

Genetic Variation of Whitebark Pine (*Pinus albicaulis*) Provenances and Families From Oregon and Washington in Juvenile Height Growth and Needle Color

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Abstract—A three year common garden study was conducted on whitebark pine (*Pinus albicaulis*) which included 215 families from the eight provenances or seed zones in Oregon and Washington. Total height and needle color were assessed. Height differed significantly among provenances and families, and was primarily associated with source elevation, longitude, and precipitation. A moderate to high heritability was estimated for total height. Seedling needle color differed among provenances and was associated with temperature:moisture indexes and source elevation. Height growth along the Cascade mountain range (USA), representing four adjoining provenances or seed zones, appeared to be similar and clinal in nature. The four remaining provenances, representing seed zones from a more moderate or extreme environmental settings, differed significantly from the Cascade provenances for one or more traits that were examined. It would seem prudent to restrict seed transfers to within each of these four seed zones respectively. This study also supports the need to plan gene conservation collections within each seed zone for whitebark pine in the Pacific Northwest region.

Introduction

Understanding geographic patterns of genetic variability in adaptive traits will be a key to successful restoration and genetic conservation of whitebark pine (*Pinus albicaulis*). A large common garden study was conducted with seedlings at the Dorena Genetic Resource Center (DGRC) and used to assess family and provenance variation in height growth, foliage color, and resistance to white pine blister rust (the assessment of blister rust is still in progress). This summary reports on data from collections in Oregon and Washington, and complements the few other studies that have been reported to-date for this species.

Materials and Methods

The study included 215 families from eight provenances in Oregon and Washington. The eight provenances represent the current seed zones (geographic subdivisions) associated with whitebark pine in the Pacific Northwest region (Figure 1; Aubry and others 2008); the number of parents (seedling families) varied from seven to 51 per provenance (Table 1). Wind-pollinated seeds collected in natural stands were stratified in November 2006. Germination occurred in a germination chamber, and emerging seedlings were

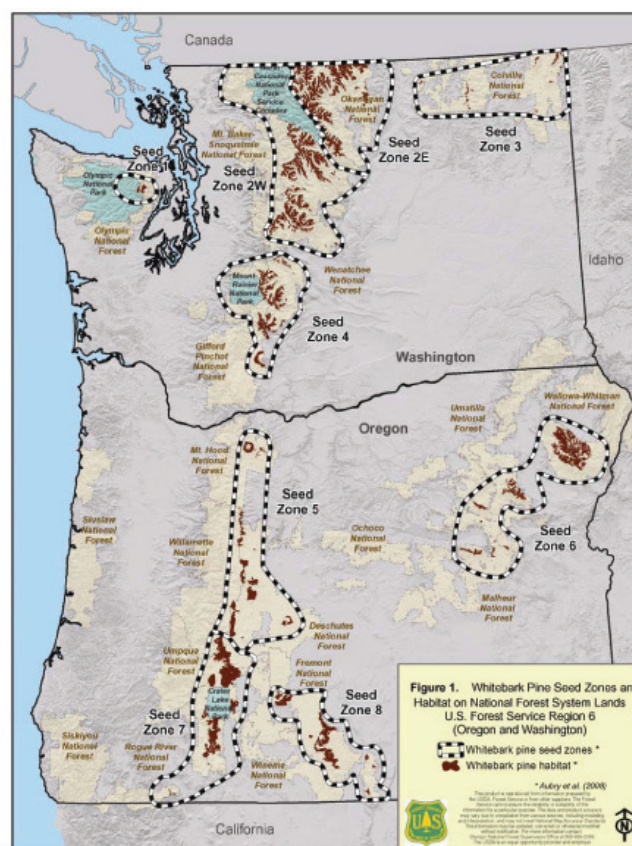


Figure 1. Whitebark pine seed zones and habitat on National Forest system lands in U.S. Forest Service Region 6 (Oregon and Washington). See Table 1 for listing of provenances.

transplanted into 164 cm³ tubes and placed in a greenhouse at DGRC (Cottage Grove, Oregon) in March 2007. The seedlings were randomized into complete blocks in August 2007. They were inoculated with blister rust in fall 2008, and then transplanted to a common garden raised bed (in the same complete block randomization) for future assessment of disease resistance and other traits (Figure 2).

The experimental design was a randomized complete block with six blocks. For the blister rust inoculation, the experiment was divided into two separate trials; where each trial was inoculated with a different geographic source of blister rust spore inoculum in early fall 2008. Each of the six blocks consisted of 215 families planted in row-plots (up to 10 trees/family/plot).

Table 1. Provenance locations and sample size (number of parent trees) in the common garden study^a.

Provenance	Latitude (°N)	Longitude (°W)	Elevation (m)	Sample Size (N)
Washington				
1. Olympic	47.8	123.1	1792-1981	7
2. N. Cascades	46.9	121.2	1792-2084	51
3. Northeastern	49.0	117.1	1755-2072	9
4. S. Cascades	46.2	121.5	1896-2118	16
Oregon				
5. N. Cascades	44.0	121.7	1676-2457	56
6. Blue Mountains	44.8	118.2	2219-2469	22
7. S. Cascades	43.4	122.1	2011-2446	33
8. Southeastern	42.5	122.1	2286-2535	20

^a The common garden locale was located near Cottage Grove, Oregon; 43.7° N latitude, 122.9° W longitude, 128 m in elevation.

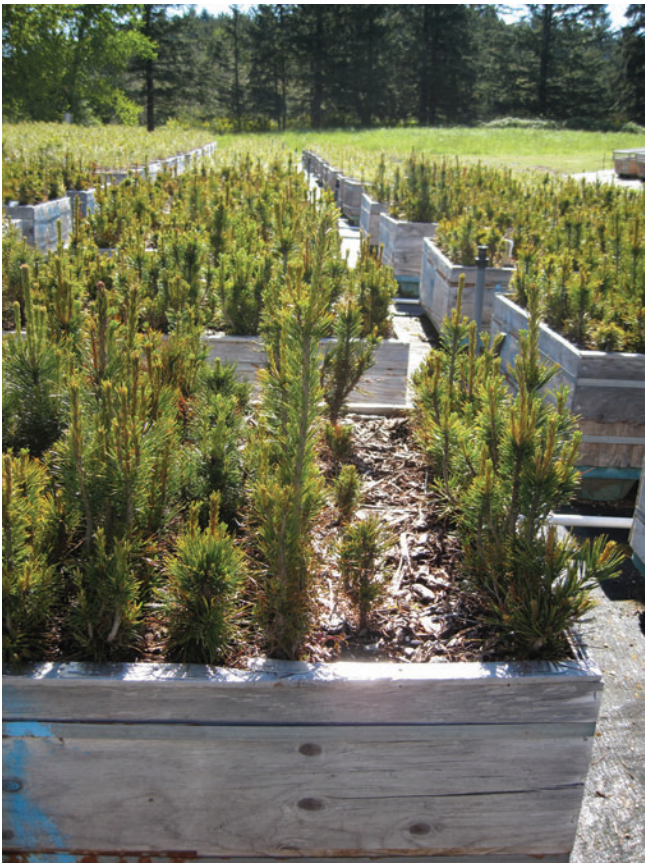


Figure 2. Family row plots of whitebark pine in common garden study. Note variation in height.

Total height after the second (prior to spore inoculation in 2008) and third growing season (fall 2009) were recorded for all seedlings. The correlation between the two measurements was 0.97 (family mean basis) and only total height after the third growing season is reported in this paper. The affect of inoculating seedlings with blister rust in 2008 on the third year height growth (2009) and color assessment (2010) is assumed to be indeterminate (with unknown bias) and negligible. Color of the 2009 foliage was assessed in 2010; five seedlings per family row-plot were assessed for color. One person performed the color assessments



Figure 3. Variation in foliage color; some individuals and families are 'blue' and others are 'green'.

to minimize the variation, where the color categories were based on a subjective assessment of color differences. Four initial color categories were assessed : green, intermediate green, intermediate blue, and blue color. After an initial look at frequencies, the colors were combined to form two classes (Figure 3): green (green + intermediate green) and blue (blue + intermediate blue).

The ClimateWNA v4.60 program (Wang and Hamann 2010) was used to derive climate variables on the basis of each parent tree's source location (latitude, longitude, elevation).

The mean, minimum, and maximum temperatures on an annual, seasonal, and monthly basis were obtained in addition to the annual, seasonal and monthly precipitation data. Various indexes were also utilized from the ClimateWNA program. The annual heat:moisture index is defined as the (mean annual temperature (degrees C) + 10) / (mean annual precipitation (mm)/1000)). The summer heat:moisture index is defined as the ((mean warmest month temperature (degrees C)) / (mean annual summer (May to Sept.) precipitation (mm)/1000)). These climate variables and source location variables were used in the regression on height and color frequency data.

Statistical analysis procedures were conducted using SAS (SAS Institute Inc. 2008). The analysis of variance (ANOVA) of height was based on family-plot means using Proc Glimmix. Provenance was considered as a fixed effect, while trial, block within trial, family within provenance, and associated interactions were considered as random effects in the generalized linear mixed model. Proc Glimmix was used to estimate variance components for use in the estimation of individual tree heritability for selected provenances where individual tree data was used. Individual tree heritability was estimated as: $3(\sigma_f^2) / (\sigma_f^2 + \sigma_{fr}^2 + \sigma_w^2)$ where σ_f^2 , σ_{fr}^2 , σ_w^2 are the components of variance for family (f), plot (fr), and within-plot (w) respectively. The value of $3(\sigma_f^2)$, used in the numerator to estimate the additive genetic variance, reflects the likelihood that offspring from an open-pollinated parent are related to a greater degree than half-sibs (Campbell 1986). Standard errors of heritability were calculated according to Becker (1984). Proc Reg (selection = stepwise with default probability settings) was used in the regression of height and foliage color on source location and environmental variables. Proc Surveyfreq was used to summarize the color frequency data (green-blue distribution per provenance) and to determine if a significant association exists between the provenances and color frequency distribution.

Results

Height

Significant differences among provenances were observed in the ANOVA ($F = 9.6$, $P < .004$) and the pair-wise provenance contrasts are exhibited in Figure 4. There was substantial variation among families within provenances ($Z = 8.7$, $P < .0001$) as well (Figure 5), with family means varying from 6.2 to 21.3 cm across provenances. The range of family means within provenances is striking with mean height ratios (largest family mean/smallest family mean) ranging from 1.5 to 2.9. If provenance had been considered as a random variable along with the remaining variables, it would have accounted for 20 percent of the total observed variation with family within provenance accounting for an additional 31 percent. The variation accounted for by family within provenance is a high value for a biometric trait in conifers. Provenance mean heights ranged from 12.4 to 18.6 cm. Height differences were significant among geographic areas as shown in the following selected contrasts. The Olympic provenance (# 1; mean = 18.6 cm) differed significantly ($P < .05$) from six of the seven provenances in the pair-wise contrasts (Figure 4). It was not, however, significantly different than the Washington – S. Cascades provenance (# 4; mean = 16.0 cm). The Cascade region provenances (combined # 2, 4, 5, 7, 8; mean = 15.0 cm) differed significantly from the northeastern Washington and Blue Mountains region (combined # 3, 6; mean = 12.5 cm). In addition, the Oregon – S. Cascades provenance (# 7; mean = 13.1 cm) differed significantly from the rest of the combined Cascade provenances (combined # 2, 4, 5, 8; mean = 15.4 cm).

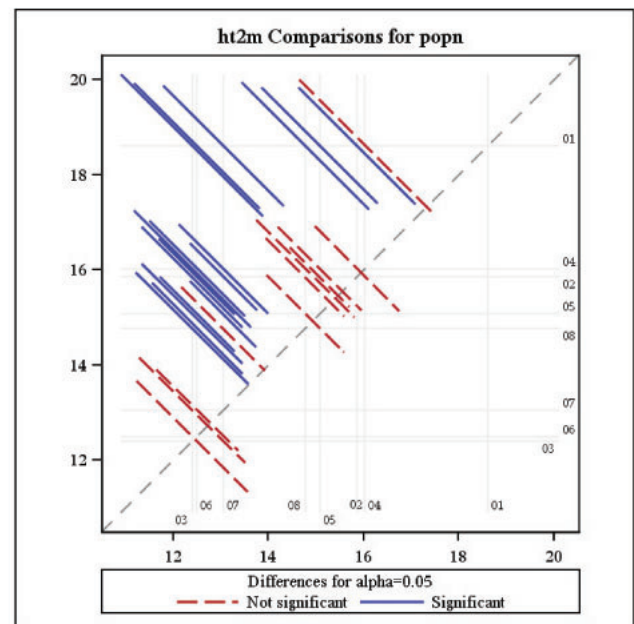


Figure 4. Statistical significance ($P < .05$) among provenance means for total height. Height shown on vertical and horizontal axis (cm.). See Table 1 for listing of provenances (popn).

Elevation, longitude, temperature, and precipitation explained only 35 percent of the variation in height (regression model $df = 182$). The final set of variables in the regression model were: elevation, longitude, temperature difference between mean warmest and coldest monthly temperature, October precipitation, June precipitation, May precipitation, and mean annual precipitation. Elevation, as a singular variable, was associated with height to the greatest extent ($r = -0.41$, Figure 6). A subset of the data was also analyzed in a separate regression analysis; where the subset designates the higher elevation sources that are greater than 2286 m. Longitude and precipitation in the month of June explained 50% of the variation (regression model $df = 35$). Elevation was not a significant variable in the regression model for this subset.

Individual tree heritability was estimated for three of the eight provenances (# 2, 5, 7) where sample sizes ($n = 51, 56, 33$) were deemed sufficient. Heritability (and standard errors) estimates were 0.57 (0.10), 0.56 (0.10), and 0.83 (0.16) for the respective provenances. Thus, height appears to possess a moderate to high degree of heritability even after considering the high standard errors and single common garden test site.

Foliage Color

There was a significant association between the eight provenances and green-blue frequency distribution (Rao-Scott chi-square, $P < .0001$). The percentage of green seedlings ranged from 64 to 99 percent (Figure 7). The two lowest percentages (64 and 76) were associated with the provenances from sources representing the highest elevations in Oregon

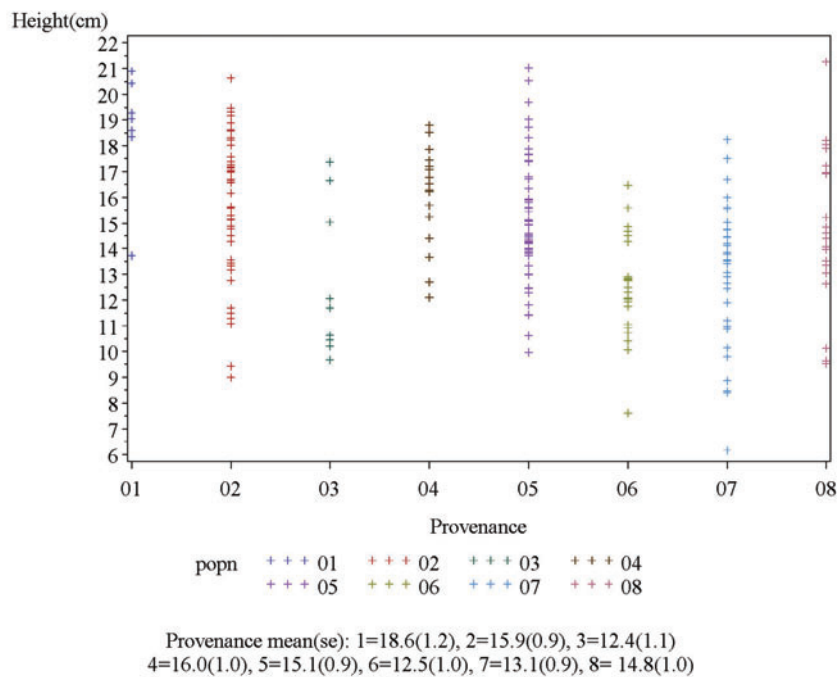


Figure 5. Family mean heights by provenance. See Table 1 for listing of provenances.

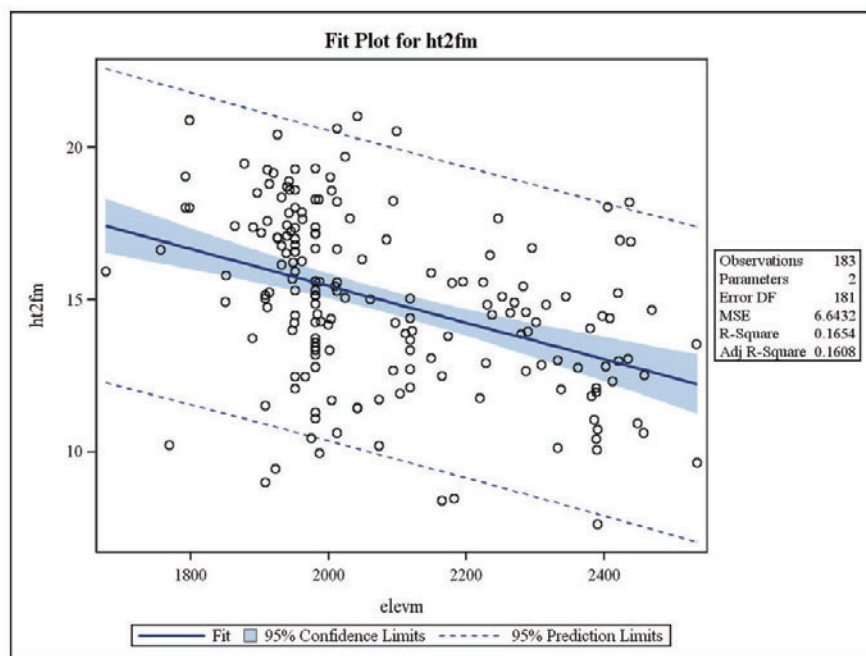


Figure 6. Family mean heights (cm) by elevation (m) across provenances.

(# 6, 8), while the two highest percentages (98 and 99) were associated with provenances from two lower elevation sources in Washington (#1, 3).

A total of 132 families were 100 percent green while one family was 100 percent blue. The remaining 82 families segregated into blue and green seedlings. The frequency of color segregation among families varied considerably among provenances. Only one seedling scored as blue in the Olympic provenance where one family out of seven segregated into green and blue seedlings. In stark contrast, 36 percent of the seedlings scored as blue in the southeastern Oregon provenance (# 8) where 13 of the 20 families segregated into

green and blue seedlings and one family was 100 percent blue. The vast majority (83 percent) of blue seedlings in the four lower elevation provenances (# 2, 3, 4, and 5) occurred in families where the parent tree source elevation was equal to or greater than 1981 m. The individual family segregation proportions (expressed as the proportion of blue seedlings) were wide ranging within the respective provenances; where proportion of blue seedlings in a family ranged from 0 to 100 percent. Figure 8 displays the segregation proportions for those families (82 in total) which segregated into blue and green seedlings in addition to the single family that was 100 percent blue.

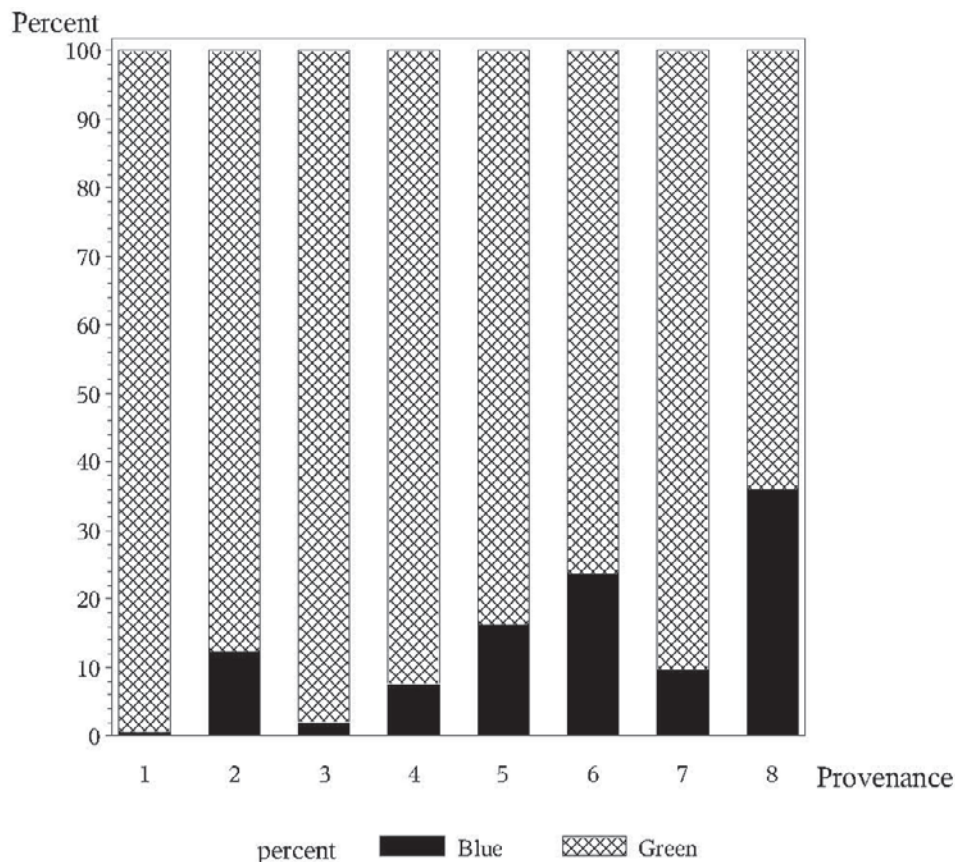


Figure 7. Percent of green/blue needle color by provenance (see Table 1).

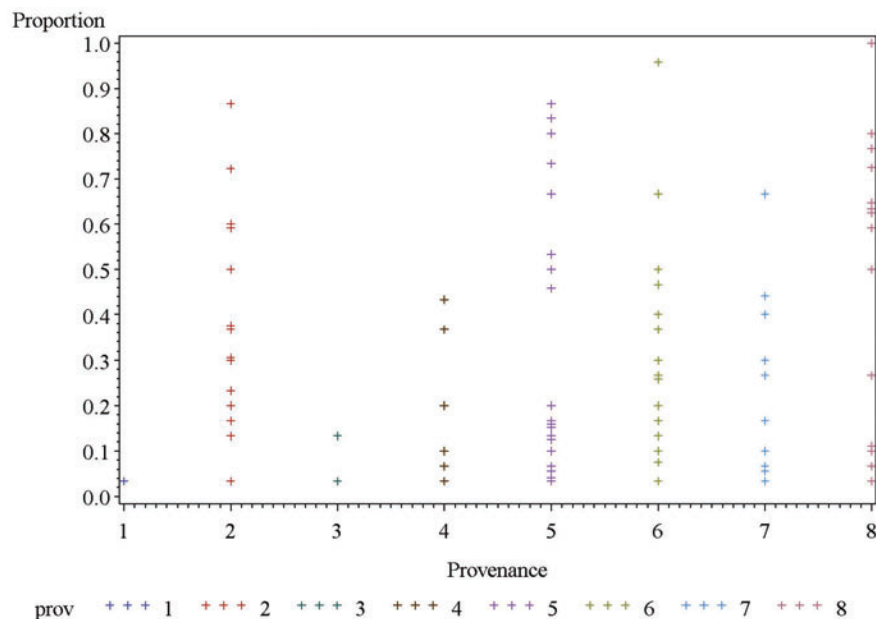


Figure 8. Proportion of blue seedlings per family that segregated into blue and green seedlings. See Table 1 for listing of provenances.

For the 83 of 215 families that segregated into blue/green or 100 % blue color.

A suite of 14 environmental and location variables explained 57 percent of the variation in color frequency (family mean basis, regression model $df = 188$). The first three variables entered into the model explained the majority of the variation (38 percent): annual heat:moisture index, elevation, and summer heat:moisture index. The remaining variables

(eight of which related to monthly or seasonal precipitation values) explained a relatively low percentage of the variation as they entered the model. A higher annual heat:moisture index, higher elevation, and lower summer heat:moisture index were associated with a relatively higher percentage of blue seedlings.

Discussion

The results from this study confirm some of the general trends that were found in an earlier study which summarized the third year growth increment of seedlings for four of the sampled provenances in this study (# 4, 5, 6, 7; Hamlin and others 2008). The families and sampled geographic areas differed in the two studies, but both studies sampled within the four respective provenances. Similar height growth trends were depicted for provenance differences and tier rankings. In the earlier study, provenances representing the Washington - S. Cascades (# 4) and Oregon - N. Cascades (#5) were the tallest (top tier group) and differed significantly from the Blue Mountains (#6) and Oregon - S. Cascade (#7) provenances (shorter, lower tier group). A similar general trend of declining height increment with increased elevation was evident. Precipitation amounts explained 34 percent of the variation in height increment in the earlier study. Height increment also exhibited a moderate degree of heritability (0.27) in the earlier study. Thus, the two studies exhibit a repeatable difference (relative tier ranking) among the sampled provenances and a measureable degree of genetic adaptation across the region for height growth.

In the earlier study (Hamlin and others 2008), growth initiation and completion were estimated for provenances (# 4,5,6,7) on the basis of one season's growth. The results indicated a difference in phenology among the provenances where the Blue Mountains provenance (# 6) initiated growth at the earliest date in the spring and completed growth at an earlier date. The remaining provenances (# 4, 5, 7) were more similar in their phenology pattern, although a low elevation subset (1676 m) within provenance # 5 exhibited later dates for growth initiation and growth cessation. This additional trait suggests a difference between the higher elevation eastside provenance (# 6) and the relatively lower elevation (with warmer temperature and higher precipitation) Cascade provenances. Such differences in phenology can affect the hardiness and potential for cold injury when moving sources to more extreme environments (Bower and Aitken 2006).

The results from this study indicate an association between the provenances and foliage color (green-blue frequency distribution) which in turn are associated to a degree with source elevation and temperature to moisture ratios. The color differences (green to blue) in a myriad of other studies of conifers have been attributed to differences in surface wax on the needles (Hanover and Reicosky 1971), and there has been speculation that this may be a physiological response to certain ecological habitats; example, as an adaptive character response to higher UV irradiation levels (Clark and Lister 1975). UV radiation increases with elevation, and decreases with cloud cover (Noaa/National Weather Service 2006). The Blue Mountains (#6) and southeastern Oregon (#8) provenances represent the two higher elevation ranges within the study, and the higher source elevations (with correspondingly higher UV irradiation levels) represent a logical causal factor for the higher blue frequency distribution in these two provenances.

The Blue Mountains and southeastern Oregon provenances also experience the larger annual heat to moisture ratios which would affect the basic physiological response to moisture deficits.

Hanover and Reicosky (1971) suggested that these waxes represent an additional resistance to the diffusion of water, while Clark and Lister (1971) have also suggested the importance of waxes in relative reflectance of UV light which may aid in dealing with high irradiation levels, lower relative humidities, and low soil moisture levels.

A few general observations in reference to the current seed zones might be garnered from this small study and previous studies. Variation patterns among the eight provenances (current seed zones) differ, depending on the trait studied. A previous wide ranging study of whitebark pine (Bower and Aitken 2008) noted the importance of date of needle flush in seed transfer. They also noted the clinal nature for height growth within the study area and correlation to length of growing season. In addition, previous and current studies at DGRC suggest there may be geographic trends such as higher rust resistance in the northern areas or hotspots for rust resistance in the region (Snieszko and others 2007; and Snieszko, personal communication). Height growth along four of the Cascade provenances (# 2, 4, 5, 8) appear to be more similar and clinal in nature; where growth appears to be associated to an extent with elevation, temperature, and precipitation gradients. Therefore, one should be cognizant of the differences in the respective environmental gradients prior to determining a seed transfer within or among these four provenances. The remaining four provenances (# 1, 3, 6, 7) differ significantly from the aforementioned Cascade provenances for one or more traits that were examined in either this or the previous study (Hamlin and others 2008). The Olympic provenance (#1) differs in growth rate from the majority of provenances and is located in a more moderate climate. The annual rainfall (mean = 2621 mm in the provenance sample area) is approximately two to three times that of the other provenances and relatively lower summer temperatures exist within the Olympic provenance. The northeastern Washington provenance (# 3) differs in growth rate from the Olympic (# 1) and Cascades (# 2, 4, 5, 8), and is separated a great distance from the Blue Mountains provenance (#6). The phenology of the Blue Mountains provenance (# 6) differs substantially from the Cascades and height growth is substantially slower than the Cascades (#2, 4, 5, 8). The Oregon - S. Cascades provenance (#7) was unique in that growth rates were similar to the east-side provenances (# 3, 6) while phenology and foliage color were similar to the northern Cascade provenances (# 2, 4, 5). Thus, on the basis of these few studies and traits examined, it would seem prudent to restrict seed transfers to within each of these four provenances (# 1, 3, 6, 7) and seed zones respectively.

This short term study suggests the need to utilize seed zone delineations to an extent when considering seed deployment. This study also suggests that there exists a large amount of genetic variation among families within any single provenance. This large amount of genetic variation would

be desirable when deploying seed/seedlings into a planned restoration effort, and may serve as a desirable buffering in future climate change scenarios. Additional short or long-term studies of additional fitness traits are still desirable and would be useful in furthering knowledge of the species genecology. Any additional studies should also try to increase the sampling of parent trees where sample sizes have been less than desirable (for example, provenance # 1 and # 3 in this study).

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