig.2). When samples were grown in warm soil, latitude became an important predictor (Fig.1). For example, calculated dry weights of two sources from 600 m, 50 km inland - one from 44°N, the other from 48°N - were 4.6 and 3.1 g, respectively, a difference of 1.5 g or 39 % of the mean dry weight.

For several traits population-sample performances were complexly related to latitude, distance from the ocean and elevation. In warm soil and cool air, extension period decreased by about 2.5 and 1 day per degree of increasing latitude for sources near (Coastal) and distant (Cascades) from the ocean, respectively (Fig.3a). This is mainly accounted for by a delay in bud burst in north Coastal sources in warm soil. The discrepancy in mean bud-burst dates between Coastal and Cascades sources is greatest in warm soil, warm air (Fig.8) and is negligible in cool soil, cool air. In this latter environment, population distance from the ocean is no longer a predictor of bud burst (Table 3, equation 6a, and Fig.9).

The latitudinal clinal pattern for dry top-weight was similar to the pattern for extension period shown above. Dry top-weight of Coastal populations grown in warm soils decreased by about .4 g per degree of increasing latitude (Fig.1). Based on the heaviest population-sample, this is a decrease of 6.6 % per degree. For Cascades sources, the corresponding decrease is approximately 2.4 %. Average dry weights and extension periods of provenances were correlated, with extension period accounting for 15 % to 40 % of the variation in dry weight. Correlations were weakest in cool environments and strongest in warm environments.

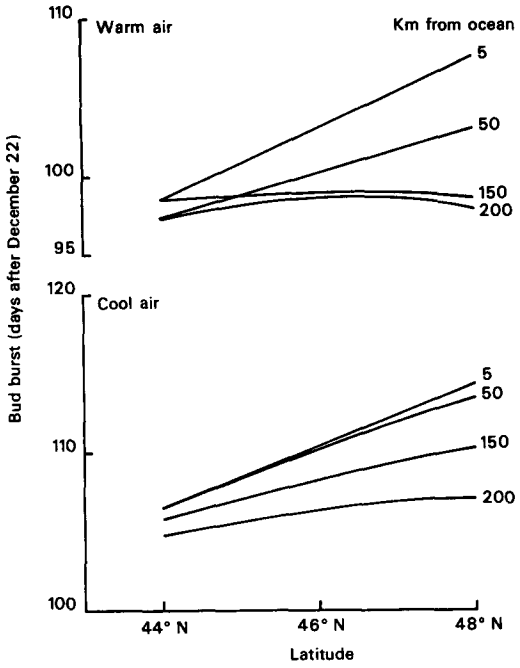

Fig.8. Clines in bud-burst as established in warm-soil test environments

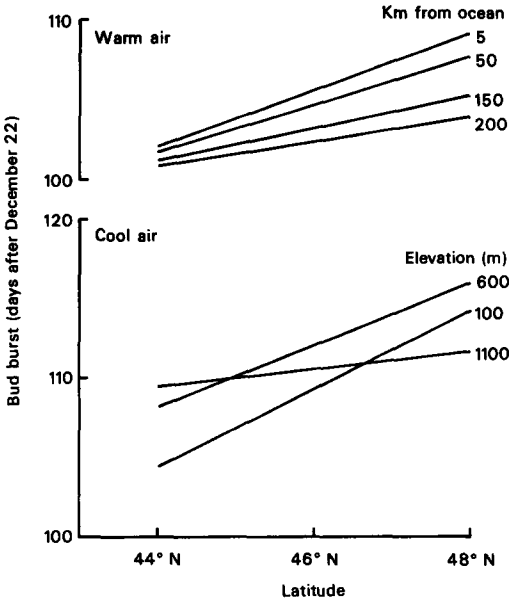

Fig.9. Clines in bud-burst as established in cool-soil test environments

b. Adaptive Significance of Trait Response

Within any single test environment a large part of the variation among population-samples was clinally patterned. On the average, regression accounted for about 50 % of the variation among samples, though means were subject to sampling error and regression models probably did not include all pertinent predicting variables for some traits. Interaction effects also could be attributed largely to differences in clinal patterns; ie: the individual manner in which the average genotype of a population-sample was expressed in contrasting environments was also patterned according to population origin.

Such a multidimensional structuring of genetic variation is difficult to explain except as a reflection of differential selection pressures in native environments, especially since both growth and growth-rhythm traits were involved. Also, such a structuring is equally difficult to explain if it is hypothesized that the test environments were irrelevant to environments in which populations evolved. We therefore infer that the responses in our test environments have indicated adaptive differences among populations and that responses were not artifacts exposed in an "unnatural" environment.

c. Interactions and Test Environments

The correlation between dry top-weight and extension period and the concommitant association of growth and phenological traits with population latitude were strongest in warm environments. Since effects related to latitude of populations usually can be ascribed to photoperiodic control, this suggests a basis for the significant interactions in this experiment and reasons for emphasizing some components of environment, especially, in the choice of test environments.

To explain population-specific responses to soil and air temperatures, we offer a three part hypothesis. First, photoperiod and temperature are major information sources serving to synchronize developmental periodicity to annual climatic cycle. Second, not only are overt phenological events (e.g., bud burst, bud set) programmed to occur at particular times in the annual cycle, but so also are other features of the growth process (Krueger 1966). Third, the temperature regimes created in this test have tended to put growth processes of some population-samples "out of phase" in reference to photoperiodic information being received as a consequence of their being adapted to different photoperiod-temperature regimes. Thus, some stages of their growth may have occurred in

conditions which were not optimal, giving rise to population-sample × temperature interactions in growth traits as well as in phenological traits.

Photoperiod as a synchronizing mechanism has been repeatedly discussed (e.g., Wiersma 1963, Heslop-Harrison 1964, Langlet 1967). Reports which specifically deal with the remainder of the hypothesis in respect to perennial plants are not common, but support may be inferred from examples.

In Coastal Douglas-fir, soil temperature influences spring rate of shoot development (Lavender et al 1973) and also dry weight accumulation (Lavender and Overton 1972). In the latter capacity, soil temperature optima for growth are strongly affected by thermoperiod. Plant activities affected by soil temperature are also affected by photoperiod, in much the same way. Photoperiod influences spring rate of development (Campbell and Sugano 1975); it affects the number of needles initiated (Irgens-Moller 1962) and the lateral meristem activity (Lavender and Hermann 1970), both presumably essential aspects of dry-weight accumulation; and, at any given photoperiod within the normal range, spring rate of development is also modified by thermoperiod (Campbell and Sugano 1975). Thus, longer photoperiods appear to compensate, in part, for higher soil temperatures, whether the response is in traits of growth or of growth rhythm. Further, in most of the reports cited, responses to temperature or photoperiod were population-specific. This suggests that for Douglas-fir in the region we sampled, appropriate combinations of temperature and photoperiod may be needed to adjust growth processes of a population to its native environment.

Additional evidence that synchronization to seasonal cycle can require appropriate temperatures in combination with photoperiod comes from a study of timothy strains with different growth rhythms (Evans et al. 1935). The order of flowering in vegetatively early strains depended mainly on temperature, heading starting first in warmer southern stations and progressing regularly to northern sites where temperature was the limiting factor. In contrast, late strains were limited by requirements for longer photoperiods in southern stations, at a time when temperature was not limiting. Flowering of these started first at northern latitudes in response to the earlier long days in late spring and early summer.

Knight (1971) described contrasting growth patterns of natural populations of a perennial pasture grass from two contrasting climatic regions. In one, populations were summer-growing and winter-dormant and in the other the reverse. These disparate annual growth rhythms (expressed as yield in successive harvests) persisted even when the presumed causative agent (seasonal moisture pattern) in the local environment was altered. Differences in rhythm were apparently maintained by relative growth rates and leaf area ratios which were population-specific in response to air temperature (Eagles 1973).

Since we did not directly control photoperiod in our experiment, we cannot propose that our population-sample × temperature interactions had causes identical to those described for grasses. However, based on the points discussed and on our results, we do suggest that such interactions are more likely to accompany changes in factors associated with the annual climatic cycle (soil and air temperature, photoperiod) than changes in factors not so directly associated with the cycle (e.g., fertilizer, competition). Therefore, photoperiod and temperature regimes are likely to be critical components in any satisfactory set of test environments.

d. Test Environments and a Provisional Model

We propose that reasonable first steps in developing a model can be based on nursery or growth-chamber tests. In this way, environments can be produced which will tend to maximize the expression of adaptive genetic variation and, at the same time, permit evaluation of patterning among interaction effects.

Although future studies may show moisture regimes to be a complicating factor, based on our experience we suggest a set of contrasting temperature- and photo-regimes as test environments. We believe these may foster interactions in growth rhythm responses which will, in turn, bring to light adaptively significant growth differences. These differences might otherwise be measurable only in unusually precise short-term field tests, or in "rotation-length" tests, or in exceptional "disaster" years.

In the envisioned procedure, this initial model is used to stratify the region into units of similarity based on adaptive response among population-samples.

Presumably, similarity in response patterns implies similarity of environments within which populations evolved. The model provides provisional transfer rules, i.e., it provides an index of the relative risk involved in transferring seed from one environmental unit to another. A long-term field test is then used to evaluate the practical, field manifestations of lack of adaptation. In this way the model is validated or improved. Over the course of a rotation, relative risks can be quantified in terms of actual losses in growth and survival. In the field test fewer test sites will be required because seed transfers can be allocated, for efficiency, at design points along and across clinal gradients within the model framework.

e. Seed-Transfer Inferences

In this experiment, clines could not be described with sufficient precision to justify their use in devising transfer rules. Also, the test environments we used did not sample the range available in the region, particularly the cooler, drier segments. Nor did provenances sample all topographic points represented in figures 1 to 9 - e.g. the experiment included no provenances from 1,100 m at 5 km from the ocean, or from 100 m at 200 km from the ocean. Therefore, although in general the lines in figures reflect an adequate sample, there is some extrapolation. Nevertheless, if we use dissimilarity of population performance as reflected in steepness of clinal regressions as a measure of increased risk in population transfer, several tentative inferences can be made regarding seed transfer in western Washington and Oregon: (1) There is more risk in moving seeds east-west than north-south. East-west clines for most traits are quite steep. (2) The risk increases as elevation of populations is higher. In most cases, east-west clines become steeper as elevation becomes higher. (3) The risk is greater with elevational seed transfers in the Cascades than in the Coast Ranges. Clinal regression lines on elevations are generally steepest when close to the crest of the Cascades, i.e., 150 to 200 km from the Pacific Coast. (4) North-south transfers appear to involve smaller risks than do transfers east-west or in elevation, although the risk is greater for north-south transfers in the Coast Ranges. North-south clines are not steep for most traits.

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R.K. Campbell, Principal Plant Geneticist
USDA Forest Service
Forestry Sciences Laboratory
Corvallis, Oregon 97331 (USA)