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**VARIATION IN COLD HARDINESS AMONG POPULATIONS  
OF *PSEUDOTSUGA MENZIESII* VAR. *GLAUCA***

**Gerald E. Rehfeldt**

**USDA Forest Service Research Paper INT-233  
INTERMOUNTAIN FOREST AND RANGE EXPERIMENT STATION  
Forest Service • U.S. Department of Agriculture • Ogden, Utah 84401**

## THE AUTHOR

GERALD E. REHFELDT is a plant geneticist at the Forestry Sciences Laboratory, Moscow, Idaho. His research includes studies of the ecological genetics of Northern Rocky Mountain conifers, primarily Douglas-fir.

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# RESEARCH SUMMARY

Freezing tests were conducted to follow cold acclimation in seedlings representing 51 populations of *Pseudotsuga menziesii* var. *glauca*. For each of six dates between August and December, twigs from 2-year-old seedlings growing in a common environment were frozen to each of six test temperatures. Injury from freezing was scored by leaf discoloration.

Hardiness developed at a rate associated with the minimum temperature. During the early stages of cold acclimation, variation in the hardiness of populations was related to geographic and ecologic factors of the seed source. Multiple regression analyses accounted for 38 percent of the variance among populations and showed that populations of greatest hardiness in early autumn were from relatively high latitudes and high elevations. But, during late stages of cold acclimation, variation in hardiness among populations could not be related to factors of the seed source.

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# INTRODUCTION

Cold acclimation in woody plants from temperate zones typically develops in two phases (Weiser 1970). The first phase is associated with the cessation of growth and development. In most tree species of the northern temperate zone, this phase coincides with the onset of winter dormancy or rest period that cannot be broken until chilling requirements have been satisfied (Glerum 1973). The second phase of cold acclimation results in the development of tolerance to extreme cold (Weiser 1970). This phase is triggered by the first frost, and hardiness develops in general response to the minimum temperature (Levitt 1972). Autumn frost injuries occur during the first phase of cold acclimation when phenological events are not synchronized with the local climate; injuries occur during the second phase when dormant tissues have failed to harden sufficiently to withstand the minima of autumn and winter.

The actual levels of hardiness that develop in response to given environmental stimuli are under genetic control. For example, geographic races of *Cornus stolonifera* (Smithberg and Weiser 1968), *Liquidambar styraciflua* (Williams and McMillan 1971) and *Quercus rubra* (Flint 1972) acclimate differently. Throughout cold acclimation, progenies of the coastal variety of Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) are of lesser hardiness than those of the Rocky Mountain variety (*P. m.* var. *glauca*) (Rehfeldt 1977). And for the Rocky Mountain variety, genetic variation in hardiness exists among populations and among trees within populations (Rehfeldt 1979).

Cold acclimation in populations of Douglas-fir from northern Idaho and eastern Washington is the primary concern of the present research. In this area, tolerance to cold temperatures is required for survival. Freezing temperatures can occur during any month, and temperatures as low as  $-40^{\circ}\text{C}$  are not rare. Previous research has shown that tolerance to freezing is generally greater in Douglas-fir seedlings from western Montana than in those from northern Idaho and eastern Washington (Rehfeldt 1979). Differentiation of populations in cold hardiness may reflect ecological adaptations that should be considered in limiting seed transfer for reforestation. The present study emphasizes the effects of variation in hardiness on the delineation of seed zones for northern Idaho and eastern Washington.

## MATERIALS AND METHODS

Cold acclimation was studied in 2-year-old seedlings from 51 populations of Douglas-fir (fig. 1). Each population was represented by seeds from at least 10 trees. Most of these populations were from northern Idaho and eastern Washington, but populations from northeastern Oregon and north central Washington were also included. Populations were selected to fully represent the geographic distribution and ecological amplitude of the species in the region of study; longitude, latitude, elevation, and habitat type were variables used to characterize a seed source. Habitat type was classified according to the system of Daubenmire and Daubenmire (1968).

About 100 seedlings from each population were grown for 2 years in plastic containers ( $150\text{ cm}^3$ ) in a shadehouse at Moscow, Idaho. In order to promote branching, seedlings were decapitated during the first growing season.

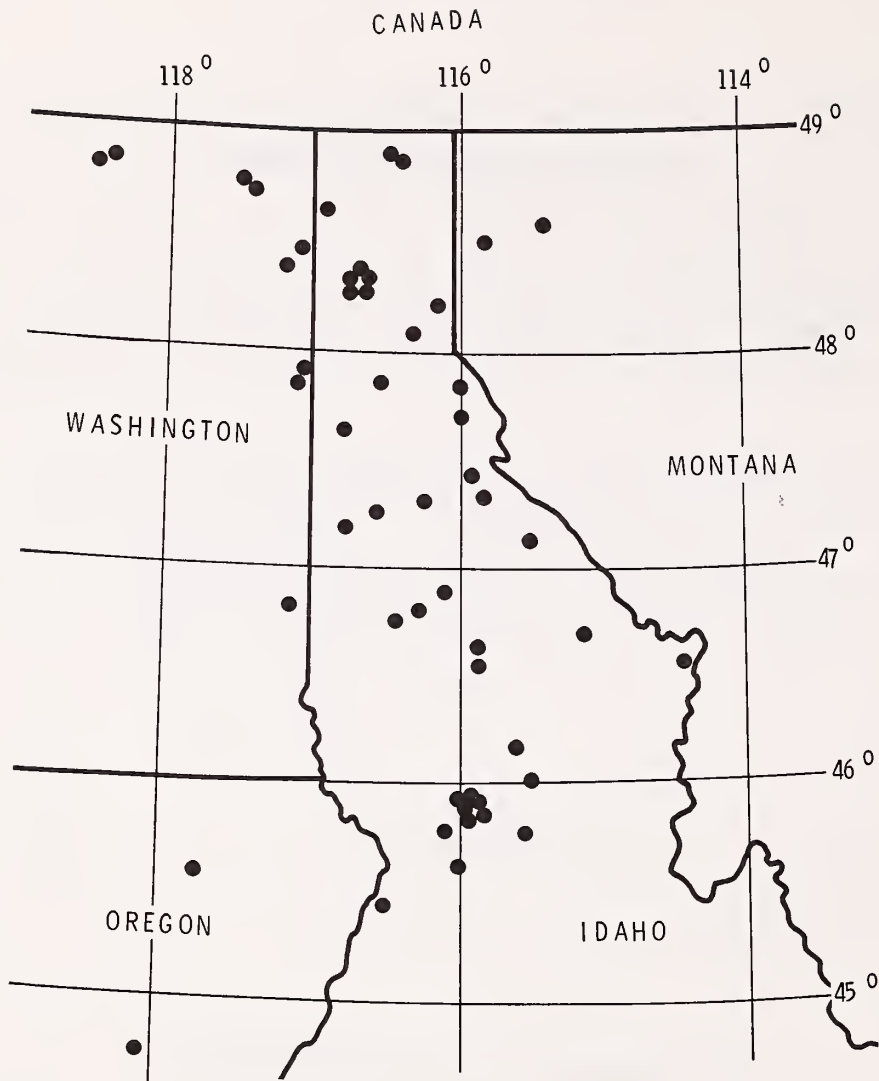


Figure 1.--Location of populations (dots).

Freezing tests were made to determine levels of hardiness of each population for six dates between August and December of the second growing season. These tests generally followed the procedures outlined by Levitt (1972). For each sampling date, six sets of 10 lignified twigs (5 to 10 cm in length) were cut from the current growth of seedlings representing each population. Twigs in each set were moistened and packaged in a plastic bag. Although each set contained twigs from 10 different seedlings, all sets did not necessarily contain twigs from the same 10 seedlings. About 15 seedlings from each population were needed to supply sufficient twigs for a single sampling date.



Twigs were stored overnight at 3°C. The following morning, one set of twigs from each population was frozen at a rate of 5°C/h to one of six test temperatures. The six test temperatures for a given date spanned a temperature range of 10° to 12°C. Each test temperature was maintained for 1/2 hour; twigs were thawed for 24 hours at 2°C, and basal portions of twigs were placed in water within plastic drinking cups. After 5 to 7 days, freezing injury to each twig was scored according to discoloration of leaves. For each population, the number of twigs exhibiting leaf injury was recorded for each test temperature.

Tolerance of populations to freezing was assessed by regression analyses that were made for each population according to the logistic model. This model, suitable for proportional data between values of zero and one (Johnson and Kotz 1970), is of the form:

$$Y_{ij} = \frac{1}{1 + be^{-rX_j}}$$

which is represented by the linear model:

$$\ln \left( \frac{1}{Y_{ij}} - 1 \right) = -rX_j + \ln b$$

where:

$Y_{ij}$  = proportion of twigs injured for population  $i$  in treatment severity  $j$ ,

$X_j$  = index of freezing severity = percentage of twigs from all populations injured in freezing treatment  $j$ ,

$b = \frac{1}{y_0} - 1$  where  $y_0$  is the predicted damage if no freezing treatment is applied ( $X_j = 0$ ),

$r$  = rate of increase in damage associated with an increase in severity index.

Separate analyses were made for each population on data obtained before the first frost (phase one of cold acclimation) and after frost (phase two). Since there were only two sampling dates before the first frost, regressions for phase one were based on 12 observations, and those for phase two were based on 24 observations.

Tolerance of each population to freezing during both phases of cold acclimation was expressed as the injury predicted from the logistic model when freezing severity ( $X_j$ ) was 50 percent (50 percent of the twigs from all populations exhibited injury). In fact, throughout this paper, these estimates of injury are used as expressions of the hardiness of populations during cold acclimation.

Differentiation of populations in relation to latitude, longitude, elevation, and habitat type was assessed by multiple regression analyses. These analyses were made to relate differentiation to geographic and ecologic variables of the seed source so that hardiness could be used as a variable in delineation of seed zones for northern Idaho and northeastern Washington. However, the tests included populations from the Okanogan Mountains in north central Washington and from the Blue Mountains in northeastern Oregon. Since these populations could represent adaptive provinces that differ from the northern Idaho province in manners similar to the differentiation of the latter province from those of western Montana (Rehfeldt 1979) and southern

Idaho (Wright and others 1977), the four populations of greatest longitude (fig. 1) were excluded from the following analyses.

The hardiness of 47 populations for each phase of cold acclimation was used as a dependent variable in the following model:

$$Y_i = b_0 + b_1X_{i,1} + \dots + b_7X_{i,7}$$

where:

$Y_i$  = predicted injury for population  $i$ ,

$X_{i,1}$  = degrees latitude at the origin of population  $i$ ,

$X_{i,2}$  = degrees longitude at the origin of population  $i$ ,

$X_{i,3}$  = meters ( $1 \times 10^{-2}$ ) elevation at the origin of population  $i$ ,

$X_{i,4}$  to  $X_{i,7}$  = constant terms (values of zero or one) that respectively code the *Pseudotsuga menziesii*, *Abies grandis*, *Thuja occidentalis*, and *Tsuga heterophylla* series of habitat types.

Under this model, effects of the *Abies lasiocarpa* series of habitat types are contained within the intercept ( $b_0$ ). Consequently, values of regression coefficients for constant terms ( $b_4$  to  $b_7$ ) are deviations from the mean value for seedlings from *Abies lasiocarpa* habitat types.

Because multiple regression models are partially interpretable from inter-correlations among independent variables, it is instructive to note that significant associations were apparent between latitude and longitude ( $r = 0.56$ ) and between longitude and elevation ( $r = -0.32$ ). For the populations represented, habitat types accounted for significant proportions of variance in none of the other independent variables.

In accordance with the techniques of Morgenstern and Roche (1969), concepts of selection were used to estimate population change associated with the geographic and ecologic variables. This technique uses the expression for genetic gain:

$$R = ih\sigma_A$$

where:

$R$  = selection response,

$h^2$  = heritability,

$\sigma_A^2$  = additive genetic variance,

$i$  = selection intensity.

The selection intensity is estimated by assuming that  $h^2 = 1$ ,  $\sigma_A$  is estimated by the standard error of regression ( $s_{y \cdot x}$ ), and  $R$  is estimated by the regression coefficient ( $b$ ). Because the intensity of selection depends only on the proportion of the population in the selected group, the proportion of a population that is similar to a neighboring population one geographic unit distant is estimated directly from the selection intensity (fig. 11.3, Falconer 1960).

# RESULTS

In order to relate cold acclimation to weather at Moscow, the temperature associated with injury to 50 percent of the twigs was estimated for each sampling date. That cold acclimation is closely related to the minimum temperature (Levitt 1972) is well illustrated in figure 2. Low levels of hardiness developed after the first frost on October 3. Hardiness increased little during a relatively warm period in late October, but increased greatly after the first cold wave in mid-November.

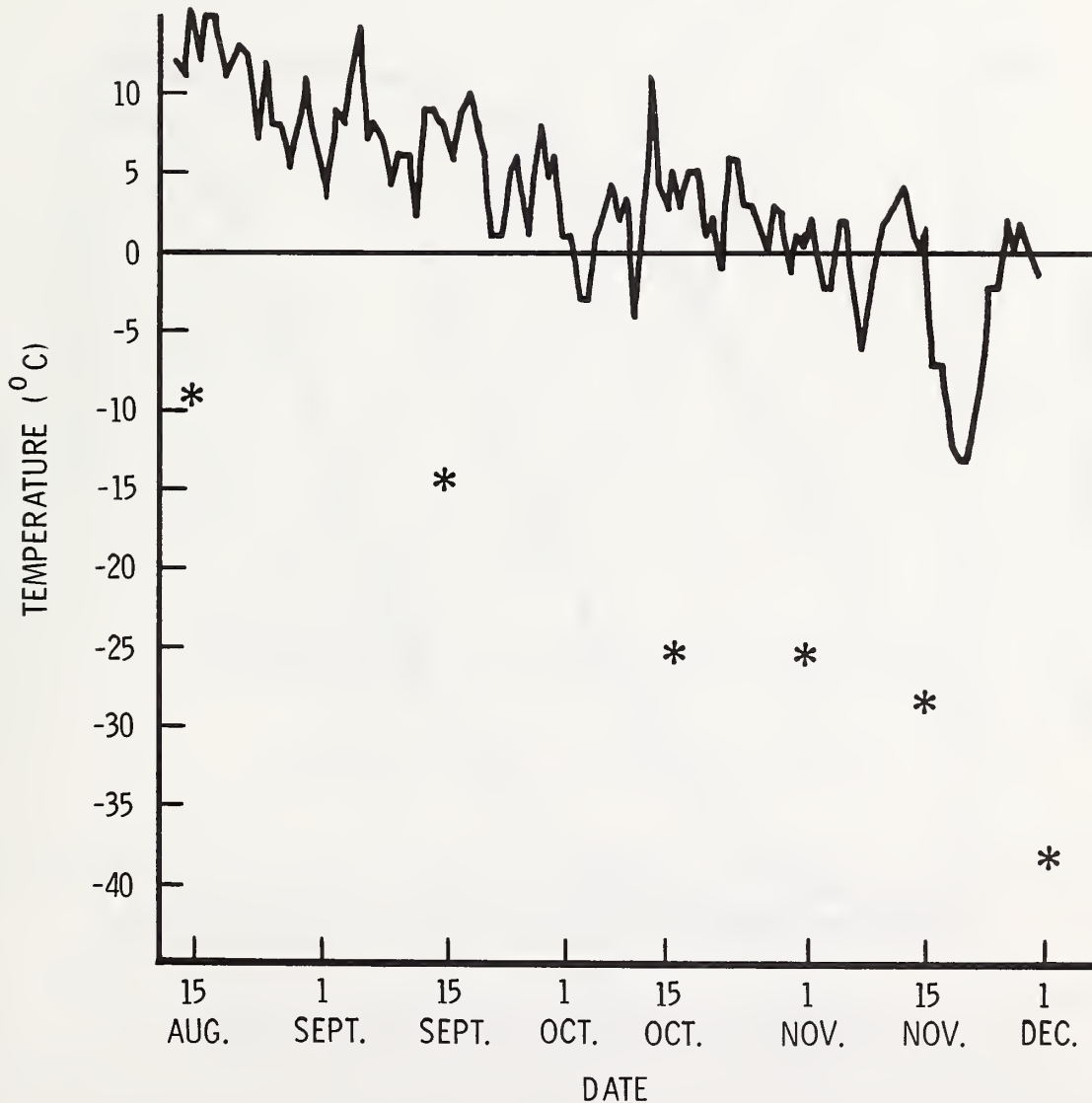


Figure 2.--Development of hardiness in relation to daily minimum temperatures. Stars denote the temperature associated with injury to 50 percent of the twigs at each sampling date.

Regressions of freezing tolerance for data from phase one of cold acclimation were statistically significant (1 percent level) for all populations and accounted for 64 to 96 percent of the variance. Response curves for three populations illustrate the variety of observed responses to freezing severity (fig. 3). Each of the curves presented in figure 3 differs significantly (5 percent level) from each other for either  $r$  or  $b$  in the regression equation.

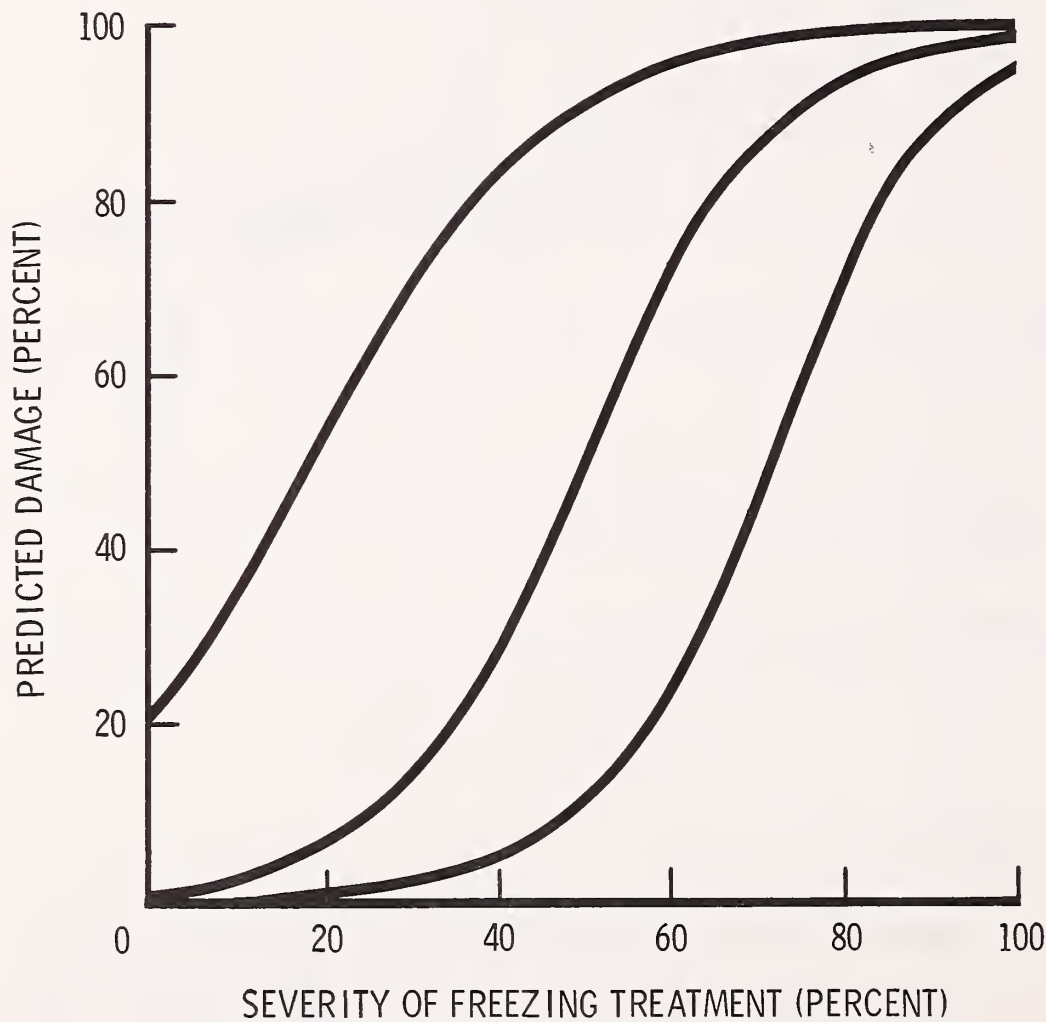


Figure 3.--Response curves of freezing damage according to severity of the freezing treatment for three populations during the first phase of cold acclimation.

Regressions of freezing tolerance for phase two were also significant (1 percent level) for each population. Values of  $R^2$  ranged from 0.45 to 0.81. Figure 4 illustrates the variety of observed responses; but, only the response curves of maximum contrast differ significantly (5 percent level) for either  $r$  or  $b$ .

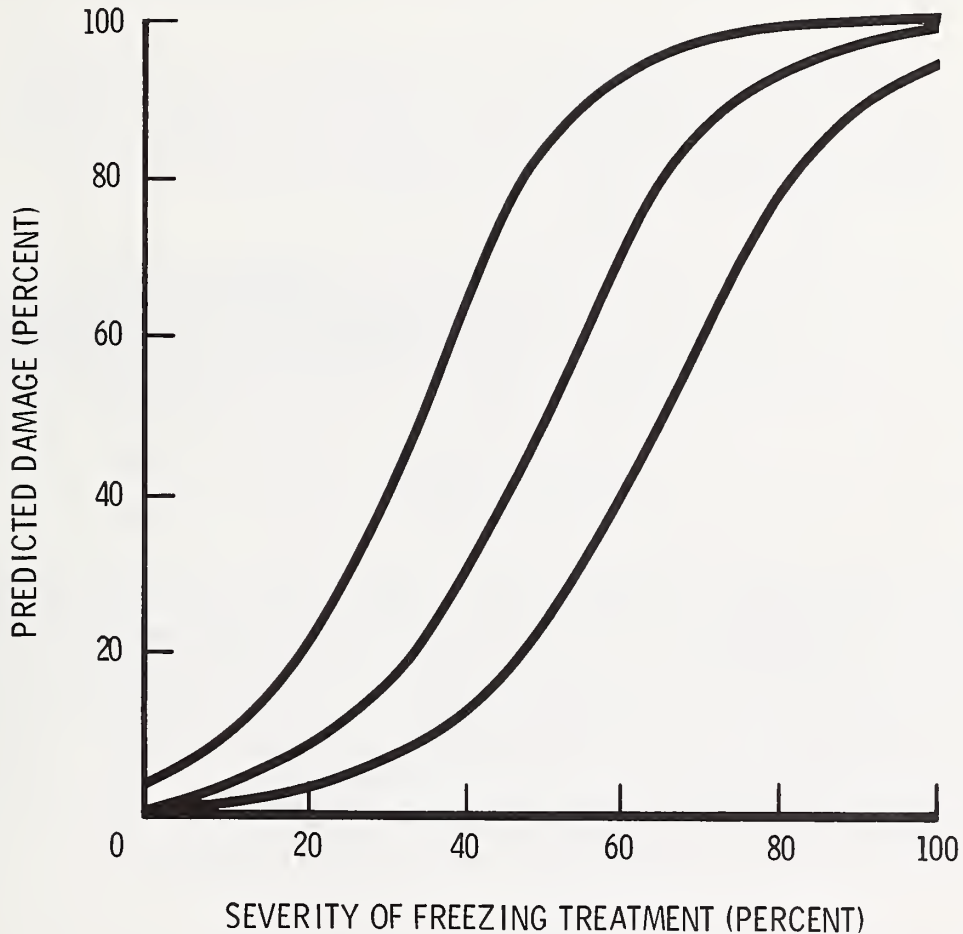


Figure 4.--Response curves of freezing damage according to severity of the freezing treatment for three populations during the second phase of cold acclimation.

The curves in figures 3 and 4 express a greater range in response and a consequent higher variance among populations for cold tolerance during phase one than during phase two. Previous studies (Rehfeldt 1979) have also suggested that population differentiation is readily detected for acclimation during autumn, but that relatively little genetic variance exists among populations for hardiness during deep winter dormancy. In concurrence with previous studies (Rehfeldt 1979), the curves in both figures that illustrate maximum tolerance to freezing depict populations from cool subalpine environments at relatively high elevations (above 1,300 m).



Multiple regressions for assessing the effects of geographic and ecologic variables on the hardiness of populations were made separately for data from two phases of cold acclimation (table 1). Whereas the regression for phase one accounted for a statistically significant (1 percent level) 38 percent of the variance, that for phase two accounted for a nonsignificant 15 percent. In accordance with relatively little variance among populations, variation in hardiness during the second phase of cold acclimation could not be related to geographic or ecologic variables of the seed source.

Table 1.--Results of multiple regression analyses of hardiness during phases one and two of cold acclimation on geographic and ecologic variables. Variables are defined in the text. Results are presented in terms of regression coefficients ( $b$ ) and standardized partial regression coefficients ( $b'$ )

Variable	Hardiness during:			
	Phase one		Phase two	
	$b$	$b'$	$b$	$b'$
Latitude $X_1$	-9.27	-0.59	0.51	0.03
Longitude $X_2$	2.03	.08	-2.96	-.12
Elevation $X_3$	-3.05	-.37	.82	.11
Habitat type:				
<i>Pseudotsuga menziesii</i> $X_4$	.96	-.02	14.74	.41
<i>Abies grandis</i> $X_5$	-4.66	-.13	21.60	.64
<i>Thuja occidentalis</i> $X_6$	-.62	-.02	20.92	.64
<i>Tsuga heterophylla</i> $X_7$	-2.09	-.04	11.91	.28
-----				
$b_0$	111.40		29.26	
$R^2$	.38		.15	
$s_{y \cdot x}$	14.35		15.18	

By contrast, a comparison of standardized partial regression coefficients reflects the relative importance of the continuous independent variables in predicting hardiness during phase one. Elevation and latitude control differentiation (table 1). Longitude has little influence. Since regression coefficients for the constant terms ( $b_4$  to  $b_7$ ) are deviations from the intercept  $b_0$ , under the present model the constants reflect deviations in mean hardiness of seedlings representing the indicated habitat types from that of seedlings from the *Abies lasiocarpa* habitat types. None of the coefficients for the constant terms are statistically significant.

The multiple regression analyses pertaining to hardiness during phase one have direct application in delineating seed zones that reflect adaptive differentiation in the cold hardiness of populations. Methods described by Morgenstern and Roche (1969) were used to obtain estimates of selection intensity ( $i$ ) for latitude (0.65), longitude (0.14), and elevation (0.21). The resultant percentages of similarity among populations at various units of distance involving one degree latitude, one degree longitude, or 100 meters elevation are:

Units of geographic distance	Percent similarity for		
	Latitude	Longitude	Elevation
0.5	82	98	96
1	63	95	90
2	28	88	75
3	8	75	60
4	--	56	50

Morgenstern and Roche (1969) show that their assumptions ( $h^2 = 1$  and  $s_{y \cdot x} = \sigma_A$ ) introduce bias that maximizes estimates of the selection intensity ( $i$ ). Accordingly, the percentages of similarity presented above are minimal estimates.

## DISCUSSION

Freezing tests showed variation in hardiness among populations of Douglas-fir during the two phases of cold acclimation. Yet hardiness of populations during the first phase (autumnal dormancy) was not related ( $r = -0.05$ ) to hardiness during the second phase, which is characterized by low levels of cold hardiness. Moreover, regression models accounted for significant proportions of the variance in hardiness for only phase one. Consequently, it appears that selective differentiation of populations is systematically related to geography and ecology for physiological conditioning to the first frost (phase one). Little variance differentiates populations in cold acclimation after the first frost (phase two), and most of that variance seems to be random. As such, the variation could be related to random events within the history of the population rather than to environmental conditions systematically related to geography and ecology.

The latitude and elevation of the seed source seemed to control differentiation of populations for hardiness during phase one. Longitude and habitat type had little effect. For habitat types to have little influence on differentiation is contradictory to previous results (Rehfeldt 1974, 1979) that related population differentiation to *Abies lasiocarpa* habitat types. However, one of the populations in the present study represented an *Abies lasiocarpa* habitat type from a small frost pocket at a relatively low elevation (980 m). Contrary to a previous report (Rehfeldt 1974), levels of hardiness for this population corresponded to those of populations from similar latitudes and elevations rather than to those from subalpine environments at relatively high elevations. Finally, that none of the regression coefficients for constant terms (table 1) were statistically significant also suggests a lack of effects of habitat types on genetic differentiation in cold acclimation.

Populations from areas peripheral to the central area of study were also included in the present tests. The Okanogan Mountains in north central Washington (longitude 118.5°) were represented by two populations. Both populations were from relatively high elevations (1,460 m). Cold hardiness of these populations evidently approached maximum values in mid-November. Unlike populations from farther east, hardiness in populations from the Okanogan Mountains failed to develop greatly in response to the cold temperatures of late November. On the other hand, populations from the Blue Mountains in northeastern Oregon (longitude 118°) hardened similarly to populations from northern Idaho.

Current and previous studies on the hardiness of Douglas-fir populations have direct application in delineating seed zones in northern Idaho and eastern Washington that reflect adaptive variation in cold acclimation. In contrast with previous results (Rehfeldt 1979), present data provide no evidence that the cold *Abies lasiocarpa* series of habitat types should represent a seed zone that is independent of elevation. However, development of seed transfer guidelines associated with continuous variables requires acceptance of a level of genetic similarity that minimizes risk of maladaptation but that remains economically and administratively practical. For the present data, a 50 percent genetic similarity implies that seeds transferred 1-1/2 degrees latitude or 400 m elevation have a probability of only 0.5 of producing seedlings as cold hardy at the alien site as seedlings derived from local seeds. Thus, it seems intuitively sound biologically to limit seed transfer by 75 percent genetic similarity, even though the associated probability of maladaptation is 0.25. However, it should be recalled that the estimates of similarity derived by the techniques employed represent minimal estimates (Morgenstern and Roche 1969). Consequently, probabilities of maladaptation are maximal.

If a 75 percent genetic similarity limits seed transfer, seed for reforestation of northern Idaho and northeastern Washington should not be transferred more than 1 degree latitude, 3 degrees longitude, or 200 m elevation. However, seed zones must be based on numerous adaptational features. Consequently, this paper is one of a continuing series on the ecological genetics of Douglas-fir in the Northern Rockies.



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