Ecological Adaptations in Douglas-fir (*Pseudotsuga menziesii* var. *glauca*). IV. Montana and Idaho Near the Continental Divide

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ABSTRACT. *Seventy-seven seedling populations of Douglas-fir (*Pseudotsuga menziesii *var.* glauca*) sampled from near the Continental Divide in Idaho and Montana exhibited pronounced genetic differences when compared in three common environments. Differentiation involved several traits that are components of an annual developmental cycle that must be completed within a growing season of finite length. Consequently, the elevational and geographic clines of genetic variation tend to parallel variation in the length of the growing season at the seed source. Such clines suggest that maladaptation in artificial reforestation can be controlled by limiting the transfer of seeds. While geographic transfers can be relatively liberal, elevational transfers should be limited to ±90 m (300 ft) from the seed source at elevations below 1,400 m (4,600 ft), to ±125 m (410 ft) for elevations between 1,400 and 2,000 m (4,600 and 6,550 ft), and ±200 m (560 ft) for elevations above 2,000 m (6,550 ft).*

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Populations of coastal and inland varieties of Douglas-fir are adapted to a relatively small portion of the mosaic of environmental gradients (Sorensen 1983, Campbell 1986, Rehfeldt 1978). When grown on the same sites, seedling populations display genetic differences that reflect adaptation to the environment at the seed source. In the mountains of central Idaho, for example, genetic variation occurs along steep elevational clines: populations separated by merely 200 m altitude within the same mountain ranges are detectably different genetically (Rehfeldt 1983b).

When studied in young trees, adaptive differences are commonly expressed in traits that are components of an annual cycle of developmental events. For trees from temperate climates, bud burst, shoot elongation, leaf development, cambial expansion, bud development, lignification, and cold acclimation are sequential events that must be completed within the frost-free period. For populations within a species to differ in the timing and duration of such events implies that natural selection has attuned the entire cycle to the local climate (Dietrichson 1964, Rehfeldt 1983a, Campbell 1986). Patterns of genetic variation in Douglas-fir thus parallel variation in the length of the growing season (Rehfeldt 1983b, Sorensen 1983, Campbell 1986), a period that is truncated by frost but often interrupted by drought.

Patterns of adaptive variation are used to estimate the distance that seeds can be transferred before maladaptations begin limiting the productivity of artificial regeneration. This paper describes patterns of genetic variation among populations of Douglas-fir and presents seed transfer guidelines for the lands that flank the Continental Divide in Idaho and Montana.

METHODS

Genetic variation was studied in seedlings representing 77 populations (Figure 1). Seventy-six of these sampled the geographic, elevational, and ecological distribution of the species in the region of study; one represented northern Idaho and will be used to compare the general performance of populations from the two provinces. In each population, a sample of cones was collected from several squirrel caches to assure that each collection represented a large number of parental trees. The minimum size of a collection area was roughly 4 ha (10 ac).

Seedlings were grown in plastic tubes (65 cm^3) for 5 months in a shadehouse at Moscow, Idaho (46.5° latitude, 116.7° longitude) and were fall-planted in randomized complete blocks at three test sites. One of these sites was at Moscow (700 m elev), where frost-free periods average 130 days. Two sites were in the Priest River Experimental Forest (48.5° latitude, 117° longitude), at 670 m elev, where frost-free periods average 90 days; and at 1,500 m elev where the frost-free period averages about 60 days. At all sites, 10 seedlings from each population were planted in row
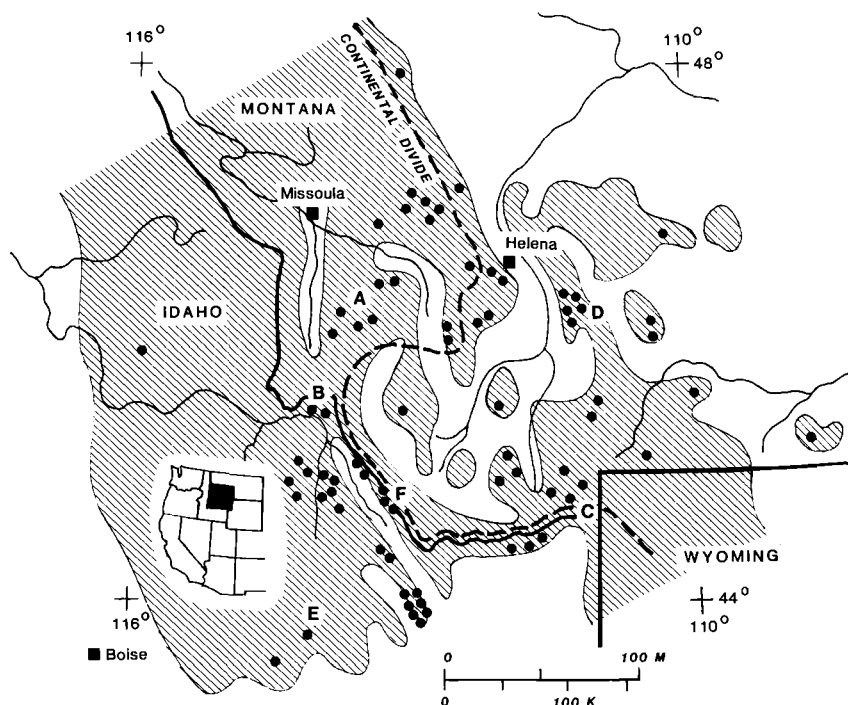

Figure 1. Geographic distribution of Douglas-fir and location of sampled populations. Letters A to F locate the elevational clines presented in Fig. 2.

plots in each of three blocks Rows were separated by 13 cm, 9 cm separated seedlings within rows.

Populations were compared according to six traits, all of which were recorded as plot means:

1. *Spring frost:* the proportion of trees on which more than 50% of the buds were damaged by a spring frost during mid-May of the third year of growth at Moscow. Injury was recognized by wilting and browning of succulent tissues.
2. *Height at 670 m:* the 3-yr height of trees growing at Priest River, 670 m elev.
3. *Two flushes:* the proportion of trees on which the terminal bud flushed a second time early in the third growing season at Priest River, 670 m elev. The second flush was recognized in mid-June by (1) bud scars on the current year's stem, and (2) immature (light-green) leaves and stem units in contrast to blue-green mature tissues of the spring flush.
4. *Lammas:* the proportion of trees that produced lammas, proleptic or syleptic growth by late August during the third growing season at Priest River, 670 m elev. Late season growth involved elongation of winter buds which had been macroscopically visible since early July and was recognized by either or both immature (light-green) leaves and stem units in late August.
5. *Height at 1,500 m:* the 3-yr height of trees growing at Priest River, 1,500 m elev.
6. *Summer frost:* the proportion of trees that suffered freezing damage to terminal shoots during a July freeze at Priest River, 1,500 m elev. Injury was recognized by wilting and browning of succulent tissues.

Height of trees at Moscow was not analyzed because of the confounding effects of severe frost damage. Statistical analyses of each trait included an analysis of variance for assessing the main effects of populations and stepwise multiple regression analyses (SAS 1982) for relating genetic variation to the elevation and geographic origin of the seed. Independent variables for the regression analyses included the geographic terms of latitude, longitude, northwest departure, southwest departure, their squares, and cubes. Northwest and southwest departures, calculated respectively as (1) the product of latitude and longitude, and (2) the product of longitude and the inverse of latitude, were included in the model to accommodate the possibility that geographic patterns were oblique to latitude and longitude. Elevation of the seed source was considered as linear and squared

terms. Thus, 14 independent variables were screened by regression analyses. All analyses were made on the original units of measure.

The best stepwise model was selected as that which produced (1) a statistically significant value of R^2, (2) a random relationship between residuals and the independent variables, (3) a Mallow's statistic that was the first to equal to the number of variables in the model (Draper and Smith 1981) and (4) a matrix of independent variables that could be inverted without singularity. However, because of the large number of independent variables, the best models could be overfit—the fitting of a model to individual samples rather than to the group as a whole (Draper and Smith 1981). Consequently, the biological significance of the patterns of variation described by the models is judged relative to the least significant difference (Steel and Torrie 1960) at the 80% level of probability (lsd 0.2), which was calculated from the error mean square in the analyses of variance. A relatively low level of probability is used to guard against accepting no differences among populations when differences actually exist.

RESULTS

Planting environments had a pronounced effect on growth and development (Table 1). Trees were nearly 70% taller and differences among populations were twice as large at 670 m than at 1,500 m. Nearly 12% of the trees at 670 m flushed twice before the middle of June and 31% produced lammas growth during the middle of August. Few trees either flushed twice or produced lammas growth at 1,500 m, partially because nearly half of the trees suffered freezing injury to terminal shoots in July. At Moscow, a May freeze damaged at least 20% of the developing buds on nearly all trees, and damaged more than 50% of the buds on 9% of the trees.

In response to these strong environmental effects, genetic differences among populations were pronounced. Populations from near the Continental Divide differed by as much as 40% in two flushes, 63% in lammas, 68% in

summer frost damage, 48% in spring frost damage, 20 cm in height at 670 m elev, and 10 cm in height at 1,500 m (Table 1). By comparison, the population from low elevation (1,000 m) in northern Idaho expressed adaptation to a relatively mild climate: a tall stature, a high proportion of seedlings with two flushes, and high susceptibility to injury from frost. For all traits, differences among populations were statistically significant (Table 2).

Population means were intercorrelated (Table 3). Populations that were tall at 670 m also were tall at 1,500 m elev, tended to flush twice, produced lammas growth, and became injured from either spring or fall frosts.

Of the multiple regression models tested, those summarized in Table 4 accounted for statistically significant proportions of variance in the dependent variables and produced a Mallow's statistic (C_p) that suggested the model was the least biased of all models capable of being developed from that particular group of independent variables. Models that included both squared and cubed geographic variables produced singular matrices upon inversion. Since models incorporating cubed values generally were superior to those using the squares, only the former are presented in Table 3. Statistical significance of individual regression coefficients is superfluous to the primary objectives, which were to describe geographic and physiographic patterns of variation rather than to assess the effects of specific environmental variables.

The regression models (Table 4) accounted for as much as 63% of the variance among populations. Patterns of variation described by the models are illustrated in Figures 2 and 3 for height at 1,500 m, the variable for which differentiation was both pronounced and most amenable to presentation. For all geographic localities, height at 1,500 m decreased nonlinearly as the elevation of the seed source increased (Fig. 2). Geographic patterns of variation, illustrated in Figure 2 by the distance between regression lines, are presented in Figure 3 as isopleths, contours of uniform performance. As suggested in Table 3,

Table 1. Mean values and range of means of 76 populations from near the Continental Divide and one population from northern Idaho.

| Trait | Continental Divide | | | Northern Idaho |
| | Mean | Range | | Mean |
		Low	High	
Spring frost (%)	9	0	48	30
Height at 670 m (cm)	20	12	32	41
Two flushes (%)	12	0	40	35
Lammas (%)	31	0	63	25
Height at 1,500 m (cm)	13	9	19	19
Summer frost (%)	49	23	91	84

Table 2. Results of the analysis of variance for testing the effects of populations and values of the least significant difference at the 80% level of probability (lsd 0.2) for each trait.

Trait	Mean squares		
	Populations	Error[1]	lsd 0.2
Spring frost	0.0388**	0.01123	0.11
Height 670 m	66.1128**	5.4847	2.48
Two flushes	0.0252*	0.0145	0.13
Lammas	0.0485*	0.0277	0.18
Height 1500 m	18.6645**	1.7662	1.41
Summer frost	0.0623*	0.0415	0.21

[1] Interaction of blocks and populations.
* Statistical significance of the F-value at the 5% level.
** Statistical significance of the F-value at the 1% level.

Table 3. Simple correlations among population means for six traits. Coefficients with an absolute value <.20 lack statistical significance.

	Height 670 m	Two flushes	Lammas	Height 1,500 m	Summer frost
Spring frost	0.67	0.19	0.17	0.72	0.44
Height, 670 m		0.33	0.36	0.87	0.35
Two flushes			0.68	0.34	0.02
Lammas				0.35	0.13
Height, 1,500 m					0.56

Table 4. Summary statistics and independent variables in the stepwise regression models that best described genetic variation among populations for six traits. EL = elevation, LT = latitude, LN = longitude, NW = northwest departure, SW = southwest departure.

Independent variable	TRAIT					
	Spring frost	Height 670 m	Two flushes	Lammas	Height 1,500 m	Summer frost
EL	X	X	X	X	X	X
(EL)²		X	X		X	X
LT	X	X			X	X
(LT)³	X	X			X	X
LN		X	X			X
(LN)³	X		X	X	X	X
NW	X				X	X
(NW)³	X	X			X	X
SW	X	X		X	X	X
(SW)³		X		X	X	
R²	0.56	0.63	0.27	0.26	0.54	0.38
Cp	5.3	8.4	0.2	0.7	9.0	8.6
Residual mean square	0.006	6.9	0.007	0.013	3.12	0.014

patterns of variation for height at 670 m essentially duplicated Figure 2. Patterns for spring frost, summer frost, and two flushes were similar to height at 1,500 m, but were of lesser resolution. The model for lammas failed to predict population differences that exceeded lsd 0.2.

DISCUSSION

Genetic variation among populations of Douglas-fir from near the Continental Divide was detected for six traits that expressed growth and development in three environments. Population means tended to be intercorrelated. Patterns of variation were described by mathematical models that related genetic variation to elevational and geographic gradients.

Genetic variation along the elevational gradient, the elevational cline, showed that height at a common test site decreased as the elevation of the seed source increased; consequently, two flushes also decreased, but tolerance of frost increased. These clines are nonlinear: differentiation at low elevations occurs across a smaller elevational interval than at high elevations. By using the solution for the quadratic equation, one can calculate the elevational interval that must separate two populations in order that the amount of differentiation equals lsd 0.2 (Fig. 4). These intervals imply that for elevations below 1,400 m, populations separated by about 180 m elevation are genetically different with a probability of about 80%. At elevations between 1,400 m and 2,000 m, 250 m subtends differentiated populations; above 2,000 m, 400 m differentiates populations. These intervals are essentially the same as those estimated for adjacent geographic regions (Rehfeldt 1979, 1982, 1983b).

For the intercept of the elevational

cline to vary geographically (Fig. 2) illustrates a geographic component to adaptive differentiation which is represented by the distance between regression lines. Like the elevational clines, geographic variation is most pronounced for tree height. In Figure 3, geographic patterns of genetic variation are presented for an elevation of 2,000 m (6,550 ft), the mean elevation of all seed sources represented in the study. In this figure, the distance between isopleths (contour lines) is scaled to a value of ½lsd 0.2. Areas separated by two contours thereby support populations that are genetically different with a probability of about 80%. This figure shows that when populations from the same elevation are compared, the shortest come from either the northeast or southwest portions of the region. The tallest tend to be from the northwest. Geographic clines for the other variables were similar to Figure 4, but the slopes of the clines were less steep.

For genetic variation to be occurring across the landscape along both elevational and geographic clines means that populations of similar genetic constitution recur at different elevations in different geographic localities. For example, populations that produced 14 cm of height at the site at 1,500 m came from 1,850 m at locality B; 1,900 m at D; 2,200 m at A; and 2,050 m at C. But at E and F, similar genotypes would be expected at an elevation (<1,800 m) where Douglas-fir does not occur.

These clines, like those for Douglas-fir in adjacent regions (Rehfeldt 1979, 1982, 1983b), follow general trends in the length of the growing season. The elevational cline parallels an environmental gradient along which the frost-free period decreases by 80 days for each 1,000 m increase in elevation (Baker 1944). Likewise, Figure 5 reveals that populations of greatest height generally come from geographic regions with about 90 frost-free days; those of lowest growth come from the south where frost-free periods are generally less than 60 days. To the east and southeast, however, precipitation becomes scant (<30 cm on the valley floor). Since drought also reduces the length of the growing season, the effects of drought and frost on the adaptation of populations are similar in that growth and development must be completed before the occurrence of adverse environmental conditions. To the east, therefore, the amount of precipitation and the length of the frost-free period interact to produce a relatively short growing season regardless of elevation. Consequently, elevational clines flatten (Fig. 2), and populations with very low stature can come from areas

Figure 4. Elevational interval associated with a difference in height (at 1,500 m elev) among populations that equals lsd 0.2 as plotted by the elevation of the seed source.
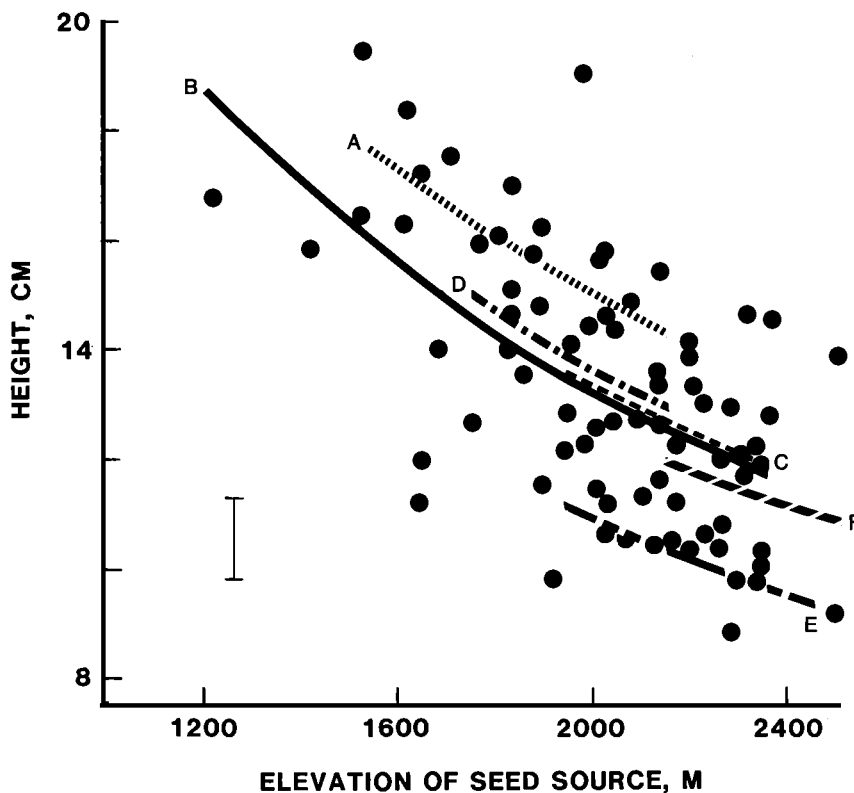

Figure 2. Elevational clines for 3-year height at 1,500 m as predicted by regression models at five geographic localities, A to F, which are keyed to Fig. 1. Brackets quantify lsd 0.2. The length of the regression line reflects the elevational distribution of Douglas-fir at each locality.
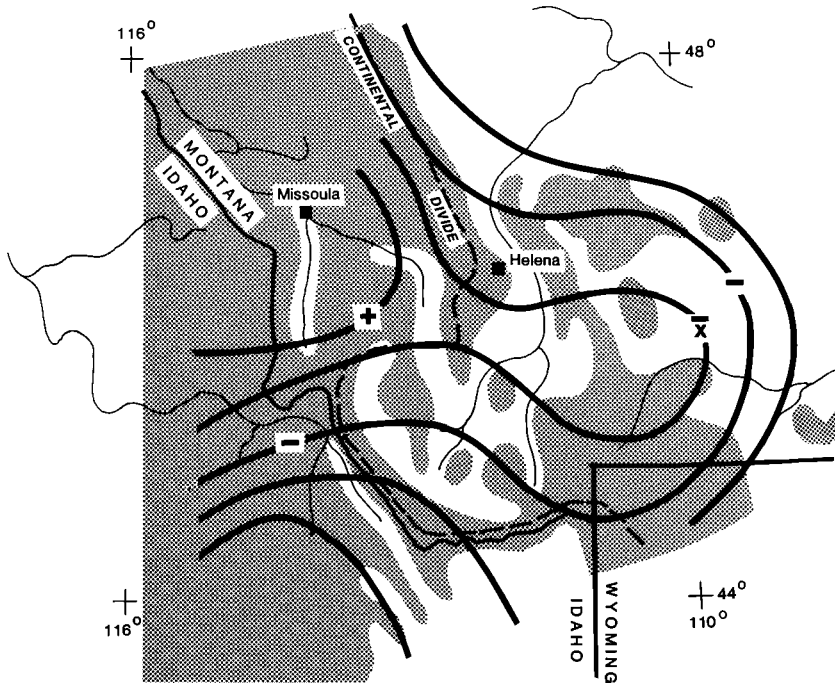

Figure 3. Geographic patterns of variation in height at 1,500 m as predicted for populations from 2,000 m elevation. The contour interval is scaled to ½lsd 0.2.

studies will be combined in a single model of genetic variation among populations of Douglas-fir in all of Idaho and Montana.

PRACTICAL APPLICATION

Optimal productivity of artificial regeneration requires that planted trees be adapted to the planting site. Because individual populations are adapted to only some sites, the distance that seeds are transferred must be limited if adaptedness is to be maintained and maladaptation is to be controlled. To control maladaptation, seed transfer guidelines must reflect patterns of adaptive variation.

Because adaptive variation is a result of developmental events being attuned to the climate, seed transfers of a given distance along a cline impose a risk regardless of direction (Rehfeldt 1983b, Campbell 1986). Whenever transfers result in a lack of synchronization between development and climate, productive losses accumulate from both biotic and abiotic environmental effects (Dietrichson 1964). In the Inland Northwest, transfers of seeds from harsh to mild sites are accompanied by losses in productivity because transferred populations have a lower growth potential than local ones; transfers from mild to severe sites risk damages such as frost injury or snow breakage to which the transferred population is vulnerable (Rehfeldt 1983b).

In the present paper, genetic variation among populations was described by elevational and geographic clines that tend to parallel environmental gradients. The patterns, therefore, most plausibly reflect attunement of growth and development with the local climate by means of natural selection. To reject that these clines are adaptive requires one to accept the untenable alternative conclusion that the systematic patterns of genetic variation have developed randomly. Because this alternative is unacceptable, it is on the basis of these clines that guidelines to seed transfer should be based.

of relatively long frost-free periods (Fig. 3).

The results and interpretations of this study are consistent with those of the previous studies in this series: genetic variation among populations is rampant and is related to the elevation and geographic location of the seed source. Populations are adapted to a relatively small portion of the environmental gradient. And adaptationally similar populations tend to recur in similar environments. In the final paper of this series, results of all
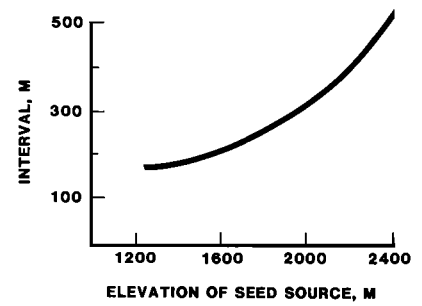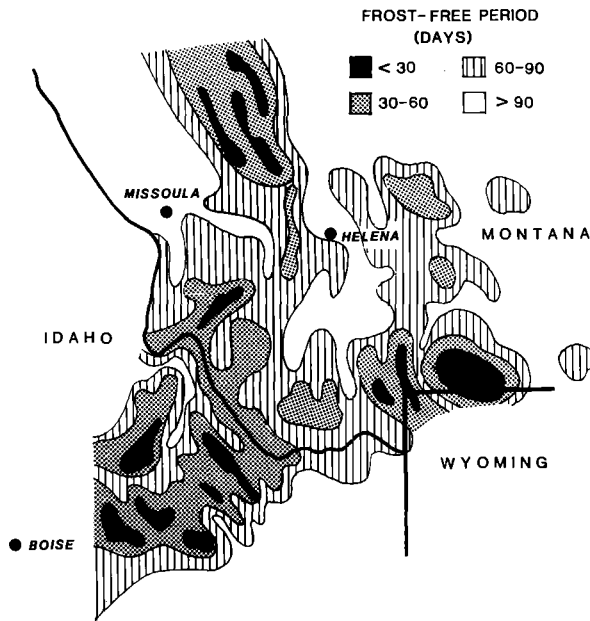
FROST-FREE PERIOD
(DAYS)

■ < 30   ▥ 60-90
▤ 30-60   ☐ > 90

Figure 5. Geographic patterns of the average frost-free period (from U.S. Department of Commerce 1968).

One way of using adaptive clines to limit seed transfer is to delineate discrete seed zones. If zonal boundaries are delimited at intervals equal to the smallest elevational or geographic distance at which differentiation can be detected (lsd 0.2), then seed zones can be constructed from Figures 3 and 4. According to Figure 3, in which contours are separated by ½(lsd 0.2), two geographic units, one for isopleths above the mean and one for isopleths below the mean, would be required; within these units, Figure 4 suggests that seed zones should be limited to about 180 m (600 ft) of elevation below 1,400 m (4,600 ft), 250 m (820 ft) for elevations between 1,400 and 2,000 m (4,600 and 6,550 ft), and 400 m (1,300 ft) for elevations above 2,000 m (6,550 ft). The total number of discrete zones needed to account for adaptive variation within the study area would be about 10.

The concept of floating transfer guidelines offers an alternative to discrete seed zones. According to floating guidelines, seeds can be

transferred across isopleths (Fig. 4), but when they are, the elevations at which they are to be used should be adjusted. When transferring across isopleths from high to low value, seeds are being moved from a relatively mild site to a more severe one. The transferred seeds therefore should be used at an elevation lower than that of the seed source. Likewise, when transferring across contours from lower to higher value, the elevational interval at which the seed is used should be adjusted upward. For example, seeds collected at 1,400 m should be used at ±90 m (±300 ft) of the elevation of their source. When these seeds are transferred across an isopleth of higher value, they should be used between 1,400 and 1,580 m (4,600 and 5,200 ft). Seeds collected at 1,800 m should be used at ±125 m (±410 ft) of their source. When transferred across an isopleth of lower value, they should be used between 1,550 and 1,800 m (5,100 and 5,900 ft). Seeds from >2,000 m (6,550 ft) should be used at ±200 m (±650 ft) of the

seed source and can be similarly floated across isopleths. Transfer intervals from seed sources at other elevations can be determined from Figure 4.

These recommendations for limiting seed transfer were developed from statistical models based on the performance of young trees under intensive culture. The models need practical verification. Verification will come from planting programs that not only restrict seed transfer, but also maintain precise records on the exact location from which planted trees originated. Productivity of such plantings will test the applicability of these guidelines.   ☐

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