Effects of genetic selection for growth on frost hardiness in western hemlock

Mats Hannerz, Sally N. Aitken, John N. King, and Sonya Budge

Abstract: Fall and spring frost hardiness was determined from electrolytic leakage of artificially frozen needle segments in 22 full-sib families of western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) from British Columbia and Washington State representing different levels of genetic gain, and of four provenance materials (stand progenies from the Queen Charlotte Islands, Vancouver Island, and Oregon Cascades, and seed-orchard progenies from the northern Oregon Coast Range). Samples for freeze testing were collected in a 5-year-old trial at Jordan River on southwestern Vancouver Island. Height and date of bud flush were recorded in the field. Genetic gain among the full-sib families was not correlated with fall frost hardiness, but high-yielding families displayed a lower spring frost hardiness and an earlier bud flush than low-yielding families. Both fall and spring frost hardiness increased and height growth decreased with the latitude of provenances. The highest growth, earliest bud flush and lowest fall and spring frost hardiness was demonstrated by the Oregon provenances, while the lowest growth, latest bud flush and highest hardiness was found for the Queen Charlotte Islands provenance.

Résumé : Les auteurs ont étudié l'endurcissement au gel de printemps et d'automne chez la pruche de l'Ouest (*Tsuga heterophylla* (Raf.) Sarg.) à partir des écoulements électrolytiques de segments d'aiguilles congelées artificiellement. Les aiguilles étaient représentatives de 22 descendances biparentales de la Colombie-Britannique et de l'État de Washington affichant des niveaux variés de gain génétique, ainsi que de quatre provenances (des descendances de peuplements des îles de la Reine Charlotte, de l'île de Vancouver et de la chaîne des Cascades en Orégon, ainsi que des descendances de vergers à graines de la chaîne Côtière du nord de l'Orégon). Les échantillons d'aiguilles pour les tests de gel ont été récoltés au sein d'un dispositif expérimental âgé de cinq ans situé à Jordan River, au sud-ouest de l'île de Vancouver. La hauteur et la date de débourrement des bourgeons ont été prises en note dans le dispositif au champ. Il n'y avait pas de corrélation entre le gain génétique affiché par les descendances biparentales et l'endurcissement au gel automnal. Cependant, les descendances à haut rendement ont démontré un plus faible endurcissement au gel printanier et un débourrement plus hâtif des bourgeons que les descendances à faible rendement. L'endurcissement au gel printannier et au gel automnal augmentait avec la latitude d'origine des provenances, alors que la croissance en hauteur diminuait avec ce même facteur. Ce sont les provenances de l'Orégon qui ont démontré la plus forte croissance, le débourrement le plus hâtif des bourgeons et les plus faibles endurcissements au gel printanier et automnal. Ce sont les provenances des îles de la Reine Charlotte qui ont démontré la plus faible croissance, le débourrement le plus tardif des bourgeons et le plus fort endurcissement au gel.

[Traduit par la rédaction]

Introduction

Western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) grows in humid areas on the North American Pacific coast and in the northern Rocky Mountains (Packee 1990). Species distribution extends from northern California to Alaska, with almost 60% of all western hemlock volume stock found in British Columbia (Beswick 1976). Western hemlock has

M. Hannerz.[1] The Forestry Research Institute of Sweden (SkogForsk), Uppsala Science Park, S-751 83 Uppsala, Sweden. e-mail: mats.hannerz@skogforsk.se
S.N. Aitken and S. Budge. Department of Forest Sciences, University of British Columbia, 3041-2424 Main Mall, Vancouver, BC V6T 1Z4, Canada. e:mail: aitken@interchange.ubc.ca and sbudge@interchange.ubc.ca
**J.N. King.** British Columbia Ministry of Forests, Research Branch, 712 Yates Street, Victoria, BC V8W 9C2, Canada. e-mail: john.king@gems7.gov.bc.ca

[1]Author to whom all correspondence should be addressed.

been subject to tree improvement in British Columbia and the U.S. Pacific Northwest since the early 1970s, and currently, over 2000 families are present in seed orchards and progeny tests (King et al. 1994). Selections based on progeny trials (backward selections of parents) have been used to establish a second round of seed orchards. The gain in these orchards, compared with unselected natural populations, is predicted to be at least 18%, based on individual tree growth in progeny trials.

Efforts made to genetically increase the gain in timber volume must be balanced with other important traits. Seed transfer to the north of provenance origin is commonly practised to increase timber production in many conifer species. However, this gain is generally accompanied by an extended growing period with delayed growth cessation and increased fall frost risk, when compared with local provenances (Rehfeldt 1992; Cannell et al. 1981). Strong clinal variation was found in western hemlock by Kuser and Ching (1980), with later bud set and later bud flush in southern or low-elevation provenances than in northern or high-altitude seed sources. Meagher (1976) also demonstrated that both fall

Table 1. Genetic materials included in the experiment.

Material	Expected gain (%)[a]	Description	Average origin		
			Latitude (N)	Longitude (E)	Elevation (m asl)
Class A	18	Elite families: eight full-sib families from southeastern British Columbia	50°00′	126°12′	167
Class B	8	Rogued seed-orchard families: eight full-sib families from Forks, Wash.	48°00′	124°24′	446
Class C	0	Unrogued seed-orchard families: three full-sib families from southeastern British Columbia	50°30′	126°48′	77
Class D	−14	Poor families: three full-sib families from southeastern British Columbia	50°18′	126°18′	297
Tillamook		Bulk collection from the unrogued Oregon Department of Forestry's Schroeder seed orchard	45°30′	123°30′	100
Cascades, Oregon		Four families each from two stands in Oregon: Blue Low and Sandy Lake	44°52′	122°11′	305
Vancouver Island		Bulked seedlot from 14 wild stands	50°00′	125°00′	120
Queen Charlotte Islands		Two families each from four stands: Bonanza, Channel, Masset, and Tlell	53°30′	132°09′	30

[a]Average predicted breeding values for volume for the parent trees (percent gain relative to unselected wild-stand materials).

Fig. 1. Locations of the Jordan River test site and geographic origins of parent trees and provenances.
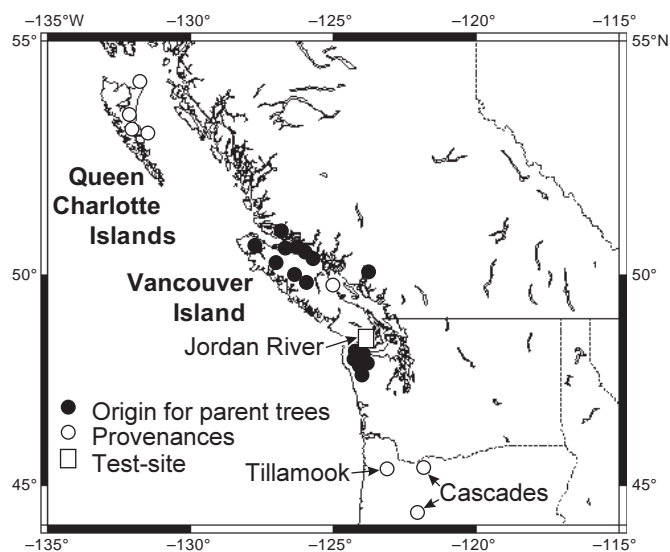
frost injury and height in western hemlock seedlings decreased with increased elevation of the seed source. These relationships indicate that provenance transfer of western hemlock to enhance growth potential also increases the risk of fall frost injury. However, as demonstrated in Norway spruce (*Picea abies* (L.) Karst.) (Mikola 1980; Skrøppa and Magnussen 1993), growth differences among families within provenances may be better explained by growth rate than duration of growth period. The relationships between frost hardiness and various growth traits likely vary with species and even populations (Aitken and Adams 1997) and, thus, need to be carefully considered to reduce the trade-off between yield and frost hardiness in tree improvement programs.

In this study we compare fall and spring frost hardiness and timing of bud flush in full-sib western hemlock families with various levels of predicted genetic gain and in provenance materials. The primary objective was to determine whether families selected for high growth exhibit lower fall or spring frost hardiness than average.

Materials and methods

The material was collected in one of the Hemlock Tree Improvement Cooperative's (HemTIC) realised-gain trials: test site No. 813.8.05 at Jordan River on southwestern Vancouver Island in British Columbia (48°25′N, 124°01′E, 75 m elevation; Fig. 1). The trial was planted in 1994 with 1-year-old seedlings representing various provenances and levels of genetic improvement. The primary materials comprised full-sib families from four levels of genetic gain (genetic classes): class A, elite families from parents selected with an intensity of approximately 1:30; class B, families from rogued first-generation seed orchards with a selection intensity of at least 1:10; class C, families from unrogued first-generation seed orchards; and class D, families of poorer than average breeding values. The A-class level corresponds to the level of improvement in the second round of seed orchards being established. The parent trees in classes A, C, and D were selected in southwestern British Columbia and class-B parents were selected in Forks, Wash. (Fig. 1). Average expected genetic gain in growth compared with unselected wild-stand material is listed in Table 1. Breeding values were based on 10- to 15-year height in 3–10 progeny tests for Washington and British Columbia materials, respectively. Thus, breeding values for the B families should be considered relative to wild-stand material from Forks, while breeding values for A, C, and D families are appropriately compared with wild-stand material from Vancouver Island. Wild-stand material from three provenances and one open-pollinated seed orchard bulk collection (Tillamook) were also included in the test. The Tillamook material is comparable with class C (unrogued first-generation seed orchard) but includes a provenance effect with parent trees originating from northern coastal Oregon. The wild-stand materials are either open-pollinated families (Queen Charlotte Islands, Oregon Cascades) or bulk collections from many stands (Vancouver Island). Tillamook and wild-stand materials are hereafter referred to as "provenances."

The experimental design is a split plot, with genetic entry (genetic classes or provenances) as main plots and families within

genetic classes as subplots. Families were completely randomized within main plots. The design was replicated in three blocks, with 3×3 m spacing in two blocks and 1×1 m in one block. The trees in the densely spaced block were visibly more yellowish than those in the normally spaced blocks in the spring of 1998, even though no differences between blocks were evident in the previous fall. The chlorosis was thought to be due to nutrient stress. Foliar samples collected in late April from three trees in a densely spaced plot and three trees of the same families from an adjacent normally spaced plot were analysed for total nitrogen. Densely spaced trees had 0.68% foliar N based on dry mass, while trees at the wider spacing had 1.18% N. It was concluded that the densely spaced trees suffered from nitrogen deficiency.

Measurements and sampling

Tree height (HT4) of all trees was measured in the fall of 1996, at 4 years of age. Frost hardiness and bud flush were assessed in the fall of 1997 and spring of 1998, at 5 years of age, on six trees per family, randomly selected from each of the full-sib plots. Each genetic class was thus represented by 6 trees $\times$ 8 families $\times$ 3 blocks = 144 trees (classes A and B) or $6 \times 3 \times 3 = 54$ trees (classes C and D). For fall hardiness testing, eight trees were sampled from each provenance plot (total 24 trees per provenance). For the spring hardiness and bud flush assessments, 16 trees per provenance plot were sampled, giving a total of 48 trees per provenance.

Test temperatures and optimal sampling period were decided based on preliminary tests. In the fall, juvenile trees on the University of British Columbia (UBC) campus were tested 2 weeks before the main test. In the spring, samples were collected in the Jordan River trial and at UBC 1 month before the main sampling and tested at five temperatures. Thereafter, the UBC trees were tested weekly to determine optimal sampling time and test temperatures.

Samples for fall hardiness tests were collected on October 27 and 28, 1997, and for spring frost hardiness on March 27, 1998. Twigs were cut from lateral branches with an easterly aspect at approximately breast height (1.3 m). The twigs were immediately placed in plastic bags, misted with water, and then the bags were sealed and stored on ice in a cooler within 1 h of collection. Within 30 h (fall) and 8 h (spring), all samples were transferred to a refrigerator with a controlled temperature of 4°C. Sampled twigs were kept refrigerated at all times when they were not being processed. Freeze tests were conducted 1–5 days after sampling.

Bud flush was assessed on three occasions: April 22, April 30, and May 12. Seven developmental stages were recorded: (0) dormant buds; (1) buds slightly swollen but not shining; (2) buds swollen and shining; (3) bud scales starting to open and expose needle tips; (4) needles elongated to twice the length of swollen bud; (5) needles elongated to three times bud length; (6) needles elongated to more than three times bud length, not yet spread; (7) needles elongated as in stage 6 but starting to spread. Date of bud flush (defined as the day when stage 3 was reached) was calculated for each tree by interpolation between the observations, assuming a linear development between stages over time. In a few cases, bud-flush date had to be extrapolated beyond the observation point.

Freeze-testing

Frost hardiness was assessed by measuring electrolytic leakage from needles exposed to different freezing temperatures. The methods for preparation of samples and assessment of leakage followed procedures developed by the B.C. Ministry of Forests (Sylvia L'Hirondelle, personal communication) with slight modifications. Twigs were rinsed with deionized water. Needles from the first-order lateral stem were cut off at the base, then one 5-mm segment was cut per needle. All segments had both ends cut. Needles from the distal 5 cm of the twig, as well as needles with visible damage, were excluded. From each tree, five needle segments were placed in one plastic vial for each of the test temperatures, and one, for an unfrozen control. Deionized water (0.2 mL) was added to each vial prior to freezing. To promote ice nucleation, a minuscule amount (≈2 mg) of silver iodide was added before the vials were sealed. Freezing was performed with a programmable Forma Scientific 8359 chest freezer with a Bio freezer CH/P Performance control unit. The starting temperature was 4°C when samples were placed in the freezer, and they were kept at 4°C for several hours to equilibrate. The temperature was then reduced at a rate of 4°C/h. When the first preset test temperature was reached, temperature was held constant for 1 h. Sample vials for that temperature were then transferred back to the refrigerator for thawing at 4°C. Freezing of the remaining vials then continued with a temperature reduction of 4°C/h down to the next test temperature, and the procedure was repeated. After 2 h in the refrigerator, an additional 3.5 mL deionized water was added to each vial. The vials were kept refrigerated for 20– 24 h and then placed on a gravity shaker at room temperature for 1 h. Electrolytic conductivity was measured with a Cole Parmer 1481-61 conductivity meter. After the first assessment of conductivity, the vials were placed in a water bath at 95°C for 1.5 h to heat kill the tissues, then placed at 4°C for 20–24 h. The heat-killed samples were then shaken at room temperature for 1 h, and the conductivity measurements were repeated. For the control samples, the same procedure was followed, except that the freezing step was omitted.

In the fall, full-sib families were tested in separate batches at –8 and –10°C during two consecutive days, starting 3 and 4 days after field sampling was completed. For the provenances, freezing was performed 5 days after field sampling in one batch at –6 and –10°C. A small sample of trees were included in all freezing batches, including a repeated freeze test 8 days after field sampling. From these results, it was clear that hardening continued during cold storage; therefore, it was not possible to compare the results for the full-sib families and the provenances (see Discussion).

In the spring hardiness test, full-sib families and provenances were tested together, with test temperatures of –9 and –14°C achieved in the same freezing batch. Blocks were tested on three separate successive days, starting with block 1 and ending with block 2. All freezing in the spring was thus carried out 1–3 days after field sampling.

Statistical analyses

For each sampled tree, the controls were given a conductivity value of zero and the heat-killed samples a value of 100. The conductivities of the frozen samples were then related to that range to calculate an index of injury (I), as described by Flint et al. (1967):

$$[1] \qquad I_t = \frac{100(R_t - R_0)}{(1 - R_0)}$$

where $R_t = L_t/L_k$, $R_0 = L_0/L_d$, I_t is the index of injury (percent) resulting from exposure to temperature t, R_t is the relative conductivity from the sample exposed to temperature t, R_0 is the relative conductivity of the unfrozen control sample, L_t is the conductance of leachate from the sample frozen at temperature t, L_k is the conductance of leachate from the sample frozen at temperature t and then heat killed; L_0 is the conductance of leachate from the unfrozen sample, and L_d is the conductance of leachate from the corresponding, heat-killed, unfrozen control sample.

One index was estimated for each temperature. The temperatures with the highest resolution among sources were used in the statistical analysis. These were –10°C for the fall test and –14°C for the spring test.

Table 2. Range of estimated family means and variance components for fall (IF-10) and spring (IS-14) index of injury, date of bud flush (BFDAY) after April 1, and height (HT4) for full-sib families in classes A, B, C, and D in the Jordan River trial.

	Range of family means	Variance components[a]				Family %[b]
		$\sigma^2_{f(\text{class})}$	σ^2_b	$\sigma^2_{b \times \text{class}}$	σ^2_e	
IF-10	19.4–62.8	80.2***	0	0	829.3	8.8
IS-14	22.9–64.0	45.6***	5.5**	23.2**	194.1	17.0
BFDAY	30.8–38.6	0.758	19.91***	1.23	25.43	1.6
HT4	178–250	38.8	9.4*	58.5	1861.4	2.0

[a]Significance levels from F test with $\sigma^2_{b \times \text{class}}$ as denominator for $\sigma^2_{f(\text{class})}$ and σ^2_b, and σ^2_e as error-term for $\sigma^2_{b \times \text{class}}$. ns, not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.
[b]Family variance percentage of total variance (intraclass correlation) attributable to differences among families.

Table 3. Block mean values and Tukey's test groupings for fall (IF-10) and spring (IS-14) frost injury index, bud flush day (BFDAY) after April 1, and height (HT4) for full-sib families in classes A, B, C, and D in the Jordan River trial.

Block (spacing)	IF-10	IS-14	BFDAY	HT4
1 (3×3 m)	40.6a	36.2b	33.3b	210.1ab
2 (3×3 m)	38.3a	36.8b	30.9b	198.5b
3 (1×1 m)	41.1a	46.5a	41.2a	219.6a

Differences between genetic classes were analysed in the SAS general linear methods (GLM) procedure with the following linear model:

$$[2] \qquad y_{ijkl} = \mu + a_i + b_j + f_{ik} + ab_{ij} + e_{ijl}$$

where y_{ijkl} is the observed value for tree l in family k in class i in block j, μ is the general mean, a_i is the fixed effect of class i, b_j is the random effect of block j with expected variance σ^2_b, f_{ik} is the random effect of family k within class i with expected variance σ^2_f, ab_{ij} is the random effect of interaction between class i and block j with expected variance σ^2_{ab}; and e_{ijkl} is the random residual error of tree l in family ik in block j. Tukey's test for differences between classes and blocks was run with the interaction effect ab_{ij} as the denominator. Family and interaction effects were tested with the residual as the error term. Variance components were derived using the REML method in SAS procedure VARCOMP. Family components were not transformed to heritabilities because of the nonrandom grouping in genetic classes and the small numbers of families in each class. Comparisons involving provenances were analysed with the following model:

$$[3] \qquad y_{ijk} = \mu + a_i + b_j + ab_{ij} + e_{ijk}$$

where y_{ijk} is the observed value for tree k in provenance i in block j, μ is the general mean, a_i is the fixed effect of provenance i, b_j is the random effect of block j with expected variance σ^2_b, ab_{ij} is the random effect of interaction between provenance i and block j with expected variance σ^2_{ab}; and e_{ijk} is the random residual error of tree k in block j. Differences between provenances were analysed with Tukey's test with the interaction effect ab_{ij} as the denominator.

The analysed variables were HT4 (height at 4 years), IF-10 (injury index in fall for the test temperature $-10°C$), IS-14 (injury index in spring, $-14°C$), and BFDAY (estimated number of days after April 1 for bud flush). Pearson's correlation coefficients were calculated on family means and individual trees among the full-sib families in the SAS procedure CORR.
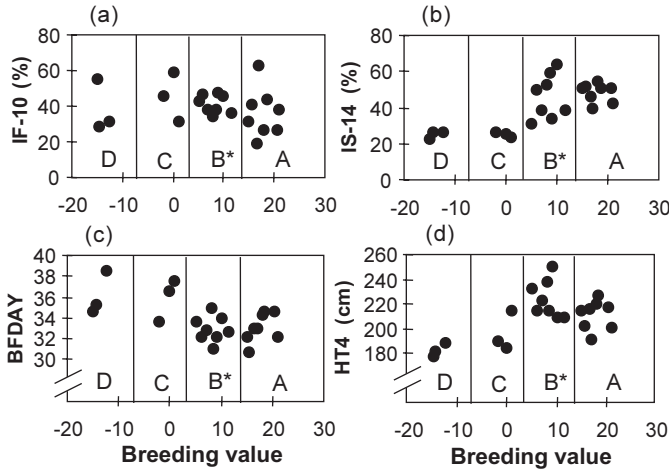
Results

Full-sib families

Analysis of variance showed a significant effect of family on both fall and spring index of injury but not on height and date of bud flush (Table 2). The block effect was significant for spring frost hardiness, bud-flush day, and height. The densely spaced block 3 had significantly later bud flush and higher spring index of injury (Table 3). The family-within-class component of total variation in injury index was approximately 9% in the fall and twice as high in the spring. There were no significant differences among genetic classes in fall index of injury (Fig. 2, Table 4). In the spring, A and B classes were significantly less frost hardy than both the C and D classes. Bud flush followed the same trend as spring frost hardiness, with the A and B classes flushing first, on average 3 days before C and D. However, the difference was not significant. Spring frost hardiness and date of bud flush were significantly correlated with both height and breeding value. There were no significant correlations between fall frost hardiness and height or spring phenology (Table 5). The correlation between breeding value and spring frost hardiness was stronger than the correlation between breeding value and actual height in the trial.

Provenances

Significant differences were found among provenances for all of the tested variables (Table 4). Frost hardiness increased with the latitude of provenances. The Oregon provenances (Tillamook and Cascades) had higher injury levels than the Vancouver Island and Queen Charlotte Islands provenances in both the fall and spring freezing tests (Table 4). The same pattern was demonstrated for bud flush, with trees from the Cascades and Tillamook flushing first, and from the Queen Charlotte Islands flushing last, 12 days after the Cascades. A similar trend with latitude was evident for 4-year-height.

It was not possible to compare the fall frost hardiness values of provenances with those of full-sib families, because they were tested in separate freezing batches with different periods of hardening prior to testing. In the spring, classes A and B dehardened and flushed at approximately the same time as Cascades and Tillamook, while classes C and D were as hardy as the Queen Charlotte Islands provenance but flushed on average at the same time as Vancouver Island wild-stand material.

Fig. 2. Injury index, date of bud flushing, and height for full-sib families in Jordan River trial related to breeding values of the parents (percent gain in height growth compared with unselected material). Bars and letters show the four genetic classes. Pearson's correlation coefficients for family mean values indicated below with p values in brackets. (*a*) Injury index from fall freeze test at –10°C (IF-10). $r = -0.17$ ($p = 0.46$). (*b*) Injury index from spring freeze test at –14°C (IS-14). $r = 0.71$ ($p = 0.0002$). (*c*) Bud flushing day after April 1 (BFDAY). $r = -0.55$ ($p = 0.008$). (*d*) Four-year height (HT4). $r = 0.52$ ($p = 0.012$). Class B parents come from Forks, Wash. Classes A, C, and D parents origin from southwestern British Columbia.

Table 4. Mean values and Tukey's test groupings for fall (IF-10) and spring (IS-14) frost injury index, bud-flush day (BFDAY) after April 1, and height (HT4) for full-sib family classes and provenances in the Jordan River trial.

	IF-10	IS-14	BFDAY	HT4
Genetic class				
A	36.2*a*	48.4*a*	33.0*a*	211.1*ab*
B	41.3*a*	46.0*a*	33.1*a*	223.8*a*
C	45.5*a*	25.3*b*	36.0*a*	196.2*ab*
D	38.7*a*	25.3*b*	36.1*a*	182.4*b*
Provenance				
Tillamook	51.5*a*	48.3*a*	32.1*a*	239.3*a*
Cascades	43.5*ab*	45.4*ab*	31.6*a*	205.6*b*
Vancouver Island	28.9*bc*	40.3*ab*	36.5*ab*	196.6*b*
Queen Charlotte Islands	21.0*c*	28.0*b*	43.5*b*	187.9*b*

Note: Tukey's studentized range tests were run separately for genetic classes and provenances. Class or provenance means followed by the same letter were not significantly different at the 5% level.

Table 5. Family mean correlations (above the diagonal) and individual tree correlations (below the diagonal) for fall (IF-10) and spring frost injury index (IS-14), bud flush day (BFDAY), height at 4 years (HT4), and breeding value for height (BV) for 22 full-sib families in genetic classes A, B, C, and D, with an average of 16.6 individuals per family.

	BV	IF-10	IS-14	BFDAY	HT4
BV	—	–0.17	0.71***	–0.55**	0.52*
IF-1	–0.05	—	–0.24	–0.09	–0.27
IS-14	0.47***	–0.05	—	–0.52*	0.43*
BFDAY	–0.15**	–0.10	0.06	—	0.28
HT4	0.21***	–0.07	0.22***	0.20***	—

*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$.

Discussion

Fall frost hardiness

The key question addressed by this study was whether selection for growth in western hemlock leads to negative effects on the frost hardiness. Western hemlock is an indeterminate species with a relatively late cessation of shoot elongation compared to determinate species such as Douglas-fir (*Pseudotsuga menziesii* (Mirb) Franco), grand fir (*Abies grandis* (Dougl.) Lindl.) and western white pine (*Pinus monticola* Dougl. ex D. Don) (Buckland 1956; Griffith 1960; Walters and Soos 1963). Western hemlock seedlings also have a slower hardening rate in the fall than seedlings of coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) (Timmis 1979), and it is presumed that fall frost hardiness is an important adaptive trait in western hemlock.

The results of this study suggest that gains in growth achieved through provenance transfer of western hemlock will be accompanied by significant reductions in fall frost hardiness, yet gains achieved through selection of families within provenances should not alter the average fall frost hardiness. A prolonged duration of growth and thus a reduced fall frost hardiness as a result of provenance transfer from milder or more southern environments has been demonstrated in numerous conifer species including Norway spruce (Skrøppa and Magnussen 1993), Sitka spruce (*Picea sitchensis* (Bong.) Carr.) (Cannell and Sheppard 1982), inland Douglas-fir (*Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco) (Rehfeldt 1983), Scots pine (*Pinus sylvestris* L.) (Persson and Ståhl 1990), and western hemlock (Meagher 1976; Kuser and Ching 1980). Strong selec-

tion for growth in breeding programs might lead to a similar adverse effect on adaptation, as was demonstrated in ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) (Rehfeldt 1992) and in inland Douglas-fir (Rehfeldt 1983). Other studies have found that selection for growth among parent trees within a population has little or no effect on fall frost hardiness or duration of growth (Scots pine, Nilsson and Andersson 1987; Norway spruce, Skrøppa and Magnussen 1993; Ekberg et al. 1994; Johnsen and Østreng 1994; silver birch (*Betula pendula* Roth), Wang and Tigerstedt 1993, 1996). In western hemlock, Walters and Soos (1963) found no correlation between amount of leader growth and duration of growth between trees from the same region. This may indicate that rate of growth is a greater determinant of height differences among families of western hemlock than duration of growth.

Spring frost hardiness and date of bud flushing

According to this study, gains in growth achieved either through provenance transfer or family selection within provenances will be associated with a cost in terms of reduced spring frost hardiness and early bud flush. The fast-growing

classes A and B dehardened and flushed first, and the low-yielding classes C and D retained hardiness longer and flushed 3 days later. There was also a strong negative correlation between spring frost hardiness and both height growth and breeding value.

Among the provenances, the low-yielding sources were also the most hardy and latest flushing. This is opposite to the strong clinal trend in western hemlock found by Kuser and Ching (1980), where seedlings from the Queen Charlotte Islands flushed about a week earlier than the coastal Washington and 2 weeks earlier than the low-elevation Cascade provenances. The results of this study could be explained by the elevational differences between the materials. Queen Charlotte Islands sources were from close to sea-level, Vancouver Island from over 100 m and Cascade from 300 m. In the study by Kuser and Ching (1980), the Queen Charlotte Islands material were from 300 m above sea level, while coastal Washington materials were from 100–200 m.

In other Pacific coast conifer species, a north–south trend in bud flushing has sometimes been demonstrated; however, the results of various studies are far less consistent than those for growth cessation and bud set. Campbell and Sugano (1979) found later bud flush with more northern origin in coastal Douglas-fir, and Campbell (1974) determined that flushing date is more strongly related to elevation and distance from the ocean than to latitude. In a study with 30 provenances of coastal Douglas-fir from northern Washington to central California, Sweet (1965) did not find any relation between date of bud flush and latitude of origin. In Sitka spruce, Burley (1966) found a weak but inconsistent trend with earlier bud flush in northern provenances. In the same species, Cannell and Sheppard (1982) found that southern provenances dehardened first, but all provenances flushed at the same day. Finally, Meagher (1976) did not find any relation between flushing date and latitude in western hemlock seedlings, but they were from a narrower distribution than those in the present study.

The family component of variation was much higher for spring than for fall frost hardiness, suggesting that frost hardiness is under stronger genetic control during deacclimation than during acclimation. This is also in accordance with findings in coastal Douglas-fir (Aitken and Adams 1997).

Experimental design

The experimental design puts some restrictions on the interpretation of the results. First, families were not a random population sample; instead, they were selected to demonstrate the full range of genetic levels in the tested plus-tree population. This is also the reason why no genetic parameters were calculated.

The most densely spaced block displayed a nitrogen deficiency, the effect of which was not detected for height growth or fall frost hardiness but was expressed during spring deacclimation. Trees in the dense spacing had significantly lower spring frost hardiness and flushed 7–9 days later than those in the wider spacing. A later bud flush in nutrient-poor conditions was demonstrated in western hemlock seedlings by Meagher (1976). Delayed bud flush might also have been caused by lower soil temperature in the densely spaced block (Sorensen and Campbell 1978). It is interesting that low spring hardiness and late bud flush were associated,

while typically low spring hardiness is associated with early bud flushing (e.g., Aitken and Adams 1997). General observations made by the authors also suggest that stressed trees, whether the stress is induced by nutrient deficiency or by frost injuries, respond with delayed bud flushing.

Methodological considerations

Freeze-induced electrolyte leakage of shoots and needles has been demonstrated to reliably measure the hardiness level in Douglas-fir, Engelmann spruce (*Picea engelmannii* Parry) and ponderosa pine (Burr et al. 1990); Sitka spruce (Nicoll et al. 1996); and yellow cypress (*Chamaecyparis nootkatensis* (D. Don) Spach.) (Hawkins et al. 1994). The technique gives a quick result compared with visual scoring after whole-plant freezing. In this study, we found quite large variation between freezing batches and within trees, despite precise control of needle sampling. This could result from the fact that, true to its species name *heterophylla*, western hemlock needles are very heterogeneous in size and shape. The variation between freezing batches in the fall could result partly from continued hardening during storage in the refrigerator. When the same set of 21 trees were freeze tested following 4 and 8 days of refrigeration after field collection, injury index decreased from 29 to 20%. In another set of 15 trees, index decreased from 26% on testing at day 5 to 15% on day 8. In the spring, a corresponding dehardening continued during storage. In the same 20 trees tested 2 days and 11 days after field sampling, injury index increased from 40 to 77% after freezing to –14°C. This shows the importance of reducing storage time after field sampling, especially if tests are made during periods of rapid hardening or dehardening.

With limited resources and a need for large-scale hardiness assessment in tree breeding, it would be advantageous to increase the number of genotypes at the expense of samples per genotype. Flint's index, used in this paper, requires that a control set is run for each tested tree. If the control samples are excluded, comparisons can instead be made for relative leakage, R_t in eq. 1. We did not find any differences among classes or provenances in the control leakage values, and the error component of total variation was equal between relative leakage and Flint's injury index both in fall and spring. Hence, we would have come to similar conclusions by analysing relative leakage instead of injury index, which would have decreased the time for sample preparation and measurements. On the other hand, if control leakage values are affected by other factors (e.g., a natural frost before field sampling), it is important to determine the magnitude of this effect (see Redfern and Cannell 1982). This could be tested on a smaller sample, and if the natural frost has caused severe damage, it is doubtful whether the artificial freezing test should proceed.

Practical implications

The Jordan River experimental site is located close to the ocean, with an expected low risk of frost events. The results show the potential for using genetic material transferred from milder, or more southern, environments to improve growth, as seedlings originating from the seed orchard Tillamook demonstrated superior height growth to other sources. On the other hand, Tillamook had the lowest frost

hardiness both in the fall and spring, and this might have been reflected in reduced growth on a harsher site. There was a tendency to higher growth of class-B families over class-A families at the Jordan River site, despite the higher estimated breeding values of class A. This likely reflects provenance differences in the base populations, with breeding values estimated in two different geographic areas: class-A families originating and tested in southwestern British Columbia and class-B families in Forks, Wash.

The importance of these findings depends on the risk of fall or spring frost events in western hemlock plantations or stands. In general, frost risk is low in coastal British Columbia. Cannell (1984) showed that mean date for the last spring frost (below –2.5°C) was late February in Vancouver and late March on the Queen Charlotte Islands. In the fall, the average date for the first damaging frost (below –4.5°C) occurs in early to middle December in coastal British Columbia (Cannell 1985). Arnott (1976) reported that the most important causes of mortality in western hemlock plantations in British Columbia are drought, competing vegetation, and soil erosion. However, when plantations are located at high elevations or further from the coast, frost risk becomes more important.

Observations made in short-term genetic field tests do not necessarily sample extreme climatic events. With regard to fall and winter frost, single catastrophic events can set the thresholds for adaptive traits. A severe frost event in November 1955 caused heavy mortality in 30- to 40-year-old stands in western Washington (Duffield 1956). Western hemlock was the most damaged species, followed closely by western red cedar (*Thuja plicata* Donn ex D. Don). Douglas-fir was also damaged, but to a lesser extent. According to Duffield (1956), serious damage to cambium and phloem were observed in mature western hemlock. Even minor frost injuries can reduce photosynthesis and growth (Lundmark et al. 1988), and decrease vigour, making trees more susceptible to pathogens or insect herbivory (Waring 1991).

Conclusions

This study suggests that genetic selection of western hemlock families for growth in field tests can be made without negative effects on the average fall frost hardiness. However, fast-growing families display lower spring frost hardiness and earlier bud flush. Elite material presently selected for establishment of new seed orchards in British Columbia are thus likely to suffer more from spring frost damage than unselected material. On the other hand, increasing gain through provenance transfer is accompanied by reduced frost hardiness both in the fall and in the spring. With proper methods for screening genotypes for adaptive traits, the outlook is good for selecting families that combine rapid growth with a low risk of frost injury.

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