Genetics of fall and winter cold hardiness of coastal Douglas-fir in Oregon[1]

S.N. Aitken and W.T. Adams

Abstract: Genetic variation in fall cold hardiness was studied in two western Oregon breeding populations of coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco), one on the west slope of the Cascade Mountains and the other in the Coast Range. On six sampling dates (September, October, and November of 1992 and January, September, and October of 1993), shoot cuttings from 40 open-pollinated families in each of two progeny test sites for each breeding zone were subjected to artificial freezing at two test temperatures. Damage on each shoot was recorded as visible injury to needle, stem, and bud tissues separately. Considerable family variation was found for cold injury scores in all tissues in early fall to midfall, but differences were often smaller or nonsignificant in late fall and midwinter. Individual heritability estimates for needle cold injury were low (<0.40) and generally decreased in late fall and midwinter. Family rankings for fall cold hardiness, however, are expected to be relatively consistent over sites and years, although needles appear to display more family-by-site interaction than stems or buds. Genetic correlations between tissues in cold injury varied considerably and were sometimes weak, indicating that the evaluation of a single tissue is probably not adequate for assessing overall cold hardiness of genotypes. Fall and winter cold hardiness seem to be largely under separate genetic control since genetic correlations between hardiness at these two stages were weak. This study confirms earlier results in Washington breeding populations and shows that coastal Douglas-fir families can be effectively ranked for fall cold hardiness by conducting artificial freeze tests on cut shoots in midfall (October) and scoring damage to stems and at least one other tissue.

Résumé : Les auteurs ont étudié la variabilité génétique de l'endurcissement au froid automnal chez deux populations d'amélioration de l'Ouest de l'Orégon de sapin de Douglas de la côte (*Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii*), l'une représentative d'une pente ouest de la Chaîne des Cascades et l'autre située au sein de la Chaîne Côtière. Pour chacune des six dates d'échantillonnage (septembre, octobre et novembre 1992, janvier, septembre et octobre 1993), des pousses furent prélevées sur les 40 descendances uniparentales établies à chacun des deux sites d'expérimentation représentatifs des deux zones d'amélioration. Les pousses ont été soumises à deux températures distinctes en deça du point de congélation. Les dommages observés sur chaque pousse furent notés selon qu'ils affectaient les aiguilles, la tige ou les bourgeons. Pour tous les tissus, une variation familiale considérable des observations de dommage dû au froid fut notée du début jusqu'au milieu de l'automne, mais les différences devenaient moindres ou non significatives à la fin de l'automne et au milieu de l'hiver. Les estimations d'héritabilité individuelle des dommages aux aiguilles étaient faibles (<0,40) et diminuaient généralement à la fin de l'automne et au milieu de l'hiver. En ce qui concerne l'endurcissement au froid automnal, les rangs des descendances devraient être relativement constants d'un site à l'autre et d'une année à l'autre, quoique les dommages aux aiguilles ont démontré plus d'effet d'interaction entre les descendances et les sites que les dommages aux tiges ou aux bourgeons. Les corrélations génétiques entre les dommages infligés aux différents tissus variaient beaucoup et étaient souvent faibles, indiquant que l'évaluation des dommages à partir d'un seul type de tissu n'est probablement pas suffisante afin de déterminer adéquatement l'endurcissement général au froid des génotypes. Les faibles corrélations génétiques entre les données d'endurcissement au froid automnal et celles d'endurcissement au froid hivernal semblent indiquer que ces deux stades phénologiques soient sous un contrôle génétique distinct. Les résultats de cette étude confirment les résultats précédents d'une étude effectuée à partir de populations d'amélioration de l'État de Washington. Ils démontrent qu'il est effectivement possible d'ordonner les familles de sapin de Douglas quant à l'endurcissement au froid automnal, en réalisant des tests artificiels de congélation sur des pousses récoltées au milieu de l'automne (octobre) et en déterminant les dommages à la tige et au moins un autre tissue.

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Introduction

The growth, dormancy, and cold acclimation cycles of woody perennials must be synchronized with seasonal changes in local climates to ensure sufficiently long growing seasons for competitive growth yet guarantee adequate levels of cold hardiness during low temperature periods (Sakai and Larcher 1987). It is well established that levels of cold hardiness vary among geographical sources of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) in both the coastal (var. *menziesii*) and interior (var. *glauca*) varieties (Campbell and Sorensen 1974; Larsen 1978*a*, 1978*b*; Loopstra and Adams 1989; Rehfeldt 1979, 1986; Schuch et al. 1989; White 1987). What has not been thoroughly investigated is the amount of within-population genetic variation for cold hardiness and the relevance of this variation for tree breeding programs. Although significant family variation for midfall cold hardiness has been demonstrated within populations of Douglas-fir (Aitken et al. 1996; Aitken and Adams 1995*a*, 1995*b*; Wheeler et al. 1990; White 1987), the degree to which family differences are evident over the course of the acclimation process is unclear.

Fall frost events in the Pacific Northwest can result in injury, loss of productivity, and mortality in Douglas-fir (Timmis et al. 1994). The single-most damaging freeze event documented in the region was in November, 1955, when even some mature trees were killed (Duffield 1956). Timmis et al. (1994) modelled frost injury risk to young Douglas-fir plantations in western Washington and Oregon by comparing seedling frost acclimation with weather station records and concluded that the period of greatest risk from fall cold injury is during October and the first half of November. They also concluded that, while across the region the risk of frost injury is two to three times greater on average in the spring than in the fall, for some specific locations the risk of injury or death due to cold events is greatest in the fall.

Because tree improvement programs typically select trees using relatively short-term field tests on mild sites, the ability to survive infrequent, extreme cold events is often not adequately determined. Thus, artificial freeze testing of stem tissues may provide the only reliable means of assessing fall cold hardiness. For fall freeze testing to be of practical value in tree improvement programs, several conditions must be met. First, screening methods must allow for the evaluation of many individuals in a short period of time. In addition, there must be adequate genetic variation for hardiness traits, and hardiness must be under sufficient genetic control. Finally, the ranking of families in the test environment must predict injury or mortality over a range of operational planting environments.

In a previous investigation, genetic variation of fall cold hardiness in two Washington breeding populations of coastal Douglas-fir was examined, based on artificial freeze testing done in October of one year (Aitken et al. 1996). In the present study, we extend this examination of the genetics of fall cold hardiness to coastal Douglas-fir breeding populations in Oregon and further quantify the inheritance of this trait and the degree to which family rankings for fall cold hardiness vary across test sites and different shoot tissues. In addition, because cold hardiness sampling in the present study included multiple dates in the fall of 2 years, as well as a midwinter assessment, we report on the consistency of family cold hardiness across fall sampling dates and years, as well as genetic variation in acclimation rates and midwinter cold hardiness. Additional reports address the genetics of spring cold hardiness, relationships between cold hardiness and bud phenology, and relationships between cold hardiness and stem growth (Aitken et al. 1996; Aitken and Adams 1995*a*, 1995*b*; and unpublished data.)

Materials and methods

Breeding zones and test sites

Two lower-elevation breeding zones in western Oregon were included in this study, one in the Coast Range on the Siuslaw National Forest (covering approximately 500 km^2) and the other on the lower west slope of the Cascade Mountains in Bureau of Land Management Santiam Breeding Unit 33 (approximately 150 km^2). Within each zone, 7-year-old open-pollinated progeny (families) of phenotypically selected parent trees were measured. Parent trees in the Coastal zone were located within 50 km of the Pacific Ocean, between 65 and 340 m (average 230 m) in elevation. In the Cascade zone, parent tree locations ranged from 300 to 840 m (average 550 m) in elevation. Families in both these zones were divided into sets of 30 for testing purposes. Twenty families from each of two sets (40 families total) in each zone were sampled in this study. Sets and families chosen for sampling were based entirely on the availability of seed in storage for subsequent seedling projects.

Families were measured on a high- and low-elevation test site in each zone: Canal Creek (Coast-L, elevation 250 m) and Flynn Table (Coast-H, 500 m) in the coastal zone and Prospect Mountain (Casc-L, 400 m) and House Mountain (Casc-H, 650 m) in the Cascade zone. Different test elevations were chosen within zones to be able to compare family development and expression of cold hardiness under different environments.

The experimental design on each of the test sites in the coastal zone is a split plot, with 30-family sets as main plots and families within sets as subplots. At planting, each family subplot consisted of four randomly located individuals (i.e., four-tree noncontiguous subplots). Four replications of this design (blocks) are found at Coast-L and five at Coast-H. In the Cascade zone, each 30-family set was planted as a separate randomized complete block design, with 5 replications. Within each block, families were represented by four-tree noncontiguous plots.

By age 7 years, mortality ranged from 6% at Coast-L to 20% at Casc-H. The Casc-H site had high mortality due to several large frost pockets within the site, in which trees had suffered repeated cold injury resulting in slow growth or death. Average 5-year height was 2.28 m at Coast-L, 1.62 m at Coast-H, 1.85 m at Casc-L, and 1.26 m at Casc-H. Daily minimum, maximum, and mean temperatures were recorded on each site over the study period using Omnidata Datapod 212 continuous temperature recorders.

Artificial freeze tests

Freeze tests were conducted on cut shoots collected from all test trees in the four sites in each of six sampling periods: September, October, and November in 1992 and January, September, and October in 1993 (fall and winter 1992 sampling dates shown in Fig. 1). In each sampling period, two cut shoots from each tree were subjected to a different freeze temperature, followed by visual scoring of needle, stem, and bud tissues for cold damage. The two freeze temperatures chosen for each sampling date were determined from preliminary samples taken 1 week earlier (see below).

When it is of interest to accurately determine cold hardiness in a small number of seedling stocks, varieties, or cultivars, detailed assessments involving freeze testing at five or more test temperatures is recommended (e.g., DeHayes and Williams 1989). Cold hardiness can then be estimated as the temperature at which 50% of seedlings are killed or 50% of tissue injured (LT_{50}) or the temperature at which significant cold injury is first detected, termed the critical temperature (Burr et al. 1990; DeHayes and Williams 1989). Such labor intensive assessment is not feasible in tree improvement programs, where the need is to screen thousands of progeny to rank hundreds of parent trees (or families) for cold hardiness. Screening at numerous test temperatures, however, is necessary only if family rankings for cold hardiness are sensitive to changes in test temperatures (i.e., when there is family-by-test temperature interaction). Such rank changes are manifested if families differ widely in their response (i.e., tissue damage) to different freeze temperatures at a particular point in time (DeHayes and Williams 1989). Evaluation of 60 coastal Douglas-fir families for cold injury response over a range of test temperatures showed that families differ primarily in the intercept (i.e., minimum temperature at which there is detectable injury) and not on the shape of the response curve (R. Timmis, Weyerhaeuser Company, Tacoma, Wash., personal communication). Thus, one test temperature may be adequate to rank cold hardiness on each sampling date. Nevertheless, we used two test temperatures to increase the probability of obtaining adequate cold injury to detect family differences and to decrease sampling error (Aitken et al. 1996; and see below). Furthermore, with two test temperatures we could test whether family ranking for cold hardiness is sensitive to temperature.

On each sampling date, two shoot tips 5 cm long were collected from second-order (usually unshaded) laterals on the east side of trees, at a fixed midcrown height at each site (1.0–1.7 m). Second-flushed shoots were avoided where possible, but some (about 5%) were sampled in the fall of 1993 because second flushing was more frequent that year. All shoots for each sampling period were collected on a single day at each site, placed in plastic bags, and transported to Corvallis, Oreg., in ice chests. Samples were stored in a 2°C walk-in cooler for a maximum of 4 days (usually <24 h) before freeze testing. This short period of storage at a temperature similar to daily minimum temperatures on the test sites should not have significantly affected hardiness of shoot samples (DeHayes et al. 1990).

Prior to freeze testing, groups of 50 shoots were wrapped first in damp cheesecloth, then in aluminum foil in flat packets. The packets were placed on a thick aluminum shelf (to facilitate cooling through conduction rather than convection) in a computer-controlled Forma Scientific Model 8270/859M freezer with a West M3750 temperature controller and held overnight at −2°C, a temperature determined to be adequate to freeze extracellular water. The following morning, the temperature was lowered 3–5°C/h (Glerum 1985) (with the faster cooling rates used in late fall and winter), until the selected test temperature was reached. The test temperature was held constant for 1 h, after which the packets were removed from the freezer and put into a 2°C refrigerator overnight to thaw slowly. They were then placed at room temperature for 1 week to allow for visible signs of cold injury to develop in the dark, humid environment inside the moist aluminum foil packets (Burr et al. 1990). Only a single freezer run was needed to test all the samples from an individual test site for each freezing temperature.

Preliminary samples taken from each test site 1 week before the main test dates consisted of shoots from 15 random trees. These shoots were tested at four freezing temperatures (except in January, when three temperatures were evaluated), and by interpolation, two temperatures (2–5°C apart) expected to result in intermediate (20–80%) damage averaged across all tissues were chosen for the main test. In September and October, needle, stem, and bud tissues had similar levels of cold hardiness; thus, selection of test temperatures was fairly simple. After October, however, while needles and stems continued to increase in hardiness, buds reached a hardiness plateau, such that temperatures causing only intermediate damage to needles and stems in late fall and midwinter caused severe damage to buds (Fig. 2). Selected test temperatures ranged from −10°C in September to −40°C in January (Fig. 1).

Scoring freeze damage

Visual discoloration is an effective indicator of loss of tissue viability after freezing (Calkins and Swanson 1990) and was used in this study to score cold injury in needles, stems, and buds. Each sample was inspected through an illuminated three diopter magnifying lens, with stems cut lengthwise to reveal approximately 2 cm of phloem and cambium, and terminal buds bisected lengthwise to reveal primordial shoot tissues (Burr et al. 1990; Aitken et al. 1996). Cold injury in the three tissues was assessed independently but in the same manner, with the percentage of damaged tissue estimated to the nearest 10%. Needle damage was evidenced by reddish-brown needles or needle loss. Damage to the stem (cambium and phloem scored together) or buds was evidenced by browning or yellowing of normally greenish tissues. All scoring was done by two individuals, with all samples from the same replication scored by a single person.

Statistical analysis

For individual test dates, preliminary analyses were conducted on cold injury at each of the two test temperatures separately, as well as for the average injury score over both temperatures. Plotting of residuals indicated nonnormality of injury scores, especially when mean scores over all samples were particularly low (under 20%) or

high (over 80%). Therefore, injury scores were converted to proportions and subjected to the arcsine square root transformation before analysis. After transformation, all traits conformed well to the assumptions of analysis of variance (Steel and Torrie 1980). In Results, means are reported for nontransformed scores, while all other estimates are based on analysis of transformed data.

To test the significance ($p < 0.05$) of family differences and family-by-test site interaction, analyses of variance were applied to the paired test sites of each breeding zone using type III sums of squares in the SAS GLM procedure (SAS Institute Inc. 1988). The following linear model was used to represent individual-tree values for each trait in the Cascade breeding zone:

$$[1] \quad Y_{ijklm} = \mu + t_i + s_j + st_{ij} + b_{ijk} + f_{jl} + ft_{ijl} + e_{ijkl} + w_{ijklm}$$

where

Y_{ijklm} is the transformed injury score for the mth tree in the lth family in the kth block within the jth set in the ith test site

μ is the overall mean

t_i is the random effect of the ith test site, $E(t_i) = 0$, $\text{var}(t_i) = \sigma_t^2$

s_j is the random effect of the jth set, $E(s_j) = 0$, $\text{var}(s_j) = \sigma_s^2$

st_{ij} is the random interaction effect of the jth set with the ith test site, $E(st_{ij}) = 0$, $\text{var}(st_{ij}) = \sigma_{st}^2$

b_{ijk} is the random effect of kth block within the jth set and ith test site, $E(b_{ijk}) = 0$, $\text{var}\ (b_{ijk}) = \sigma_b^2$

f_{jl} is the random effect of the lth family within the jth set, $E(f_{jl}) = 0$, $\text{var}(f_{jl}) = \sigma_f^2$

ft_{ijl} is the random interaction effect of lth family within the jth set with the ith test site, $E(ft_{ijl}) = 0$, $\text{var}(ft_{ijl}) = \sigma_{ft}^2$

e_{ijkl} is the random plot error of the lth family in the kth block of the jth set of the ith test site (plot error), $E(e_{ijk}) = 0$, $\text{var}\ (e_{ijkl}) = \sigma_e^2$

w_{ijklm} is the random tree error of the mth tree in the $ijkl$th plot, $E(w_{ijklm}) = 0$, $\text{var}\ (w_{ijklm}) = \sigma_w^2$

and the covariances between all pairs of factors were assumed to be zero.

The model differed somewhat for the coastal zone as family sets were nested within blocks, rather than blocks within sets:

$$[2] \quad Y_{ijklm} = \mu + t_i + s_j + st_{ij} + b_{ik} + sb_{ijk} + f_{jl} + ft_{ijl} + e_{ijkl} + w_{ijklm}$$

where all variables are as defined above except

b_{ik} is the random effect of the kth block within the ith test site, $\text{E}(b_{ik}) = 0$, $\text{var}\ (b_{ik}) = \sigma_b^2$;

sb_{ijk} is the random interaction of the jth set with the kth block in the ith test site, $E(sb_{ijk}) = 0$, $\text{var}(sb_{ijk}) = \sigma_{sb}^2$.

To assess family-by-year interaction in September and October cold injury, a single large, pooled analysis of variance was conducted in each breeding zone across sites and years for each month. The above models were used, with the addition of random effects for year and interactions of year with site, set, and family.

Variance components were estimated from the appropriate mean squares using the restricted maximum likelihood (REML) estimator in SAS procedure VARCOMP (SAS Institute Inc. 1988). The amount of genetic variation in cold hardiness traits was quantified by estimating family variances and testing their significance ($p < 0.05$). Individual tree heritabilities of these traits were estimated as

$$[3] \quad h^2 = \frac{3\sigma_f^2}{\sigma_f^2 + \sigma_{ft}^2 + \sigma_e^2 + \sigma_w^2}$$

The additive genetic variation (numerator of h^2 equation) was estimated as three times the family variance, rather than four times the family variance as appropriate for half-sib progeny, as open-pollinated progeny are more closely related than half-sibs (Campbell 1979). This heritability estimate is appropriate when individual-tree traits are corrected for block means and selections are made only among trees within sets. The standard errors of heritability estimates were calculated following Becker (1984).

To evaluate genetic relationships between injury scores at different temperatures for the same tissues, and between different tissues on the same sampling date, type A genetic correlations (r_A) were calculated whenever family differences were significant (Burdon 1977):

$$[4] \quad r_A = \frac{\text{cov}_{fxy}}{(\sigma_{fx}^2 \times \sigma_{fy}^2)^{1/2}}$$

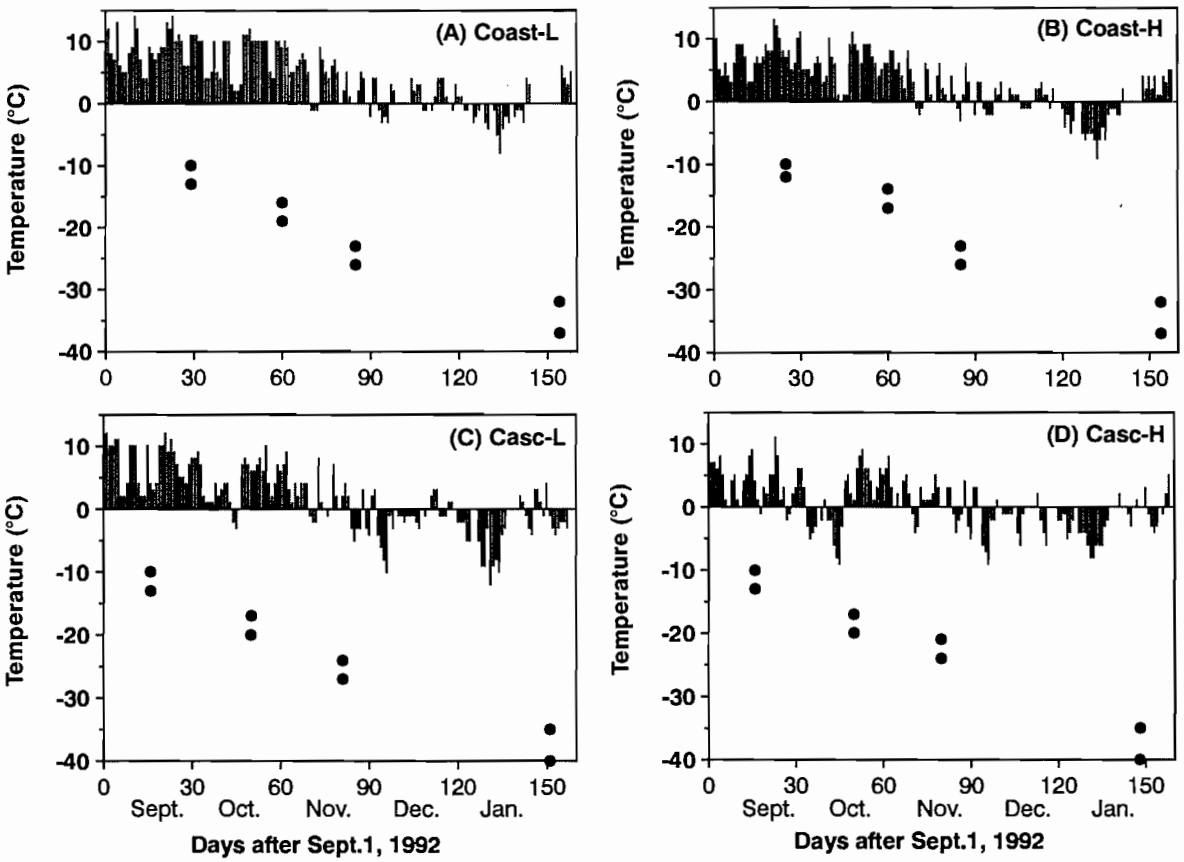
where cov_{fxy} is the family covariance between two traits (or pairs of measurements of the same trait on different sampling dates) and σ_{fx}^2 and σ_{fy}^2 are the family variances of the first and second traits. When $r_A \approx 1$, traits are under identical genetic control. Standard errors of genetic correlations were estimated following Becker (1984).

Type A genetic correlations between cold injury scores observed in the same tissue frozen at two different test temperatures on any particular sampling date were very high, never below 0.79 and averaged 0.96 over all sampling date and tissue combinations for which estimates could be calculated (total of 17). In addition, individual heritabilities were higher when cold injury scores were averaged over the test temperatures on a sampling date, than when treated separately. Both of the above findings indicate that genetic rankings for cold injury were not sensitive to the two different test temperatures used on a particular sampling date; thus, our remaining analyses are based entirely on average cold injury scores for individual trees.

Type A correlations between injury scores of the same trait on different sampling dates within the 1992 and 1993 fall acclimation periods were used to examine the degree to which families acclimate to cold at different rates. A high genetic correlation between sampling dates indicates that different families are developing hardiness at similar rates.

Genetic correlations were also estimated to quantify the magnitude of family-by-site and family-by-year interactions

Fig. 1. Minimum daily temperatures (Sept. 1, 1992, through Feb. 5, 1993; vertical bars) and artificial freeze-testing sampling dates and test temperatures (solid circles) for each of the progeny test sites included in the study.



(Burdon 1977). Type A genetic correlations between injury scores for the same tissue assessed in the same month in two different years measures family-by-year interaction. The magnitude of family-by-site interactions was evaluated by estimating type B genetic correlations (r_B; Burdon 1977) between injury scores assessed in the same month on different sites within a breeding zone:

$$[5] \quad r_B = \frac{\sigma_f^2}{\sigma_f^2 + \sigma_{fl}^2}$$

When there is no family-by-site interaction, families will rank identically across years or sites, and genetic correlation estimates are expected to be near 1.

Results

Temperature variation over sites and years

Differences in thermoperiod between the low- and high-elevation sites from September 1992 through January 1993 were less pronounced in the coastal zone than in the Cascade zone. Daily minimum temperatures throughout this period were typically one or at most just a few degrees lower at Coast-H than at Coast-L. In contrast, the two test sites in the Cascades differed considerably in temperature. For example, the lower elevation site (Casc-L) had only two nights in October with minimum temperatures below freezing, while the higher elevation site (Casc-H) had 11 subfreezing nights (Fig. 1). While on average the higher elevation site was cooler than the lower site, there were exceptions, e.g., the lowest minimum temperature in January 1993 was recorded at the Casc-L site.

Test temperatures were selected in preliminary tests, for each site separately, to inflict intermediate (20–80%) damage averaged across all three tissue types. The chosen test temperatures were at least 7°C lower than recorded on any of the test sites at or close to the time of testing, and this temperature differential steadily increased from early fall to midwinter (Fig. 1). During this period, cold damage was not observed in situ at any of the test sites, although trees at Casc-H showed signs of repeated past frost injury and were visibly damaged by spring frost events in 1992 (S.N. Aitken and W.T. Adams unpublished data).

The summers of 1992 and 1993 were very different in terms of weather. May through August 1992 was unusually warm and dry with a mean temperature of 18.7°C and total precipitation measuring only 71 mm in Corvallis (within 25 km of the coastal zone and 50 km of the Cascade zone). The same period in 1993 was cool and wet, with an average temperature of 16.8°C and a total of 196 mm of rain. Thus, conditions were ideal for testing family-by-year interaction due to year-to-year climatic variability. Tests were conducted on approximately the same dates

Table 1. Estimated means, family ranges, variance components, individual heritabilities (h^2), and type B genetic correlations between sites within breeding zones (r_{B_I}) for cold injury scores (% tissue damaged) in Oregon Coastal and Cascade breeding zone (BZ) populations of Douglas-fir, in three fall months of 1992 and January 1993.

Month and year	Tissue	BZ	Mean (family range)	Variance components[a,b]				h^2	r_{B_I}
				σ_f^2	σ_{fi}^2	σ_e^2	σ_w^2		
Sept. 1992	Needle	Coastal	29.5 (15.4–49.1)	0.004 05**	0.002 28*	0	0.063 30	0.17	0.64
		Cascades	17.1 (4.2–40.2)	0.002 83*	0.001 36	0.000 84	0.053 55	0.15	0.68
	Stem	Coastal	30.3 (15.1–43.7)	0.006 07***	0.000 39	0	0.081 68	0.21	0.94
		Cascades	10.7 (2.4–26.2)	0.003 63***	0	0.002 23	0.046 31	0.21	1.0
	Bud	Coastal	15.8 (5.3–40.6)	0.011 85***	0	0.002 89	0.118 83	0.27	1.0
		Cascades	12.4 (0.7–49.9)	0.007 06***	0	0.007 07	0.093 77	0.20	1.0
Oct. 1992	Needle	Coastal	50.6 (31.7–76.1)	0.007 93***	0.000 05	0.000 88	0.058 14	0.36	0.99
		Cascades	53.1 (17.4–87.0)	0.008 29***	0.002 47**	0.001 18	0.067 88	0.31	0.77
	Stem	Coastal	24.6 (12.8–39.8)	0.005 08***	0.002 16	0	0.053 80	0.26	0.96
		Cascades	23.9 (7.6–58.6)	0.003 57**	0.000 74	0.000 43	0.062 12	0.16	0.83
	Bud	Coastal	17.3 (5.1–36.2)	0.009 90***	0.001 42	0	0.107 26	0.25	0.87
		Cascades	22.4 (5.5–47.0)	0.000 87	0	0.000 94	0.074 90	—[c]	1.00
Nov. 1992	Needle	Coastal	69.2 (51.5–84.5)	0.006 67***	0.002 08	0	0.082 42	0.22	0.76
		Cascades	49.3 (17.2–87.7)	0.003 47**	0.003 09***	0	0.047 57	0.19	0.53
	Stem	Coastal	19.0 (9.1–29.8)	0.002 84***	0.000 35	0.001 17	0.057 30	0.14	0.89
		Cascades	13.2 (3.7–32.2)	0.000 08	0.001 93***	0	0.033 17	—[c]	0.40
	Bud	Coastal	86.0 (64.6–100)	0.003 01	0.003 50	0	0.219 70	—[c]	0.46
		Cascades	82.8 (52.5–100)	0.002 05*	0	0.001 46	0.133 44	0.05	1.0
Jan. 1993	Needle	Coastal	71.6 (55.7–83.3)	0.006 84***	0	0	0.051 80	0.35	1.0
		Cascades	62.8 (27.8–100)	0.003 31*	0.003 12**	0	0.058 86	0.15	0.51
	Stem	Coastal	17.1 (10.6–26.6)	0.001 15***	0	0.000 32	0.029 76	0.11	1.0
		Cascades	14.6 (8.8–25.2)	0.000 42*	0.000 15	0.000 34	0.010 49	0.11	0.74
	Bud	Coastal	99.5 (98.0–100)	0.000 05	0	0	0.005 10	—[c]	1.00
		Cascades	99.5 (94.6–100)	0	0.000 01	0.000 06	0.006 58	—[c]	0.00

Note: Means and family ranges are based on the original injury scores, while all other estimates are based on analysis of transformed scores (see text).

[a]Variance components were estimated using restricted maximum likelihood (REML). Asterisks indicate whether corresponding *F*-statistics for sources of variation were significant at 0.05 (*), 0.01 (**), or 0.001 (***) when tested using the SAS GLM procedure.

[b]σ_f^2, family variance; σ_{fi}^2, family-by-site variance; σ_e^2, plot (error) variance; σ_w^2, tree-within-plot variance.

[c]Individual heritabilities were not estimated if family variation for cold injury was not significant. Standard errors of h^2 estimates ranged from 0.049 to 0.167 (mean 0.109).

in September and October of both years and at the same test temperatures, but damage was considerably higher in 1993 than in 1992. For example, in the coastal zone needle injury averaged 29.3% in September 1992 and 47.4% in September 1993.

Family variation in fall injury scores

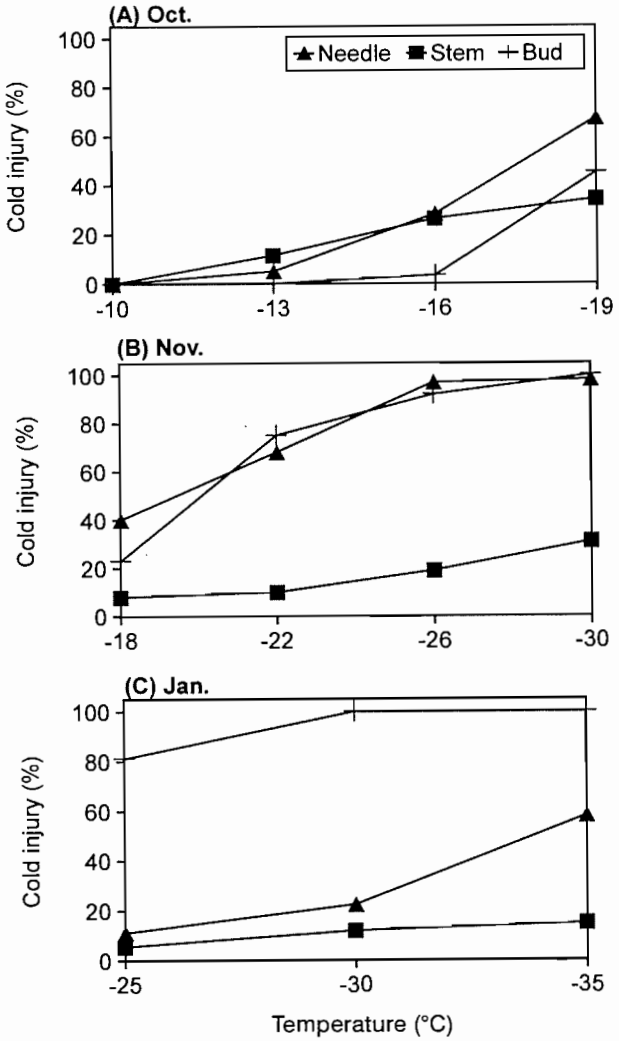
Genetic variation in cold hardiness is illustrated with cold injury data for the three fall months sampled in 1992 and for January 1993 (Table 1). September and October (early fall) cold injury results were very similar in 1992 and 1993; thus, we present the complete early fall results only for 1992. Sufficient cold injury was obtained for all tissues in most cases to analyze genetic differences in hardiness; however, mean bud injury was higher than desired in late fall (November) and winter because buds were extremely sensitive to the low test temperatures that caused only low to intermediate levels of damage in needles and stems (Fig. 2). In September, buds were hardier than needles and stems, but later in the fall and in midwinter, stem tissues were the hardiest and bud tissues were the least hardy.

In early fall, family ranges in cold injury scores were large in both the coastal and Cascade zones, and family differences were significant ($p < 0.05$) in all but one case (Table 1). In late fall, ranges in family means decreased for stem cold hardiness but remained high for needles and buds, although family variation was not significant for bud damage in the coastal zone. Individual heritability estimates for cold injury scores were low but relatively consistent across tissues in early fall (mean $h^2 = 0.21$; Table 1). Heritabilities of cold injury remained at approximately the same level for needles in late fall, but were considerably lower or could not be estimated for stems and buds.

Genotype-by-environment interactions

Family-by-site interactions in 1992 were largely nonsignificant for stem and bud injury scores (Table 1). Needle

Fig. 2. Mean cold injury score (percent) in preliminary tests for the higher elevation coastal zone test site, illustrating relative hardiness of needle, stem, and bud tissues. Preliminary tests were conducted 1 week before the main sampling dates to choose appropriate test temperatures for the main samples. Test temperatures selected were −15 and −18°C in October, −22 and −25°C in November, and −32 and −37°C in January.



injury exhibited more interaction, with significant ($p < 0.05$) interaction variance in the coastal zone in September and in the Cascade zone in October and November. Type B genetic correlations for injury to the same tissue from different test sites were relatively consistent and strong in early fall, averaging 0.90 in the coastal zone and 0.88 in the Cascade zone. These correlations were less consistent in late fall and were generally lower (mean coastal $r_{B_f} = 0.70$, Cascades $r_{B_f} = 0.64$).

There was significant ($p < 0.05$) family-by-year interaction for September assessment of needle and stem injury in both breeding zones, as well as for bud injury in the Cascades. Variance components for family-by-year interaction averaged about twice as large as family-by-site interaction in September. Genetic correlations between years for September averaged 0.86 ($0.71 \leq r_A \leq 1.0$). By October, there was no family-by-year interaction ($p < 0.05$) in either breeding zone, with very high genetic correlations between years ($0.89 \leq r_A \leq 1.0$).

Genetic correlations between fall sampling dates

Genetic correlations (r_A) between fall sampling dates in 1992 were generally high (Table 2). Buds were not included in the assessment of consistency of family rankings on different dates because of the lack of significant family variation for bud injury score in late fall and midwinter. The strong correlations between consecutive sampling dates indicate that families maintain similar rankings for cold hardiness during the acclimation process, at least over short periods of time. Correlations were somewhat stronger between September and October (average $r_A = 0.97$) and October and November (0.84) than between September and November (0.75) (Table 2).

Genetic correlations between tissues

Estimated genetic correlations between injury scores of different tissues ranged from weak to strong in the fall, depending on the tissue and sampling date, but averaged 0.56 over breeding zones, tissues, and sampling dates (Table 3). In early fall when family differences in injury scores were greatest, genetic correlations were strongest between stems and buds (mean $r_A = 0.85$) and weakest between needles and stems (0.60).

Genetic control of midwinter versus fall cold injury

Significant differences among families in needle and stem injury were observed in midwinter (January), but average bud injury scores were very high and family differences in bud injury were not significant in either breeding zone (Table 1). Ranges in family means were lower in winter than in the fall for stem and bud injury but remained considerable for needle injury. Heritability estimates for needle injury score were similar to the fall but were lower than the fall for stems (Table 1). Genetic correlations between fall and midwinter injury scores varied widely and were much weaker, on average ($r_A = 0.32$), than genetic correlations between fall dates (0.86) (Table 2).

Discussion

We have documented considerable within-population variation for fall cold hardiness in both Washington (Aitken et al. 1996) and Oregon breeding populations of coastal Douglas-fir. This has implications for both tree improvement and for the response of populations to climatic change. In a tree improvement program, there is potential to improve fall cold hardiness through selection or to change hardiness indirectly through selection of correlated traits (Aitken and Adams 1995*b*). Because of relatively low individual heritabilities, individual (mass) selection for fall cold hardiness is not expected to be very effective, but family selection alone or combined individual plus family selection should result in significant increases in average cold hardiness (Aitken and Adams 1995*b*; Aitken et al. 1996).

Adequate cold hardiness of all aboveground tissues (needles, stems, and buds) is important to the health and

Table 2. Estimated genetic correlations of transformed cold injury scores between pairs of sampling dates in 1992 for needles and stems.

	Needles		Stems	
Months	Coastal	Cascades	Coastal	Cascades
Within-fall r_A				
Sept.–Oct.	0.89	1.0	1.0	1.0
Sept.–Nov.	0.56	1.0	0.68	—[a]
Oct.–Nov.	0.84	0.73	0.95	—[a]
Fall–winter r_A				
Sept.–Jan.	0.02	0.86	−0.08	0.71
Oct.–Jan.	0.31	0.08	−0.10	0.96
Nov.–Jan.	0.22	0.21	0.32	—[a]

Note: Standard errors for genetic correlations averaged 0.22 (range 0.05 to 0.55).

[a]Family differences for stem injury score in November were not significant ($p > 0.05$) so genetic correlations were not estimated.

Table 3. Estimated genetic correlations between transformed injury scores for different tissues on the same sampling dates.

	Breeding zone	
Month and trait pair	Coastal	Cascade
September (1992, 1993)[a]		
Needle–stem	0.37	0.50
Needle–bud	0.77	0.51
Stem–bud	0.90	0.72
October (1992, 1993)[a]		
Needle–stem	0.72	0.84
Needle–bud	0.85	—[b]
Stem–bud	0.92	—[b]
November (1992)		
Needle–stem	0.64	—[b]
Needle–bud	0.42	0.52
Stem–bud	−0.04	—[b]
January (1993)		
Needle–stem	0.49	0.32
Needle–bud	−0.04	—[b]
Stem–bud	—[b]	—[b]

Note: Standard errors for genetic correlations averaged 0.24 (range 0.08 to 0.63).

[a]Genetic correlations reported for September and October are mean estimates across two sample years (1992 and 1993).

[b]Family differences in transformed injury scores for one or both tissues were not significant so genetic correlations were not estimated.

productivity of young stands. Trees will die if they suffer a high degree of stem injury, but losses of needles and buds are expected to lead to reduced growth, even if trees survive. Correlations between different tissues in fall cold injury were variable and sometimes weak in this study, as also observed in Washington breeding zones (Aitken et al. 1996). Thus, sampling a single tissue for cold hardiness, such as conducting electrolytic leakage testing on needle or stem tissue alone, or evaluating only needle browning in field tests following a natural frost event, may be inadequate for assessing overall hardiness of genotypes. Visual scoring allows for the evaluation of hardiness of different tissues on the same sample and, while subjective, produces repeatable results. It also makes possible the efficient scoring of a large number of individuals, which is necessary in tree improvement programs. It is important, however, that visual scoring be conducted in a highly controlled manner with the same individual scoring all samples from a single block to minimize experimental error due to scoring differences among personnel. The degree of correspondence between laboratory-determined cold hardiness and actual survival and growth of coastal Douglas-fir families in the field is not yet established; however, tests of this relationship are planned in the near future (Annual Report 1994–1995, Pacific Northwest Tree Improvement Research Cooperative, Oregon State University, Corvallis).

Bud injury scores were significantly different among families only in early and middle fall. The lack of family differences in January is due to the extreme sensitivity of this tissue to the test temperatures used, such that nearly all samples were 100% injured (Fig. 2). Buds never acclimated to below approximately −25°C, whereas needle and stem tissues achieved hardiness levels of −40°C or lower. If genetic variation in bud injury is to be evaluated midwinter, warmer freeze temperatures than those adequate for testing needle and stem tissues must be employed.

The explanation for why buds have a much higher critical temperature for cold injury than needle and stem tissues may lie in the rapid rate of cooling (3–5°C/h) used in this study. Sakai (1982) documented a freezing tolerance mechanism for primordial shoots (buds) of 23 coniferous species, not including Douglas-fir. By freezing shoot cuttings at different rates of cooling and using both differential thermal analysis and visual inspection of injury, Sakai determined that buds of these conifers were supercooling, rather than tolerating extreme cold through extracellular freezing, the mechanism of cold tolerance in needle and stem tissues. Sakai also found that the ability of buds to supercool increased greatly as the rate of cooling was decreased, resulting from the formation of an ice lens in the shoot crown immediately below the primordial shoot, a phenomenon Sakai termed extraorgan freezing. As the bud is further dehydrated by the loss of water to extraorgan freezing, the minimum temperature at which the buds can supercool (the minimum subzero temperature tissues can withstand without freezing) is decreased. If freezing occurs rapidly, bud dehydration is limited and maximum supercooling will not be achieved. Under the relatively rapid cooling rate used in this and other artificial freeze-testing studies, buds may suffer cold injury at much higher temperatures than would occur under slower cooling rates.

While extraorgan freezing has not been documented for Douglas-fir, we have repeatedly observed significant genetic

variation for the presence of a line of tissue discoloration in the shoot crown immediately below the primordial shoot (unpublished data). This discoloration may result from ice formation during extraorgan freezing. A test of bud injury involving a cooling rate of 5°C/day rather than per hour may reveal stronger family differences in bud cold hardiness than were found under the freezing protocol in this study, and detect midwinter cold hardiness levels for buds that are more comparable with those of needles and stems. It is unlikely in the relatively maritime climate of the Pacific Northwest that midwinter temperatures sufficiently low to inflict substantial cold damage in buds would occur (below −25°C), so this mechanism is probably of little importance in this region. However, the mechanism of continued bud acclimation following extraorgan ice development may be important for Douglas-fir in colder, more continental climates where very low temperatures may develop slowly and persist.

Genotype-by-environment interaction for fall cold hardiness appears to be minor. The significant family-by-year interactions in September cold injury were most probably due to the large differences in air temperature and moisture regimes between the 1992 and 1993 growing seasons. However, the average type B genetic correlation between years remained quite high (0.86) indicating these interactions reflect mostly scale effects and not changes in family rankings over years. No significant family-by-year interactions in cold injury were detected in October. Thus, it appears that ranking families for fall cold hardiness in one year will predict their hardiness in other years. Likewise, genetic correlations in cold injury between test sites were strong, especially in early fall months, indicating that family rankings (in this study) remained stable across test sites as well. Needles exhibited more genotype-by-site interaction than stems or buds. In our earlier study of October cold injury in Washington State we found considerable family-by-site interaction for needle cold injury in one of two breeding zones studied (Aitken et al. 1996). This indicates that stem or bud cold injury may rank families more reliably in early fall and that basing family cold hardiness ranking on needle injury alone should be done with caution.

Differences among families in levels of cold hardiness and the consistency of these differences between sampling dates during the fall (i.e., little genotype-by-sampling date interaction) indicate that acclimation rates do not vary greatly among families. Family differences in injury scores during the acclimation period likely result from genetic variation in the timing of initiation of the acclimation process, particularly in early to middle fall, when cold injury is most likely to occur (Timmis et al. 1994). Different families appear to harden at fairly similar rates, but likely initiate hardening at different times. They may also start the acclimation process with different minimum hardiness levels, but we did not assess hardiness sufficiently early (e.g., in August) to test this hypothesis. However, the fact that the correlation between dates was somewhat stronger between adjacent months, (i.e., September–October and October–November higher than September–November) suggests that acclimation rates may vary slightly among families over longer periods of time. It also may indicate that some families are continuing to harden in late fall while others have stopped. Nonetheless, these results indicate that scoring hardiness on a single date, ideally in midfall, will provide good information on family differences for fall hardiness.

One factor affecting fall cold hardiness that was not investigated in this study is the occurrence of secondary (lammas) growth. There was essentially no second flushing in 1992, presumably because of the very low available late summer soil moisture. In 1993, approximately 10% of all terminal buds on first-order lateral shoots second flushed in the coastal zone and 19% in the Cascade zone. Needle and stem tissues on lateral shoots that have second flushed are significantly less cold hardy in midfall than on shoots with only a single flush on the same tree (S.N. Aitken and W.T. Adams, unpublished data; Larsen 1978*a*).

Genetic correlations of midwinter cold injury with cold injury in the fall were generally low (Table 2), suggesting that fall and midwinter cold hardiness are largely under separate genetic control. In addition, midwinter cold hardiness is weakly inherited (Table 1). Thus, selection for fall cold hardiness is expected to have little or no impact on hardiness to extreme temperatures in the winter. As coastal Douglas-fir suffers little cold injury midwinter in the Pacific Northwest (Timmis et al. 1994), there is probably no need to consider midwinter hardiness as a separate trait in breeding programs.

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