Genetic selection for cold hardiness in coastal Douglas-fir seedlings and saplings[1]

Gregory A. O'Neill, Sally N. Aitken, and W. Thomas Adams

Abstract: Genetic control of cold hardiness in two-year-old seedlings was compared with that in 7-year-old saplings of 40 open-pollinated families in each of two breeding populations (Coast and Cascade) of coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco) from western Oregon. In addition, the efficacy of bud phenology traits as predictors of cold hardiness at the two stages was explored. Fall and spring cold hardiness were assessed using artificial freeze testing. Similar genetic control of cold hardiness in seedlings and saplings is suggested by strong type-B genetic correlations (r_B) between the two ages for fall and spring cold injury traits ($r_B \geq 0.78$) and by similar trends in individual tree heritability estimates (h_i^2), e.g., h_i^2 was greater in spring ($\bar{h}_i^2 = 0.73$) than in fall ($\bar{h}_i^2 = 0.36$) and greater in the Coast population ($\bar{h}_i^2 = 0.69$) than in the Cascade population ($\bar{h}_i^2 = 0.40$) at both ages. Strong responses to direct selection are expected for spring cold hardiness at both ages and for fall cold hardiness in seedlings, even under mild selection intensities. Similar heritabilities in seedlings and saplings, and strong genetic correlations between ages for cold-hardiness traits, ensure that selection at one age will produce similar gains at the other age. Type-A genetic correlations (r_A) between fall and spring cold hardiness were near zero in the Cascade population ($r_A = 0.08$ and –0.14 at ages 2 and 7, respectively) but were moderate and negative in the Coast population ($r_A = –0.54$ and –0.36, respectively). Bud-burst timing appears to be a suitable surrogate to artificial freeze testing for assessing spring cold hardiness in both seedlings and saplings, as is bud set timing for assessing fall cold hardiness in seedlings, but bud set timing is a poor predictor of fall cold hardiness in saplings.

Résumé : Les auteurs ont comparé le contrôle génétique de la résistance au froid chez des semis de deux ans et des jeunes arbres de sept ans, représentatifs de 40 descendances issues de pollinisation libre pour chacune de deux populations d'élevage (zone côtière et zone des Cascades) de douglas de Menzies (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco) de l'ouest de l'Oregon. De plus, les auteurs ont étudié l'efficacité des caractères phénologiques associés aux bourgeons pour prédire la résistance au froid à ces deux stades de développement. La résistance printanière et automnale au froid a été évaluée à l'aide de tests de congélation artificielle. Les résultats suggèrent l'existence d'un contrôle génétique similaire de la résistance au froid chez les semis aussi bien que chez les jeunes arbres. Ainsi, de fortes corrélations génétiques de type B ont été observées entre les deux âges pour les caractères de dommages dus au froid automnal et printanier ($r_B > 0{,}78$) et par des tendances similaires au niveau des estimés d'héritabilité individuelle (h_i^2) : par exemple, h_i^2 était plus grande au printemps ($\bar{h}_i^2 = 0{,}73$) qu'à l'automne ($\bar{h}_i^2 = 0{,}36$) et plus grande au sein de la population de la zone côtière ($\bar{h}_i^2 = 0{,}69$) qu'au sein de la population de la zone des Cascades ($\bar{h}_i^2 = 0{,}40$) et ce, pour chacun des deux âges. De fortes réponses à la sélection directe sont anticipées pour la résistance printanière au froid à chacun des deux âges, et pour la résistance automnale au froid chez les semis et ce, même pour des valeurs modérées d'intensité de sélection. Les héritabilités similaires entre semis et jeunes arbres ainsi que les fortes corrélations génétiques observées entre les deux âges pour les caractères de résistance au froid garantissent que la sélection à un âge donné produira des gains similaires à l'autre âge. Les corrélations génétiques de type A (r_A) entre la résistance printanière et automnale au froid étaient quasi nulles chez la population de la zone des Cascades ($r_A = 0{,}08$ et –0,14 aux âges de 2 et 7 ans, respectivement), mais elles étaient modérées et négatives chez la population de la zone côtière ($r_A = –0{,}54$ et –0,36). La date de débourrement des bourgeons semble être un substitut adéquat aux tests de congélation artificielle pour évaluer la résistance printanière au froid tant chez les semis que les jeunes arbres, tout comme l'est la date d'aoûtement des bourgeons pour évaluer la résistance automnale au froid chez les semis. Cependant, la date d'aoûtement des bourgeons n'est pas une mesure adéquate pour prédire la résistance automnale au froid chez les jeunes arbres.

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G.A. O'Neill[2] and W.T. Adams.[3] Department of Forest Science, Oregon State University, 321 Richardson Hall, Corvallis, OR, 97331-5752, U.S.A.
S.N. Aitken. Department of Forest Sciences, University of British Columbia, Forest Sciences Centre, 3041-2424 Main Mall, Vancouver, BC V6T 1Z4, Canada.

[1]Paper 3402 of the Forest Research Laboratory, Oregon State University, Corvallis.
[2]Corresponding author. Current address: Department of Forest Sciences, University of British Columbia, Forest Sciences Centre, 3041–2424 Main Mall, Vancouver, BC V6T 1Z4, Canada. e-mail: goneill@interchange.ubc.ca
[3]Author to whom reprint requests should be sent.

Introduction

As coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco) breeding programs advance into the second generation in the Pacific Northwest, there is increasing interest in enlarging or combining breeding zones and in utilizing the most elite genotypes. The prospect of deploying improved genotypes over larger geographical areas or of developing varieties adapted to particular stress environments, requires methods to evaluate genotypes for traits related to adaptation (Wheeler et al. 1990). Cold hardiness is an adaptive trait of considerable importance, as spring and fall frosts can cause extensive damage to planted seedlings (Timmis et al. 1994), saplings (van der Kamp and Worrall 1990; Aitken and Adams 1997) and even mature stands (Duffield 1956) of Douglas-fir and other species (e.g., van Haverbeke 1979; Strimbeck et al. 1995; Hannerz 1994, 1998).

Cold hardiness may be more important for consideration in breeding programs at the seedling stage than at older stages. Because of their small size, closer proximity to ground level where temperatures are coldest (Landsberg 1986), tendency to continue growing through free or lammas growth into late summer or early fall (Campbell and Sorensen 1973; Pollard and Logan 1976; Li and Adams 1993), and to burst bud earlier in spring than older trees (Büsgen and Münch 1929; Irgens-Moller 1967), seedlings are more vulnerable to frost injury than older age-classes of trees (van Haverbeke 1987; Wheeler et al. 1990).

Because of the relatively small area of land needed, the uniformity of nursery environments, and the relatively short duration of nursery evaluations, artificial freeze testing (or artificial freeze tests, AFT) at the seedling stage may provide cheaper, earlier, and more precise estimates of cold hardiness than similar testing of trees in field trials. For example, in the *Picea abies* (L.) Karst. breeding program in Sweden, genotypes are initially scored for cold hardiness, phenology, and growth in early tests, then promising genotypes are cloned and tested in field trials (Aitken and Hannerz 2000). On the other hand, shoot cuttings can easily be collected from the many sapling-aged progeny tests in existence. If genes controlling cold hardiness of seedlings and saplings were largely shared, the evaluation of cold hardiness at one stage could be used to effectively rank families or genotypes for cold hardiness at the other stage.

Strong genetic relationships between timing of shoot growth and cold hardiness would allow bud phenology to be used as a surrogate to AFT for assessing cold hardiness. In saplings, the genetic association between spring cold hardiness and bud-burst timing appears to be strong, but the relationship between fall cold hardiness and timing of bud set is weak (Aitken and Adams 1997; Aitken et al. 1996), likely because bud set in saplings occurs in late spring or early summer, long before fall cold hardening. One might expect, however, that fall cold hardiness and bud set are more strongly associated in seedlings than in saplings, because bud set in seedlings occurs much later than in saplings, closer to the initiation of fall hardening (Ununger et al. 1988; Li and Adams 1993).

The first of the two reports in this series (O'Neill et al. 2000) described fall and spring cold hardiness in seedlings of the same 80 families investigated at the sapling stage in an earlier Pacific Northwest Tree Improvement Research Cooperative study (Aitken and Adams 1995, 1996, 1997). The main objectives of the present report are to (*i*) compare genetic control of cold hardiness traits at the two stages (seedling and sapling); (*ii*) evaluate the breeding implications of these results, including the potential for early testing of these traits; and (*iii*) compare the relationships between cold hardiness and bud phenology in seedlings and saplings.

Materials and methods

Materials

Genetic materials included in the sapling field trial and seedling nursery test were the open-pollinated progeny (families) of 40 wild parent trees from each of two low-elevation Douglas-fir breeding zones in western Oregon, U.S.A. One breeding zone is located west of Corvallis in the Coast Range, and the second is located northeast of Corvallis on the lower west slope of the Cascade Mountains. The 40 families within each zone were chosen from among the families represented in progeny field tests (20 families from each of two 30-family sets), based on seed availability in storage for the nursery test. Details on the experimental design, cold hardiness testing procedures, and shoot phenology assessments were described previously for the saplings (Aitken and Adams 1995, 1996, 1997) and seedlings (O'Neill et al. 2000). Thus, only a summary of the methods will be provided in this report.

Sapling field tests

Progeny field tests were established in 1987 at seven sites in the Coast breeding zone and at six sites in the Cascade breeding zone using 1–0 seedlings. Families were sampled repeatedly at one high- and one low-elevation test site within each zone over a 2-year period in the sapling cold hardiness study to evaluate the consistency of family ranking for cold hardiness and phenology traits in different environments and seasons (Aitken and Adams 1996, 1997). The experimental design in the Coast zone was a split plot, with 30-family sets as main plots, families within sets represented by four-tree noncontiguous subplots, and four or five replications per site. In the Cascade zone, each 30-family set was planted as a separate randomized complete block design with five replications at each site. Within each of these replications, families were represented by four-tree noncontiguous plots. By age seven (1992), when cold hardiness assessments were initiated, survival at the four sampled test sites averaged 91% (range 80–95%).

Seedling nursery test

Stratified seed from the 80 families were sown at 8 × 10 cm spacing into two raised nursery beds in Corvallis, Oreg., in April 1995 (O'Neill et al. 2000). The experimental design was a split plot with four randomized complete blocks. The main plots were soil moisture treatments (well-watered and mild drought) applied during the second summer after sowing. Each main plot was subdivided into two replicate sub-blocks (sub-blocks A and B), both containing the 80 families, randomly allocated to four-tree row plots. Replicate sub-blocks were required to accommodate the large number of shoot samples needed for cold hardiness testing. In total, 4 seedlings/family-row subplot × 80 family-row subplots/sub-block × 2 sub-blocks/moisture regime × 2 moisture regimes/block × 4 blocks = 5120 test seedlings were established.

Seedling survival at the end of the experiment was 98%; however, symptoms of Lygus bug (*Lygus hesperidus* Hahn) attack (deformed and scarred apical shoots and buds) appeared on 4% of the seedlings at the end of the first summer (1995), and a natural frost in early November 1995 killed the apical buds of 28% of the seedlings (O'Neill et al. 2000). Artificial freeze tests were applied to

nondamaged lateral shoots after the second growing season (fall 1996 and spring 1997). The natural frost event in fall 1995 is not expected to have affected cold hardiness or bud phenology of nondamaged shoots the following year (O'Neill et al. 2000).

Measurements

Methods of AFT and assessment of bud phenology were similar at the two ages. Cold hardiness testing was performed on all saplings in the field test after the sixth and seventh growing seasons, i.e., on five fall dates (September, October, and November of 1992 and September and October of 1993), one winter date (January 1993), and three spring dates (March and April 1993, and April 1994). Cold hardiness testing in the nursery experiment was performed on all seedlings in one sub-block of each main plot (i.e., on 16 seedlings per family in each moisture treatment), four times after the second growing season, i.e., on three fall dates (September, October, and November of 1996), and one spring date (March of 1997). However, cold injury scores from only one fall and one spring test date at each age are considered here because of the need to simplify analyses, and because genetic correlations of cold injury scores between months in the same season were strong in both saplings (Aitken and Adams 1996, 1997) and seedlings (O'Neill et al. 2000). Also, previous investigations of cold hardiness in April 1993 and 1994 indicated that genetic correlations of cold hardiness between years were strong ($r_A \geq 0.95$) and genetic parameter estimates were similar, despite fairly different spring weather in the 2 years (accumulated heat sum above 5°C between January 1 and April 30 was 787 degree-days in 1993 and 952 degree-days in 1994) (Aitken and Adams 1997). October AFT scores (i.e., October 1992 for saplings and October 1996 for seedlings) were used to assess fall cold hardiness, because estimates of individual heritabilities were highest in October for both ages. Sapling heritabilities for spring cold injury were greatest in April, so AFT scores for April 1993 were used for saplings. Spring cold hardiness in seedlings was represented by March 1997 AFT scores, the only spring month in which seedling cold hardiness was tested.

Cold hardiness testing on each test date (month) began with sampling a 5-cm shoot tip from each of two lateral branches from approximately the same crown height and degree of exposure on each tree. Samples were individually labeled, then transported on ice to the freeze-testing laboratory. The two shoot samples from each tree were frozen at different test temperatures in a programmable freezer. Samples were subjected to –2°C for 7–10 h; the temperature was then decreased 3–5°C/h until the test temperature was reached. After 1 h at the test temperature, samples were placed in a cooler (2°C) for at least 6 h to thaw and then placed on laboratory benches at room temperature for 6–8 days to allow symptoms of cold injury to develop.

Cold injury was assessed visually (Rehfeldt 1980; Burr et al. 1990) by recording, for needles, stems, and buds, the percentage of discolored tissue to the nearest 10%. The goal of AFT was to achieve intermediate levels of mean cold injury to the shoot tissues (i.e., 30–70% tissue injury) so that family differences in cold injury would be maximized (Aitken and Adams 1996; O'Neill et al. 2000). To increase the likelihood of achieving this goal, two test temperatures were applied to all seedlings sampled on each test date (one temperature to each of the two replicate shoot cuttings from each tree). Test temperatures were selected on the basis of preliminary artificial freeze tests (AFTs) performed the week prior to the experimental AFTs. Temperatures selected for the experimental AFTs were –12.5 and –15.5°C in October and –15 and –19°C in March for seedlings, and –17 and –20°C in October and –10 and –14°C in March for saplings. Intermediate injury levels were typically achieved at one or both temperatures, and because family differences were greatest when injury scores at the two temperatures were averaged, mean scores were utilized in all analyses (Aitken and Adams 1996, 1997; O'Neill et al. 2000).

Sapling data from both the high- and low-elevation field sites were used, because genotype by environment (G × E) interaction effects for cold injury were generally nonsignificant in saplings (Aitken and Adams 1996, 1997), and using data from both sites for both populations increased the precision of family mean cold injury estimates. To further simplify analyses and to evaluate the feasibility of early testing for cold hardiness, data from seedlings grown only in the wet moisture regime were used. Genetic correlations for AFT traits between moisture regimes were strong ($\bar{r}_A$ = 0.89, O'Neill et al. 2000), and using only the seedlings in the wet treatment provided a sample size close to that commonly used in nursery and field genetic tests (i.e., 16 seedlings/family). Also, previous analyses have shown that 16 seedlings/family is clearly sufficient to reliably rank families for cold hardiness. Furthermore, a uniformly wet nursery environment is easier to maintain, and provides higher heritabilities ($\bar{h}_i^2$ = 0.49) for AFT traits than a uniformly dry environment ($\bar{h}_i^2$ = 0.36) (O'Neill et al. 2000). As a final simplification, only damage to stems was considered, because (*i*) at both ages, heritabilities of AFT injury scores were stronger for stems than for needles or buds; (*ii*) genetic correlations between stems and other tissues were always positive (in most cases >0.50 in fall tests and >0.80 in spring tests) (Aitken and Adams 1996, 1997; O'Neill et al. 2000); and (*iii*) stem cold injury is more likely than needle or bud injury to result in serious plant injury or plant death.

Bud phenology was observed preceding each of the selected fall and spring cold hardiness periods indicated above (i.e., bud set in 1992 and bud burst in 1993 in the saplings; bud set in 1996 and bud burst in 1997 in the seedlings). Bud set (BS) and bud burst (BB) were recorded biweekly on the terminal bud of a single, marked, secondary shoot of all trees in the sapling test, and on the apical bud of all seedlings in the nursery test. The timing of bud phenology on terminal and lateral buds has been shown to be highly correlated (Li and Adams 1993). Buds were scored as either "set" (smooth, well-developed, brown scales visible) or "burst" (new needles visible) on each assessment, and the date of bud set and bud burst were estimated as the Julian dates on which these events were first noted. When lammas growth (second flushing) was observed (i.e., on <1% of the saplings, and on 14% and 12% of the Coast and Cascade seedlings, respectively), bud set was recorded as the date of the last bud set. Seedling bud set date was scored on individuals in both replicate sub-blocks (i.e., on 32 seedlings per family), but bud burst was recorded on only one sub-block in each main plot (i.e., on 16 seedlings per family) because one sub-block in each main plot was harvested prior to bud burst.

Statistical analysis

Data for each breeding zone and assessment age were analyzed separately. All analyses were conducted on single-tree observations. All variables were assumed to be random in both seedling and sapling analyses.

Statistical models and analysis of percent cold injury in fall (FCI) and spring (SCI) for saplings and seedlings were described in detail previously (Aitken and Adams 1996, 1997; O'Neill et al. 2000). The main difference between the experimental design of the Coast and Cascade breeding zone field sites was that family sets were nested within blocks at the Coast sites, while blocks were nested within sets at the Cascade sites. In the nursery experiment, families were not blocked by family sets, and preliminary analyses indicated that the 20-family sets did not differ statistically within each zone (O'Neill et al. 2000). Thus, family sets were ignored in the seedling analyses. FCI and SCI scores in saplings were subjected to arcsine square-root transformation prior to analysis. Residual values were normally distributed in all other traits and, thus, were analyzed without transformation.

All traits utilized in this report were shown in earlier analyses to vary significantly ($p < 0.05$) among families (Aitken and Adams

1995, 1996, 1997; S.N. Aitken and W.T. Adams, unpublished data; O'Neill et al. 2000). Earlier analyses also provided restricted maximum likelihood (REML) estimates of variance components using the SAS VARCOMP procedure (SAS Institute Inc. 1996) for sapling-age traits and the SAS MIXED procedure for seedling-age traits.

The similarity of genetic control of cold hardiness and bud phenology traits was evaluated at the two ages by comparing estimates of individual heritabilities of the corresponding traits and genetic correlations between them. Likewise, genetic correlations between seedlings and saplings for each trait were estimated to evaluate genetic relationships between the two ages. The additive genetic variance (σ^2_A) was estimated as three times the family variance ($3\sigma^2_F$), because open-pollinated Douglas-fir progeny are expected to be more closely related than half-sibs (Squillace 1974; Campbell 1979). Individual heritability estimates for FCI and SCI in saplings were obtained from previous reports (Aitken and Adams 1996, 1997). Individual heritability estimates of BS and BB in saplings were estimated from variance components provided by Aitken and Adams (1997 and unpublished data). Individual heritabilities for sapling traits were estimated as

$$[1] \qquad h^2_i = \frac{\sigma^2_A}{\sigma^2_F + \sigma^2_{FT} + \sigma^2_e + \sigma^2_w}$$

where σ^2_F is the family variance (seedlings) or family within set variance (saplings), σ^2_{FT} is the family-by-test site interaction, σ^2_e is the plot error (family-by-block interaction), and σ^2_w is the within family-plot error.

To estimate h^2_i for seedling traits, σ^2_{FT} was set to zero. To be consistent in terms of the number of observations per family in seedling traits, BS, the only trait recorded in both sub-blocks, was analyzed separately by sub-block, and the two resulting individual heritability estimates averaged. Standard errors of heritability estimates were calculated according to Dickerson (1969, pp. 49–50), using the asymptotic variances of variance components derived in the VARCOMP procedure.

Genetic correlation estimates were obtained in two ways. When measurements of both traits were made on the same individuals, type-A genetic correlations were calculated as

$$[2] \qquad r_A = \frac{Cov_{F_{1,2}}}{\sqrt{\sigma^2_{F_1} \times \sigma^2_{F_2}}}$$

where $\sigma^2_{F_1}$ and $\sigma^2_{F_2}$ are the estimated family (or family within set) variances of traits 1 and 2, and $Cov_{F_{1,2}}$ is the estimated family covariance between traits 1 and 2 (Falconer 1986).

Type-B genetic correlations (r_B) were calculated to evaluate genetic relationships between traits measured on different individuals in the same families (Burdon 1977). This occurred when seedlings were measured for different traits in different replicate sub-blocks of the same main plot or when measurements were made at different ages. To calculate r_B, $Cov_{F_{1,2}}$ in eq. 2 was replaced by $Cov_{\bar{F}_{1,2}}$, the covariance of family means for traits 1 and 2.

Assessment of BS on seedlings in both sub-blocks of each main plot allowed both type-A and -B genetic correlations to be estimated between seedling BS and the other seedling and sapling traits. The two genetic correlations (r_A and r_B) were averaged to improve the precision of the correlation estimates.

The potential genetic response in saplings to early selection for cold hardiness, and the potential response in seedling cold hardiness following selection for sapling cold hardiness, were evaluated by calculating expected correlated responses to selection (Falconer 1986, p. 286). The response in cold hardiness to selection of bud phenology as a surrogate for cold hardiness was also estimated. It was assumed in all selection scenarios that the "best" 20% of parent trees in each breeding zone are selected on the basis of the performance of their open-pollinated progeny (e.g., those with the lowest mean cold injury, earliest BS or latest BB) at either the seedling or sapling stage. It was further assumed that the selected parents are placed in a clonal seed orchard to produce offspring by random mating. Expected correlated response (CR) in the seed orchard offspring was estimated according to Shelbourne (1969):

$$[3] \qquad CR_y = 2i(h^2_{f_x})^{0.5}(h^2_{f_y})^{0.5}\, r_{A_{x,y}} \sigma_{\bar{p}_y}$$

where i is the selection intensity expressed in standard deviations (= 1.40), $h^2_{f_x}$ and $h^2_{f_y}$ are estimated family heritabilities for the selected (x) and response (y) traits, $r_{A_{x,y}}$ is the estimated genetic correlation between the selected and response traits, and $\sigma_{\bar{p}_y}$ is the estimated phenotypic standard deviation of family means for the response trait.

Family heritabilities for sapling traits (either x or y) were calculated as

$$[4] \qquad h^2_f = \frac{0.25\sigma^2_A}{\sigma^2_{\bar{p}}}$$

where

$$[5] \qquad \sigma^2_{\bar{p}} = \sigma^2_F + \frac{\sigma^2_{FT}}{t} + \frac{\sigma^2_e}{tb} + \frac{\sigma^2_w}{tbn}$$

and t is the number of sites (2), b is the number of blocks (4.5 and 5 in the Coast and Cascade zones, respectively), and n is the harmonic mean number of saplings per plot (3.5 in the Coast and 3.2 in the Cascades). Equation 4 was also used to estimate family heritabilities for seedling data, but with $\sigma^2_{FT} = 0$, $t = 1$, $b = 4$, and $n = 3.9$. Family heritability for BS was analyzed separately by sub-block, and the two resulting individual heritability estimates averaged, as for individual heritability of BS (above).

The expected response to direct selection (R_y) of the traits at each age was also calculated:

$$[6] \qquad R_y = 2i(h^2_{f_y})\sigma_{\bar{p}_y}$$

and compared with the correlated response. Direct and correlated responses for FCI and SCI at age 7 were back-transformed because of the use of arcsine square-root transformed injury values in the estimation of $\sigma_{\bar{p}}$ for these traits.

Results and discussion

Genetic control of cold hardiness and implications for selection

Genetic variation in cold injury was large for both seedlings and saplings. For example, in the Coast population the range in sapling family mean SCI was 5–88% (Table 1). Individual heritabilities were strong in both seedlings ($\bar{h}^2_i$ = 0.60) and saplings ($\bar{h}^2_i$ = 0.49) (averaged over populations and seasons), and similar trends in heritability estimates were observed for cold injury traits between seasons and between breeding zones at both ages. Heritability estimates were greater in spring ($\bar{h}^2_i$ = 0.69 for seedlings and 0.77 for saplings) than in fall ($\bar{h}^2_i$ = 0.50 for seedlings and 0.21 for saplings) (averaged over populations), and greater in the Coast breeding zone ($\bar{h}^2_i$ = 0.74 for seedlings and 0.63 for saplings) than in the Cascade zone ($\bar{h}^2_i$ = 0.45 for seedlings and 0.35 for saplings) (averaged over seasons). The slightly lower heritabilities, on average, for cold hardiness in the saplings may be attributable, in part, to genotype × environment interaction, which was not estimated in the seedling

Table 1. Estimated population means, ranges in family means, individual (h_i^2) and family (h_f^2) heritabilities, and phenotypic standard deviations of family means ($\sigma_{\bar{p}}$) for fall (FCI) and spring (SCI) stem cold injury scores (% of tissue damaged) after artificial freeze testing, and timing (Julian date) of bud set (BS) and bud burst (BB), at ages 2 and 7 in Coast and Cascade populations of coastal Douglas-fir.

	Coast					Cascade				
Trait	Population mean	Range of family means	$h_i^{2\,a}$	$h_f^{2\,b}$	$\sigma_{\bar{p}}$	Population mean	Range of family means	$h_i^{2\,a}$	$h_f^{2\,b}$	$\sigma_{\bar{p}}$
Age 2										
BS	245.3	225.0–260.4	0.30[c]	0.41[c]	8.96	240.1	226.2–256.3	0.32[c]	0.43[c]	8.481
FCI	69.4	33.7–97.2	0.66	0.56	16.30	50.5	23.3–84.0	0.35	0.45	14.30
BB	114.9	104.1–123.4	1.00[d]	0.67	4.26	112.5	104.0–119.3	0.78	0.62	3.481
SCI	58.2	40.4–88.1	0.83	0.62	12.37	64.5	48.3–86.8	0.55	0.58	9.888
Age 7										
BS[e]	132.3	127.1–146.4	0.54	0.66	3.47	130.4	127.6–134.5	0.30	0.47	1.682
FCI[f]	24.6	12.8–39.8	0.26	0.56	0.086	23.9	7.6–58.6	0.16	0.38	0.077
BB[e]	134.8	121.6–145.8	1.00[d]	0.70	4.01	133.5	126.2–137.0	0.70	0.65	2.481
SCI[f]	43.4	5.1–88.3	1.00[d]	0.70	0.244	53.1	23.8–83.8	0.54	0.63	0.176

[a]Average standard error of h_i^2 = 0.16 (range 0.07–0.29).
[b]Average standard error of h_f^2 = 0.23 (range 0.23–0.25).
[c]Values were estimated separately by sub-block and then averaged.
[d]Estimate exceeded 1.00.
[e]BS values are for 1992; BB values are for 1993.
[f]Heritablities and phenotypic variances are based on arcsine transformed injury scores; means and family ranges are based on original scores.

test, as seedling cold hardiness was considered in only one environment. Lower heritabilities for cold hardiness in fall than in spring may be due to the greater number of environmental cues regulating fall hardening compared with spring dehardening, resulting in greater environmental "noise" influencing fall cold hardiness (Weiser 1970; Alden and Hermann 1971; Jonsson et al. 1986). These results cannot be explained by simpler genetic control of spring cold hardiness than fall cold hardiness, as the number of significant quantitative trait loci are similar for fall and spring cold hardiness in Douglas-fir (Jermstad et al. 2000). However, these results are consistent with those of other temperate conifers for which fall cold hardiness and phenological traits have lower heritabilities, on average, than corresponding traits in the spring (Aitken and Hannerz 2000).

Differences in genetic control between the two populations may be related to the trade-off between height growth and cold hardiness. Late bud burst or early bud set will reduce the risk of frost injury but at the expense of height growth (Kuser and Ching 1980; Rehfeldt 1983, 1989; Hannerz et al. 1999). Natural selection for cold hardiness targets the least cold-hardy genotypes, while natural selection for height growth targets the most cold hardy. Selection for cold hardiness is greater in harsher and more temporally and spatially variable Cascade environments, than in milder, less variable coastal and low-elevation environments. Selection for height growth, on the other hand, is greater on the coast, and in low-elevation environments. Consequently, lower heritabilities for cold hardiness in the Cascade than the Coast zone may be due to a higher combined level of selection for cold hardiness and height growth in the Cascades, which results in less genetic variation in cold hardiness. Lending support to this hypothesis is the prediction of stronger selection for spring and fall cold hardiness in Cascade than in Coast environments (Timmis et al. 1994). As a second hypothesis, genotypes in the Cascades may have adapted to their harsher and more variable environment by being more sensitive to environmental variation, resulting in more phenotypic relative to additive variation, in the Cascade than in the Coast zone. As a final hypothesis, the range of environments represented by parent trees may be narrower in the geographically smaller Cascade breeding zone than in the Coast zone, resulting in less genetic variation in the Cascade zone. These hypotheses are partially supported by the data: genetic variation for cold hardiness is appreciably lower in the Cascades in spring but not in fall (see coefficients of family variation in Table 1; O'Neill et al. 2000). In two Washington populations of Douglas-fir, heritabilities and coefficients of additive genetic variation of fall cold hardiness (calculated from Table 1; Aitken et al. 1996) were both greater in the Coast than in the Cascade population.

Strong genetic relationships were observed between seedling and sapling cold hardiness in both fall and spring. Families that sustained little FCI (or SCI) at age 2 also sustained little FCI (or SCI) at age 7 ($r_B \geq 0.78$; Table 2), despite differences in the year of testing, growth environments and location, sampling design, and scoring personnel. These results attest not only to the similarity of the genetic regulation of cold hardiness at the different ages but to the reliability of methods of AFT used to evaluate cold injury in both seedling nursery and sapling field tests.

Direct selection for cold hardiness

Strong genetic control and significant family variation for SCI in both seedlings and saplings indicate that genetic improvement of spring cold hardiness would be highly effective at both ages, even under relatively weak artificial selection, as shown in expected response values (Table 3). For example, selecting the 20% of Coast parents with the most spring cold-hardy offspring (i.e., lowest cold injury scores) at age 2 is predicted to reduce spring cold injury (SCI) of 2-year-old seedlings from a mean of 58.2% damage

Table 2. Estimated genetic correlations among stem cold hardiness (fall cold injury (FCI) and spring cold injury (SCI)), and bud phenology (Julian dates of bud set (BS) and bud burst (BB)) traits at age 2 (above diagonal) and age 7 (below diagonal) in the upper portion of the table and between ages 2 (traits listed in columns) and 7 (traits listed in rows) in the lower portion of the table, for two western Oregon breeding populations (Coast and Cascade) of Douglas-fir.

	Coast				Cascade			
	BS	FCI	BB	SCI	BS	FCI	BB	SCI
Age 2 vs. age 2 and age 7 vs. age 7								
BS		0.96*[a]	0.60*	–0.52*		0.65*	0.19	0.03
FCI	0.28*		0.75*	–0.54*	0.38*		0.11	0.08
BB	0.88*	0.46		–0.90*	0.90*	0.48		–0.82*
SCI	–0.85*	–0.36*	–0.94*		–0.96*	–0.14	–0.90*	
Age 7 vs. age 2								
BS	0.76*	0.70*	0.79*	–0.49*	0.11	0.29	1.00*[b]	–0.70*
FCI	0.47*	0.80*	0.64*	–0.38	0.56	1.00*[b]	0.42	–0.17
BB	0.81*	0.87*	0.93*	–0.73*	0.11	0.26	0.91*	–0.67*
SCI	–0.71*	–0.71*	–0.88*	0.87*	–0.09	–0.08	–0.76*	0.78*

[a]Correlations with asterisks are those for which corresponding family mean correlations are significant ($p < 0.05$). In all cases, genetic correlations were similar to, or slightly stronger, and in the same direction, as family mean correlations.
[b]Genetic correlation estimate exceeded 1.00.

in the current generation to a mean of 36.9% damage in the next generation (i.e., a –21.3% response). Likewise, direct selection of Coast parents with the most fall cold-hardy offspring at age 2 is predicted to result in a –25.7% response in fall cold injury (FCI) at age 2. Expected responses in fall and spring cold injury at age 2 were nearly as strong in the Cascade population (–16.1 and –18.0%, respectively).

Predicted responses in spring cold injury at age 7 from direct selection at age 7 were of similar magnitude as the direct responses predicted for spring cold injury at age 2 (Table 3). Predicted responses from direct selection for fall cold injury, however, were considerably smaller at age 7 (–1.8% for Coast, and –0.7% for Cascade population) than at age 2 (–25.7% for Coast, and –18.0% for Cascade population). Weak responses to direct selection for FCI in saplings reflect the small family variation in sapling FCI, which may be due to the attainment of fairly low FCI values, and underscores the importance of choosing AFT temperatures that inflict intermediate levels of mean cold injury.

Correlated responses in cold injury of saplings to selection at the seedling stage and vice versa

Regulation of cold hardiness traits by similar sets of genes in seedlings and saplings implies that selection for alleles that control hardiness at one age will also select alleles that control hardiness at the other age. For example, the expected response in spring cold injury of saplings in the Coast population is –21.3%, if selection is based on AFT at the same age, and –14.6% if selections are made at age 2 (Table 3). Predicted direct and correlated responses in spring cold injury of saplings are less than half the magnitude in the Cascade population (–9.2 and –5.3%, respectively). Predicted responses of fall cold injury of saplings are poor, regardless of whether selections are made at age 2 or age 7. Predicted responses in seedlings to selection for fall or spring cold injury, however, are strong and of similar magnitude, regardless of whether selections are made at the seedling or sapling stage.

Therefore, early selection can be used to quickly and inexpensively screen large numbers of individuals grown in common gardens or nurseries for cold hardiness at the seedling stage when trees are most frost susceptible, while assuring gains in hardiness at the sapling stage approach those that would be obtainable had selection been delayed to the later age. Alternatively, selection for cold hardiness in Douglas-fir progeny tests already established for tree improvement programs in the Pacific Northwest and elsewhere (e.g., Canada, France, Germany, and New Zealand), would improve the cold hardiness of deployment populations in both older trees and at the seedling stage.

Indirect responses of cold hardiness in one season to selection for cold hardiness in the other season

The effect of selection for cold hardiness in fall or spring on cold hardiness in the other of these seasons was addressed previously (O'Neill et al. 2000). In the present report, the impacts of indirect selection are explored more broadly, in particular, to include indirect responses at both ages, and indirect responses at one age when selection is applied at the other age.

In both seedlings and saplings, estimated genetic correlations between fall and spring cold injury (FCI–SCI) were negative and moderate (r_A = –0.54 and –0.36 at ages 2 and 7, respectively) in the Coast population but negligible (r_A = 0.08 and –0.14 at ages 2 and 7, respectively) in the Cascade population (Table 2). These differences between populations may be due to substantially lower individual heritabilities for both FCI and SCI at both ages in the Cascade than in the Coast population (Table 1). Consequently, in the Cascade breeding zone, fall and spring cold hardiness appear to be genetically independent of each other. Thus, if improvement is desired for both traits, both traits must be selected; however, selection for cold hardiness in one season will have no impact on cold hardiness in the other season.

In the Coast breeding zone, fall and spring cold hardiness are not controlled independently; selection for one trait is expected to have a detrimental impact on the other unless both traits are selected. This is illustrated most strongly at age 2, where the predicted response from direct selection for spring cold hardiness is expected to reduce SCI by 21.3%

Table 3. Predicted direct (with asterisks) and correlated responses expected in fall (FCI) and spring (SCI) stem cold injury scores (% of tissue damaged) at ages 2 and 7 when 20% of parent trees are selected on the basis of cold hardiness or bud phenology of their open-pollinated offspring.

Selection trait[a]	Selection age[a]	Response age	Coast response[b]		Cascade response[b]	
			FCI	SCI	FCI	SCI
BS	2	2	–20.9	9.0	–11.5	–0.4
FCI	2	2	–25.7*	11.0	–18.0*	–1.1
BB	2	2	21.0	–20.0	2.3	–13.5
SCI	2	2	14.5	–21.3*	–1.6	–16.1*
BS	7	2	–19.5	10.8	–5.3	10.1
FCI	7	2	–20.5	7.7	–16.7	2.3
BB	7	2	24.8	–16.5	5.6	–11.3
SCI	7	2	20.3	–19.8	1.8	–13.0
BS	7	7	–0.2	14.8	–0.1	6.4
FCI	7	7	–1.8*	2.4	–0.7*	0.1
BB	7	7	0.5	–18.9	0.3	–7.9
SCI	7	7	0.3	–21.3*	0.0	–9.2*
BS	2	7	–0.3	6.5	–0.2	0.0
FCI	2	7	–1.2	9.0	–0.8	0.0
BB	2	7	0.9	–16.2	0.2	–5.3
SCI	2	7	0.3	–14.6	0.0	–5.3

[a]Selection for early bud set (BS) or low FCI in the fall and for late bud burst (BB) or low SCI in the spring.
[b]Negative response means reduced cold injury. Age 7 values have been back transformed.

but will increase FCI by 14.5% (Table 3). The detrimental response in FCI to selection for SCI is not as great at age 7 as it is at age 2, because both family variation in FCI and the FCI–SCI genetic correlation are weaker at age 7 than at age 2. Because there is greater risk of cold injury to Douglas-fir in spring than in fall in the majority of areas of the Pacific Northwest (Timmis et al. 1994) and in Europe (Heois 1994; Aitken and Hannerz 2000), spring cold hardiness should probably receive greater emphasis in most Douglas-fir tree improvement programs. Nevertheless, despite the negative genetic correlation between fall and spring cold hardiness in the Coast population, there is still considerable family variation for fall cold hardiness among the families that are most cold hardy in spring. Consequently, families that are cold hardy in both spring and fall should not be difficult to identify if cold hardiness is evaluated in both seasons.

Expected correlated responses to selection may also be used to examine indirect cold hardiness response in one season and age, to cold hardiness selection applied in the other season and age. Because of the lack of correlation between FCI and SCI in the Cascade population, little response in one trait is predicted if the other trait is selected, regardless of which age selections are made (Table 3). In the Coast population, responses in cold hardiness to selection across seasons and ages are always detrimental (i.e., reduced cold hardiness) but are large only at age 2.

Bud phenology as a surrogate for cold hardiness selection

Strong negative genetic correlations between BB and SCI were estimated for both seedlings (r_A = –0.90 for Coast and –0.82 for Cascade population) and saplings (r_A = –0.94 for Coast and –0.90 for Cascade population) (Table 2), indicating that BB timing can be an effective surrogate for spring AFTs at both ages. For example, selecting for delayed BB at age 2 in the Coast population is expected to reduce SCI damage by 20.0% (i.e., from 58.2% damage to stems to 38.2% damage to stems) at age 2 and by 16.2% at age 7. Direct selection for spring cold hardiness based on AFT is expected to be only marginally better at reducing SCI (i.e., response = –21.3% at both ages).

Bud phenology also appears to be an effective predictor of fall cold hardiness in seedlings (BS–FCI r_A= 0.96 for Coast and 0.65 for Cascade population), but not in saplings (BS–FCI r_A = 0.28 for the Coast and 0.38 for the Cascade population) (Table 2). While selection on earlier BS in the Coast population at age 2 is expected to reduce FCI at age 2 in the next generation by 20.9%, selection on earlier BS at age 7 in this population is expected to reduce FCI at age 7 by only 0.2%. (Table 3).

An explanation of the difference between seedlings and saplings in BS–FCI genetic correlations likely resides in the striking difference in timing of bud set at the two ages; bud set in saplings occurred, on average, on May 12 and 10, 1992 (Coast and Cascade, respectively), far in advance of stem hardening in late summer or early fall. In contrast, bud set in seedlings occurred, on average, on September 2 and August 28, 1996 (Coast and Cascade, respectively), closer to the time of stem hardening. Considerably earlier bud set in 15-year-old saplings (June 3) than in two-year-old seedlings (September 6) was also documented in Douglas-fir from another breeding zone in coastal Oregon (Li and Adams 1993).

It thus appears that bud phenology assessment provides a suitable surrogate for AFT cold hardiness evaluation for seedlings in fall and spring, and for saplings in the spring. AFTs require availability of a freezer with a precise, programmable temperature controller. Assessing bud phenology, on the other hand, requires no specialized equipment, but repeated visits to nursery or field sites are necessary. While bud burst is easily observed, accurate assessment of bud set

date requires careful visual examination of the developing bud, which is often small and obscured by needles. Second flushing can also complicate the assessment of cold hardiness using both AFT and bud set timing, as second-flushed shoots are less cold hardy than shoots that have not second-flushed (Anekonda et al. 1998). Therefore, efforts should be made in the nursery to reduce the incidence of second flushing by minimizing irrigation at the time of bud set and to ensure that moisture conditions are uniform. Second-flushed shoots should also be avoided, if possible, when sampling for AFTs, and bud set date should be recorded as the date of the final bud set of the year.

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