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Adaptive Variation in Pinus ponderosa *from Intermountain Regions. I. Snake and Salmon River Basins*

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ABSTRACT. Genetic differentiation of 64 populations from central Idaho was studied in field, greenhouse, and laboratory tests. Analyses of variables reflecting growth potential, phenology, morphology, cold hardiness, and periodicity of shoot elongation revealed population differentiation for a variety of traits. Regression models related as much as 61 percent of the variance among populations to the elevation and geographic location of the seed source. Clinal patterns of adaptive variation provide the basis for developing seed transfer guidelines that will control maladaptation in reforestation. In central Idaho, for example, seed transfer should be limited to within 180 m of the seed source. FOREST SCI. 32:79–92.

ADDITIONAL KEY WORDS. Genetic variation, population differentiation, ecological genetics, genecology.

AS THE DOMINANT FOREST TREE on the semi-arid sites that border the steppe, ponderosa pine (*Pinus ponderosa*) was readily accessible to the settlers of the mountainous West. As noted by Steele and others (1981), accessibility by early farmers, ranchers, lumbermen, and miners prompted misuse and destruction by fire. Grazing pressure on droughty soils of low fertility limited natural regeneration. Consequently, the pine received an early emphasis in forest management activities, including artificial reforestation. Because successful artificial reforestation requires adapted planting stock, a ponderosa pine provenance test was established in 1911 (Kempff 1928) as the oldest provenance test of forest trees in North America (Wang 1977).

This and subsequent provenance tests have revealed considerable genetic diversity within the species. Variation among populations from diverse geographic regions is pronounced for a variety of traits reflecting growth potential, phenology, morphology, and other environmental adaptations (Squillace and Silen 1962; Hanover 1963; Wells 1964a, b; Steinhoff 1970; Read 1980, 1983). Adaptive differentiation of populations within geographic regions also occurs along elevational gradients in the Sierra Nevada (Callaham and Liddicoet 1961, Conkle 1973), Colorado Front Range (Mitton and others 1977), Northern Rockies (Madsen and Blake 1977), and the Salmon River Mountains of central Idaho (Rehfeldt 1980b).

Even though previous tests have described geographic and altitudinal patterns of adaptive differentiation, they were of insufficient scope and power for estimating the distance that seeds from wild trees can be transferred before maladaptation begins limiting productivity. Although seed transfer guidelines for ponderosa pine in central Idaho were developed from the performance of 16-year-old trees on

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four planting sites (Rehfeldt 1980b), the tests involved only 37 populations and results seemed to be influenced greatly by large, random, biotic and abiotic environmental variances. Consequently, a series of studies was begun to reassess adaptive variation in central Idaho.

Distribution and Ecology

In the mountains of central Idaho (Fig. 1), macro- and microenvironmental heterogeneity produce extreme ecological diversity (Steele and others 1981). During winter and early spring, gentle rainfall and a relatively heavy cloud cover produce a moderate climate with definite maritime components. In late spring and summer, the maritime influence is replaced by an arid continental climate (Ross and Savage 1967), characterized by warm days, cool nights, and precipitation that is delivered in brief downpours. The maritime influence is pronounced in the northwest, becoming depressed toward the south and east. In fact, at Stanley (Fig. 1), the climate is entirely continental and extremely arid. Furthermore, the elevation of valley floors increases from west to east, a trend associated with increasing environmental severity. Microenvironmental variations associated with mountainous terrain, combined with climatic transitions, produce extremely heterogeneous environments in central Idaho.

Within this region, ponderosa pine displays a broad ecologic amplitude. The distribution of the species (Fig. 1) adjoins the steppe at low elevations, is limited to river valleys within much of the northern mountains, and reaches its limits of distribution with the high and arid lands to the east. Thus, ponderosa pine occurs at elevations ranging from 900 m to 1,900 m. It is an ecological climax in the relatively pure stands that border the steppe but is seral first to *Pseudotsuga menziesii* and then to *Abies grandis* as moisture supplies increase. Precipitation ranges from 38 cm near the steppe to 90 cm under mesic conditions.

Materials and Methods

Population differentiation was studied in seedlings from 64 populations representing three physiographic regions (Fig. 1). These populations sampled the geographic distribution and ecological amplitude within which the species is of commercial importance in central Idaho. Each population was represented by an equal number of wind-pollinated seeds from five trees. Seedlings were used to study: (1) growth and development in a field test, (2) periodicity of shoot elongation in a greenhouse, and (3) cold hardiness in the laboratory.

Growth and Development.—Seedlings were grown for 6 months in plastic containers (65 cm^3) in a shadehouse at Moscow, Idaho (lat. 48.5°N, long. 116.7°W). In the fall, seedlings were transplanted to a single test site at 670 m elevation at the Priest River Experimental Forest, which is located 190 km north of Moscow and presents a frost-free period of about 90 days. The site was cleared of rocks, roots, and competing vegetation, then tilled before planting. Nine seedlings, separated by ⅓ m, were planted in row plots, spaced at ½ m, in each of five blocks.

Population performance in all blocks was described by (1) height: average seedling height after 3 years; (2) early growth: average amount (mm) of 3-year elongation that occurred by May 24 (week 1 of shoot elongation); (3) late growth: average amount (mm) of the 3-year predetermined shoot produced after June 16 (week 4); (4) leaf length: the average (mm) length of a leaf near the center of the 3-year shoot; (5) fall growth: the sum of the lammas shoot and bud length; and (6) adjusted height: 3-year height adjusted by regression on 2-year height, a value relatively independent of previous environmental (such as transplanting shock)

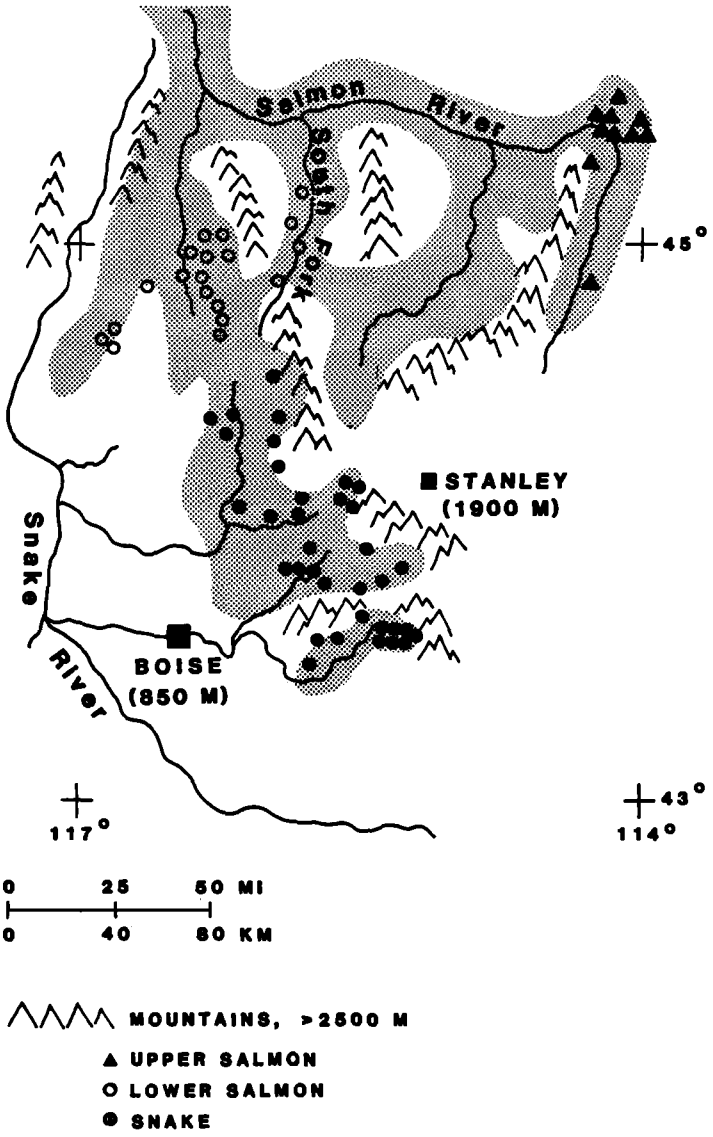

FIGURE 1. Geographic distribution of ponderosa pine (shading) within the region of study, location of sampled population (symbols) according to major mountain ranges and river drainages.

and genetic effects and, thereby, capable of reflecting the adaptive response of populations to a particular environmental stress in a relatively short time.

Measurements were analyzed according to a model of random effects:

$$Y_{ij} = \mu + p_i + b_j + g_{ij}$$

where Y_{ij} = the performance of population i in block j; μ = the overall mean; p_i = the effect of population i; b_j = the effect of block j; and g_{ij} = the residual, the interaction of populations and blocks.

Periodicity of Shoot Elongation.—Seedlings from 64 populations were grown for 2 years in plastic containers (740 cm^3) in a shadehouse at Moscow. Each population

was represented by nine seedlings in each of three blocks. In late March of the second growing season, seedlings were moved into a greenhouse before shoot elongation had begun. Greenhouses were maintained under natural photoperiods and light intensities; temperatures were held at about 21°C during the day and 13°C at night. All seedlings were measured three times each week until elongation of the preformed shoot had ceased.

As described by Rehfeldt and Wykoff (1981), shoot elongation of individual trees was expressed by a modified logistic function with a hyperbolic time term:

$$Y = \frac{1}{1 + be^{(-rX+c/X)}}$$

where Y is the proportion of total increment attained by day x, and b, r, and c are regression coefficients.

Regression statistics allowed calculation of the following variables for describing periodicity in shoot elongation of individual seedlings: (1) initiation of growth: the day on which 2 mm of growth had occurred; (2) cessation of growth: the day on which all but 2 mm of growth had occurred; (3) duration of growth: the number of days between initiation and cessation; (4) rate of growth: elongation per day during the period of maximum elongation; and (5) total elongation.

Population differentiation was assessed according to least squares analyses of random effects:

$$Y_{ijk} = \mu + p_i + b_j + g_{ij} + d_{ijk}$$

where Y_{ijk} = performance of individual k from population i in block j; μ = overall mean; p_i and b_j = the effects of population i and block j, respectively; g_{ij} = the interaction of population i in block j; and d_{ijk} = the residual, the deviation of seedling k from the effect of population i in block j. A harmonic mean of 8.81 reflects the number of seedlings from each population in each block.

Cold Hardiness.—Environmental stresses sufficient for differentiating populations according to cold hardiness did not occur while field tests were in progress. Consequently, laboratory tests of freezing tolerance were made according to the general procedures of Levitt (1972). That laboratory tests corroborate field estimates of cold hardiness is well established in horticulture (Levitt 1972) and is illustrated in forest trees for *Pseudotsuga menziesii* (Rehfeldt 1977, 1979b), *Pinus contorta* (Jonsson and others 1980), *Abies sachalinensis* (Eiga and Sakai 1984), and conifers in general (Sakai and Okada 1971). Soon after shoot elongation ceased, seedlings which had been used in studies of shoot development were moved from the greenhouse to a shadehouse. Mature leaves were collected in late September from near the center of the 2-year shoot. By using mature leaves at a time when frosts were expected, freezing tests assessed the relative cold hardiness of populations in early autumn.

Four sets of nine leaves were collected from each nine-tree plot in all blocks; each set contained one leaf from each tree. Leaves were moistened, packaged in plastic bags, and stored at 2°C for 3 days. One set of leaves from each population was exposed to a test temperature by cooling at the rate of 5°C/h to a temperature between −14°C and −25°C. Four test temperatures were nested within each block such that sets within a block received a range of temperatures. Leaves were removed from the freezer according to remote sensing of internal air temperatures, were thawed at 2°C for 24 hours, and were placed on a shaded greenhouse bench for 1 week. After removal from plastic bags, leaves were examined visually for injury. Injured leaves were identified by the presence of discolored and flaccid tissue (Rehfeldt 1980a).

Population differentiation was assessed from the model of random effects:

TABLE 1. Mean values and results from analyses of variance for 12 traits in 64 populations of ponderosa pine. Effects of populations (σ^2_P) are presented as the proportion of the phenotypic variance ($\sigma^2_W + \sigma^2_{BP} + \sigma^2_P$), where σ^2 = variance components and P, BP, *and* W *code the effects of populations, the interaction of populations and blocks, and variance within plots.*

Variable	Mean	Range in population means	Analysis of variance: Intraclass correlation for populations	Analysis of variance: Error mean square
Growth and development				
Early growth	20 mm	13 mm	0.06	34.62
Late growth	81 mm	43 mm	.31**	163.82
Leaf length	152 mm	26 mm	.28**	53.93
Fall growth	48 mm	26 mm	.16**	71.82
3-year height	414 mm	158 mm	.44**	1,195.85
Adjusted height	414 mm	68 mm	.29**	458.11
Periodicity of shoot elongation				
Initiation	2 d	2 d	.05	.46
Cessation	34 d	8 d	.44**	2.87
Rate	4 mm/d	2 mm/d	.21**	.16
Duration	32 d	9 d	.38**	3.50
Elongation	90 mm	52 mm	.25**	86.37
Cold hardiness				
Injury	42%	60%	.13**	.10

** Statistical significance (1 percent level) of the *F*-value associated with the effects of populations.

$$Y_{ijk} = \mu + p_i + b_j + t_{jk} + d$$

where Y_{ijk} = the percentage (arcsin $\sqrt{\%}$) of leaves injured for population i in block j at temperature k; μ = the overall mean; p_i and b_j = the effects of populations i and block j, respectively; t_{jk} = the effect of temperature k within block j; and d = the residual, composed of interactions of (1) blocks × populations and (2) temperatures within blocks × populations.

Patterns of Variation.—Geographic patterns of genetic variation were assessed from a series of regression analyses of the general form:

$$Y_i = b_0 + b_1X_{1i} + b_2X_{2i} \ldots + b_nX_{ni}$$

where Y_i = the mean performance of population i for variables reflecting growth and development, periodicity of shoot elongation, and cold hardiness; b's = regression coefficients; and X's = independent variables describing the origin of population i.

The sequence of regression analyses concentrated first on the relationship between performance and elevation of the seed source, and second on geographic patterns of variation that were independent of elevation. Adequacy of a model was judged according to the goodness of fit (R^2), residual variance ($s_{y.x}$), and geographic or ecologic patterns displayed by residuals (Draper and Smith 1966). The series of analyses is summarized by three regression models:

Model 1 considered genetic variation in relation to the elevation of the seed origin. Deviations from linear regression were scanned visually for departure from linearity and for geographic effects on the slope of regression lines.

Model 2 considered geographic patterns of variation that were relatively in-

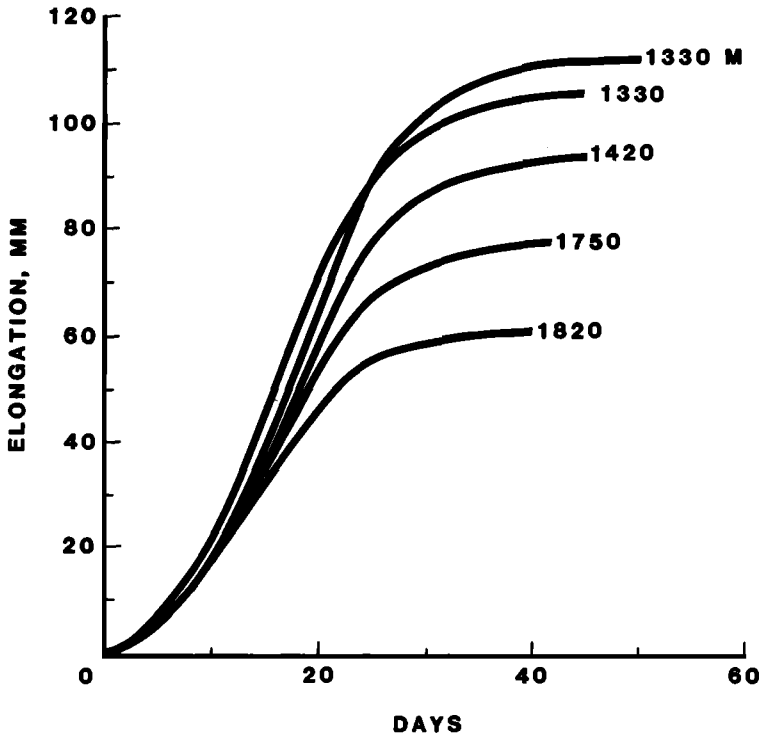

FIGURE 2. Periodicity of shoot elongation for populations from a range of elevations which illustrate maximum differences in response.

dependent of elevation. Thus, deviations from model 1 were used as dependent variables. Independent variables included latitude, longitude, arcs of a circle centered at latitude 44° and longitude 114°, northwest departure, and southeast departure. These last two variables were generated by rotating the latitudinal and longitudinal grid by 45°. Independent variables plus their squares were represented both independently of and nested within three geographic regions (Fig. 1): (1) the Snake River drainage, (2) the lower Salmon drainage, and (3) the upper Salmon. The regions, having been subjectively delineated, were not included as dummy variables. Thus, 30 independent variables were included in the model, which used a stepwise regression program for maximizing R^2 (SAS 1982).

In model 3, elevation of the seed source was added to the independent variables of the "best fitting" regression of model 2 to estimate the joint determination of performance by geography and elevation.

RESULTS

Growth and Development.—Variation among populations accounted for approximately one-third of the phenotypic variance for all variables except for the amount of 3-year elongation that took place during the first week (Table 1). Thus, populations began 3-year shoot elongation at about the same time but diverged greatly in both the amount and the manner by which total elongation was achieved (Table 1).

Shoot elongation was obviously underway at Priest River by May 24, the date when early growth was measured. By June 16, some populations had achieved nearly all (less 5 cm) of the 3-year predetermined elongation while others had

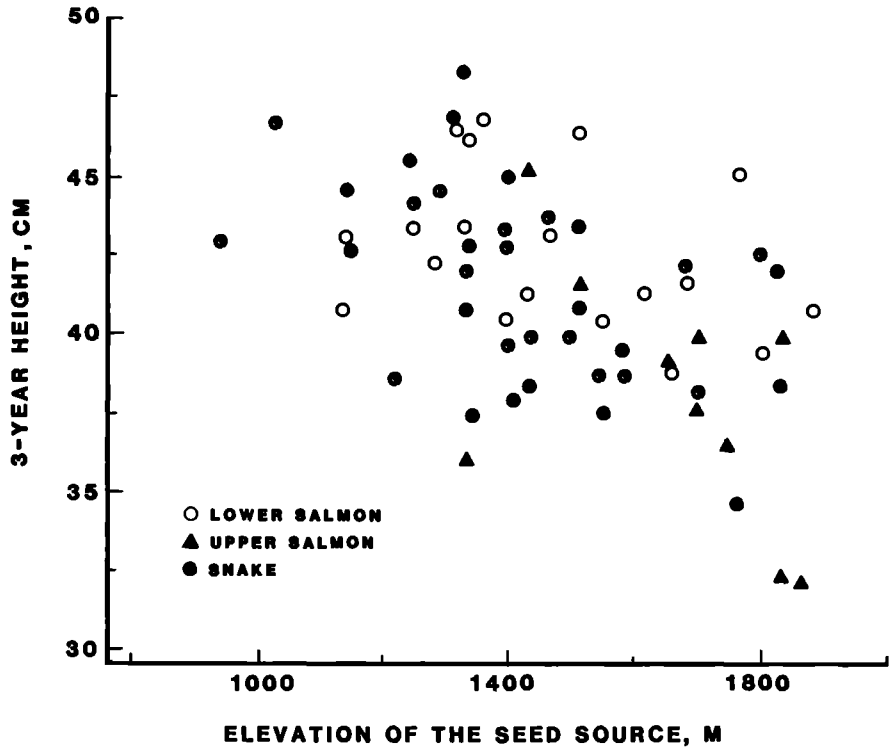

FIGURE 3. Three-year height of populations plotted by elevation of the seed source.

achieved only half. Essentially all populations produced lammas growth in late August and early September, but the amount produced varied greatly. Thus, a value for fall growth of 4 cm described a population for which actual lammas growth was small; the measurement, therefore, primarily represented the length of the bud. But some populations produced lammas growth to such an extent that fall growth accounted for 23 percent of the 3-year shoot. Populations also differed greatly in 3-year height, leaf length, and adjusted height. Variation in adjusted height indicates that differences in 3-year height of populations would have been apparent even if all populations had been the same height at age 2.

Periodicity of Shoot Elongation.—Shoot elongation of all seedlings was completed between 23 and 60 days after being placed in the greenhouse. This means that 9 to 18 observations were available for regression analyses. The logistic function described periodicity of shoot elongation of individual trees nearly perfectly. Values of R^2 ranged from 0.91 to essentially 1.0, averaging 0.99. Thus, as shown previously (Rehfeldt and Wykoff 1981), greenhouse conditions allowed precise measurement of shoot elongation which developed with little environmental perturbation. As a result, elongation of the preformed shoot was represented nearly perfectly by mathematical models.

Analyses of variance detected significant differences among populations for the duration, rate, cessation, and amount of shoot elongation (Fig. 2). For these variables, main effects of populations accounted for 20 to 44 percent of the phenotypic variance and were reflected in mean differences among populations as large as 9 days in cessation, 9 days in duration, 1.5 mm/day in rate, and 50 mm total elongation. Even though individual seedlings differed by as much as 9 days

TABLE 2. Coefficients of determination and residuals for three regression models applied to 12 traits in 64 populations.

Variable	Model 1		Model 2		Model 3	
	R^2	$s_{y.x}$	R^2	$s_{y.x}$	R^2	$s_{y.x}$
Growth and development						
Early growth	0.01	2.99	0.28	2.76	0.29	2.78
Late growth	.32**	8.52	.29**	7.67	.53**	7.72
Leaf length	.17**	5.14	.44**	4.17	.56**	4.08
Fall growth	.09*	5.10	.37**	4.39	.41**	4.42
3-year height	.26**	29.52	.39**	25.36	.56**	25.45
Adjusted height	.09*	16.33	.24*	15.59	.26	15.69
Periodicity of shoot elongation						
Initiation	.01	.42	.33	.39	.34	.39
Cessation	.25**	1.56	.44**	1.23	.61**	1.21
Rate	.05	.30	.30**	.27	.35**	.27
Duration	.25**	1.58	.49**	1.27	.61**	1.26
Elongation	.22**	7.57	.47**	6.04	.60**	6.00
Cold hardiness						
Injury	.13**	.14	.36**	.12	.44**	.12

* Statistical significance at the 5 percent level.
** Statistical significance at the 1 percent level.

in the initiation of shoot elongation, no differences could be detected among populations; mean values for populations differed by a maximum of only 1.8 days.

Cold hardiness.—Because test temperatures had been selected to induce both a low and high proportion of injury, effects of test temperatures dominated the analysis of variance. These effects are illustrated by averages of 11 percent injured leaves at −14°C but 90 percent at −25°C. Effects of populations accounted for a significant but rather low proportion (13 percent) of the phenotypic variance. Experimental errors (interactions of blocks × populations plus temperatures within blocks × populations), however, were large. Mean differences among populations, amounting to 60 percent injury, were detected with relatively low statistical power (Table 1).

Patterns of Variation.—Regression model 1 related significant proportions of variance among populations in most traits to the elevation of the seed origin (Fig. 3). For only those variables reflecting the initiation and rate of shoot elongation was the regression not significant (Table 2). As illustrated for 3-year height (Fig. 3), the slope of the regressions did not vary greatly for populations representing different portions of the region under study. Regression coefficients were strongly negative for all variables except cold injury.

By including as many as 13 independent variables, model 2 accounted for significant proportions of the variance among populations for all variables except those describing the initiation of shoot elongation. In fact, geographic variables plus elevation (model 3) accounted for as much as 61 percent of the variance among populations.

Geographic patterns of genetic variation (model 2) are presented in Figure 4 for several traits. These patterns were generated from values predicted from model 2. The contour interval is scaled to a value equaling one-half the least significant

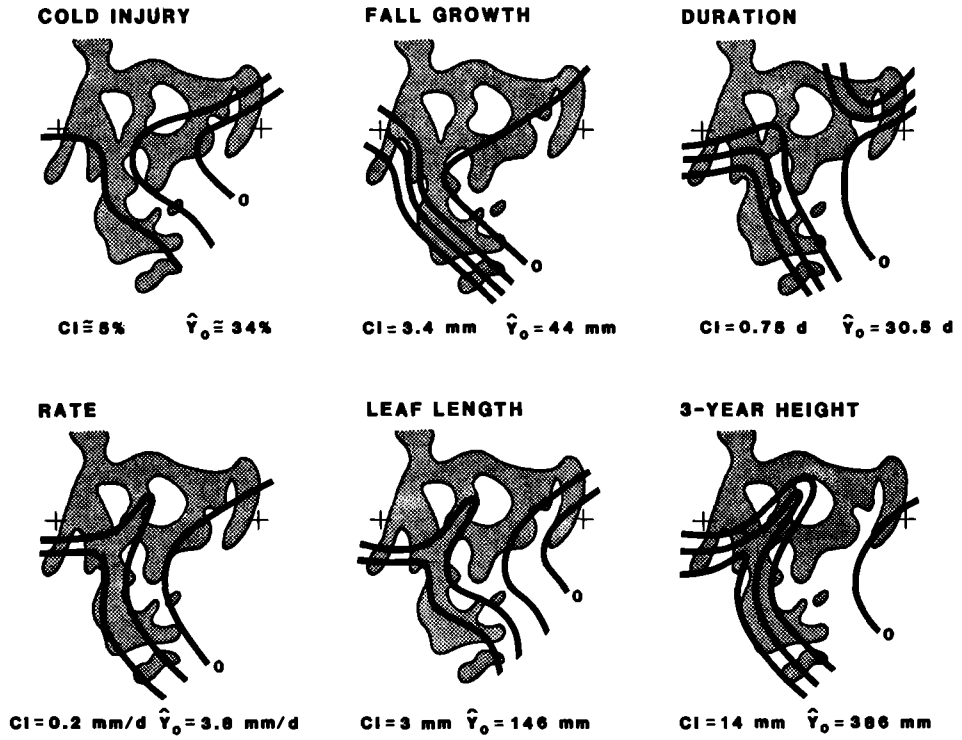

FIGURE 4. Geographic patterns of variation that are independent of elevation. C.I. = the contour interval which is scaled to a value of ½ *lsd* 0.2. $\hat{Y}_0$ = the predicted value for the contour of lowest mean performance.

difference (Steel and Torrie 1960) among populations at the 80 percent level of probability [½*lsd*(0.2)]. Because values of *lsd* were calculated from the analysis of variance (Table 1), contours represent about one-half the geographical distance associated with population differentiation at the 80 percent level of probability. Contouring was begun with the zero deviation from the elevational regression, but patterns are presented in relation to the contour of lowest predicted performance.

Even though the variables selected for Figure 4 illustrate patterns of greatest divergence, all variables for which model 2 was significant presented variations on the same pattern. Similar patterns are an obvious and expected result of intercorrelations among dependent variables (Table 3). Populations located adjacent to the steppe in the southwest were tallest, grew at the fastest rate, ceased elongation latest, and suffered the most freezing damage. From this area, clines radiate toward the north, east, and northeast. Variations in this general pattern arise from the degree by which contours of relatively high numeric value protrude into the South Fork of the Salmon River.

DISCUSSION

In central Idaho, populations of ponderosa pine display genetic diversity for a variety of traits reflecting growth and development, periodicity of shoot elongation, and cold hardiness. Similar patterns of differentiation (Fig. 4) and strong correlations among traits (Table 3) suggest parallel selection in several traits to

TABLE 3. Matrix of simple correlations among 12 traits for 64 populations.

Variable	Code	LG	LL	FG	H3	AH	INT	C	R	D	EL	INJ
Growth and development												
Early growth	EG	0.14	−0.02	0.25	0.29	0.23	0.30	0.11	−0.13	0.04	0.01	0.15
Late growth	LG		0.57	0.41	0.84	0.34	0.10	0.60	0.27	0.57	0.53	0.16
Leaf length	LL			0.37	0.60	0.08	0.01	0.38	0.31	0.37	0.43	0.14
Fall growth	FG				0.65	0.53	0.07	0.36	0.25	0.34	0.43	0.16
3-year height	H3					0.37	0.13	0.56	0.37	0.52	0.58	0.11
Adjusted height	AH						0.06	0.15	−0.04	0.14	0.16	0.09
Periodicity of shoot elongation												
Initiation	INT							0.05	−0.22	−0.19	−0.15	−0.04
Cessation	C								0.29	0.97	0.77	0.29
Rate	R									0.34	0.79	−0.08
Duration	D										0.79	0.30
Elongation	EL											0.16
Cold hardiness												
Injury	INJ											

All coefficients of absolute value >0.23 are statistically significant at the 5 percent level.

produce coherent (Clausen and Hiesey 1960) genetic systems. Coherence, like that in *Pseudotsuga menziesii* (Rehfeldt 1983b) and *Pinus contorta* (Rehfeldt 1983a) from the Rocky Mountains, involves traits that are part of an annual sequence of developmental events. This sequence begins with dehardening in the spring, includes shoot elongation, leaf expansion, bud development, lignification, and concludes with cold acclimation. Because the entire sequence must fit into a frost-free period of finite length, components are intercorrelated. Consequently, populations that were tall after 3 years of field testing also tended to have long leaves, a high proportion of the predetermined shoot that continued elongation into midsummer, and a large amount of fall growth; populations that were short completed developmental events early (Table 3).

Coherence, however, is not complete. Early growth in the field and the initiation of shoot elongation in the greenhouse were independent of the network of intercorrelated traits, and cold hardiness was only weakly associated with other traits. The initiation of shoot elongation apparently represents an environmental rather than a genetic response in ponderosa pine, a result in common with other conifers from the Rocky Mountains: *Pseudotsuga menziesii* (Rehfeldt 1979a, 1982b, 1983b), *Larix occidentalis* (Rehfeldt 1982a), and *Pinus contorta* (Rehfeldt 1983a). But, for cold hardiness to be poorly related to the intercorrelated network counters results with other species. These low correlations will be discussed subsequently.

Population differentiation was closely related to the elevation of the seed source. As elevation increases, the length of the frost-free period rapidly decreases. Consequently, populations from low elevation exhibit a high growth potential that is associated with a long duration and late cessation of elongation. Populations from high elevation express a low growth potential largely because developmental events must be completed within a short frost-free period. These trends are also apparent, in varying degrees, in *Pseudotsuga menziesii* (Rehfeldt 1979a, 1982b, 1983b), *Larix occidentalis* (Rehfeldt 1982a), and *Pinus contorta* (Rehfeldt 1983a). Again, cold hardiness was only weakly related to elevation and was poorly related to other traits.

Geographic patterns of genetic variation that are relatively independent of elevation (Fig. 4) were pronounced, consistent, and uniform. Populations of highest growth potential tend to occur in those environments at relatively low elevations that are adjacent to the steppe. It is, moreover, exactly in these environments that the species reaches its ecological dominance. Toward the north, northeast, and east, the climate is subject to strong orographic effects of particularly massive mountains. Here, growth potential tends to be low while cold hardiness is high. Variations in these general patterns result from the performance of populations from the South Fork of the Salmon River. As illustrated in Figure 4, populations from the South Fork exhibited a high growth potential and a relatively high cold hardiness. This combination develops because the high growth potential is more dependent on a high rate of growth than on a long duration (Fig. 4). Because developmental events are synchronized, an early cessation automatically conveys relatively high tolerance to frosts in the early fall. It is these populations from the South Fork that primarily are responsible for low correlations (Table 3) involving cold hardiness.

Even though the regression of 3-year height on 2-year height accounted for 70 percent of the variance in the dependent variable, population differentiation was evident for adjusted height. Some populations grew more than expected according to their 2-year height while others grew less than expected. Thus, differences in the height of populations still would have been apparent after 3 years, even if all trees had been of the same height at age 2. Patterns of variation and simple correlations (Table 3) suggest that differential performance resulted from an in-

crease in the differences that were observed at age 2 rather than flip-flops in performance between ages 2 and 3.

Positive relationships occur among variables reflecting similar developmental events measured under different conditions. Thus, 3-year height in the field was strongly correlated with greenhouse elongation, and late growth in the field was closely linked with the cessation and duration of elongation in greenhouse studies. Even early growth in the field and the initiation of elongation were significantly correlated despite a lack of detectable genetic variation. And finally, strong correlations link juvenile tests with long-term performance trials. Nineteen of the populations represented in this study were also included in field tests for which the performance after 16 years already has been analyzed. Although the power of statistical analyses of 19 observations is low, significant simple correlations involve the 16-year height of populations at four planting sites with 3-year height ($r = 0.68$), late growth (0.55), and greenhouse elongation (0.46).

Adaptive variation among populations has direct implications in artificial reforestation. To limit maladaptation in plantings of wild trees, seed transfer guidelines must reflect patterns of adaptive variation. One estimate of an appropriate limit to seed transfer involves the smallest geographic or elevational interval across which differentiation can be detected (Rehfeldt 1979a). This interval is estimated by the ratio $lsd(0.2)/b$, where b is the regression coefficient and $lsd(0.2)$ is derived from analysis of variance as the least significant difference among population means at the 80 percent level of probability. (A rather low level of probability is used to avoid accepting no differences when differences actually exist.)

Variables for which model 1 (Table 3) was significant provide an example. Elevational intervals associated with $lsd(0.2)$ ranged from 1,500 m for adjusted height to 360 m for 3-year height. To accept the smallest interval suggests that the transfer of seed from a single source should be limited to approximately ± 180 m. Geographic patterns of variation (Fig. 4) were scaled to a value of $\frac{1}{2}lsd(0.2)$. Thus, lateral transfers of seed should be limited to about ± 1 contour. Again, acceptance of the most limiting of these patterns is to base geographic transfers of seed on the patterns illustrated for 3-year height. According to these limits, either two or three geographic zones would be sufficient for central Idaho. The appropriate size of seed zones, however, should be practical administratively and profitable economically. Thus, limits to seed transfer must accommodate biology, economics, and administration.

Populations from the South Fork of the Salmon River that combine high growth potential with high hardiness is of tremendous practical value to both artificial reforestation and tree improvement. Such genotypes are capable of expressing a high growth potential in both mild and severe environments. The potential practical value of these genotypes is supported from the 16-year results of long-term field tests: families from South Fork populations were among the tallest for their elevational zones at four test sites (Rehfeldt 1980b).

Regardless, genetic diversity among populations in central Idaho is readily interpretable as adaptive variation. These results, together with those from the Sierra Nevada (Callaham and Liddicoet 1961), the eastern Rockies (Read 1980, 1983), and the mountains of northern Idaho and western Montana (Madsen and Blake 1977) demonstrate that populations of ponderosa pine have become genetically differentiated in response to environmental selection within restricted geographic localities.

Literature Cited

Callaham, R. Z., and A. R. Liddicoet. 1961. Altitudinal variation at 20 years in ponderosa and Jeffrey pines. J For 59:814–820.

CLAUSEN, J., and W. M. HIESEY. 1960. The balance between coherence and variation in evolution. Proc Nat Acad Sci 46:494–506.

CONKLE, T. 1973. Growth data for 29 years from the California elevational transect study of ponderosa pine. Forest Sci 19:31–39.

DRAPER, N. R., and H. SMITH. 1966. Applied regression analyses. Wiley and Sons, New York. 407 p.

EIGA, S., and A. SAKAI. 1984. Altitudinal variation in freezing resistance of Saghalien fir (*Abies sachalinensis*). Can J Bot 62:156–160.

HANOVER, J. W. 1963. Geographic variation in ponderosa pine leader growth. Forest Sci 9:86–95.

JONSSON, A., ERIKSSON, G., and I. DORMLING. 1980. A summary of studies of frost hardiness of *Pinus contorta* seedlings grown in climatic chambers. *In Pinus contorta* as an exotic species, p 75–81. Swedish Univ Agric Sci, Res Note 30, 353 p. Garpenberg, Sweden.

KEMPFF, G. 1928. Nonindigenous western yellow pine plantations in northern Idaho. Northwest Sci 2:54–58.

LEVITT, J. 1972. Responses of plants to environmental stresses. Academic Press, New York. 697 p.

MADSEN, J. L., and G. M. BLAKE. 1977. Ecological genetics of ponderosa pine in the northern Rocky Mountains. Silvae Genet 26:1–8.

MITTON, J. B., LINHART, Y. B., HAMRICK, J. L., and J. S. BECKMAN. 1977. Observations on the genetic structure and mating system of ponderosa pine in the Colorado Front Range. Theor Appl Genet 51:5–13.

READ, R. A. 1980. Genetic variation in seedling progeny of ponderosa pine provenances. Forest Sci Monogr 23, 59 p.

READ, R. A. 1983. Ten-year performance of ponderosa pine provenances in the Great Plains of North America. USDA Forest Serv Res Pap RM-250, 17 p. Rocky Mt Forest and Range Exp Stn, Fort Collins, CO.

REHFELDT, G. E. 1977. Growth and cold hardiness of intervarietal hybrids of Douglas-fir. Theor Appl Genet 50:3–15.

REHFELDT, G. E. 1979a. Ecological adaptations in Douglas-fir (*Pseudotsuga menziesii* var *glauca*) populations. I. North Idaho and northeast Washington. Heredity 43:383–397.

REHFELDT, G. E. 1979b. Variation in cold hardiness among populations of *Pseudotsuga menziesii* var *glauca*. USDA Forest Serv Res Pap INT-233, 11 p. Intermt Forest and Range Exp Stn, Ogden, UT.

REHFELDT, G. E. 1980a. Cold acclimation in populations of *Pinus contorta* from the northern Rocky Mountains. Bot Gaz 141:458–463.

REHFELDT, G. E. 1980b. Genetic gains from tree improvement of ponderosa pine in southern Idaho. USDA Forest Serv Res Pap INT-263, 9 p. Intermt Forest and Range Exp Stn, Ogden, UT.

REHFELDT, G. E. 1982a. Differentiation of *Larix occidentalis* populations from the northern Rocky Mountains. Silvae Genet 31:13–19.

REHFELDT, G. E. 1982b. Ecological adaptations in Douglas-fir populations. II. Western Montana. USDA Forest Serv Res Pap INT-295, 28 p. Intermt Forest and Range Exp Stn, Ogden, UT.

REHFELDT, G. E. 1983a. Adaptation of *Pinus contorta* populations to heterogeneous environments in north Idaho. Can J Forest Res 13:405–411.

REHFELDT, G. E. 1983b. Ecological adaptations in Douglas-fir (*Pseudotsuga menziesii* var *glauca*) populations. III. Central Idaho. Can J Forest Res 13:626–632.

REHFELDT, G. E., and W. R. WYKOFF. 1981. Periodicity of shoot elongation among populations of *Pinus contorta* from the northern Rocky Mountains. Ann Bot 48:371–377.

ROSS, S. H., and C. N. SAVAGE. 1967. Idaho earth science. Idaho Bur of Mines and Geol, Moscow, Idaho. 271 p.

SAS INSTITUTE, INC. 1982. SAS user's guide: basics. SAS Inst, Cary, N C. 923 p.

SAKAI, A., and S. OKADA. 1971. Freezing resistance of conifers. Silvae Genet 20:91–97.

SQUILLACE, A. E., and R. R. SILEN. 1962. Racial variation in ponderosa pine. Forest Sci Monogr 2, 27 p.

STEEL, R. G. D., and J. H. TORRIE. 1960. Principles and procedures of statistics. McGraw-Hill, New York. 481 p.

STEELE, R., R. D. PFISTER, R. S. RYKER, and J. A. KITTAMS. 1981. Forest habitat types of central Idaho. USDA Forest Serv Gen Tech Rep INT-114, 138 p. Intermt Forest and Range Exp Stn, Ogden, UT.

STEINHOFF, R. J. 1970. Northern Idaho ponderosa pine racial variation study—50-year results. USDA Forest Serv Res Note INT-118, 4 p. Intermt Forest and Range Exp Stn, Ogden, UT.

WANG, C. 1977. Genetics of ponderosa pine. USDA Forest Serv Res Pap WO-34, 24 p. Wash, D C.

WELLS, O. O. 1964a. Geographical variation in ponderosa pine. I. The ecotypes and their distribution. Silvae Genet 13:89–103.

WELLS, O. O. 1964b. Geographical variation in ponderosa pine. II. Correlation between progeny performance and the characteristics of the native habitat. Silvae Genet 13:125–132.

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Eastern Dwarf Mistletoe Seed Storage, Germination, and Inoculation of Spruce Seedlings

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ABSTRACT. Eastern dwarf mistletoe seed used for inoculation of *Picea mariana* and *P. glauca* had better germination (i.e., appearance of a red radicle) and radicle growth when stored at subfreezing temperatures (−10°C vs. +4°C) and high humidity for at least 10–12 weeks, and such seed remained viable for at least 40 weeks. Usually 25 percent or less of the seeds had radicle growth longer than 1 mm. In greenhouse inoculations, most spruce seedlings (57 of 61) became infected if the radicle made contact with the stem. FOREST SCI. 32:92–96.

ADDITIONAL KEY WORDS. *Arceuthobium pusillum, Picea glauca, Picea mariana.*

INOCULATIONS with western species of dwarf mistletoe (*Arceuthobium*) on conifer seedlings have been successful (Scharpf 1969, 1972; Knutson 1974), but similar attempts with eastern dwarf mistletoe (*A. pusillum* Pk.) have not been reported. Spruce seedlings infected with eastern dwarf mistletoe would be useful in pathological and physiological studies requiring a controlled environment. This paper describes techniques for infecting black (*Picea mariana* (Mill.) B.S.P.) and white spruce (*P. glauca* (Moench) Voss) seedlings with eastern dwarf mistletoe in the greenhouse.

Eastern dwarf mistletoe seeds needed for inoculation are easily collected and germinated (Bonga 1965). However, proper conditions for storage and inoculations have not been published. Seed of other dwarf mistletoe species decreased in viability when stored at temperatures below 1°–5° C (Knutson 1971, Scharpf 1970). However, eastern dwarf mistletoe seeds are exposed to subfreezing temperatures during the winter months in northern Minnesota, and this study tested whether viability could be improved if storage temperatures were below 1°–5° C. The study also determined the success rate for infecting spruce seedlings with eastern dwarf mistletoe.

MATERIALS AND METHODS

Eastern dwarf mistletoe seeds (14,000) were collected from infected black spruce in Carlton Co. (3 stands) and Pine Co. (1 stand), MN. Cheesecloth was loosely wrapped around 23 witches-brooms in early September 1981, and was brought back to the laboratory 4 weeks later after natural dispersal of the seed. Seeds were removed from the moistened cheesecloth, surface sterilized in 3 percent hydrogen peroxide for 30 minutes on a shaker to prevent

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