Genetic Variation in Needle Traits of Whitebark Pine (*Pinus albicaulis*) Seedling Families: Within-Population Variation at Crater Lake National Park

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Abstract—Genetic variation in needle traits (number of rows of stomata, areal density of stomata, and density of stomata in a row, as well as needle length and needle width), seedling height growth, and early measures of blister rust (causal agent: *Cronartium ribicola*) resistance were examined among 22 open-pollinated families of whitebark pine (*Pinus albicaulis*) from Crater Lake National Park (CLNP) in Oregon. Needle traits were examined for both the adaxial and abaxial sides. Needle traits were also assessed for 19 of 22 parent trees from CLNP. The adaxial side of the needle on average had more rows of stomata (3.3 versus 2.0 rows), a greater density of stomata within a row (12.3 versus 11.2 stomata per mm of row), and a much higher areal density of stomata (45.60 versus 22.00 stomata per mm^2) than the abaxial side for the seedling families. Statistically significant variation among families was noted for many traits, particularly needle traits from the adaxial side of the needles as well as for height and rust infection (number of needle spots and number of stem symptoms, approximately 8 and 15 months post-inoculation). Individual tree heritabilities were low to moderate for needle traits (adaxial side), moderate for height, and very high for number of stem infections. Family mean correlations between stomate variables were generally statistically significant and moderate to high, with somewhat lower correlations between traits across the two surfaces. Needle length was uncorrelated with any of the other traits examined. The density of stomata (adaxial side) within a row showed a moderate correlation (0.47) to the number of needle spots; however, neither of these measures was correlated with the percentage of seedlings in a family with stem infections. The number of needle spots was only moderately correlated with the number of stem infections. Correlations between progeny and parental traits were generally significant and moderate to high. The parent–progeny mean correlations were highest for some of the traits on the abaxial side. There was evidence of significant differences in some traits between the 4 families from the east side (drier) and the 18 families from the west side of Crater Lake National Park, with families from the east side tending to have more stomata, shorter needles, shorter height, more stem infections, and a much higher percentage of infection 1 year after inoculation. The level of within-population variation was similar to that observed among populations from much of the range (seedlings grown in the same common garden study). This genetic variation may help buffer the Crater Lake population of whitebark pine against some biotic and abiotic factors in the future. Recently established genetic field trials at CLNP will provide a means of determining whether the characterized genetic variation contributes to maintenance of this population.

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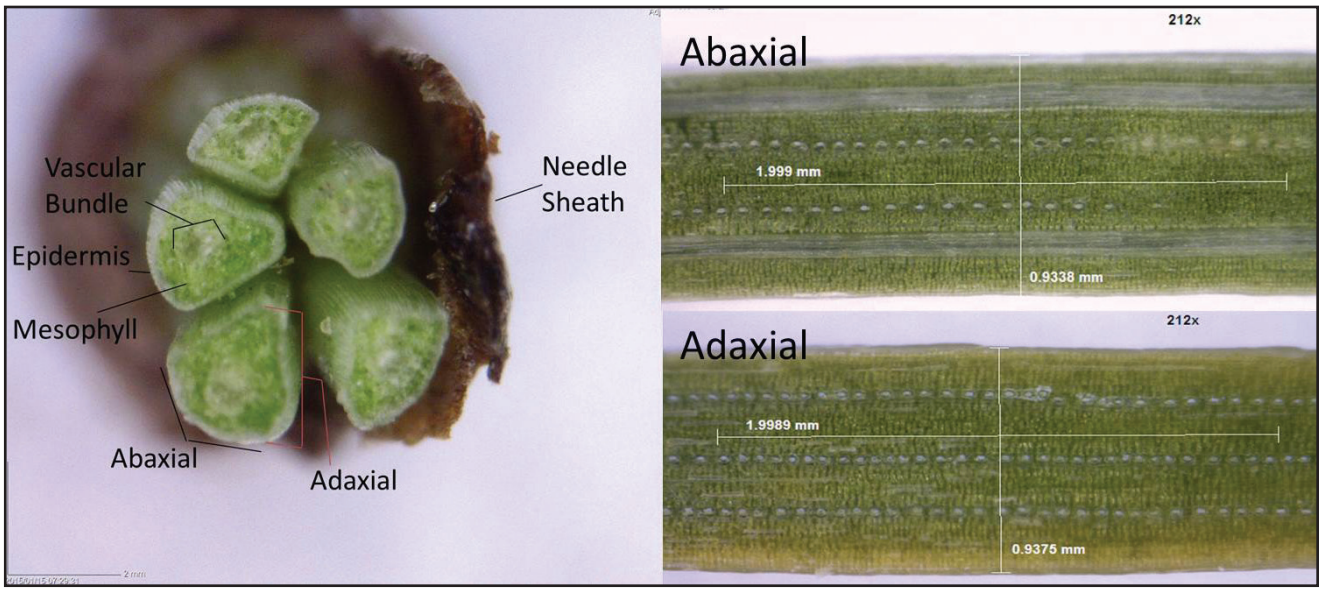

Figure 1—Cross section of a whitebark pine fascicle, illustrating how the one abaxial and two adaxial sides of each needle fit together to form the five-needle bundles, at approximately 205x magnification (left). The abaxial side at 212x magnification, exhibiting the typical two resin channels on either side of the stomatal rows (family CL04, top right). On the adaxial side at 212x magnification, the lack of resin channels can be noted along with an increased amount of stomatal rows (family CL22, bottom right).

INTRODUCTION

Whitebark pine (*Pinus albicaulis*) is a wide-ranging conifer in high elevation forest ecosystems of western North America (Tomback and Achuff 2010). It is highly susceptible to white pine blister rust, caused by the nonnative fungal pathogen *Cronartium ribicola*. Mortality due to blister rust, mountain pine beetle (*Dendroctonus ponderosae*), and a changing climate have raised concerns about the future viability of many populations of whitebark pine in both the United States and Canada (Committee on the Status of Endangered Wildlife in Canada 2010; U.S. Fish and Wildlife Service 2011). Genetic conservation efforts for whitebark pine are underway (Man and Moltzan, this proceedings; Sniezko et al. 2011b).

Genetic variation in a species is the cornerstone to its potential to evolve in response to biotic and abiotic challenges. Genetic variation in a limited number of traits has recently been examined in whitebark pine (Bower and Aitken 2006, 2008; Hamlin et al. 2011; Kegley et al. 2012; Mahalovich et al. 2006; Sniezko et al. 2007, 2011a; Warwell, this proceedings, *Adaptive Variation among Whitebark Pine (Pinus albicaulis) Populations from the Interior Northwestern United States in Relation to Climate*; Warwell, this proceedings, *Phenotypic Selection on Growth Rhythm in Whitebark Pine (Pinus albicaulis) in Low Elevation Common Gardens*), but there is little or no information to date on variation in needle traits. Needles provide the critical photosynthetic surface of whitebark pine, and stomata on needles are the entryway to infection for the *C. ribicola* pathogen (Patton and Johnson 1970). Stomata also regulate the flow of gases such as carbon dioxide and water vapor in and out of needles and are a key experimental tool to examine plant responses to a changing environment (Hetherington and Woodward 2003). Genetic variation in needle traits may help whitebark pine mitigate the impacts of a changing climate, or may aid the understanding of some of the genetic resistances to white pine blister rust.

Whitebark pine typically has stomata on both the abaxial (AB) and the adaxial (AD) sides of needles (fig. 1), which can be useful in distinguishing it from other five-needle pine species such as western white pine (*P. monticola*) (Hoff 1993). The AB side is the rounded outer side of the needle; a whitebark pine needle has one AB side and two AD sides (fig. 1). The AB surface generally has two resin channels running down the

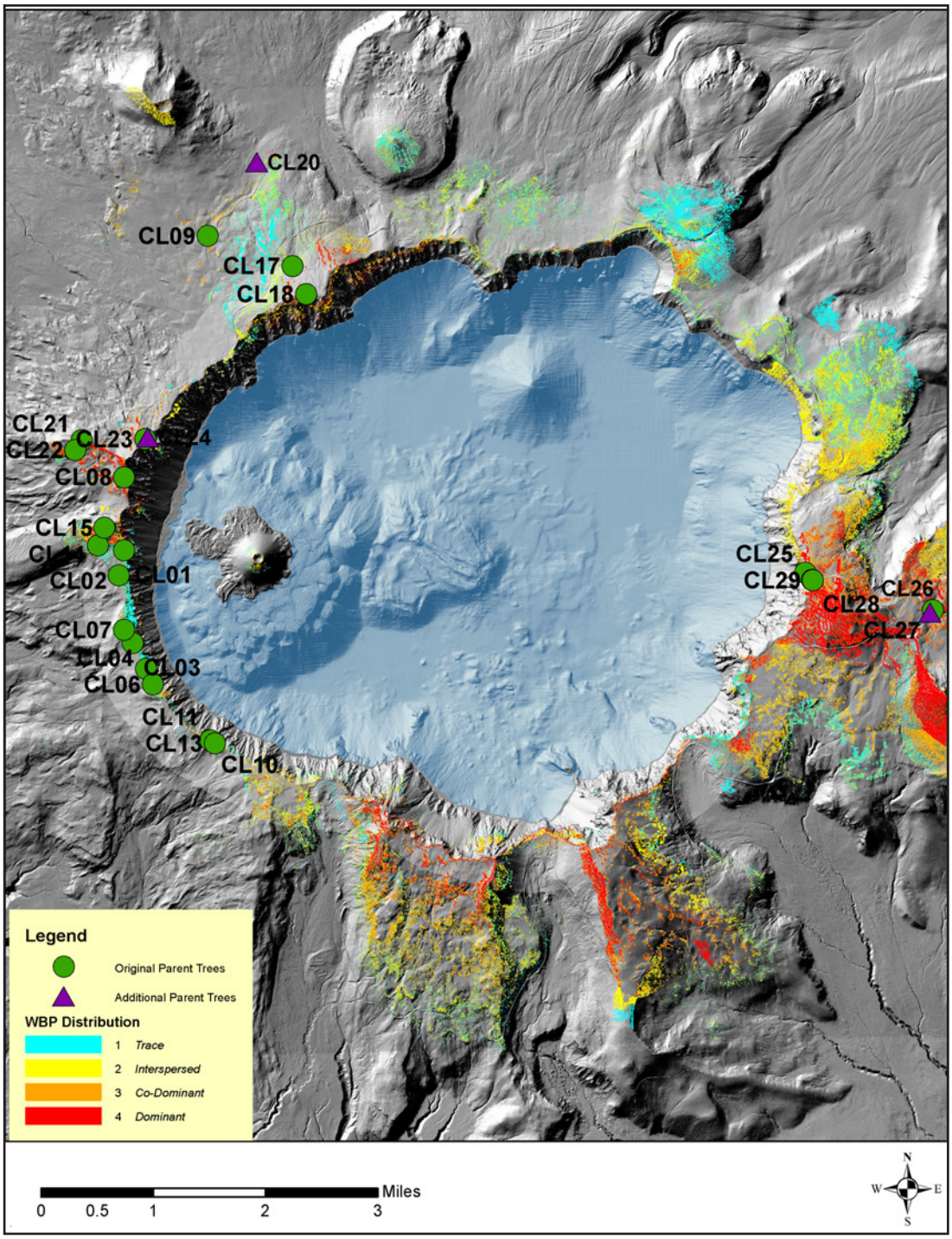

Figure 2—Map of Crater Lake National Park, showing the locations of the 25 whitebark pine parent trees whose progeny are in the 2007 common garden study. Also shown is the natural density of whitebark pine throughout the Park. Green circles signify the location of the subset of 22 families used for the stomate measurements in this study.

needle on both sides of the stomatal rows and generally has only one to three rows of stomata (fig. 1). The AD side usually has three or four rows of stomata and usually lacks resin channels (fig. 1).

A common garden study of whitebark pine seedling families from a wide range of geographic sources (populations) offers a unique opportunity to examine structural and anatomical aspects of needles within

and among populations. In this study we assess needle length and needle width as well as the frequency and distribution of stomata on both the adaxial and abaxial sides of needles among a sample of whitebark pine seedling progenies from parent trees at Crater Lake National Park (CLNP) in southern Oregon (USA) (fig. 2). We also examine the relationship between these seedling traits and the same traits assessed on needles of the parent trees located at CLNP, as well as the relationship of the seedling needle traits with early height growth and infection with white pine blister rust following artificial inoculation. This report complements a separate report in which we examine genetic differences in these traits among families from a wide segment of the geographic range of this species (Bennett et al., this proceedings).

METHODS

Samples collected from two sets of whitebark pine materials of CLNP origin were used in this study: (1) needles from 22 open-pollinated seedling families that were part of a larger common garden study involving 225 families sown in 2007 at the U.S. Department of Agriculture, Forest Service, Dorena Genetic Resource Center, Cottage Grove, Oregon (see Hamlin et al. 2010 for background on the study); and (2) needles from a subset of 19 of the 22 parent trees collected in the natural population at CLNP (fig. 2).

The seedlings, in 10-in^3 (164 cm^3) containers, were placed into a randomized complete block (RCB) design prior to inoculation with *C. ribicola*, with six blocks. The seedlings were inoculated in 2008, after their second growing season. Three blocks were inoculated in early September, and the other three blocks in early October; a different geographic source of rust was used for each inoculation. Seedlings from all 225 families were transplanted in October 2008 to wooden boxes (0.9 m × 1.2 m × 0.3 m) in the same RCB design with 12 families per box, and 20 boxes per block. Families were in row plots (up to 10 seedlings per family row plot).

Two seedlings from each of the 22 CLNP families were sampled from each of the 6 blocks (12 seedlings total per family) except for 1 seedling family that experienced poor germination and growth (9 seedlings total collected for this family). Effort was taken to collect from the third and eighth seedlings in the family row plot; samples were collected from alternate positions within the row-plot if the seedling had died or lacked tissue for sample collection. Needles from each seedling were collected during late spring 2010 and placed in individual small labeled paper envelopes. These envelopes were then bundled into family groups with rubber bands, placed into plastic bags, sealed, and stored in a freezer (–20 °C) until assessment, which occurred between August 2010 and June 2011.

For assessment, needles from two to four fascicles per seedling were separated and affixed to white index cards using adhesive tape. Needle lengths (NL) were measured with a ruler to the nearest millimeter (mm) for four randomly selected needles of each seedling. Needles were examined and segments photographed under a digital microscope at approximately 200× magnification (Dino-Lite model Pro-AM413ZT; AnMo Electronics Corp., Taipei, Taiwan). The microscope was calibrated using a 10-mm scale; the magnification was input, allowing the software to automatically correct length and width measurements on the segment photographs. Segments were about 2 mm long, and these lengths were accounted for in density calculations.

Needles from 19 parent trees were collected at CLNP on September 7, 2009. Three fascicles per tree were collected, and an effort was made to collect needles from low, middle, and high branches. However, in the case of family CL07, all fascicles originated on one branch. The parent needles were stored in the same location in the freezer as the seedling needles. Needles from parent trees were measured after all of the seedling needle measurements were complete and used the same protocols as the seedling samples.

For both sets of needle samples, data were collected from the 2009 cohort of needles. Data on needle width (NW), the number of stomate rows (nROWS), and the number of stomata per row (nSR) were collected (counts were made from the photographs) for both the AD and AB sides. Judgments on which needle side was being examined were made on the basis of the AB side having two resin channels. Stomate rows ending or beginning in the photographed segment were commonly observed; in those cases, an estimate to the closest tenth of a row was made for nROWS.

Typically, four AD and four AB measurements were made for each seedling sample. There were several instances where fewer or more measurements were made for a seedling, as a second observer reviewed every photo and changed some AD or AB designations. The total number of stomata per approximately 2-mm section of the needles was determined (tSTOM). The 2-mm sections examined were generally toward the center section of each needle; the tips and basal sections of needles were not examined. Although efforts were made to take each measurement from a different needle, due to logistical difficulties (notably twistiness of needles), occasionally two measurements were made on different regions of the same needle.

The counts and measurements taken were used to calculate several other stomate variables, including lineal density (LDEN), areal density (ADEN), and stomate frequency within rows (RDEN). LDEN was calculated as the number of stomata divided by the length of the needle segment in the photo. ADEN was calculated as LDEN divided by the needle width in the center of the segment photo. RDEN was calculated as LDEN divided by the number of rows of stomata in the photo. The average of the individual assessments for each trait was calculated to create a mean for each individual seedling.

For the seedling family means, a mean of means procedure was applied in which the means from the two seedlings in a block were averaged to get a family-by-block average, which was then averaged across the six blocks to get the overall family average for each trait. For the parent trees, there was no replication, so the mean is the average of the individual measurements for each tree.

Second-year height (HT2, height through the 2008 growing season), third-year height (HT3), and data on blister rust symptoms were also recorded for the seedlings. Number of blister rust needle spots at first assessment (summer 2009) (nSPOT1), the number of early stem infections present on all trees (nSS2) and on trees with stem infections in late fall 2009 (nSS2i), and the percentage of seedlings with stem infections at second inspection (pSS2) were determined. Only the subset of seedlings (maximum 12 seedlings per family) that had needle measurements was used here for the mean calculations of rust and growth traits.

Statistical analysis procedures were conducted using SAS® 9.4 (SAS Institute Inc., Cary, North Carolina, USA) and R 3.0.2 (R Foundation for Statistical Computing Vienna, Austria). Family means and parent means were used to calculate Pearson correlations between all traits by using PROC CORR. Linear models were fit by using PROC GLIMMIX. For count and proportion data, quasi-Poisson and quasi-binomial models were used, respectively. The east side of CLNP is drier than the west side (about 8 km apart), and separate analyses were set up to examine this contrast. Geographic source of rust, families, and side of CLNP were treated as fixed effects; blocking variables were treated as random effects. Individual tree heritabilities (h^2_i) were calculated from variance components in the same manner as Hamlin et al. (2011). For heritabilities of count data, square root transformations were applied to the individual tree data. Heritability using offspring-parent regression was estimated as described in Falconer and Mackay (1996).

RESULTS

Family Variation

Needle Variation: Adaxial Versus Abaxial Side

Although the abaxial side of the needle was wider than the adaxial side by a factor of 1.19 for seedlings from the 22 families, the AB side averaged fewer rows of stomata (2.0 vs. 3.3), a lower density of stomata within rows (11.2 vs. 12.3), and a much lower areal stomate density (22.0 vs. 45.6) relative to the AD side (table 1). Although there was no statistically significant family variation in the needle traits for the AB side (although NW and ADEN were close to significantly different, with $P < 0.10$), all traits showed significant family differences for the AD side (table 1). For the AD side, minimum and maximum family means varied by greater than 17 percent for NW, 14 percent for RDEN, and more than 38 percent for ADEN.

Growth and Rust Infection Traits

There were statistically significant differences among families in seedling height (both HT2 [$P < 0.0001$] and HT3 [$P < 0.0001$]), nSPOT1 ($P < 0.0001$), nSS2i ($P < 0.0001$), and pSS2 ($P = 0.0005$). Families showed very large variation in these traits. For example,

Table 1–Overall means and range in family means for needle traits, growth, and blister rust infection measures for seedlings of 22 *Pinus albicaulis* families. Needle traits are shown separately for the adaxial (AD) and abaxial (AB) side of the needles except for needle length; height and rust means are shown here under the adaxial means. See the text for a description of traits.

	Adaxial (AD)				Abaxial (AB)			
	Mean	**Minimum**	**Maximum**	**p-val**	**Mean**	**Minimum**	**Maximum**	**p-val**
nROWS	3.3	2.7	4.0	<.001	2.0	1.6	2.3	0.142
tSTOM	84.0	67.1	98.7	<.001	48.5	38.6	56.5	0.338
LDEN	40.5	32.3	47.5	<.001	23.4	18.6	27.2	0.338
ADEN	45.6	38.8	53.7	<.001	22.0	18.2	26.5	0.069
RDEN	12.3	11.5	13.1	0.017	11.2	10.2	12.4	0.280
NW	0.89	0.81	0.95	0.009	1.06	0.98	1.10	0.066
NL	39.1	33.9	44.2	0.059	-	-	-	-
HT2	10.2	6.5	15.0	<.001	-	-	-	-
HT3	14.6	7.9	19.8	<.001	-	-	-	-
nSPOT1	89.8	30.5	164.1	<.001	-	-	-	-
nSS2	6.1	0.4	18.3	<.001	-	-	-	-
nSS2i	9.4	1.7	19.5	<.001	-	-	-	-
pSS2	60.5	16.7	100.0	<.001	-	-	-	-

Table 2–Overall means and range in parental means for needle width and stomate traits for 19 *Pinus albicaulis* parent trees at Crater Lake National Park. Needle traits are shown separately for the adaxial (AD) and abaxial (AB) side of the needles, except for needle length which is shown here under adaxial. See the text for a description of traits.

	Adaxial (AD)			Abaxial (AB)		
	Mean	**Minimum**	**Maximum**	**Mean**	**Minimum**	**Maximum**
nROWS	3.3	2.0	4.3	1.9	0.8	2.7
tSTOM	79.7	55.0	105.8	42.8	17.3	68.0
LDEN	38.4	26.5	50.9	20.6	8.3	32.7
ADEN	42.5	31.3	51.5	18.6	8.0	27.7
RDEN	11.6	10.1	13.6	10.6	6.8	12.5
NW	0.90	0.74	1.10	1.10	0.90	1.20
NL	50.5	42.0	67.5	-	-	-

family means for HT2 ranged from 6.5 to 15.0 cm, and nSPOT1 ranged from 30.5 to 164.1 spots (table 1). The artificial inoculation of the seedlings was very effective, as 100 percent of the seedlings from CLNP families had needle spots (no family variation present in this trait). Evidence of family differences in NL ($P = 0.06$) was suggested.

Parent Variation (Geographic Variation)

There was large variation in all needle traits for the 19 parent trees (table 2). Although the abaxial side of the needle was wider by a factor of 1.21, as with the seedling families, the AB side of parent tree needles had fewer rows of stomata (1.9 vs. 3.3), a lower density of stomata within rows (10.6 vs. 11.6) and a much lower areal stomata density (18.6 vs. 42.5) than the AD side (table 2). There was large variation in means for all needle traits (table 2), and the minimum and maximum parental means on the AD side varied by greater than 49 percent for NW, 35 percent for RDEN, and more than 64 percent for ADEN. Although the needles from the parent trees tended to be longer and slightly wider than their seedling progeny, the needles from the parent trees tended to have fewer stomata than their progeny (tables 1 and 2; fig. 3).

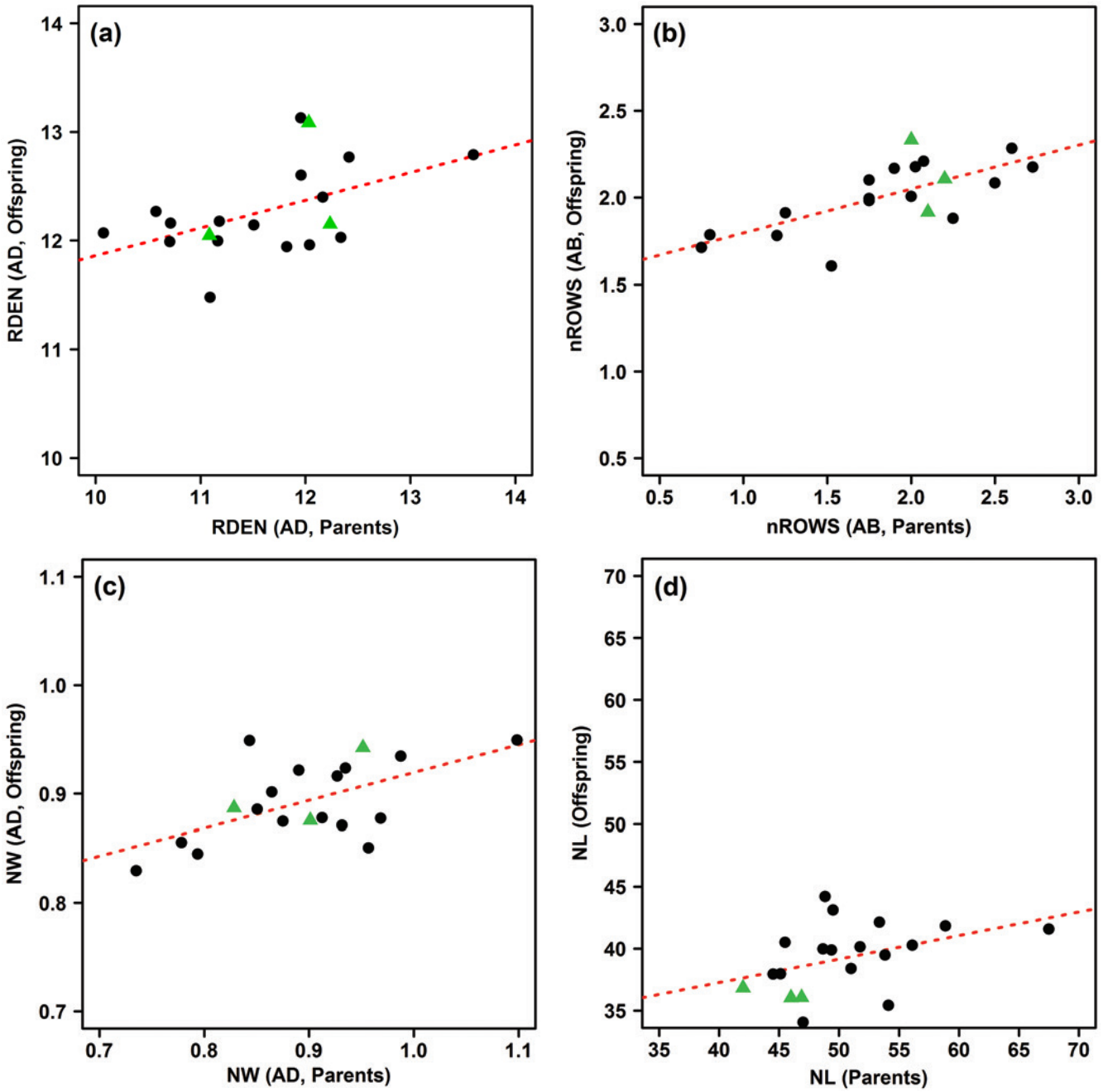

Figure 3—Relationship between offspring (y-axis) and parents (x-axis) for a variety of needle traits for 19 seedling families in a common garden test and the corresponding parent trees at Crater Lake National Park (CLNP). (a) stomatal density in rows (RDEN) on the adaxial (AD) side (r = 0.50); (b) number of stomatal rows (nROWS) on the abaxial (AB) side (r = 0.70); (c) needle width (NW) on the adaxial (AD) side (r = 0.59); (d) needle length (NL, r = 0.41). Green triangles represent parents on the east side of CLNP, while black circles represent the west side. The dotted red line shows the least-squares regression line. Significance at the α = 0.05 and 0.1 levels occurs for correlations with absolute values greater than 0.46 and 0.39, respectively. Note the smaller needle length of the three parents and their progenies from the drier east side of Crater Lake National Park.

Correlation Between Traits: Progeny

Family mean correlations (22 families) between the stomate variables on the AD and AB sides for the seedling families were generally significant and moderate to very high. For example, nROWS on the AD side was significantly correlated with NW, tSTOM, LDEN, and ADEN of stomata (r = 0.51, 0.93, 0.93, and 0.79, respectively) on the AD side. Correlations between

Table 3–Correlations for stomate traits between abaxial (AB) and adaxial (AD) sides for seedlings of 22 *Pinus albicaulis* families from Crater Lake National Park. Significance at the α = 0.05 and 0.1 levels occurs for correlations with absolute values greater than 0.42 and 0.36, respectively. See the text for a description of traits.

	Adaxial (AD):					
Abaxial (AB):	**nROWS**	**tSTOM**	**LDEN**	**ADEN**	**RDEN**	**NW**
nROWS	0.59	0.49	0.49	0.40	-0.24	0.29
tSTOM	0.50	0.54	0.54	0.54	0.11	0.10
LDEN	0.50	0.54	0.54	0.54	0.11	0.10
ADEN	0.37	0.44	0.44	0.58	0.21	-0.19
RDEN	0.11	0.29	0.30	0.44	0.50	-0.24
NW	0.33	0.20	0.20	-0.19	-0.30	0.89

the AD and AB sides of the needles for these variables were also generally statistically significant, but somewhat lower (r = 0.33, 0.50, 0.50, and 0.37 for nROWS with NW, tSTOM, LDEN, and ADEN, respectively (the first and last correlation being nonsignificant at the 0.05 level) (table 3).

Needle width was moderately correlated with tSTOM and LDEN on the AD side, but not on the AB side (and not with ADEN on either side). Needle width was negatively correlated with height on both the AD side (r = –0.51, –0.54 with HT2 and HT3) and AB side of needles (r = –0.57, –0.59 with HT2 and HT3) but was not significantly correlated with measures of blister rust infection such as nSPOT1, nSS2i and pSS2, except for a moderate, negative correlation with nSPOT1 on the AB side (r = –0.43). There was a negative, but non-significant, correlation (r = –0.29) on the AD side.

Needle length was uncorrelated with any trait, although the correlation with HT3 was nearly significant (r = 0.40, $P = 0.06$). HT2 and HT3 were significantly correlated with nSPOT1 (r = 0.63 and 0.66, respectively) and nSS2i (r = 0.45 and 0.48, respectively), but not with nSS2 or pSS2.

The density of stomata within rows (RDEN) was correlated with nSPOT1 (r = 0.47) for the AD side, but not for the AB side (r = 0.14) (figs. 4 and 5). The number of stem symptoms (both nSS2 and nSS2i) was only moderately correlated with nSPOT1 (r = 0.53 and 0.57, respectively). However, nSS2i was not significantly correlated with pSS2 (r = 0.33).

Family mean correlations between needle traits between the AD and AB side were significant (or nearly significant) for many needle traits (table 3). They were generally highest for the same trait on AD versus AB: NW (0.89), followed by nROWS (0.59), ADEN (0.58), LDEN (0.54), tSTOM (0.54), and RDEN (0.50).

Correlation Between Traits: Parents

Correlations between traits for the 19 parent trees were generally significant and moderate to high. For example, nROWS on the AB side was correlated with NW (0.67), tSTOM (0.97), ADEN (0.96), and RDEN (0.54) on the AB side and also, to a lesser degree, with those traits on the AD side (though not correlated with AD RDEN). Needle width (both AB and AD) also showed moderate-to-high correlation with most measures of stomate frequency (except RDEN).

Correlation Between Traits: Parents versus Progeny

The parent-progeny correlations were significant for the AB but not the AD sides for nROWS (r = 0.70 and 0.44, respectively) (fig. 4). The correlation between parent-offspring for ADEN was also significant for the AB side but not the AD side (r = 0.59 and 0.23, respectively). None of the parental needle traits was correlated with either seedling height or rust infection traits except for ADEN on the AB side with pSS2 (r = 0.48).

East Side Versus West Side

As a group, families from the drier east side (n = 4) differed significantly (at $P = 0.05$) from those on the west side (n = 18) for many traits and nearly so ($P < 0.10$) for several other traits (table 4), particularly for the AD side of the needles. The difference was

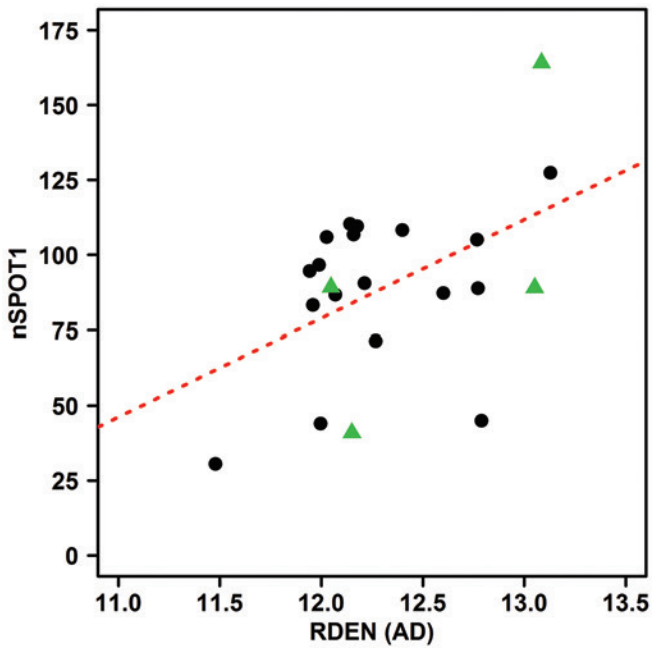

Figure 4—Relationship between 22 family means for stomatal density in rows (RDEN) on the adaxial (AD) side of the needle (x-axis) and the number of needle spots (nSPOT1, y-axis) after inoculation with blister rust spores (r = 0.47, *P* = 0.026). Green triangles represent parents on the east side of CLNP, while black circles represent the west side. The dotted red line shows the least-squares regression line.

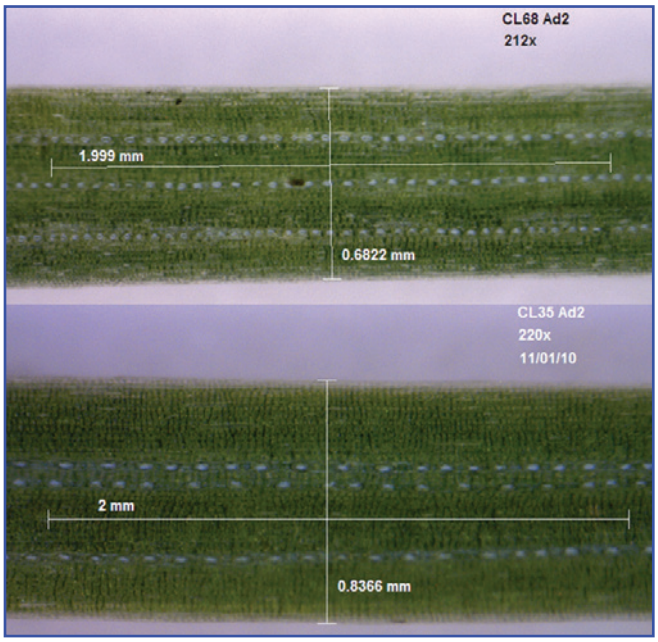

Figure 5—Micrographs of adaxial needles from the families with the highest (top) and lowest (bottom) within row density of stomates (RDEN). Family CL25 (top, 212x) had the highest stomatal row density as well as high spotting after inoculation with blister rust. Family CL02 (bottom, 220x) had the lowest stomatal row density as well as the lowest number of needle spots.

particularly notable for needle length, areal density, and number of stomate rows. The east and west side families did not significantly differ for needle width (table 4).

Narrow-Sense Individual Tree Heritability

Heritability was calculated by using two techniques, one involving variance components and the other relying on parent-offspring regression. Individual tree heritability (h_i^2) calculated from variance components was generally higher for the AD side, ranging from 0.23 to 0.73 (table 5), than for the AB side. Height was also very heritable (h_i^2 = 0.69 and 0.78 for second- and third-year height, respectively), as was the number of stem symptoms at second inspection (h_i^2 = 0.92). The parent-offspring regression resulted in more-equal heritabilities between the AD and AB sides. The mean of all needle trait heritabilities using this method was 0.37, compared to 0.29 with the variance component method.

DISCUSSION

There is large within-population variation for needle traits, seedling height, and measures of rust infection among the 22 seedling families for the CLNP population of whitebark pine. This large genetic variation provided an excellent opportunity to examine the relationships among these traits.

In general, the relationships between needle traits and seedling growth were not significant. An exception was a moderate one for needle width (both AD and AB) and height; taller families tended to have narrower needles. There was also a moderate negative relationship between number of rows of stomata on the abaxial side of needles and height.

In this study of whitebark pine, needle traits were generally not significantly correlated with rust infection traits, with the exception of density of stomata within rows of the adaxial side of needles and number of needle spots (r = 0.47; fig. 5). Bennett et al. (this proceedings) reported a significant correlation between these two traits for both the AD and AB sides for the 2008 needle cohort and a nearly significant correlation for the AD side for the 2009 needle cohort. Thus, families with more stomata per millimeter within a

Table 4–East side means (n = 4 families), west side means (n = 18 families), and p-values for the significance of side of Crater Lake for seedlings of 22 *Pinus albicaulis* families from Crater Lake National Park. See the text for a description of traits.

	Adaxial (AD)			Abaxial (AB)		
	East Mean	West Mean	p-value	East Mean	West Mean	p-value
nROWS	3.45	3.26	0.018	2.14	1.98	0.090
tSTOM	89.9	82.9	0.002	51.8	47.9	0.086
LDEN	43.3	39.9	0.002	25.0	23.0	0.087
ADEN	48.3	45.1	0.003	23.4	21.8	0.084
RDEN	12.6	12.3	0.082	11.0	11.2	0.532
NW	0.90	0.89	0.496	1.06	1.06	0.701
NL	35.7	39.8	0.006	-	-	-
HT2	9.3	10.4	0.054	-	-	-
HT3	12.9	14.9	0.010	-	-	-
nSPOT1	95.8	88.4	0.845	-	-	-
nSS2	9.9	5.3	<.001	-	-	-
nSS2i	10.9	9.1	0.071	-	-	-
pSS2	85.4	55.4	<.001	-	-	-

Table 5–Narrow sense individual tree and parent-offspring heritabilities and standard errors for needle traits, growth, and blister rust infection traits for seedlings of 22 *Pinus albicaulis* families from Crater Lake National Park. See the text for a description of traits.

	Variance Components		Parent-Offspring Regression	
Trait	Heritability	S.E.	Heritability	S.E.
NL	0.18	0.15	0.38	0.20
NW (AB)	0.14	0.13	0.23	0.20
NW(AD)	0.26	0.16	0.51	0.17
tSTOM(AB)	0.03	0.09	0.35	0.12
tSTOM(AD)	0.61	0.25	0.35	0.24
LDEN(AB)	0.03	0.09	0.35	0.12
LDEN(AD)	0.61	0.25	0.35	0.24
ADEN(AB)	0.14	0.12	0.48	0.16
ADEN(AD)	0.63	0.27	0.37	0.37
RDEN(AB)	0.04	0.09	-0.07	0.18
RDEN(AD)	0.23	0.17	0.51	0.21
nROWS(AB)	0.09	0.11	0.51	0.13
nROWS(AD)	0.73	0.29	0.45	0.22
HT2	0.69	0.31	-	-
HT3	0.78	0.34	-	-
HT inc	0.37	0.22	-	-
nSPOT1	0.39	0.24	-	-
nSS2	0.92	0.37	-	-

row tended to have more needle spots, but the number of rows or density of stomata over the needle surface showed no correlation to the level of needle spotting.

At the time of sampling in 2010, the 2008 cohort of needles (which were present at the time of inoculation) was not available for the CLNP population, but there were significant and moderate-to-high correlations between most needle traits for the 2008 and 2009 needle cohorts in the related study (Bennett et al., this proceedings). There was also a significant negative correlation ($r = –0.43$, $P = 0.045$) between needle width (AB side) and number of needle spots in this study, but not in the Bennett et al. (this proceedings) study.

In earlier studies with a different five-needle pine species, western white pine (*P. monticola*) (Gansel 1956; Woo et al. 2001), no significant differences were found for stomate frequency traits (or needle width and needle length) in pairwise comparisons among susceptible and resistance groups, but needles of susceptible families had significantly wider and larger stomata (greater area) than did those of resistant or seed orchard lots (Woo et al. 2001). Size of stomata was not assessed in this study; perhaps some measures of both stomate size and stomate density would help clarify the relationship with the frequency of needle spotting in whitebark pine seedlings.

Number of spots in this study was correlated with the number of stem infections but not with the percentage of seedlings infected in a family, so other traits play an important role in determining which families are most resistant (at least for the canker-free trait). The heritability for number of stem infections was much higher than that of number of needle spots. Field trials will be needed to ascertain the potential merit of reduced needle spotting in some seedling families following artificial inoculation.

There was a significant correlation between second-year seedling height and number of needle spots ($r = 0.63$), with taller families having more needle spots. This relationship may be due to several factors including potentially more needles on taller seedlings, more needles exposed to rust spores (less clumping), or possibly difficulty counting a high number of needle spots on the smallest seedlings. In another study (Bennett et al., this proceedings) involving families sampled across much of the range of whitebark pine, the correlation between these traits was low ($r = 0.21$) and nonsignificant. This relationship will be examined in more detail using the larger number of families (225) in the common garden study and other inoculation trials. It is also notable that although the taller families had more needle spots, they did not tend to have significantly more stem symptoms ($r = 0.24$ for nSS2). Further investigations and field trials may clarify the underlying relationship.

Nearly all whitebark pine seedlings (99.9 percent) developed needle spots in the trial from which the CLNP seedlings were sampled, as well as in other trials at DGRC (Sniezko et al. 2007; Sniezko, unpublished data, on file at DGRC), indicating few escapes at the inoculation stage and little capacity of whitebark pine for a "no spots" trait. However, families varied greatly in the number of needle spots per seedling; some seedlings had more than 600 needle spots. There was a significant family mean correlation between number of needle spots and number of stem symptoms, but not with the percentage of seedlings with early stem symptoms. In this population, the resistances resulting in stem symptom-free seedlings (at 15 months post-inoculation) under heavy inoculum pressure appear to be unrelated to the number of needle spots. Although having fewer needle spots did not result in a lower percentage of seedlings exhibiting stem symptoms in this study, lower levels of spotting have been postulated to play a more significant role in reducing cankering in *P. monticola* in the field (Hoff and McDonald 1980). Field studies are needed to confirm the efficacy of this trait in both *P. monticola* and whitebark pine.

The very large differences among CLNP families in percentage of seedlings showing early stem infection show great promise in identifying parents with resistance to blister rust, but this trait appears to be unrelated to any needle traits examined here. Some families with a low percentage of stem symptoms at this early stage also showed a marked increase in the percentage of stem symptoms over time (R. Sniezko, unpublished data).

Several restoration trials have been established at CLNP since 2009 using many of the seedling families in this study. The data from these field sites will allow for examination of the relationship of genetic variation in these traits in the seedling trial with survival, growth, cone production, blister rust resistance, and other traits in the field over time.

The statistically significant differences between some of the trait means of progeny of east-side versus west-side parents were somewhat surprising and may be due to selection in the different environments (east side being drier) or limitations of the sample set (i.e., sampling of four families from the east side vs. 18 families from the west side of CRLA). Further examination is needed to confirm the trends.

For any additional future efforts to examine needle traits, methods for micrographing and analyzing the needles may need to be reexamined and standardized. Although storage in the –20 °C freezer did not seem

to affect the clarity of stomata, needle conformation could be affected (Wykoff 2002). The twistiness of the needles posed some problems. Several micrographs appeared to be needles with resin channels on the adaxial side; further investigation suggested that most of these needles had a reflective ridge on the needle that looked very similar to a resin channel. Others showed an unusually high number of stomate rows on the AD side. Although this may be an entirely natural event, it is also possible that because the angle between the two AD sides varies, both of the adaxial sides were captured and analyzed in a single micrograph. It is also possible that needle curvature caused part of the abaxial side to be captured as part of an adaxial photograph. Each photograph was examined by at least two people at different times and obvious problems were removed from the analysis. However, it is possible that some of these unusual observations were naturally occurring. In future investigations of needle properties, greater care should be taken to ensure only one side of the needle is being micrographed.

An entire needle typically has hundreds to over 1,000 stomata depending on side. Our subsamples were in the center portion of the needle only. Although there was genetic variation for the 2-mm subsamples, the 2-mm subsample data cannot be used to estimate the total number of stomata on a needle.

Whitebark pine showed large heritable variation among families from the CLNP population. The large genetic variation in whitebark pine needle and white pine blister rust infection traits in the CLNP population is encouraging and may help the species to persist under pressure of at least some biotic and abiotic challenges.

Future studies should consider including other traits such as stomate size. A greater understanding of the genetic variation in whitebark pine adaptive traits and how it varies within and among populations can help guide conservation and restoration strategies.

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