

Genetic variation in cold hardiness of Douglas-fir in relation to parent tree environment¹

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Abstract: The extent to which parent trees within breeding zones of coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco) are locally adapted to their native environments was evaluated. Forty families from each of one Coastal and one Cascade breeding population in Oregon were assessed for cold hardiness and growth phenology, and family means for traits were summarized using principal components analysis (PCA). Composition of the first two principal components (PCs) was consistent between breeding zones, years, and test sites. PC-1, describing 39–46% of trait variation, represents a suite of traits related to spring phenology and spring cold hardiness. PC-2, describing 20–22% of trait variation, consists of cold-hardiness traits not associated with phenology. The first two PCs from each population, as well as univariate traits, were regressed on parent tree location variables and modeled climatic indices separately. In the Coastal zone, PC-1 was weakly but significantly related to temperature and moisture regimes ($0.176 \leq r^2 \leq 0.235$), varying with elevation and distance from the ocean. PC-2 was related to temperature and moisture regimes in both populations ($0.087 \leq r^2 \leq 0.249$). These relatively weak geographic patterns for adaptive traits within breeding zones suggest that current zone size is not excessive and could likely be increased north or south.

Résumé : Les auteurs ont évalué le niveau d'adaptation locale d'arbres parents de *Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco à leur environnement d'origine à l'intérieur même des zones d'amélioration. Pour ce faire, 40 descendances représentatives de chacune de deux populations d'élevage en Orégon, l'une de la région de la Côte et l'autre de la région des Cascades, ont été évaluées pour l'endurcissement au froid et la phénologie de la croissance. Afin de résumer la variation observée, les moyennes familiales pour ces caractères ont été soumises à l'analyse en composantes principales. La structure des deux plus importantes composantes principales était similaire entre les deux zones d'amélioration, entre les années et entre les sites d'étude. La plus importante composante était responsable de 39 à 46% de la variation des caractères. Elle était largement constituée par la phénologie printanière et l'endurcissement au froid printanier. La seconde composante expliquait de 20 à 22% de la variation des caractères. Elle était principalement constituée des caractères d'endurcissement au froid qui n'étaient pas associés à la phénologie. Les deux premières composantes de chacune des populations ainsi que des caractères univariés particuliers ont été soumis à l'analyse de régression séparément à partir de deux ensembles de paramètres : des variables résumant l'origine géographique des arbres parents et des indices climatiques issus de la modélisation. Pour la population de la Côte uniquement, la première composante était faiblement mais significativement liée à la température et au régime d'humidité ($0,176 \leq r^2 \leq 0,235$). Cette tendance s'accompagnait d'une variation selon l'élévation et l'éloignement de l'océan. La seconde composante était liée à la température et à l'humidité chez les deux populations ($0,087 \leq r^2 \leq 0,249$). Ces patrons de variation géographique relativement faible des caractères adaptatifs observés à l'intérieur même des zones d'amélioration suggèrent que la taille actuelle des zones n'est pas excessive et qu'elle pourrait possiblement être élargie vers le nord ou vers le sud.

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Introduction

Close synchronization of the annual growth and dormancy cycle to the local climate is important for the survival and productivity of perennial plants. Plants initiating growth too soon in the spring risk damage from early spring frost events (Christophe and Birot 1979), whereas plants continuing growth far into the summer risk damage from drought and

early fall frost (Loopstra and Adams 1989). Thus, accurate timing of growth, dormancy, and associated cold acclimation and deacclimation represents a delicate balance between maximizing growth under favorable conditions and minimizing frost or drought damage during unfavorable periods (Rehfeldt 1989; Schuch et al. 1989a, 1989b).

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The first stage of cold acclimation in most woody perennials is induced by shortening photoperiod (Irgens-Moller 1958; van den Driessche 1969; Weiser 1970; Schuch et al. 1989a); low night temperatures further this process in the second phase of acclimation, winter rest (Lavender et al. 1968; van den Driessche 1969). During winter rest, plants cannot be induced to grow until their chilling requirements have been met (Weiser 1970). After the chilling requirement is met, buds enter the winter quiescent phase, in which growth resumes under favorable conditions (i.e., warm temperatures). Deacclimation and budbreak are induced by warm temperatures in late winter (van den Driessche 1969; Fuchigami et al. 1982; Schuch et al. 1989b).

Genetic variation in cold hardiness within woody species appears to result mostly from variation in timing of acclimation and deacclimation, rather than variation in absolute levels of cold hardiness achieved or in the fundamental patterns of acclimation and deacclimation (Weiser 1970; Oohata and Sakai 1982; Eriksson 1982; Aitken and Adams 1996). Furthermore, geographic variation in cold hardiness appears to be an adaptive response to factors of the operational environment, especially temperature, moisture and photoperiod. Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) has strong clines in growth, phenology, and cold hardiness traits observed along geographical gradients in both the coastal (var. *menziesii*; Campbell and Sorenson 1973; White et al. 1979; White 1987) and interior (var. *glauca*; Rehfeldt 1979, 1989) varieties.

Evidence also exists in Douglas-fir for adaptive differentiation among populations (or stands) on a microgeographical scale (Hermann and Lavender 1963; Campbell 1979, 1986; Sorenson 1983). However, the extent to which such microgeographical adaptations are reflected in parent tree selections within operational breeding zones of Douglas-fir tree improvement programs (often such zones are less than 150 000 acres in size (1 acre = 0.405 ha); Silen and Wheat 1979) is unclear. If the adaptability of offspring of first-generation selections is strongly associated with the source environment of the parent trees, parent tree environment could be an important consideration in the deployment of seeds for reforestation and in breeding for adaptability in future generations.

Family variation in both fall and spring cold hardiness traits was found to be extensive among the offspring of first-generation selections in two breeding zones of coastal Douglas-fir in western Oregon (Aitken and Adams 1996, 1997). Here, we examine the association of family differences in these traits with the environments of the parent trees and discuss the implications of these associations with regards to spring and fall cold adaptation in Douglas-fir and its application in tree improvement programs.

Materials and methods

Study region and test sites

The two breeding zones in this study are found at approximately 44°30'N, one west of Corvallis in the Coast Range (Coastal) and the other east of Corvallis on the lower western slope of the Oregon Cascades (Cascade). The Coastal zone occupies an area of 500 km², with parent trees selected at elevations ranging from 65 to 340 m. The Cascade zone occupies a smaller area (150 km²), but parent trees were selected over a broader elevational range

(300–840 m). Open-pollinated seed was collected from each parent tree (open-pollinated families), and in 1987, one-year-old seedlings from each family were planted into progeny test sites located within their respective zones. Prior to planting, the families in both zones were divided into sets of 30 with families randomly allocated to sets in the Coastal zone, and families grouped geographically in sets in the Cascades. Twenty families from each of two sets per zone were chosen for inclusion in this study (40 families total per zone). Sets and families were selected entirely on the availability of seed in storage for subsequent seedling projects.

Families were measured at one higher and one lower elevation progeny test site in each breeding zone to allow for the study of cold hardiness expression in different environments. In the Coastal zone, the test sites were Flynn Table (elevation 500 m, moderate northwest-facing slope, 17 km from the ocean) and Canal Creek (250 m, steep west-facing slope, 10 km from the ocean). In the Cascade zone, the test sites were House Mountain (650 m, gentle east-facing slope, 122 km from the ocean) and Prospect Mountain (400 m, flat, 105 km from the ocean). Although mean monthly temperatures are higher on average at the lower elevation sites in each zone, precipitation levels predicted by models are somewhat greater at the highest site in the Coastal zone and the lowest test site in the Cascades (Balduman 1995). The House Mountain site has numerous frost pockets containing dead or stunted trees.

The two breeding zones selected were part of the breeding programs of two different organizations that utilized somewhat different field designs. In the Coastal zone, the tests were planted in a split-plot design with four (Flynn Table) or five (Canal Creek) replications, with sets as main plots and families within sets as subplots. In the Cascade zone, each set consisted of parent trees from a restricted area within the zone. Sets in this zone were planted as separate randomized complete block experiments, each with five replications. Within blocks, families were represented by four-tree noncontiguous plots in both zones. At the time of cold-hardiness assessments (ages 6–7 from seed) survival was 80% at House Mountain and exceeded 94% at the other sites. Thus, on average, family means of each site were based on measurements of 15 or 16 individuals.

Measurements

Growth phenology and cold hardiness were assessed over a 2-year period (May 1992 through May 1994) to sample year-to-year variation in weather conditions and its influence on the expression of these traits. Weather patterns varied greatly between the 2 years, with the growing season warmer and drier than average in 1992, and cooler and wetter than average in 1993 (Aitken and Adams 1996). In addition to growth phenology and cold-hardiness measurements, data on individual tree heights at 5 years of age (HT) were available from the landowners.

Growth phenology was evaluated each growing season by measuring three traits, timing of bud burst (BB), bud set (BS), and shoot elongation on a single marked branch at a fixed height (varying among sites from 1 to 1.7 m) on the east side of the crown of each tree. A branch was used for assessing bud and elongation phenology as most trees were too tall for the terminal shoot to be measured, and terminal and lateral shoot phenology is strongly correlated (Li and Adams 1993). BB was recorded as the Julian date at which new needles were first observed through the bud scales of the terminal bud and BS as the date when brown bud scales first became visible at the branch tip. Both BB (1993, 1994) and BS (1992, 1993) were recorded twice weekly. Shoot elongation was measured every 1 or 2 weeks after bud break over the course of the growing season. Percent elongation (EP) was calculated as the percentage of total growth completed by a given date in midseason when approximately 50% of total elongation had occurred on a site, on average, to provide an estimate of shoot elongation phenology rather than amount of lateral growth. The percentage of lateral

shoots that second flushed was visually estimated at the end of the 1993 growing season (second flushing was negligible (<1%) in 1992).

Cold hardiness of cut shoots (5 cm long) was assessed on all trees in each of nine sampling periods: September, October, and November 1992; January, April, May, September, and October 1993; and April 1994. Timmis et al. (1994) found that the greatest risk of frost injury in Douglas-fir in the Pacific Northwest in the fall occurs in October and November and in the spring occurs between mid-April and mid-May. Data from only September and October (shoot acclimation phase) and April (shoot deacclimation phase) in each year were utilized in this study. The two test sites in each zone were sampled within a 2-week period, with the site with the highest hardiness sampled first in the fall and last in the spring. Two shoots were collected from every tree in each sampling period, one shoot for each of two different freeze temperatures, followed by visual scoring of cold damage. The two freeze temperatures for each sampling period were determined from preliminary samples taken a week earlier and selected to result in variable damage to shoot tissues, with average damage levels between 20 and 80%. All shoots for each sampling period were collected on a single day at each site and transported on ice to Corvallis. Samples were cold-stored for a maximum of 4 days at 2°C before freeze testing. They were wrapped in damp cheesecloth and aluminum foil and then placed into a computer-controlled freezer at -2°C overnight. The freezer was programmed to reduce the temperature 3–5°C/h until the desired test temperature was met. After 1 h at this temperature the samples were removed, allowed to thaw slowly in a 2°C refrigerator overnight, and then stored at room temperature for 7 days to allow visual signs of damage to develop.

Each shoot was examined under an illuminated magnifier and the proportion (to the nearest 10%) of damaged bud, needle, and stem (cambium and phloem) tissues visually estimated. Damage to needle tissues was assessed by scoring the percentage of needles that were discolored or dropped (NEED). Shoot tissues were exposed by slicing stems lengthwise with a razor blade and scoring damage to cambial and phloem tissues based on tissue discoloration (STEM). Bud tissue was divided into two areas: the needle primordia and apical meristem (NP) and the subapical area between the bud and shoot tissue (INT). Damage recorded for INT may reflect extraorgan freezing of Douglas-fir bud tissues (Sakai 1982; Aitken and Adams 1996). Cold injury was averaged between test temperatures to estimate individual cold injury scores for each tissue for individual trees. Additional details of cold hardiness assessment methods are in Aitken and Adams (1996).

Statistical analyses

The results of conventional analyses of variance estimating genetic components of variance and heritabilities for individual cold hardiness traits are published elsewhere (Aitken and Adams 1995a, 1995b, 1996, 1997). In this study, regression analyses were used to examine the association between family means for cold hardiness and growth traits and the environments of the parent trees. Factors of the parent tree operational environments with the greatest influence on cold hardiness are those that affect site temperature and moisture (Schuch et al. 1989a; Loopstra and Adams 1989). Two types of independent variables were used to describe the temperature and moisture regimes of the parent trees: (i) direct estimates of climatic variables derived from climate models, and (ii) parent tree location variables, used as surrogates of more direct factors of parent tree environments.

Climatic variables

The eight variables chosen for investigation were mean temperatures for January, March, July, and September; temperature amplitude (the mean of the warmest month minus the mean of the coldest month; Tuhkanen 1980); an aridity index (Tuhkanen 1980);

and a topographic convergence index estimating soil water capacity (Zheng 1993).

With the exception of the topographic convergence index, only values of mean monthly temperature and precipitation were required to derive the above variables. These were estimated from climate models developed by the Environmental Protection Agency (Environmental Research Laboratory, Corvallis, Oreg.) using *X* and *Y* map coordinates (in the UTM system) of the parent trees as inputs into the models. Mean monthly temperature estimates for parent trees were obtained using a model that incorporates ten years of climate records gathered from weather stations throughout the Pacific Northwest (Turner et al. 1996). The model assumes a correlation between regional air temperature variation and topography; local climate values are integrated with topographic surface variation affecting temperature regimes and temperatures are interpolated between climate stations (at a 1-km digital elevation model (DEM) grid scale) based on topographic position relative to the nearest climate stations (J. Kimball, University of Montana, Missoula, personal communication). Estimates of mean monthly precipitation were calculated using PRISM (precipitation–elevation regressions on independent slopes model), which incorporates precipitation data compiled for at least 20 years from over 8000 weather stations in the United States. The model assumes a correlation of precipitation with elevation and interpolates precipitation at a DEM 10-km grid scale using a regression of known precipitation from orographic elevation (Daly and Neilson 1992). In the regression, greater weight is given to stations with location, elevation, and topographic positioning similar to that of the grid cell. Whenever possible, PRISM calculates a prediction interval for the estimate, which is an approximation of the uncertainty involved. The large grid scale used in PRISM reflects the low variability of precipitation on a small scale.

The aridity index (*A*; Tuhkanen 1980) was calculated as $A = P/(T + 10)$, where *P* and *T* are the mean monthly precipitation and temperature, respectively, over the three driest months of the year (July, August, and September). Because a reasonably good correlation exists between *T* + 10 and evaporation in certain temperature intervals, this index resembles precipitation–evaporation indices (Tuhkanen 1980). Low values of *A* correspond to warm, dry conditions; high values of *A* indicate cool, wet conditions.

The topographic convergence index (TCI) (Zheng 1993) represents available soil water capacity as modeled from high resolution topographic data. The model assumes a linear relationship between available soil water capacity and surface slope and drainage area, and is most reliable in areas with extreme topographic heterogeneity and nonsaline soils. TCI values are highest at the bottom of slopes or in hollows or swales. TCI values have been modeled for locations in the central Oregon Cascades but not for the Oregon Coast Range and thus were used only in the analysis of the Cascade population.

Location variables

The location variables common to the analyses in both zones were: elevation, slope aspect, and percent slope. These were determined by visiting all parent tree locations. To account for the circularity of aspect (from 0 to 360°) and the contribution of slope steepness on the effect of aspect, two new variables were created: (i) (sine of aspect × percent slope) which contrasts east versus west aspects, and (ii) (cosine of aspect × percent slope) which contrasts north versus south aspects (Stage 1976). Because of the strong west to east moisture cline in the Coast range, distance from the ocean was also included as a variable in the Coastal zone.

Principal components and regression analysis

Arithmetic means of the families in each of the four test sites were calculated for 32 traits: 12 cold hardiness assessments (4 tissue

types \times 3 measurement periods) and 3 phenology traits (BB, BS, and EP) measured in each of the 2 years, percent second flushing in 1993 and 5-year height. Because of the large number of traits, principal components analysis (PCA; SAS Institute Inc. 1988) was performed on the family means in each site to summarize the variation over all the traits. Factor scores of principal components for each family were then regressed on climatic variables of the parent tree location or their location variables. In addition, three univariate traits of particular interest to tree breeders were employed as dependent variables: 5-year height, fall cold hardiness (represented by stem damage in October 1992; STEM 1092), and spring cold hardiness (represented by stem damage in April 1993; STEM493). STEM1092 and STEM493 were found to be the best variables tested for ranking overall fall and spring hardiness, respectively. Family rankings for both early fall and spring cold hardiness were found to be quite consistent over sampling periods and years in both breeding zones in this study (Aitken and Adams 1996, 1997); thus, the representation of family cold hardiness by means based on individual sampling data was considered reasonable.

Preliminary regressions indicated that mean March and September temperatures were only very weakly related to the dependent variables. Thus, these variables were excluded from the remaining analyses. Family factor scores or means for individual traits were then regressed on all five of the remaining climatic variables and separately on the four or five location variables using multiple linear regression. For example, the full regression model for the climatic variables in both breeding zones was: $y_i = a + bTA_i + cA_i + dTCI_i + eJAN_i + fJUL_i$, where a , b , c , d , e , and f are regression parameters; y_i is the average value for the PC score or univariate trait of concern for the progeny of parent tree i ; TA_i is the temperature amplitude of the source environment of parent tree i ; A_i is the aridity index of parent tree i ; TCI_i is the topographic convergence index of family i ; JAN_i is the mean January temperature of parent tree i ; and JUL_i is the mean July temperature of parent tree i . Similar models were tested using location descriptors as independent variables. Subsequent selection of independent variables for final models was based on estimates of Mallow's C_p , which compares the full model (including all variables) to models including all possible subsets of variables (SAS Institute Inc. 1988). The formula used for calculation of Mallow's C_p is $C_p = MSE_p / MSE_{full} - (n - 2p) + 1$, where, MSE_p is equal to the mean square of a partial model (containing a subset of the variables), MSE_{full} is equal to the mean square of the full model (containing all possible variables), and p is equal to the number of independent variables in the model. C_p values for the best models approximate $p + 1$ and selection of the best models is based on models with the lowest C_p values. If C_p selection indicated the "best" model included only one variable, models including a squared term were also tested. When C_p selection indicated more than one variable should be included in the "best" model, interactions between these variables were also tested.

Initially separate analyses (both principal component and regression) were performed on years, sites, and sets in the Cascade population and years and sites in the Coastal population. Results were found to be consistent across years, sites, and sets (Balduman 1995); thus, only the results of the pooled analyses are presented here.

Results and discussion

Principal components

PC-1 and PC-2 account for 68 and 59% of the variation over all traits for the Coastal and Cascade zones, respectively, and the eigenvector loadings for these PCs are quite similar for the two zones (Tables 1 and 2). The remaining PCs individually account for less than 10% of the total variation among traits and represent combinations of traits that

are more difficult to interpret biologically. These PCs will not be discussed further.

Eigenvector loadings for PC-1 primarily reflect stem growth phenology differences among families and cold hardiness that is most closely associated with timing of growth (spring cold hardiness). Families with larger factor scores for PC-1 tend to break and set bud later, have less shoot elongation completed by midseason and less spring cold damage, yet somewhat greater fall cold damage. The fact that the eigenvector loadings for BB and BS are all positive and strong suggests a strong positive genetic correlation between BB and BS timing in Douglas-fir of this age, as other studies have also found (Li and Adams 1993; Aitken and Adams 1996). The traits with strong eigenvector loadings for PC-1 tend to be those under strong genetic control. Individual heritabilities averaged 0.85 for date of BB, 0.46 for date of BS, and 0.61 for spring cold hardiness traits across the two breeding zones (Aitken and Adams 1996, 1997).

PC-2 largely reflects cold hardiness that is unassociated with stem growth phenology, primarily fall cold hardiness. Families with larger factor scores for PC-2 tend to have greater fall cold damage and somewhat greater spring cold damage. PC-2 may reflect absolute levels of tissue hardiness not related to timing or speed of acclimation or deacclimation. Fall cold injury is under fairly weak genetic control than spring cold injury, with an average individual heritability of 0.21.

Strong genetic correlations have been reported in coastal Douglas-fir between BB and BS (Li and Adams 1993) and between spring cold hardiness and BB (Aitken and Adams 1995). The sign and magnitude of the loadings for these traits in PC-1 are consistent with these correlations. Fall cold damage, on the other hand, has weaker and less consistent genetic correlations with phenology traits (BB, BS, or EP). The weaker loadings for fall damage in PC-1 are, therefore, also consistent with earlier observations. Thus, PC-1 is largely a composite trait reflecting growth phenology and spring cold hardiness, which is most closely associated with the timing of bud phenology. This association of traits represents a balance between adaptation for high cold hardiness in severe environments and high growth rates in milder environments. PC-2 reflects cold hardiness of families in both spring and fall that is not strongly associated with bud phenology. Thus, it mostly reflects fall cold hardiness but also includes components of spring cold hardiness that are not associated with bud phenology.

Family-by-year interactions and family-by-fall assessment period interactions for bud phenology and cold hardiness traits appear to be of little importance as the sign and magnitude of loadings for most of these traits are consistent over these time periods, similar to results for analyses of variance (Aitken and Adams 1996, 1997). Height and second flushing have little influence on the first two PCs. Individual heritabilities averaged 0.26 for height and 0.40 for the proportion of lateral shoots that second flushed (unpublished data).

Regressions

Table 3 shows the results of regressions using both climatic and location variables for PC-1 and PC-2 as well as univariate traits for both Coastal and Cascade zones. Although four or five independent variables were available for

Table 1. Eigenvectors, eigenvalues, and percent of total variation explained among open-pollinated family means of the first five principal components (PCs) for the Coastal zone (pooled over family sets, test sites, and years).

Trait	PC-1	PC-2	PC-3	PC-4	PC-5
5-year height	0.0800	0.0172	0.2354	-0.1775	0.5912
Bud set 92	0.2055	-0.0583	-0.2573	0.1791	0.0745
Elongation P.92	-0.2115	0.1327	0.1627	-0.149	0.0144
NEED 992	0.0785	0.2428	-0.1383	-0.2636	-0.129
STEM 992	0.1856	0.1268	0.2054	0.0569	-0.036
NP 992	0.1584	0.1079	0.2714	0.1455	-0.264
INT 992	0.2145	0.0674	0.1874	0.1034	-0.194
NEED1092	0.1005	0.2444	-0.0892	-0.4023	0.101
STEM1092	0.1736	0.1955	0.0643	-0.2334	-0.196
NP 1092	0.1766	0.1955	0.1075	-0.0275	-0.112
INT 1092	0.1996	0.1562	0.1980	0.1034	-0.220
NEED 493	-0.2012	0.1795	-0.0443	0.1500	-0.119
STEM 493	-0.2151	0.1779	0.0476	0.1537	0.0424
NP 493	-0.206	0.1949	0.0141	0.0586	-0.044
INT 493	-0.2081	0.2000	-0.0433	0.0956	-0.015
Bud burst 93	0.2389	-0.1190	-0.0511	0.0694	-0.031
Bud set 93	0.1864	-0.0658	-0.2310	0.2283	0.0226
Elongation prop. 93	-0.2273	0.0568	0.0750	-0.2097	0.0104
Second flushing 93	0.0112	-0.0244	0.3397	0.2045	0.5171
NEED 993	0.0724	0.2500	-0.3808	-0.0661	0.1122
STEM 993	0.1258	0.1955	-0.2803	0.1970	0.2247
NP 993	0.1923	0.1751	-0.0852	0.2246	0.1171
INT 993	0.1706	0.1840	-0.1909	0.1862	0.1335
NEED 1093	0.0950	0.2914	-0.0939	-0.3297	0.1139
STEM 1093	0.1333	0.2637	0.1283	-0.0310	0.0640
NP 1093	0.1140	0.1898	0.3547	0.1060	0.0492
INT 1093	0.2083	0.1705	0.0802	0.0743	-0.016
NEED 494	-0.1797	0.1826	-0.0671	0.1778	-0.039
STEM 494	-0.2011	0.2000	-0.0091	0.1816	0.0360
NP 494	-0.1863	0.1842	0.0237	0.2267	-0.048
INT 494	-0.1781	0.2384	-0.0541	0.0945	0.0339
Bud burst 94	0.2396	-0.1153	-0.0484	0.0107	-0.019
Eigenvalue	14.73	7.04	2.32	1.37	1.21
% Variation	46.0	22.0	7.3	4.3	3.8

Note: NEED, STEM, NP and INT refer to cold injury to needles, stem tissues, bud apical meristems, and needle primordia, and the bud-shoot interface, respectively. Values after traits refer to sampling month and (or) year.

use in the regressions and there was the possibility of adding polynomial and interaction variables to the models, the best fitting models contain only one or two independent variables. In all cases where models were significant, linear terms were adequate, and no significant interactions between independent variables were detected. Even the best fitting models accounted for only about 17–35% of the variation in the dependent variables.

Coastal zone

In the Coastal zone, PC-1 decreased in magnitude with increasing elevation and distance from the ocean (Fig. 1). PC-1 also increased with temperature amplitude of the parent tree site (Fig. 2). This indicates that growth phenology and the cold hardiness affiliated with it are associated with harshness of the parent tree location. Greater spring cold hardiness, associated with later BB and BS, occurs in families from milder sites (those sites with low temperature am-

plitudes, typically associated with areas closer to the ocean and at lower elevations).

There was no significant relationship between PC-2 and location variables, but a significant relationship between PC-2 and climatic variables indicated that PC-2 decreases with increasing July temperature. Thus, families from sites with hotter summers have greater fall and spring cold hardiness not associated with phenology.

No significant relationship was found between the univariate traits for spring (STEM493) and fall (STEM1092) cold hardiness and the location or climatic variables. Mean family height decreases with distance from the ocean, decreasing January temperatures, and increasing July temperatures.

Cascade zone

There was no significant relationship between PC-1 and either set of variables in the Cascade zone. PC-2 decreased

Table 2. Eigenvectors, eigenvalues, and percent of total variation explained among open-pollinated family means of the first five principal components for the Cascade zone (pooled over family sets, test sites, and years).

	PC-1	PC-2	PC-3	PC-4	PC-5
5-year height	-0.0806	0.1487	0.0301	0.4178	-0.007
Bud set 92	0.0729	-0.0148	-0.3018	0.1675	-0.213
Elongation Prop. 92	-0.1352	0.1697	0.0739	0.0051	0.4418
NEED 992	-0.0027	0.2688	-0.1775	-0.4107	-0.023
STEM 992	0.0975	0.1899	0.1988	-0.2903	0.2623
NP 992	0.1873	0.1004	-0.0298	-0.0704	0.2139
INT 992	0.1897	0.1378	-0.0766	-0.1316	0.199
NEED 1092	0.0877	0.3044	-0.243	-0.2325	0.0443
STEM 1092	0.1508	0.2255	-0.0772	0.0982	0.0517
NP 1092	0.0251	0.1391	-0.3961	0.2577	0.2518
INT 1092	0.1960	0.1845	-0.1341	0.0575	0.1137
NEED 493	-0.1489	0.2533	-0.1118	0.1356	-0.219
STEM 493	-0.2366	0.1417	0.0679	0.0488	0.0664
NP 493	-0.2307	0.1184	0.0633	0.1085	0.081
INT 493	-0.2508	0.1067	0.0860	0.0305	0.0369
Bud burst 93	0.2414	-0.1496	-0.0235	-0.1488	-0.090
Bud set 93	0.2126	-0.1581	-0.091	-0.1099	-0.037
Elongation prop. 93	-0.1836	0.1246	0.0192	-0.0103	0.3725
Second flushing 93	0.0905	0.1573	0.4171	0.0801	-0.010
NEED 993	0.0987	0.2766	0.0763	-0.2166	-0.224
STEM 993	0.1802	0.1726	0.3224	0.1418	-0.142
NP 993	0.2053	0.1158	0.3172	0.0632	-0.063
INT 993	0.2126	0.0901	0.2370	0.1311	0.1037
NEED 1093	0.1189	0.2969	-0.1348	-0.1321	-0.218
STEM 1093	0.1700	0.2251	0.1306	0.2100	-0.085
NP 1093	0.1513	0.1301	-0.223	0.3669	0.0277
INT 1093	0.2142	0.1409	-0.0496	0.1186	-0.072
NEED 494	-0.1608	0.2336	-0.0522	-0.0507	-0.259
STEM 494	-0.238	0.1574	0.1000	-0.0965	-0.079
NP 494	-0.2179	0.1154	-0.017	0.0039	-0.242
INT 494	-0.2285	0.0926	0.0592	-0.0638	-0.169
Bud burst 94	0.2381	-0.1125	0.0527	-0.0039	-0.086
Eigenvalue	12.51	6.36	1.67	1.37	1.21
% Variation	39.1	19.9	7.5	5.2	4.3

Note: NEED, STEM, NP and INT refer to cold injury to needles, stem tissues, bud apical meristems and needle primordia, and the bud–shoot interface, respectively. Traits with values refer to sampling month and (or) year. See text for details about traits.

with increasing elevation and slope (Fig. 3). This indicates that cold hardiness unassociated with phenology increases with elevation and slope. PC-2 also increased with January temperatures, but decreased with July temperatures and the aridity index. Cold hardiness unassociated with phenology appears to be greater in families from sites with lower winter temperatures, higher summer temperatures and higher summer moisture.

There was no significant relationship between either set of independent variables and STEM493. Fall cold damage (STEM1092) increased with decreasing elevation and increasing January temperatures (Fig. 4) and was also greater on east- versus west-facing slopes. Thus, fall cold hardiness is greater in families from higher elevations and west aspects (possibly related to the moisture differences between east and west aspects) and sites with colder winters. There was no significant relationship between either set of independent variables and height.

Interpretation

A small but significant proportion of family variation for cold hardiness traits was found to be associated with parent tree environment in both zones. In general, the relationships between adaptive traits and location or climatic variables were fairly weak in both populations ($0.098 < r^2 < 0.351$). These results are discussed in terms of wild stand seed movement, tree improvement programs, and genecology.

The results of this study support recent changes in seed zones for coastal Douglas-fir from native stands in Oregon. The newly designated seed zones in western Oregon now encompass longer (north–south), narrower (east–west) geographic areas than previously delineated, and this change is supported by the current study. Genetic clines are steeper east–west than north–south, and increased east–west seed movements could somewhat alter the risk of cold injury (Sorenson 1983). In the current study, progeny of Douglas-fir from the edge of the Willamette Valley burst bud on

Table 3. Table of results (for both zones) for PCs 1 and 2 using location and climatic variables associated with parent tree environments.

Zone	PC	% ^a	Traits	Location variables	<i>P</i> ^b	<i>R</i> ²	Climatic variables	<i>P</i>	<i>R</i> ²
Coastal	PC-1	46	Bud burst (+) ^c Bud set (+) Elongation (-) Spring damage (-)	Elevation (-) Distance (-)	0.028	0.176	Temperature range (-)	0.002	0.235
	PC-2	22	Fall needle and stem damage (+) 5-year height	ns ^d Distance (-)	ns 0.033	ns 0.098	July temperature (-) January temperature (+) July temperature (-)	0.001 0.008	0.249 0.279
Cascade	PC-1	39	Bud burst (+) ^c Bud set (+) Elongation ⁹³ (-) Spring damage (-)	ns	ns	ns	ns	ns	ns
	PC-2	19	Fall needle and stem damage (+) Spring needle damage (+) STEM1092	Elevation (-) Slope (-) Elevation (-) Cos (aspect) (-)	0.009 0.0003	0.224 0.351	January temperature (+) July temperature (-) Aridity index (-) January temperature (+)	0.018 0.007	0.242 0.176

Note: Cos (aspect) is the aspect transformed into cosine including an interaction with slope; distance is the distance to the ocean; July and January temperatures are means for the respective months.

^aProportion of variation over all traits accounted for by each PC.

^bSignificance of the model.

^cSigns indicate the sign of the eigenvector loadings.

^dNot significant ($p > 0.05$).

Fig. 1. First principal component scores versus parent-tree elevation and distance to the ocean (Coastal zone).

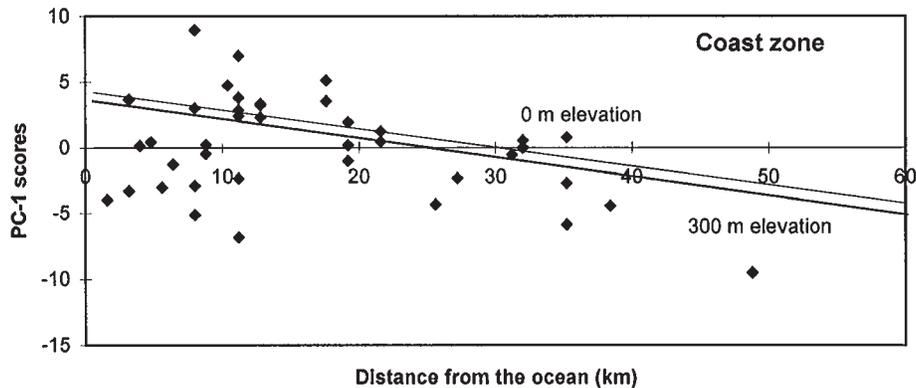
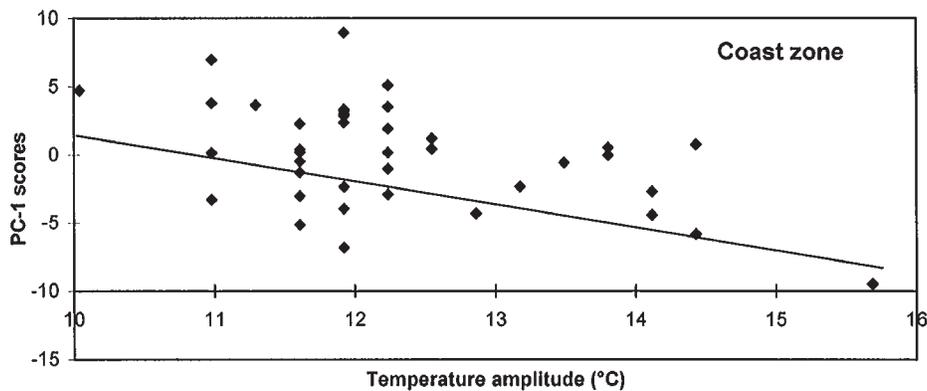


Fig. 2. PC-1 scores versus modelled temperature amplitude of parent-tree environments in the Coastal zone.



average 10 days before progeny from the coast. On one hand, movement of "inland" genetic stock too far toward the coast could result in increased spring cold damage, although the risk of frost injury in coastal areas has not been well documented. On the other hand, movement of coastal seed too far inland may result in more fall damage.

Phenology may reflect adaptation to biotic as well as abiotic stresses. There is some evidence that westward movement of seed results in increased amounts of Swiss needle cast defoliating young plantations of Douglas-fir near the coast. Investigations are underway to determine if bud phenology is involved in susceptibility to this disease (R. Johnson, U.S. Forest Service Pacific Northwest Research Station, Corvallis, Oreg., personal communication). East-west clinal variation has resulted in changes in the structure of seed zones for wild stand seed along the coast (B. Randall, USDA Forest Service, Siuslaw National Forest, Eugene, Oreg., personal communication).

Breeding zones are typically larger than wild-stand seed transfer zones because materials included in breeding populations are tested over a range of environments and selected genotypes should be more stable in performance than randomly selected genotypes. These data provide no evidence that the current size or configuration of breeding zones included in this study is inappropriate. In fact, further studies are warranted to examine the potential for increasing the size of breeding zones, particularly in the north-south direction.

The association of adaptive characteristics with parent tree environments within breeding zones is too weak to be

an effective tool for assisting with selection of trees with particular sets of adaptive characteristics. Screening families for adaptive traits would be a much more effective approach than characterizing families based on parent tree environments for identifying families with particular annual developmental cycles and levels of cold hardiness. However, the results reinforce the concept of making selections across a broad range of environments within a breeding zone to ensure a broad range of adaptiveness within the breeding population and an enhanced potential for future selection. After the first generation of breeding, geographic origin within a breeding zone becomes largely irrelevant. In future generations of breeding, testing families over a similarly broad range of environments and artificial screening for adaptive traits will ensure the maintenance of well-adapted, genetically improved populations composed of adaptational generalists rather than specialists.

While geographic trends are weak and unlikely to change tree improvement strategies, they are significantly related to variation in operational environments on a fairly fine geographic scale and can be interpreted in terms of the extent to which selection pressures differ among environments within these breeding zones. In the Coastal zone, univariate measures of both spring and fall cold damage of families were not significantly associated with location or climate variables of parent tree environments. The weak association of PC-1 with both types of variables indicates, however, that family variation in growth phenology and associated cold hardiness traits (especially spring cold hardiness) within this

Fig. 3. Second principal component scores versus slope and elevation of parent-tree environment in the Cascade zone.

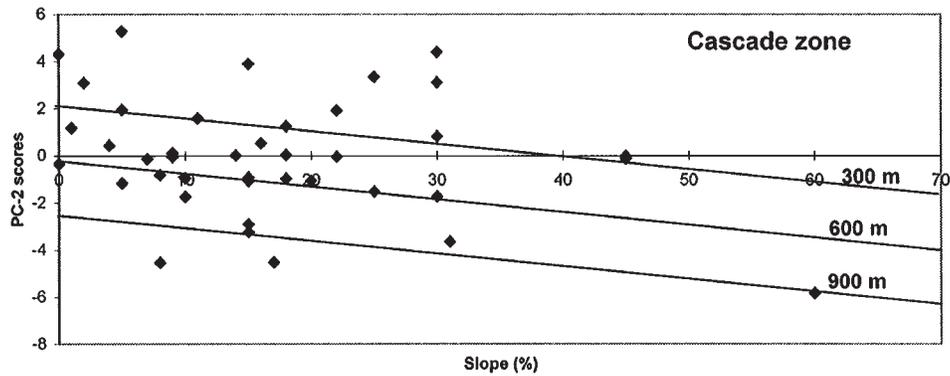
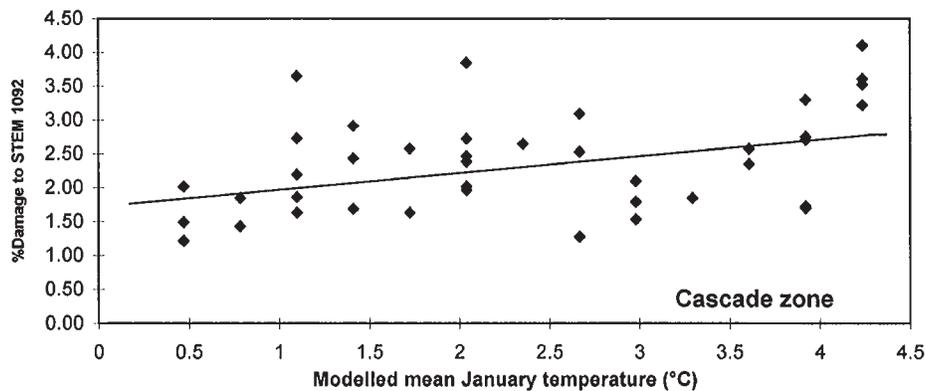


Fig. 4. Fall stem cold hardiness versus mean January temperature of the parent-tree environment in the Cascade zone.



zone at least partially reflects a selective response of parent trees to their environments. PC-1 decreased with temperature range of parent tree environments and with increasing elevation and distance to the ocean. This indicates that families from milder (more coastal and lower elevation) sites burst bud slightly later and are therefore somewhat less susceptible to cold damage in the spring.

Variation in bud phenology within this species and others along environmental gradients has previously been documented (e.g., Campbell 1974, 1979; Eriksson 1982; Steiner 1979; Dietrichson 1969; Scroppa and Magnussen 1993; Sorenson 1983). In areas with relatively short growing seasons, such as those found at high elevations or further inland (as opposed to coastal), trees have shorter chilling requirements and enter the quiescent stage of development in which growth may occur under favorable conditions sooner than trees from milder climates when planted in a common environment. This is an advantage for trees that need to utilize as much of the short growing season as possible. Spring temperatures may also be more variable at low elevations or in coastal environments (Campbell 1979); thus, it also becomes an advantage for trees from these areas to break bud well after the threat of unpredictable spring frost is over.

The negative association of PC-2 with July mean temperature indicates that families from sites with hotter summers are slightly harder to both fall and spring cold. Acclimation in the fall may be induced by moisture stress (van den Driessche 1969; Timmis and Tanaka 1976). Since moisture stress is likely to be greater in areas with higher mean summer temperatures, it is possible that trees from these areas

respond to moisture stress and acclimate sooner than trees from more coastal locations. However, this pattern was not reflected in significant associations with the aridity index.

Relationships of 5-year height in the Coastal zone with both location and climatic variables indicate early height growth is greatest in families with warmer winters and cooler summers (i.e., nearest to the coast). Joly et al. (1989) found that, despite the earlier bud burst in progeny of more inland parent trees, overall growth in these trees was lower because of slower growth rates. The more conservative growth habits in these trees may be adaptations to the droughtier conditions found in the more inland areas. No significant relationship was found between height and either climate or location variables in the Cascade zone.

Neither spring cold hardiness nor PC-1 were significantly associated with location or climate variables in the Cascade zone. This is somewhat surprising given that the finding of Timmis et al. (1994) that spring frost damage risk is fairly high in this area of the Oregon Cascades and that it increases with elevation. The smaller size of the Cascade breeding zone and the geographic clustering of parent trees into sets likely limited the detectability of adaptive trait patterns in the test trees. However, Campbell (1979) also found little relationship between bud burst timing and elevation in a Cascade watershed. Both fall cold damage and PC-2 were significantly associated with both location and climatic variables. Families harder to fall cold damage tend to come from higher elevations, more northerly aspects, and sites with colder January temperatures. Movement of material up 600 m in elevation would result in a 15% increase in fall

cold damage. Movement to contrasting aspects or dissimilar slopes may also affect fall damage. The lower night temperatures in these environments could contribute to earlier acclimation to cold (Lavender et al. 1968; van den Dreissche 1969). Fall cold hardiness and elements of spring cold hardiness unassociated with growth phenology were also positively although weakly associated with harshness of the parent tree environment.

In this study, modelled climatic variables were only slightly better than location variables in accounting for variation among the test trees. The use of location variables has been more common in past studies describing environmental variation because of the relative ease in obtaining their estimates; however, they are simply surrogates for local climatic conditions based on broad-scale relationships between geographic location and climate. Climatic variables should provide better estimates of components of the operational environments of plants as they directly measure temperature and moisture regimes. Despite this, some limitations to the use of the modeled climate variables exist, such as their differing scales of resolution (i.e., 10-km grid scale for precipitation, 1-km grid scale for temperature). Additionally, the variables used in this study attempt to describe only the mean environments to which parent trees are adapted. Environmental variability or potential climatic extremes within parent tree habitats may also play a role in determining adaptive trait patterns and thus might result in high amounts of genetic variation within a single environment. Variables tested did not account for soil differences among the parent tree sites. It is likely, however, that the general pattern of weak geographic or environmental trends will hold up even with the addition of more environmental factors because of high levels of within-source genetic variation in Douglas-fir and the use of open-pollinated seed, for which pollen may well have originated from parents in somewhat different environments.

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