Spring cold hardiness under strong genetic control in Oregon populations of *Pseudotsuga menziesii* var. *menziesii*[1]

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Abstract: Genetic variation in spring cold hardiness of shoots prior to bud break was studied in two Oregon breeding populations of *Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco, one on the west slope of the Cascade Mountains and the other in the Coast Range. In March and April 1993, and April 1994, shoot cuttings from 40 open-pollinated families in each of two progeny test sites in each breeding zone were subjected to artificial freezing. Visible cold damage to needle, stem, and bud tissues was recorded. Date of bud burst (all sites), and injury resulting from a 1992 natural frost event (one site), were also recorded. Spring cold injury varied widely among families. Individual heritabilities for spring cold injury scores averaged 0.76 in the Coastal zone and 0.42 in the Cascade zone. Genetic correlations among tissues, sites, sampling dates, and years, and between April cold injury and date of bud burst were high, in most cases over 0.80. Correlations were also strong between natural frost damage in 1992 and artificial cold injury scores in 1993. Artificial freeze testing stem tissues of cut shoots sampled in April from a single test site should effectively rank families in this region for spring cold hardiness.

Résumé : Les auteurs ont étudié la variabilité génétique pour le caractère de résistance au froid printanier des pousses avant leur débourrement au sein de deux populations d'élevage de *Pseudostuga menziesii* var. *menziesii* (Mirb.) Franco de l'Oregon, l'une du versant ouest de la chaîne de montagnes des Cascades et l'autre de la chaîne côtière. En mars et avril 1993 ainsi qu'en avril 1994, des pousses ont été prélevées et soumises au gel induit. Ces pousses étaient représentatives de 40 descendances uniparentales pour chacune de deux plantations comparatives de descendances situées dans chacune des zones d'amélioration. Les dommages visibles infligés par le froid aux aiguilles, à la tige et aux tissus des bourgeons ont été notés. Les dates de débourrement des bourgeons (pour tous les sites) et les dommages résultant d'un gel naturel en 1992 (sur un site) ont également été notés. Les dommages résultant du froid printanier variaient passablement d'une descendance à l'autre. Les héritabilités individuelles pour les indices de dommages résultant du froid printanier affichaient des moyennes de 0,76 pour la zone côtière et de 0,42 pour la zone des Cascades. Les corrélations génétiques furent estimées entre les différentes sources de tissu, entre les sites, entre les dates et les années d'échantillonnage, et entre les dommages dus au froid d'avril et les dates de débourrement des bourgeons. Ces corrélations affichaient des valeurs élevées, de plus de 0,80 dans la plupart des cas. Les corrélations étaient également élevées entre les dommages dus au gel naturel de 1992 et les indices de dommages résultant du gel induit en 1993. Les auteurs en concluent que la procédure impliquant la mesure des dommages infligés par un gel induit aux tissus de pousses récoltées en avril à partir d'une seule plantation comparative apparaît être efficace afin d'ordonner les familles de cette région quant à leur résistance au froid printanier.
[Traduit par la Rédaction]

Introduction

Most coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco) tree improvement programs in the Pacific Northwest are just completing the first generation or entering the second generation of breeding, testing, and selection (Woods 1993). In the first generation, selection focused on improving volume growth, with some attention to tree form and wood density. Breeding zones in the first generation were typically small geographically (Adams et al. 1990), and thus improvement programs started with local, presumably well-adapted populations. As these programs enter the second generation of breeding and beyond, there is interest in increasing the size of breeding zones or in moving genetic material among zones. With the prospect of deploying improved materials over greater environmental distances, there is a need to characterize genotypes for traits related to adaptation to environmental stresses (Wheeler et al. 1990). Both the possibility that selection for increased growth rate may result in indirect, but unfavorable, changes in adaptive traits (e.g., Aitken and Adams 1995*b*), and the potential for major climate change, increase the need for such screening. Methods of screening adaptive traits need to be simple because thousands of progeny are typically assessed in each generation of a tree improvement program.

One adaptive trait of considerable importance is spring cold hardiness. In some years, spring frost events cause considerable damage to young plantations of coastal Douglas-fir (Timmis et al. 1994). This injury can occur prior to bud burst, as tissues deacclimate in the spring, or after bud burst, as damage to newly flushed shoots. Bud phenology has been widely used

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as a surrogate for spring cold hardiness (Campbell 1974), and genetic variation in bud phenology has been investigated both among populations (e.g., Campbell and Sugano 1979) and among families within populations (Li and Adams 1993). What has not been adequately investigated is the genetics of spring cold hardiness prior to bud break and whether families vary significantly in the rate of deacclimation. In addition, while deacclimation of seedlings has received considerable attention (e.g., Schuch and Duryea 1989), temporal patterns of cold hardiness in older trees growing under operational forestry conditions remain relatively unexplored.

This study was undertaken to assess the potential of increasing spring cold hardiness in Oregon breeding populations of coastal Douglas-fir by screening genotypes with relatively simple, artificial freeze tests of cut shoots from sapling-age progeny tests. Of interest was the extent to which cold hardiness prior to bud burst is genetically variable and under genetic control, and the degree to which family rankings for spring cold hardiness are stable over shoot tissues, test sites, sampling dates within years, and between years. It was also of interest to evaluate how well rankings based on artificial freeze testing might predict damage from actual early spring frost events in the field. This is one of a series of studies of the genetics of cold hardiness in coastal Douglas-fir (see Aitken et al. 1996; Aitken and Adams 1995*a*, 1995*b*, 1996).

Materials and methods

Breeding zones and test sites

Details on genetic materials and artificial freeze-testing methodologies used in this study were described in Aitken and Adams (1996). We provide only a summary here. Two lower elevation breeding zones in western Oregon were investigated, one in the Coast Range (Coast) and the other on the lower west slope of the Cascade Mountains (Casc). Models developed by Timmis et al. (1994) indicate that both of these breeding zones include areas susceptible to damage from spring frost. Because of the large total number of families included in the progeny tests, families in each breeding zone were grouped into 30-family sets to allow for effective within-set comparison and selection of superior families. In the Coastal zone families were assigned to sets randomly, while in the Cascade zone families were grouped based on geographic origin within the breeding zone. In each breeding zone, 20 families from each of two 30-family sets were sampled (40 families per zone; 80 families total). Sets and families chosen for sampling were based entirely on the availability of seed in storage for subsequent seedling projects (now in progress).

Families were sampled at one higher (H) and one lower (L) elevation test site in each zone (Coast-H (500 m), L (250 m), Casc-H (650 m), L (400 m)) in order to compare family development and loss of cold hardiness under very different environmental conditions. 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 was represented by a four-tree noncontiguous subplot. 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 five replications. Within each block, families were represented by four-tree noncontiguous plots.

One-year-old seedlings were planted in both breeding zones in 1987. By age 7 (1992), mortality ranged from 6% at Coast-L to 20% at Casc-H. The higher mortality rate at the Casc-H site appeared to be due to repeated frost injury, primarily in several large frost pockets within the site, in which trees had suffered repeated cold injury resulting in slow growth or death (Balduman 1995). Considerable spring frost injury was observed on this site in 1992 (see section Natural frost damage). 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 during the study period using Omnidata Datapod 212 temperature recorders.

Artificial freeze tests

Freeze tests were conducted on cut shoots collected from all test trees in the four sites during each of three sampling periods: March and April 1993 and April 1994. In both breeding zones, the higher elevation test sites were sampled 1 to 2 weeks after the low-elevation sites in order to sample trees at similar stages of deacclimation and to facilitate the processing of large numbers of samples. In each sampling period, two shoot tips 5 cm long (one sample for each of two freeze testing temperatures) were collected from second-order (usually unshaded) laterals on the east side of trees, at a fixed midcrown height at each site (1.0 to 1.7 m). All samples from an individual site were collected in 1 day, placed in plastic bags, and transported to Corvallis 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 cool storage should not have significantly affected shoot hardiness (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. Shoots were grouped into packets by family set and block, with approximately 1.5 packets per set per block combination. The packets were placed on a thick aluminum shelf in a computer-controlled Forma Scientific Model 8270/859M freezer with a West M3750 temperature controller, and held overnight at –2°C. In the morning, the temperature was lowered 3 to 5°C per hour (Glerum 1985), until the selected test temperature was reached. The test temperature was held constant for 1 h. Packets were then removed from the freezer and placed at 2°C overnight in a refrigerator 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).

To determine appropriate test temperatures, preliminary samples were taken from each test site 1 week before the main test dates. This preliminary collection consisted of four shoots from each of 15 random trees. These shoots were tested at four freezing temperatures; and by interpolation, two temperatures (4 to 5°C apart) expected to result in intermediate (20 to 80%) damage averaged across all tissues were chosen for the main test. Sampling dates and selected test temperatures in March and April 1993 are illustrated in Fig. 1. Test temperatures in April 1994 were the same as in April 1993 (–10 and –14°C).

Scoring freeze damage

Visual discoloration was used to score cold injury in needles, stems, and buds (Calkins and Swanson 1990). 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 (Aitken et al. 1996). Cold injury in the three tissues was assessed independently, with the percentage of damaged needle, stem, or bud tissue estimated to the nearest 10% (Aitken and Adams 1996). All visual scoring was done by two individuals, with all samples from the same replication scored by a single person.

Date of bud burst

In April and May of 1993, bud burst was recorded biweekly on a single, marked branch of each tree at approximately the same crown position (height and aspect) that shoot samples for cold hardiness were collected. Date of bud burst was recorded as the Julian date at which new needles were first observed through the bud scales of the terminal bud on the marked branch.

Natural frost damage

In May of 1992, damage was observed on many trees at Casc-H resulting from a frost event earlier in the spring, likely in late April or early May. Damage mostly occurred as killed buds, but was also evident in injury to newly flushed shoots. While there is evidence that light frost events affecting the lower crown within approximately 0.5 m of the ground are relatively common on this site, particularly in frost pockets (local topographic depressions), this was the only observed event in a 2-year period that resulted in damage across the full height of crowns of many trees. The majority of trees in the plantation had some observable injury. Damage was scored in late May as the percentage of first- and second-order laterals that had frost-damaged tips. Additionally, it was noted if the damage was to unflushed buds, to newly flushed shoots, or to both buds and shoots.

Statistical analysis

For individual test dates, analyses were conducted on artificial cold injury scores at each of the two test temperatures separately, as well as for the average score over both temperatures. Plotting of residuals indicated non-normality 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 arc-sine square root transformation before analysis. Natural frost damage scores in 1992 also required the arc-sine square root transformation. After transformation, all traits conformed well to the assumptions of analysis of variance (Steel and Torrie 1980). In the 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 (genetic) differences and family by test site interaction in artificial cold injury scores, 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). Family effects were tested using family by site interaction as the appropriate error term. 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 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 overall mean

t_i is random effect of the ith test site; the expected value of t_i ($E(t_i)$) = 0 and the variance of t_i (Var(t_i)) = σ_t^2

s_j is random effect of the jth set; $E(s_j) = 0$, Var(s_j) = σ_s^2

st_{ij} is random interaction effect of the jth set with the ith test site; $E(st_{ij}) = 0$, Var(st_{ij}) = σ_{st}^2

b_{ijk} is random effect of kth block within the jth set and ith test site; $E(b_{ijk}) = 0$, Var(b_{ijk}) = σ_b^2

f_{jl} is random effect of the lth family within the jth set; $E(f_{jl}) = 0$, Var(f_{jl}) = σ_f^2

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

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

w_{ijklm} is random tree error of the mth tree in the $ijkl$th plot; $E(w_{ijklm}) = 0$, Var(w_{ijklm}) = σ_w^2

and the covariances between all pairs of factors were assumed to be zero. Natural frost damage scores for Casc-H were subjected to a similar analysis of variance, but with all terms involving test site removed from the model.

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 random effect of the kth block within the ith test site; $E(b_{ik}) = 0$, Var(b_{ik}) = σ_b^2

sb_{ijk} is random interaction of the jth set with the kth block in the ith test site; $E(sb_{ijk}) = 0$, Var(sb_{ijk}) = σ_{sb}^2

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

Analyses of variance were also conducted using the restricted maximum likelihood (REML) estimator in SAS procedure VARCOMP (SAS Institute Inc. 1988) in order to estimate variance components. Individual tree heritabilities 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 (rather than four) times the family variance, as open-pollinated progeny are more closely related than half-sibs (Campbell 1979). This heritability is appropriate when individual-tree traits are corrected for block means (in this study by including block effects when estimating variance components) and selections are made only among trees within sets. The standard errors of heritability estimates were calculated following Becker (1984).

Genetic correlations (Burdon 1977) were estimated in a number of cases when family differences were significant for both traits of interest. When both traits were measured on the same individual, type A genetic correlations (r_A) were calculated. These correlations were used to evaluate consistencies of family rankings for cold injury scores between different test temperatures on the same sampling date, between different tissues, and between the same sampling period in different years. To assess the potential for predicting susceptibility to spring frost by artificial freeze testing, r_A's between 1993 cold injury scores from Casc-H and 1992 natural frost damage scored on the same site were calculated. Type A genetic correlations between cold injury scores of the same trait assessed in March and April 1993 were used to examine the degree to which families deacclimate at different rates in the spring. A high r_A between sampling dates indicates that families are losing hardiness at similar rates. Finally, r_A's were estimated between spring cold hardiness traits and midwinter cold injury (assessed by freeze testing in January 1993; Aitken and Adams 1996), to investigate the extent to which cold hardiness in these two developmental stages are under similar genetic control. Standard errors of genetic correlations were estimated following Becker (1984).

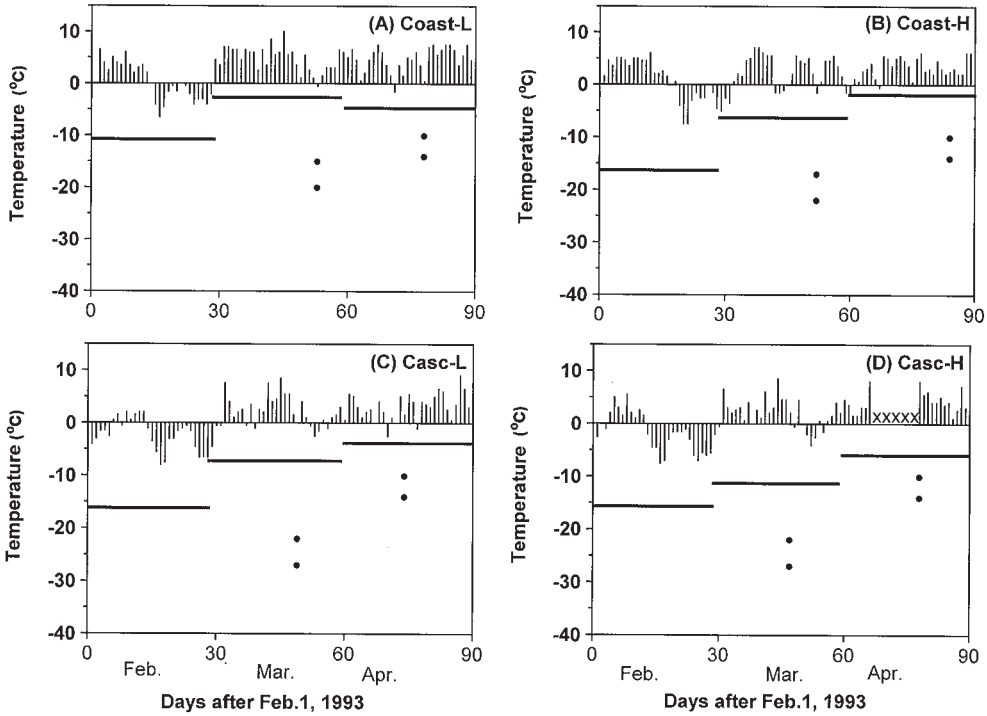
When two traits are measured on different individuals in the same family, type B genetic correlations r_B can be estimated (Burdon 1977). These correlations were used to evaluate stability of family rankings for cold injury across test sites. They were also used to investigate the degree to which cold injury scores from Casc-L (1993) and natural frost damage in Casc-H (1992) are genetically associated.

Results

Climatic conditions during the study period

The period of January through April 1993 was considerably cooler than the same period in 1994. For example, in both March and April, accumulated degree days above 10°C in Corvallis, Oregon, were almost twice as high in 1994 as in 1993. Despite this year-to-year climatic variation, results of genetic analyses, and estimates of genetic parameters for cold injury scores in April 1993 and April 1994 were very similar. Thus, we emphasize findings for spring cold hardiness assessments

Fig. 1. Minimum daily temperatures at each test site (Feb. 1 through April 30, 1993; vertical bars); artificial freeze testing sampling dates and test temperatures (filled circles); and extreme minimum monthly temperatures from 1961 through 1990, recorded at the nearest weather station to each test site (horizontal bar). Missing minimum temperature data are indicated by x. Note that the nearest weather stations (Newport for Coast-L, Mary's Peak for Coast-H, Silver Falls for Casc-L, and Cascadia for Casc-H) were, on average, 30 km from the test sites and 155 m lower in elevation.



in 1993 and discuss the 1994 results only in the context of the consistency of family rankings between years.

Sites did not vary greatly in minimum temperatures from February through April 1993, and no temperatures occurred during this period that were sufficiently low to cause substantial cold injury at the test sites (Fig. 1). Weather records for the closest long-term weather station to each progeny test site show that the 30-year extreme minimum temperatures (1961–1990) for each month during this period are generally considerably colder than the temperatures that occurred at the test sites in 1993 (Oregon Climate Service, Oregon State University); however, these extremes are warmer than the freeze test temperatures used in this study.

Family variation in spring cold injury scores

The range over families in cold injury score was usually considerable (three to four fold) for both freezing temperatures applied on any one sampling date. The higher test temperature resulted in around 20% injury, on average, while the lower resulted in around 70% injury (e.g., Fig. 2). Despite the wide spread in damage levels, estimated genetic correlations between injury scores for the two test temperatures were always high, averaging 0.97 over the 17 combinations for which estimates could be calculated (3 test periods × 2 breeding zones × 3 tissues = 18 total). In addition, individual heritability estimates were higher when cold injury scores were averaged over the two test temperatures than when treated separately. Thus, all results that follow are based on analyses of individual-tree cold injury scores averaged over two test temperatures (see also Aitken and Adams 1996, for discussion of the treatment of two test temperatures).

In all cases except one (buds, Cascade Breeding Zone, March 1993), intermediate levels of cold damage were obtained by artificial freeze testing and family differences in cold injury scores were large and significant (Table 1). Mean bud injury was higher than desired (95%) in the March 1993 samples from the Cascade Breeding Zone, while only low to intermediate levels of damage were observed in needles (64%) and stems (29%) subjected to the same freezing temperatures. Differences among families in both breeding zones were generally greatest for stem injury, and somewhat lower for needles and buds. In some cases, the hardiest family experienced almost no injury, while the least hardy family had very high levels of injury (e.g., April 1993 stem injury score in the Coastal zone).

Estimated individual heritabilities for cold injury scores in March and April were similar and strong in the Coastal zone (mean $h^2 = 0.76$, range 0.53 to 1.0). Heritability estimates were somewhat lower in the Cascade zone (mean = 0.42, range 0.36 to 0.54). Stem cold injury appears to be under the strongest genetic control of the three tissues, on average (mean $h^2 = 0.98$ across sampling dates and tissues in the Coastal zone and 0.46 in the Cascades). In the Coastal zone, estimated heritabilities were intermediate for buds (mean $h^2 = 0.75$) and lowest for needles (0.55). In the Cascade zone, needle injury scores (mean $h^2 = 0.42$) were somewhat more heritable than bud injury scores ($h^2 = 0.36$; April 1993 only).

Genotype by environment interaction

Family by site interactions in 1993 were significant in only one-quarter of the cases examined (3 of 12 possible combinations of breeding zones, tissues, and dates) (Table 1). Significant

interaction was detected for needle injury scores in March for the Coastal zone, and for stem injury in March and needle injury in April in the Cascades (Table 1). Family by site interaction appeared to result in little change in rank of families between sites for cold hardiness, as genetic correlations between sites were over 0.88, with the exception of stem cold injury in March in the Cascades ($r_{Bt} = 0.67$).

Genetic correlations between tissues

Estimated genetic correlations among injury scores for needle, stem, and bud tissues were high in both zones, in both March and April (mean $r_A = 0.89$, range 0.74 to 0.98) (Table 2*a*). This indicates that families rank similarly for spring cold hardiness when different tissues are tested and scored, and that families vulnerable to cold injury in one tissue are likely to suffer damage to other tissues as well.

Correlations between spring sampling dates in the same and different years

Cold injury scores in March and April 1993 were very strongly correlated ($r_A > 0.95$) in both breeding zones for all tissues (Table 2*b*), indicating that families deacclimate at very similar rates in the spring and thus rank similarly on different sampling dates. Despite substantial differences in spring weather between 1993 and 1994, genetic correlations between the same tissues scored for artificial freeze damage in the two years were very high (over 0.95) (Table 2*c*). This indicates that families ranked very similarly when freeze tested in different years.

Correlations between midwinter and spring

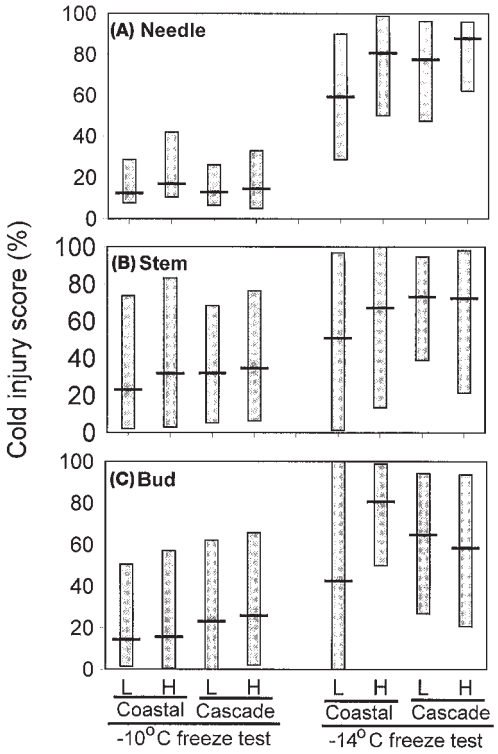
Spring cold injury scores for needles and stems appeared to be relatively uncorrelated with midwinter (maximum) cold hardiness in the Cascade zone ($-0.27 \le r_A \le 0.34$), and only moderately correlated in the Coastal zone ($0.30 \le r_A \le 0.73$) (Table 2*d*). Genetic correlations between midwinter and spring bud cold injury scores could not be estimated because midwinter bud injury scores did not differ significantly among families in either zone.

Correlations between spring cold injury and bud burst timing

The mean date of bud burst was similar in the two zones (May 14 in the Coastal zone, May 13 in the Cascade zone). However, the range among family mean dates of bud burst was much higher in the coastal zone, ranging from May 1 to May 24 in the Coastal zone, and from May 6 through May 17 in the Cascade zone. Bud burst timing was under strong genetic control in both breeding zones ($h^2 = 1.0$; SE = 0.33 in the Coastal zone; $h^2 = 0.70$; SE = 0.20 in the Cascade zone).

Genetic correlations between spring cold injury scores for all tissues and date of bud burst were strong and negative, with genotypes that break bud early having high cold injury scores, for all tissues for both the March and April sampling dates (mean $r_A = -0.90$, range –0.79 to –0.96; Table 2*e*). In the Cascade zone, correlations between date of bud burst and cold injury scores were similar to the Coastal zone in April (mean $r_A = -0.83$, range from 0.70 to 0.92) and for March stem cold injury ($r_A = -0.86$), but were considerably weaker for March needle cold injury ($r_A = -0.28$). Needle cold injury scores had, on average, weaker correlations with date of bud burst than stem and bud tissues.

Fig. 2. Means (horizontal bar) and family ranges, by test site, for cold injury scores to (A) needle; (B) stem; and (C) bud tissues of shoot cuttings after artificial freeze tests conducted in April 1993 at –10 and –14°C.



Family variation in natural frost injury at Casc-H

Seventy-two percent of the trees at Casc-H were damaged by the frost event in May 1992. On average, 11.4% of shoot tips (buds or newly flushed shoots) were injured, with damage scores varying significantly among families ($p < 0.001$) (family range: 2.3 to 42.2%). The frost event appeared to have occurred early during bud flushing, as 39% of the trees had frost-killed buds, 25% had both frost-killed buds and newly flushed shoots, and 8% had damage only to newly flushed shoots.

Estimated individual heritability for natural frost injury to shoot tips at Casc-H site was 0.56 (SE = 0.20), similar to the heritability for stem cold injury scored in April 1993 artificial freeze testing of samples from both Cascade sites. In addition, natural frost injury at Casc-H had strong genetic correlations with 1993 stem and bud cold injury scores of shoot samples from both Casc-H ($r_{A(stems)} = 0.83$ (SE = 0.08); $r_{A(buds)} = 0.90$ (SE = 0.05)) and Casc-L ($r_{B(stems)} = 1.0$; $r_{B(buds)} = 0.93$). Genetic correlations between natural frost injury and needle injury score were lower ($r_A = 0.59$ (SE = 0.15) for Casc-H and $r_B = 0.48$ for Casc-L).

Discussion

Spring cold hardiness of coastal Douglas-fir in Oregon is under strong genetic control, varies greatly among families, and can be evaluated using the relatively simple freeze testing techniques described here. Thus, there is much potential for improving spring cold hardiness in coastal Douglas-fir through selection and breeding. Using existing progeny tests to obtain

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

	Tissue	Breeding zone	Cold injury scores		Estimated variance components[a,b]					
			Mean	Fam. range	σ^2_f	σ^2_{ft}	σ^2_e	σ^2_w	h^{2c}	r_{Bt}
March 1993	Needle	Coast	39.8	23.3–62.7	0.00871***	0.00065*	0.00151**	0.03861	0.53	0.93
		Casc.	64.1	41.7–77.5	0.01010***	0.00030	0.00044	0.05798	0.44	0.99
	Stem	Coast	28.3	8.6–78.2	0.03530***	0.00072	0.00164	0.07197	0.97	0.98
		Casc.	29.5	12.1–57.1	0.00951***	0.00464***	0.00097	0.06258	0.37	0.67
	Bud	Coast	52.3	19.1–94.8	0.04846***	0.00032	0.00543*	0.14123	0.74	0.99
		Casc.	95.2	88.1–99.1	0.00064	0.00090	0.00026	0.04455	—	—
April 1993	Needle	Coast	42.3	28.3–65.8	0.00701***	0.00071	0.00046	0.02886	0.57	0.91
		Casc.	48.2	36.4–58.4	0.00352***	0.00047***	0.00037	0.02133	0.41	0.88
	Stem	Coast	43.4	5.1–88.3	0.05526***	0.00223	0.00315	0.10028	1.0[d]	0.96
		Casc.	53.1	23.8–83.8	0.02624***	0.00110	0.00060	0.11841	0.54	0.96
	Bud	Coast	29.5	22.8–72.5	0.05314***	0.00496	0.00253	0.14746	0.77	0.92
		Casc.	43.0	20.4–72.7	0.02773***	0.00071	0.00457	0.19638	0.36	0.95

Note: Means and family ranges are based on 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. The asterisks indicate whether corresponding *F*-statistics for sources of variation were significant at the 0.05 (*), 0.01 (**), or 0.001 (***) level when tested using the SAS GLM procedure.

[b]σ^2_f, family variance; σ^2_{ft}, family by site variance; σ^2_e, plot (error) variance; and σ^2_w, 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 0.15 to 0.33 (mean = 0.22).

[d]h^2 estimate over 1.0.

shoot cuttings for artificial freeze testing is less expensive than establishing new genetic tests for cold hardiness assessment and more efficient than waiting for unpredictable natural frost events to result in levels of damage adequate for detecting family differences. In addition, results are highly repeatable across sampling dates, years, test sites, and tissues; and families do not appear to differ substantially in rates of deacclimation during the spring (Table 2). This stability of family ranking over sampling dates and test sites indicates that families in this region can effectively be assessed for spring cold hardiness by artificial freeze testing of samples from an individual test site collected on a single date prior to bud break in midspring (i.e., April). As genetic correlations of cold injury scores among needles, buds, and stems appear to be high, scoring a single tissue should be adequate for ranking cold hardiness of shoot tips. We recommend scoring stems (discoloration of cambium and phloem) because stem injury consistently had a high heritability in this study (especially in the Coastal zone), and because damage to stems has greater long-term consequences for the tree than damage to either needles or buds.

The consistency of family rankings for cold injury between artificial freeze tests conducted in 1993 and natural frost injury observed at Casc-H in 1992 also indicates strong genetic control of spring cold hardiness. Natural frost injury at Casc-H not only showed a strong genetic correlation with injury scores of artificially freeze-tested shoots from the same site, but also with injury scores for shoots from the milder, more productive, low-elevation Cascade site (Casc-L). These data indicate that artificial freeze tests of shoot samples from trees grown in relatively mild environments can adequately predict vulnerability of families to spring frost damage in harsher (colder) environments.

Comparison of average hardiness levels on each site with 30-year extreme minimum temperatures during the deacclimation period indicates that for most of the deacclimation process, the risk of cold injury at the four test sites is low. The relative risk of cold injury is highest at the Casc-H site and lowest at the Coast-L site, because of both faster deacclimation of the families in the Cascade zone and colder minimum temperatures at Casc-H. For all sites, the risk of cold injury is highest late in the deacclimation process in late April and May (Timmis et al. 1994). While the majority of trees on all of the test sites would be expected to withstand the historic minimum temperatures recorded at nearby weather stations, it is important to note that the least hardy families might suffer extensive injury at these temperatures. In addition, it is important to note that long-term weather stations tend to be located in populated areas at somewhat lower elevations than many forested sites, and thus minimum temperatures recorded are typically higher than those that would be observed on harsher sites or in localized frost pockets.

In March, the trees in the Cascade zone were considerably hardier than trees in the coastal zone, requiring test temperatures 5 to 7°C lower to produce similar levels of cold injury. By the April sampling date, the situation had reversed, with trees from the Cascade breeding zone suffering higher levels of cold injury at the same test temperature as coastal genotypes growing in the Coast range. These differences in relative cold hardiness levels are likely the product of genetic as well as environmental differences. It is well documented that seed sources further from the Pacific Ocean break bud sooner than those from more coastal areas when grown in a common garden test (Campbell and Sugano 1979; Balduman 1995). In April, trees from the higher elevation test site in each zone suffered slightly more damage, on average, than those from the lower elevation sites, but this is most likely due to the fact that the low-elevation test sites were sampled 1 to 2 weeks prior to the high-elevation sites.

One prominent difference between the two breeding zones was that the Coastal zone showed a moderate, positive correlation

between midwinter and spring cold injury, while these traits were weakly and inconsistently associated in the Cascade population (Table 2). The positive genetic association in the coastal zone indicates that family rankings for cold hardiness are roughly similar in these two developmental stages. This may mean, perhaps, that the trees in the Coastal population had already begun to deacclimate by the end of January, while those in the Cascade zone were still in the depths of hardiness at that time. It also means that selection for spring cold hardiness in Coastal populations may lead to an increase in midwinter cold hardiness, but will leave midwinter hardiness of Cascade populations unchanged. Spring cold injury is of much greater concern than midwinter injury in any event, as most cold injury causing significant economic loss in coastal Douglas-fir occurs in late spring or in fall (Timmis et al. 1994).

It appears that spring cold hardiness is under considerably stronger genetic control than cold hardiness in the fall. Heritability estimates for midfall (October) cold injury scores in a study conducted with the same breeding zones and test sites were all under 0.40 and averaged 0.27 (Aitken and Adams 1996). The amount of variation among families in cold injury score is also greater in the spring than in the fall. It is not clear why spring cold hardiness is under stronger genetic control than fall or midwinter hardiness. One possible explanation lies in the different environmental factors that affect acclimation and deacclimation. Fall cold acclimation is primarily a function of genetic response to cool temperatures after cessation of growth due to photoperiod (Sakai and Larcher 1987), but can also be influenced by any environmental factor affecting the cessation of growth in the summer, including available moisture (Glerum 1985; Burr 1990). The deacclimation process is under somewhat simpler environmental control; loss of hardiness up until the time of bud burst is largely controlled by genetic response to temperature sum after chilling requirements have been fully met (van den Driessche 1969; Burr 1990). Douglas-fir genotypes have been shown to differ in both the chilling sum required to break dormancy and the heat sum necessary for bud break (Campbell and Sugano 1979).

The high heritabilities for spring cold injury suggests that fewer genes may control spring cold hardiness than fall cold hardiness. Two studies are underway that will investigate this question. Quantitative trait loci (QTL) are being mapped for Douglas-fir for fall and spring cold hardiness and bud phenology at both Oregon State University (S. Strauss and W.T. Adams) and the USDA Forest Service Institute of Forest Genetics (D.B. Neale).

The cold hardiness of needle, stem, and bud tissues are all strongly correlated in the spring, but correlations among tissues in the fall are weaker (Aitken and Adams 1996). Thus, it appears that different shoot tissues deacclimate in synchrony in response to the cumulative effects of chilling and heat sum, whereas cold acclimation rates and timing vary somewhat among tissues.

The strong correlation between timing of bud burst and cold hardiness during the deacclimation process has important practical implications. If cold hardiness is assessed in the weeks prior to bud burst, those genotypes most likely to flush early and suffer post bud burst cold injury can be accurately identified. Thus, assessing date of bud burst and artificially freeze testing shoot samples appear to be equally effective means of ranking genotypes for cold hardiness, as both methods produce scores with high heritabilities and the two methods are highly correlated. The decision to use one technique or the other in tree improvement programs will likely depend on access to equipment and accessibility of field sites. Assessing bud burst in the field requires repeated visits to field sites, but requires no specialized laboratory equipment. Artificial freeze testing requires sampling genetic tests on just a single date in the spring sometime in the 6 weeks or so prior to bud burst, but depends upon access to a cold hardiness testing freezer with a precise temperature control device. Either method will provide adequate data for ranking genotypes in a breeding program for spring cold hardiness.

Table 2. Estimated genetic correlations (r_A) of transformed cold injury scores between (*a*) different tissues scored at the same time and for the same tissue scored on (*b*) different spring sampling dates (months) in the same year; (*c*) different years, with the same sampling month; and (*d*) different seasons (midwinter vs. spring) in the same year. Also included are (*e*) genetic correlations between spring cold injury scores and date of bud burst.

		Breeding zone	
Sampling date(s)	Tissue(s)	Coast	Cascades
(*a*) Between tissues			
Mar. 1993	Needles vs. stems	0.88 (0.09)	0.74 (0.20)
	Needles vs. buds	0.78 (0.17)	—[a]
	Stems vs. buds	0.94 (0.05)	—[a]
Apr. 1993	Needles vs. stems	0.93 (0.05)	0.85 (0.12)
	Needles vs. buds	0.95 (0.04)	0.80 (0.16)
	Stems vs. buds	0.98 (0.02)	0.98 (0.02)
(*b*) Between spring sampling dates			
Mar. vs. Apr. 1993	Needles	0.95 (0.04)	1.0[b] (0.01)
	Stems	0.99 (0.01)	1.0[b] (0.01)
	Buds	0.98 (0.02)	—[a]
(*c*) Between years			
Apr. 1993 vs. 1994	Needles	0.98 (0.02)	0.98 (0.02)
	Stems	0.99 (0.01)	0.98 (0.02)
	Buds	0.96 (0.03)	0.88 (0.13)
(*d*) Between midwinter and spring			
Jan. vs Mar. 1993	Needles	0.54 (0.31)	–0.02 (0.46)
	Stems	0.73 (0.19)	0.21 (0.43)
	Buds	—[a]	—[a]
(*e*) Between spring cold injury scores and bud burst date			
Mar. 1993	Needles	–0.79 (0.17)	–0.28 (0.43)
	Stems	–0.92 (0.06)	–0.86 (0.12)
	Buds	–0.96 (0.03)	—[a]
Apr. 1993	Needles	–0.86 (0.12)	–0.70 (0.23)
	Stems	–0.92 (0.06)	–0.88 (0.10)
	Buds	–0.92 (0.06)	–0.92 (0.07)

Note: Standard errors of r_A are in parentheses.

[a]Family differences for bud injury score in both zones in January 1993, and in the Cascade zone in March 1993 were not significant ($p < 0.05$), so genetic correlations were not estimated.

[b]r_A estimate over 1.0.

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