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Family variation for fall cold hardiness in two Washington populations of coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco) ☆

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Abstract

In order to assess the genetics of fall cold hardiness in coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco), shoot cuttings were collected in October from saplings (9-year-old trees) of open-pollinated families in two progeny tests in each of two breeding zones in Washington, one in the Coast range (80 families) and one on the west slope of the Cascade Mountains (89 families). Samples from over 5500 trees were subjected to artificial freezing and visually evaluated for needle, stem and bud tissue injury. The extent to which cold injury is genetically related to tree height and shoot phenology (timing of bud burst and bud set) was also evaluated.

Significant family variation was found for all cold hardiness traits; however, individual heritability estimates were relatively low (ranging from 0.09 to 0.22). Significant family-by-test site interaction was detected for needle injury in the Cascade breeding zone, but not in the coastal zone. Genetic correlations (r_A) among needle, stem and bud tissues for cold damage were weak ($0.16 \leqslant r_A \leqslant 0.58$) indicating that genes controlling fall hardening are somewhat different for different tissues. Timing of bud burst and bud set were only weakly correlated with cold injury ($r_A \leqslant 0.49$). Thus, bud phenology is a poor predictor of fall cold hardiness in this species. There was no consistent relationship between tree height and cold injury in the coastal zone. In the Cascade zone, taller trees appeared to be more susceptible to cold injury, but the association was weak (mean $r_A = 0.38$, range 0.20–0.72).

Keywords: Artificial freeze testing; Genetic variation

1. Introduction

The ability of trees to grow competitively, yet withstand low temperature stresses, depends on the synchronization of growth and cold acclimation to the climatic cycle of the local environment (Sakai and Larcher, 1987; Hanninen et al., 1990). Trees need to be able to fully utilize the growing season, yet commence hardening sufficiently early to withstand fall frosts, and reach a sufficiently deep winter hardiness to withstand extreme cold events. The hardening process is controlled by genetically deter-

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mined responses to environmental cues (Sakai and Larcher, 1987). While the phenotypic development of cold hardiness in Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings is well understood (e.g. Burr et al., 1989; Schuch et al., 1989a, b), genetic variation in cold hardiness, particularly within-population variation, has received only limited attention.

Investigations of genetic variation in cold hardiness of Douglas-fir (either by direct observations of cold injury after natural cold events or by laboratory testing) have focused primarily on differences among populations, rather than among families within populations. Significant variation among populations has been observed in both the coastal (var. *menziesii*) (Campbell and Sorensen, 1974; Larsen, 1978a, b; White, 1987; Loopstra and Adams, 1989; Schuch et al., 1989a, b) and interior (var. *glauca*) varieties of Douglas-fir (Larsen, 1978a, b; Rehfeldt, 1979, 1986). In the few studies where cold hardiness has been investigated within populations of this species, significant family differences in cold hardiness have also been found (White, 1987; Wheeler et al., 1990).

Fall cold hardiness is of interest to tree improvement programs for a number of reasons. Fall frost events cause damage and mortality in operational plantations of Douglas-fir in the Pacific Northwest. Timmis and others (1994) modelled frost injury risk for Douglas-fir in western Washington and Oregon and concluded that while, on average, the risk of frost injury at individual sites is two to three times greater in the spring, at some locations the risk of injury or death due to cold events is greatest in the fall. The single most damaging freeze event documented in the region was in November 1955, when even some mature trees were killed by cold (Duffield, 1956). Thus, fall frost can be an important damaging agent, and the potential to reduce fall frost injury by breeding for hardier genotypes needs consideration. In addition, the relationship between fall cold hardiness and stem growth rate is not known. If this relationship is unfavorable, selection based on improved growth rate alone could lead indirectly to reduced fall cold hardiness.

Characterizing genotypes for adaptive traits in tree improvement program requires the development of simple and inexpensive procedures for screening many individuals in a short period of time. There are currently over 40 km^2 of coastal Douglas-fir progeny tests in the Pacific Northwest (Adams et al., 1990). If screening methods can be developed to evaluate cold hardiness using materials from these existing field tests, rather than establishing new seedling tests, screening improved families for cold hardiness would be more feasible.

This study was undertaken to quantify the amount of genetic variation for and degree of genetic control of fall cold hardiness in sapling-aged trees from two breeding populations of coastal Douglas-fir. In addition, we examined the relationship between fall cold hardiness and stem growth in these populations. Because bud phenology is expected to be associated with shoot hardiness (Campbell and Sorensen, 1974), we also investigated the correlations of fall cold hardiness with timing of bud burst and bud set. A final objective was to develop a relatively simple freeze-testing protocol for assessing large numbers of genotypes in existing progeny tests.

2. Materials and methods

2.1. Breeding populations

Two breeding zones from the state of Washington, one in the Coast range (Grays Harbor) and one on the lower west slope of the Cascade Mountains (Snoqualmie) were sampled. Both zones were represented by open-pollinated progeny (families) of parent trees from wild stands. In the Grays Harbor zone, the progeny of 40 parent trees in each of two family sets (80 families total) growing in two test sites (Donovan, 100 m elevation and Rehab, at sea level) were studied. The open-pollinated progeny of 30 parent trees in each of two family sets, and 29 parent trees in a third family set, were investigated in the Snoqualmie zone, also on two test sites: Hogpen (elevation 330 m) and Voight Creek (240 m). At the time of sampling in 1991, the trees in all tests were 9 years old (from seed). Sets were planted in separate randomized complete block designs on each site, with five blocks per set in the Grays Harbor test and four in Snoqualmie. Each family was represented in each block by a four-tree non-contiguous plot at the time of planting. By age 9, mean survival in the sampled sets averaged 97% in the Grays Harbor

breeding zone and 90% in the Snoqualmie zone. In total, 5589 trees were available for fall cold hardiness assessment.

2.2. Artificial freeze tests

In the second and third weeks of October 1991, samples were collected from all test trees in the four sites. This is approximately the time at which the earliest fall frosts occur in this region. A 4 cm terminal shoot was removed from a first-order lateral branch on the north side of the fifth whorl from the top of each tree. These shoots were easily collected from the ground and came from relatively exposed branches. All samples were individually labelled at the time of collection, placed in plastic bags, and transported to Corvallis in ice chests. Samples were stored in Corvallis in a 2°C walk-in cooler for a maximum of 7 days before freeze-testing.

Prior to artificial freeze-testing, groups of 50 samples were wrapped first in damp cheesecloth, then in aluminum foil in flat packets. The packets were placed on a thick aluminum shelf (to facilitate uniform cooling by conduction rather than convection) in a computer-controlled Forma Scientific Model 8270/859M freezer with a West M3750 temperature controller, and held overnight at −1.5°C to equilibrate and allow free water to freeze. The following morning, the temperature was dropped 3°C h^{-1} (Glerum, 1985) until computer-monitored thermocouples placed inside three of the sample packets had an average reading at or just below the selected test temperature. 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 (Burr et al., 1990).

Appropriate temperatures for the freeze tests were determined from preliminary samples collected 1 week before the main sampling dates. Shoots were collected from random trees at the Donovan, Rehab and Voight Creek sites, but not from Hogpen. These samples were tested at a range of four freezing temperatures and damage to needle, stem and bud tissues assessed visually (see below). A single temperature which resulted in intermediate tissue damage in the preliminary tests was chosen for each test site except for Hogpen, for which the temperature determined for Voight Creek was used. The selected temperatures were −10°C and −12°C for the Donovan and Rehab sites respectively, in the coastal breeding zone, and −17°C for both Cascade sites.

2.3. Scoring freeze damage

Each sample was inspected for needle damage through an illuminated three diopter magnifying lens. Then a tangential cut was made lengthwise along the stem to reveal approximately 2 cm of phloem and cambium tissues. Terminal buds were bisected lengthwise to reveal damage to bud tissues (Burr et al., 1990).

Cold injury to needle and stem (cambium and phloem) tissues was assessed independently, but in the same manner. The percentage of damaged tissue was estimated and scored according to one of five classes: 1, no damage; 2, 1–10% of the tissue damaged; 3, 10–40% of the tissue damaged; 4, 40–70% damage; 5, 70–100% damage. Needle damage was evidenced by reddish-brown needles or needle loss. Damage to the cambium and phloem was evidenced by browning or yellowing of the normally greenish tissues.

Bud damage tended to be either absent or total within each of three localized regions of the bud and nearby tissues. These regions were: (1) the primordial shoot, including the shoot meristem and needle primordia (NP); (2) the bud–shoot interface (INT), as seen by a narrow band of browning between bud and stem tissues; (3) below-bud shoot tissues (SH), as indicated by browning in the center of the stem in the relatively undifferentiated tissue within 5 mm of the bud–shoot interface (Fig. 1). Because the pres-

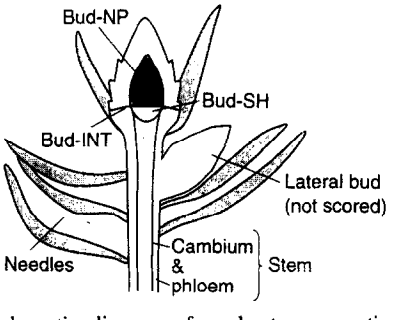

Fig. 1. Schematic diagram of a shoot cross-section showing needle, stem and bud tissues scored for cold injury.

ence of damage in one region was positively and strongly correlated with presence of damage in the other regions, we developed a cumulative bud damage score: 1, no regions damaged; 2, one region damaged; 3, two regions damaged; 4, all three regions damaged.

2.4. Growth and phenology traits

Bud phenology was recorded in the growing season immediately preceding the fall cold hardiness testing and tree heights were measured at the end of the season (Schermann, 1994). All bud phenology assessments were made at the terminal bud of one branch at the fourth whorl from the top of the tree. At this height, buds were fully exposed to sunlight, but could be easily reached from the ground. Presence or absence of bud burst (needles emerged beyond bud scales) and bud set (brown scales visible) were scored on a single day midway in the bud flushing (late April to early May) and bud setting (mid to late May) periods of each site (i.e. when between 25 and 75% of the trees on the site had burst or set buds). The percentage of trees in a family with flushed (or set) buds in this period has a strong negative genetic correlation (r_A) with the mean date of family bud burst (or bud set) ($|r_A| > 0.80$; Li and Adams, 1993). Bud set was not recorded at one of the sites, Voight Creek, because most trees on this site had set bud before it could be visited.

2.5. Statistical Analysis

Analyses of variance and covariance were carried out for the paired test sites of each breeding zone using the GLM procedure of the Statistical Analysis Systems (SAS) Institute Inc. (1988). The following linear model was used to represent individual-tree values for each trait

$$Y_{ijklm} = \mu + t_i + s_j + st_{ij} + b(st)_{ijk} + f(s)_{jl} + f(s)t_{ijl} + e_{ijkl} + w_{ijklm}$$

where μ is the overall mean; t_i is a random effect of the ith test site, $E(t_i) = 0$, $\mathrm{var}(t_i) = \sigma_t^2$; s_j is a random effect of the jth set, $E(s_j) = 0$, $\mathrm{var}(s_j) = \sigma_s^2$; st_{ij} is a random interaction effect of the jth set with ith test site, $E(st_{ij}) = 0$, $\mathrm{Var}(st_{ij}) = \sigma_{st}^2$; $b(st)_{ijk}$ is a random effect of the kth block within the jth set and ith test site, $E(b(st)_{ijk}) = 0$, $\mathrm{Var}(b(st)_{ijk}) = \sigma_b^2$; $f(s)_{jl}$ is a random effect of the lth family within the jth set, $E(f(s)_{jl}) = 0$, $\mathrm{var}(f(s)_{jl}) = \sigma_f^2$; $f(s)t_{ijl}$ is a random interaction effect of the lth family within the jth set with the ith test site, $E(f(s)t_{ijl}) = 0$, $\mathrm{Var}(f(s)t_{ijl}) = \sigma_{ft}^2$; e_{ijkl} is the random plot error of the lth family in the kth block of the jth set of the ith test site, $E(e_{ijk}) = 0$, $\mathrm{Var}(e_{ijkl}) = \sigma_e^2$; w_{ijklm} is a random tree error of the mth tree in the $ijkl$th plot, $E(w_{ijklm}) = 0$, $\mathrm{Var}(w_{ijklm}) = \sigma_w^2$; and; the covariances between all pairs of factors were assumed to be zero.

Inspection of residuals and variances indicated that all traits conformed well to assumptions of analysis of variance (Steel and Torrie, 1980), thus, untransformed variates were analyzed in all cases. Variance and covariance components were estimated from the appropriate mean squares and cross products using the restricted maximum likelihood (REML) procedure in SAS 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 (h_i^2) and family (h_f^2) heritabilities of these traits were estimated following Namkoong (1979). For estimating individual heritabilities, the additive genetic variance (numerator of h_i^2 equation) was estimated as three times the family variance, rather than four times the family variance as appropriate for half-sib progeny, as Douglas-fir 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). The breeding implications of genetic variation for cold hardiness were examined by predicting decreases in needle, stem and bud cold injury when the 20% of the hardiest parent trees are selected and randomly mated to produce progeny (Falconer, 1981).

To further quantify the genetic control of cold hardiness traits, we estimated the extent of family-by-site interaction in these traits by testing the significance of the family (within set)-by-site interaction variance component. We also estimated the Type B

genetic correlation (Burdon, 1977) of the same trait measured in different trees of the same families on two test sites as

$$r_B = \sigma_f^2 / (\sigma_f^2 + \sigma_{ft}^2)$$

When $r_B \approx 1$, families rank identically in both test sites.

To examine the relationships among cold hardiness traits and between these traits and tree height and bud phenology (timing of bud burst and bud set), we estimated genetic correlations (r_A) and the standard errors of these estimates (Becker, 1984). We also estimated family mean correlations (pooled within sets) and their significance ($P < 0.05$).

3. Results

3.1. Variation in cold hardiness

Test temperatures were chosen to inflict, on average, intermediate levels of tissue damage (i.e. with mean percentage of damaged tissues at around 50%). Thus, for needles and stems, mean test-site scores of 3–4 were expected, and for buds, mean scores between 2 and 3. The expected damage levels were achieved for needles, but stems and buds were hardier under the selected test temperatures and were damaged less than expected. On average, only about 10% of stem tissues were damaged as a result of freezing.

Mean injury levels were greatest for samples from the Snoqualmie Hogpen site, where needle, stem and bud injury averaged 4.4, 2.4, and 1.7, respectively. Mean damage in samples from each of the remaining three sites ranged narrowly for both needles (3.3–3.6) and stems (1.8–2.1), and was identical across sites for buds (1.3). Despite the less than desired levels of cold damage in stems and buds, families differed significantly in levels of cold injury in both breeding zones for all three tissues (Table 1). In general, the hardiest families had about half the mean damage score of the least hardy families.

Estimated individual heritabilities of the cold hardiness traits were low in both breeding zones (Table 1), and considerably lower than observed for 9-year-height in the same tests (0.35 (Grays Harbor) and 0.30 (Snoqualmie); Schermann 1994). Corresponding family heritabilities for hardiness traits averaged 0.55 in Grays Harbor ($0.53 \leqslant h_f^2 \leqslant 0.56$) and 0.38 ($0.26 \leqslant h_f^2 \leqslant 0.48$) in Snoqualmie. Heritabilities were consistently lower in the Snoqualmie zone than in Grays Harbor. This is not because test precision at individual sites was less in the Snoqualmie zone, because single-site estimates of heritabilities in the Snoqualmie tests were similar to those in Grays Harbor (results not shown). The lower combined-site

Table 1
Estimates of breeding zone means (family ranges), variance components and significance levels for F-statistics for family (σ_f^2) and family-by-test site (σ_{ft}^2) sources of variation, type B genetic correlations between test sites (r_B) and individual tree heritabilities (h_i^2) for cold injury to needle, stem and bud tissues resulting from artificial freeze testing. Numbers in parentheses following heritabilities are standard errors

Trait	Breeding zone [a]	Mean	σ_f^2	σ_{ft}^2	r_B	h_i^2
Needle injury (1–5)	GH	3.33 (2.23–4.10)	0.080 ***	0.007	0.92	0.20 (0.05)
	SN	3.97 (2.79–4.75)	0.027 *	0.061 ***	0.31	0.09 (0.04)
Stem injury (1–5)	GH	1.97 (1.40–3.03)	0.074 ***	0	1.0	0.20 (0.05)
	SN	2.08 (1.42–3.22)	0.026 **	0.007	0.84	0.09 (0.04)
Bud injury (1–4)	GH	1.32 (1.02–1.98)	0.030 ***	0.002	0.95	0.22 (0.06)
	SN	1.5 (1.08–2.12)	0.026 ***	0.007	0.79	0.16 (0.05)

[a] GH, Grays Harbor; SN, Snoqualmie.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 2
Estimated genetic (below diagonal) and family mean (above diagonal) correlations among cold injury traits and 9-year height, for the Grays Harbor (GH) and the Snoqualmie (SN) breeding zones. Numbers in parentheses following correlations are standard errors

	Breeding zone	Cold injury			9 year height
		Needles	Stem	Buds	
Cold injury					
Needles	GH		0.30 *	0.20	0.08
	SN		0.48 *	0.26 *	0.13
Stem	GH	0.30 (0.14)		0.65 *	−0.17
	SN	0.58 (0.33)		0.51 *	−0.12
Bud	GH	0.16 (0.16)	0.71 (0.08)		−0.02
	SN	0.46 (0.31)	0.46 (0.21)		−0.06
9-year height	GH	0.11 (0.16)	−0.17 (0.15)	0.05 (0.15)	
	SN	0.72 (0.30)	0.21 (0.22)	0.20 (0.18)	

* Family mean correlation is significant, $P \leq 0.05$.

heritabilities in Snoqualmie are due to family-by-site interaction. While estimated type B genetic correlations were never lower than 0.92 between the Grays harbor test sites, they ranged from 0.31 to 0.84 between the Snoqualmie sites. The only significant family-by-test site interaction, however, was observed for needle injury in Snoqualmie. The very low type B genetic correlation in this case suggests that this interaction results in considerable family rank changes in needle cold hardiness between test sites.

Predicted responses to selection for increased cold hardiness in the Snoqualmie breeding zone are generally low. If the top 20% of parent trees are selected and intermated, average needle cold injury would decrease from 3.97 to 3.77. This corresponds to a 6.1% decrease in injury. Predicted selection responses are higher for the Grays Harbor zone. For example, selecting the top 20% of parents for needle hardiness is expected to reduce fall cold injury by 15.1%. Similar family selection responses were predicted for stem and bud hardiness in both breeding zones.

3.2. Correlations between traits

The estimated genetic and family mean correlations in cold injury of different tissues were positive but somewhat weak (Table 2). The correlations were generally strongest between stems and buds (average $r_A = 0.59$), and weakest between needles and buds (average $r_A = 0.31$).

Table 3
Genetic (r_A) and family mean (r_F) correlations between proportions of trees per plot which have burst (BB) or set buds (BS) on a particular day and cold injury traits for the Grays Harbor (GH) and Snoqualmie (SN) breeding zones. Numbers in parentheses following correlations are standard errors

Cold injury	Breeding zone	BB		BS	
		r_A	r_F	r_A	r_F
Needles	GH	0.14 (0.14)	0.12	0.23 (0.14)	0.16
	SN	−0.06 (0.23)	−0.08	−0.02 [a] (0.20)	0.02
Stem	GH	−0.43 (0.12)	−0.38 *	−0.40 (0.12)	−0.36 *
	SN	−0.22 (0.19)	−0.20	−0.02 [a] (0.19)	0.14
Buds	GH	−0.35 (0.13)	−0.30 *	−0.33 (0.13)	−0.27 *
	SN	−0.47 (0.14)	−0.38 *	−0.49 [a] (0.14)	−0.41 *

[a] Based on data from only a single test site (Hogpen). See text.
* Family mean correlation is significant, $P \leq 0.05$.

Cold hardiness was uncorrelated with tree size in the Grays Harbor breeding zone (Table 2). In Snoqualmie, there is evidence of a weak positive relationship between tree size and needle cold injury, with larger trees suffering more cold injury in the artificial freeze tests. Although the family mean correlation between needle injury and 9 year height was near zero, the estimated genetic correlation between these traits was relatively large. The genetic correlations of 9 year height with stem and bud injury were also positive in the Snoqualmie breeding zone, but the estimates were small and no larger than their standard errors.

The proportion of trees per plot with flushed buds on a particular day (BB) and set buds on a particular day (BS) were similarly related to cold injury in the different tissues (Table 3). This is not unexpected because date of bud burst has a high positive genetic correlation with date of bud set in Douglas-fir saplings (Li and Adams, 1993). It is also expected that cold injury would be negatively associated with BB and BS, because families with lower BS (i.e. later mean date of BS) should be more susceptible to fall cold injury. This expectation was met for injury to stems and buds, but the estimated correlations between bud phenology and fall cold damage were relatively weak (Table 3). Fall needle cold injury appears to be essentially unrelated to bud phenology.

4. Discussion

There is substantial genetic variation for fall cold hardiness traits in coastal Douglas-fir; however, estimates of individual heritabilities for these traits were low. Heritabilities of fall cold hardiness traits were particularly low in the Snoqualmie breeding zone, where one trait (needle injury) showed significant family-by-site interaction and the other traits appeared to have been influenced by genotype-by-environment interaction as well. Significant genotype-by-environment interaction in sapling-stage assessments indicates that the choice of test sites may be critical to the validity of family rankings, and furthermore, results based on one or a few test sites may have little relationship to fall cold hardiness of families in operational forest plantations. The family-by-site interaction observed in the Snoqualmie breeding zone may have been due to differences in the timing of cold acclimation in the two sites. Apparently, cold acclimation was more advanced at Voight Creek, since mean cold damage was considerably lower in shoots from this site than from Hogpen when tested at the same temperature.

The topography at Voight Creek suggests that this site may have poorer air drainage and experience colder temperatures in the early fall, thus experiencing accelerated hardiness development relative to the other sites. Available soil moisture can also affect the rate of cold hardening early in the acclimation process, but we have no information to compare sites in this regard. Little genotype-by-environment interaction in fall cold hardiness was observed in two breeding zones in Oregon, despite large differences in moisture and temperature regimes between the test environments in each zone (Aitken and Adams, 1995). Thus, the amount of genotype-by-environment interaction to expect in fall cold hardiness of saplings, and its potential causes, are unclear.

The first phase of cold acclimation is mediated primarily by photoperiod in most woody perennials (Sakai and Larcher, 1987) although other factors resulting in growth cessation such as low available soil moisture also play a role (Glerum, 1985). The second phase of acclimation is initiated by low temperatures and continues largely as a function of temperature. In the Grays Harbor and Snoqualmie breeding zones, the second phase would likely be initiated in late October or early November. Rehfeldt (1979) found that genetic differences in fall cold hardiness among populations of interior Douglas-fir were greatest in the first phase of acclimation. Similarly, subsequent research indicates that differences among coastal Douglas-fir families are greatest at the end of the first phase of acclimation (S.N. Aitken and W.T. Adams, unpublished data, 1993). Thus, the sampling dates in this study (mid-October) correspond to this optimal period for detecting genetic differences among families.

The cold hardiness of different shoot tissues was only loosely related among the three tissues investigated. This suggests that those tissues are acclimating either at different rates or initiating hardening at different times. In either case, it appears that cold hardiness assessed in one tissue is only weakly predictive of hardiness in other tissues. Likewise, bud phenology appears to be a poor predictor of risk of

fall cold injury in coastal Douglas-fir. This is particularly true for needle injury. Phenological traits may be better predictors of fall cold injury in seedlings (Campbell and Sorensen, 1974; Loopstra and Adams, 1989), as seedlings typically set bud much later in the summer than saplings. Bud phenology appears to be highly predictive of spring cold injury in Douglas-fir saplings (Aitken and Adams, 1995).

The low genetic correlations between fall cold injury and tree height suggest that selection for greater tree size will have little or no negative impact on fall cold hardiness, at least in the Grays Harbor breeding zone. There may be a weak but positive relationship between fall cold injury and tree height in Snoqualmie. The extent to which this weak, but unfavorable, association might impact long-term survival and productivity is unclear. Long-term field testing of materials in a variety of environments is necessary to quantify this effect.

Discoloration of tissue is generally considered a reliable indicator of tissue viability following cold injury, and continues to be used as a control for quantitative measurements of cold injury (Calkins and Swanson, 1990). However, great care must be taken when using the subjective method of visual scoring to assess cold injury. Since different individuals may score damage slightly differently, it is important not to use more than one scorer within a replicate of samples and to score samples in a random order within a replicate. Some may argue that more objective, quantitative techniques such as the electrical conductivity method (e.g. Burr et al., 1990) would be more appropriate, in order to enhance repeatability and precision of results. Genetic screening, however, requires the assessment of large numbers of samples in limited time. The major drawback to quantitative techniques is that they are time consuming and allow only one tissue to be sampled at a time. As we have seen in this study, the cold hardiness of one tissue may not be a good indicator of susceptibility of other tissues to fall frost events. Although subjective, the methods employed in this study are repeatable, as evidenced by significant family differences in all traits assessed. The methods also allowed the processing of almost 6000 samples for injury to five different tissues in a 2 week period.

The results of this study led to modifications of the freeze-testing methodology which we formerly employed. We now collect samples from a fixed height in the crown rather than a fixed whorl, as we have found slight but significant within-crown variation in hardiness associated with sampling height, particularly in the lower crown (unpublished data). Damage levels are now recorded to the nearest 10% instead of on a 1–5 scale. We have found that scorers can discriminate more classes of damage, and there are statistical advantages to the resulting increased precision of injury assessment. We now use two shoot samples per tree at each testing date, subjecting each sample to a different freeze temperature. In this study, there was a problem in reliably achieving intermediate levels of damage as preliminary screening for setting test temperatures was done a week before sampling dates and it was difficult to predict how hardiness might change in the interim. Using two temperatures increases the likelihood that intermediate levels of damage will be achieved. Two samples also provide an element of repeated sampling from each tree, as scores are based on two observations rather than one. We have found that damage over two test temperatures results in higher heritabilities for cold injury than with a single temperature. Testing over two temperatures also makes it more likely that intermediate damage levels will be achieved in different tissues that are acclimating at different rates. A final modification in our current methods is to hold samples in the freezer for 1 h at the test temperature, rather than removing the sample as soon as the test temperature is achieved. We feel that longer exposure to the test temperature results in a more uniform freezing of samples, regardless of stem size.

Screening Douglas-fir families for fall cold hardiness can be done simply and relatively economically using the methods described here. A visual screening approach of two or more shoot tissues should provide useful information on the relative ability of different genotypes to withstand a fall frost event. However, to translate cold injury scores based on laboratory freeze testing into effects on survival and productivity will require the installation of long-term field experiments in a variety of environments. Continued work is needed in the development of methods for screening for adaptive traits to ensure the survival and growth of improved genotypes following exposure to environmental extremes.

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References

Adams, W.T., Johnson, G., Copes, D.L., Daniels, J., Quam, R.G., Heaman, J.C. and Weber, J., 1990. Is research keeping up with the needs of Douglas-fir tree improvement programs? West. J. Appl. For., 5: 135–137.

Aitken, S.N. and Adams, W.T., 1995. Screening for cold hardiness in coastal Douglas-fir. In: B.M. Ptts, N.M.G. Borralho, J.B. Reid, R.N. Cromer, W.N. Tibbits and C.A. Raymond (Editors), Eucalypt Plantations: Improving Fibre Yield and Quality. Proc. CRC/IUFRO Conf., Hobart, 19–24 February, CRC for Temperate Hardwood Forestry, Hobart, pp. 321–324.

Becker, W.A., 1984. Manual of Quantitative Genetics, 4th edn. Academic Enterprises, Pullman, WA, 190 pp.

Burdon, R.D., 1977. Genetic correlation as a concept for studying genotype–environment interaction in forest tree breeding. Silvae Genet., 26: 168–175.

Burr, K.E., Tinus, R.W., Wallner, S.J. and King, R.M., 1989. Relationships among cold hardiness, root growth potential and bud dormancy in three conifers. Tree Physiol., 5: 291–306.

Burr, K.E., Tinus, R.W., Wallner, S.J. and King, R.M., 1990. Comparison of three cold hardiness tests for conifer seedlings. Tree Physiol., 6: 351–369.

Calkins, J.B. and Swanson, B.T., 1990. The distinction between living and dead plant tissue—Viability tests in cold hardiness research. Cryobiology, 27: 194–211.

Campbell, R.K., 1979. Genecology of Douglas-fir in a watershed in the Oregon Cascades. Ecology, 60: 1036–1050.

Campbell, R.K. and Sorensen, F.C., 1974. Cold-acclimation in seedling Douglas-fir related to provenance, photoperiod, chilling, and flushing temperature. Bot. Gaz., 136(3): 290–298.

Duffield, J.W., 1956. Damage to Western Washington forests from November 1955 cold wave. Res. Note No. 129, USDA Forest Service, PNW Forest and Range Experimental Station, 8 pp.

Falconer, D.S., 1981. Introduction to Quantitative Genetics, 2nd edn. Longman Group, Barking, UK, 340 pp.

Glerum, C., 1985. Frost hardiness of coniferous seedlings: Principles and applications. In: M.L. Duryea (Editor), Proc. of a Workshop on Evaluating Seedling Quality: Principles, Procedures, and Predictive Abilities of Major Tests, 16–18 October 1984, Forest Research Laboratory, Oregon State University, Corvallis, OR.

Hanninen, H., Hakkinen, R., Hari, P. and Koski, V., 1990. Timing of growth cessation in relation to climatic adaptation of northern woody plants. Tree Physiol., 6: 29–39.

Larsen, J.B., 1978a. Untersuchungen zur Bestandesbegrundung der Douglasie. Schriften aus der Forstlichen Fakultat der Universitat Gottingen, 52: 1–136.

Larsen, J.B., 1978b. Die Frostresistenz der Douglasie (*Pseudotsuga menziesii* (Mirb.) Franco) verschiedener Herkunfte mit unterschiedlichen Hohenlagen. Silvae Genet., 27(3–4): 150–156.

Li, P. and Adams, W.T., 1993. Genetic control of bud phenology in pole-size trees and seedlings of coastal Douglas-fir. Can. J. For. Res., 23: 1043–1051.

Loopstra, C.A. and Adams, W.T., 1989. Patterns of variation in first-year seedling traits within and among Douglas-fir breeding zones in southwest Oregon. Silvae Genet., 38(5–6): 235–243.

Namkoong, G., 1979. Introduction to quantitative genetics in forestry. Tech. Bull. 1588, USDA Forest Service, Washington, DC.

Rehfeldt, G.E., 1979. Variation in cold hardiness among populations of *Pseudotsuga menziesii* var. *glauca*. Res. Pap. INT-233, USDA Forest Service, Washington, DC, 11 pp.

Rehfeldt, G.E., 1986. Development and verification of models of freezing tolerance for Douglas-fir populations in the inland Northwest. Res. Pap. INT-369, USDA Forest Service, Washington, DC, 5 pp.

Sakai, A. and Larcher, W., 1987. Frost Survival of Plants. Springer, Berlin/Heidelberg, 321 pp.

Schermann, N., 1994. Etude des parametres genetiques de trois populations de Douglas vert (*Pseudotsuga menziesii* (Mirb.) Franco). These, Institut National Agronomique Paris-Grignon, France, 117 pp.

Schuch, U.K., Duryea, M.L. and Fuchigami, L.H., 1989a. Dehardening and budburst of Douglas-fir seedlings raised in three Pacific Northwest nurseries. Can. J. For. Res., 19: 198–203.

Schuch, U.K., Duryea, M.L. and Fuchigami, L.H., 1989b. Frost hardiness as acquired by Douglas-fir seedlings in three Pacific Northwest nurseries. Can. J. For. Res., 19: 198–203.

Statistical Analysis Systems Institute Inc., 1988. SAS/STAT User's Guide, release 6.03 edition. SAS Institute Inc., Cary, NC, 1028 pp.

Steel, R.G.D. and Torrie, J.H., 1980. Principles and Procedures of Statistics: A Biometrical Approach, 2nd edn. McGraw-Hill, New York, 633 pp.

Timmis, R., Flewelling, J. and Talbert, C., 1994. Frost injury prediction model for Douglas-fir seedlings in the Pacific Northwest. Tree Physiol., 14: 855–869.

Wheeler, N.C., Stonecypher, R.W. and Jech, K.S., 1990. Physiological characterization of select families to aid in source movement decisions: Supplementing long-term performance trials. In: Proc. of the Joint Meeting of Western Forest Genetics Association and IUFRO Working Parties S2.02-05, 06, 12 and 14 Douglas-fir, Contorta pine, Sitka spruce and Abies Breeding and Genetic Resources, Olympia, WA, 20–24 August 1990. Weyerhaeuser Company, Tacoma, WA, pp. 2.362–2.377.

White, T.L., 1987. Drought-tolerance of southwestern Oregon Douglas-fir. For. Sci., 33(2): 283–293.