

Genetic Variation in Stomate Densities and Needle Traits in a Rangewide Sampling of Whitebark Pine (*Pinus albicaulis*)

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Abstract—Needle dimensions and stomate densities were measured for 300 seedlings representing a stratified sampling of 30 families of whitebark pine (*Pinus albicaulis*) from California, Oregon, Washington, Idaho, and Montana (USA), and British Columbia (Canada). The seedlings were selected from a larger common garden trial examining white pine blister rust (WPBR) resistance of 225 families from populations representing much of the geographic range of whitebark pine. The objective was to examine variation in needle characteristics, including needle width, number of stomata, and stomate density, and their relation to WPBR infection. Two cohorts of needles were sampled—2008 and 2009—and morphological characteristics on both adaxial (AD) and abaxial (AB) surfaces were measured. Averaged over families and blocks, all traits except needle width were larger for the 2009 cohort, and within a cohort, averages were greater for variables measured on the AD surface relative to the AB surface. Mean stomate densities (ADEN, stomata mm⁻²) ranged from 29.30 to 40.57 on the AD leaf surface and from 13.75 to 22.52 on the AB leaf surface for the 2008 cohort. These values were moderately correlated with stomate densities on the same needle surfaces in the 2009 cohort ($r = 0.543$, $p = 0.002$ and $r = 0.565$, $p = 0.001$ for AD and AB sides, respectively). ADEN for the 2009 needle cohort ranged from 38.77 to 52.22 on the AD surface, and from 18.82 to 29.86 on the AB surface. Family means for needle width (NW) ranged from 0.773 to 1.022 mm for the AD surface, and from 0.886 to 1.197 mm for the AB surface across cohorts. Mean numbers of rows of stomata, stomate abundance, and stomate density by needle length were significantly correlated with NW on the AD surface. Stomate density within rows (RDEN) showed a moderate and significant correlation for the 2009 cohort with number of needle spots resulting from the artificial inoculation with blister rust spores ($r = 0.543$, $p = 0.002$ and $r = 0.438$, $p = 0.015$ for AD and AB, respectively); RDEN was significantly correlated with mean temperature in the warmest month. In addition, the number of stomate rows was negatively correlated with early canker development (number of cankers) as well as growing season and spring precipitation at the seed source. These results suggest that source climate may influence rust symptom development via stomate traits that are under adaptive selection.

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INTRODUCTION

Whitebark pine (WBP, *Pinus albicaulis*) occurs at high-elevation sites across much of western North America but is currently threatened in large portions of its range due to the combined effects of climate change, mountain pine beetle (*Dendroctonus ponderosae*), and white pine blister rust caused by the invasive pathogen *Cronartium ribicola*. This project investigated genetic variation in needle traits of WBP using collections from a seedling common garden trial of families representing much of the geographic range of this forest tree species. Parent tree environmental gradients due to geographic factors such as elevation, latitude, and longitude were considered as possible drivers of morphological diversification. A parallel project underway examined within-population variation in some of the same needle traits in seedling families from Crater Lake National Park in southeast Oregon (Sniezko et al., this proceedings, *Genetic Variation in Needle Traits of Whitebark Pine (Pinus albicaulis) Seedling Families: Within-Population Variation at Crater Lake National Park*; hereafter referred to as *Genetic Variation*).

Stomata mediate the exchange of oxygen, water vapor, and carbon dioxide (CO₂) with the atmosphere. Control of transpiration allows plants to photosynthesize in a wide range of changing environments, and was most likely a driving force behind the ecological diversification of plants (Hetherington and Woodward 2003). Variations in stomate size and density are generally correlated with environmental variables such as atmospheric CO₂ concentration, as well as geographic variables such as elevation (Holland and Richardson 2009; Schoettle and Rochelle 2000). The size and density of stomata influence the rate of conductance of carbon to sites of assimilation within the leaf and regulate many other ecophysiological functions of leaf surfaces (Franks and Beerling 2009). Stomate morphology may also play a significant role in interactions with pathogens and pests. Natural openings in leaf surfaces such as stomata are important entry points for many fungal pathogens, including *C. ribicola* (Patton and Johnson 1970). Consequently, plants have evolved to regulate the stomate opening as an important part of a general response to microbial invasion (Gudesblat et al. 2009; Zeng et al. 2010).

Because many WBP populations face the imminent threat of damage by *C. ribicola*, variations in needle morphology in relation to blister rust infection are being studied together with other possible indicators of blister rust resistance. Investigation of natural variation in needle characteristics such as stomate abundance and arrangement may provide some key insights into factors influencing susceptibility to the blister rust pathogen and its geographic variation.

METHODS

Sample Selection and Inoculations

A subset of 30 families was selected from a common garden inoculation study examining resistance to white pine blister rust in 225 seedling families from much of the range of WBP. Parent trees of the seedlings examined here represent eight seed zones from throughout Oregon and Washington as well as individual populations from Idaho, Montana, California, Nevada, and British Columbia (fig. 1). The seeds were sown in 2007 (see Hamlin et al. 2011 for background on seedling culture) in 10-in³ (164-cm³) containers, which were then placed into a randomized complete block (RCB) design before inoculation with *C. ribicola*. 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; different geographic sources of rust were used for each inoculation. Infected *Ribes* leaves were placed on wire screens atop the WBP seedlings to allow basidiospore release. Basidiospore discharge was monitored on trap slides to ensure that all trees received similar inoculum exposures. Average spore densities were 3,359 and 6,013 cm⁻² for the first and second inoculations, respectively. 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).

In March 2010, needles from both the 2008 and the 2009 cohorts were collected from 2 seedlings per family per block in all 6 blocks; however, samples from only 5 of the 6 blocks were photographed and analyzed (300 total seedlings). Assessments of number of needle spots, number of stem infections (including zeros), and

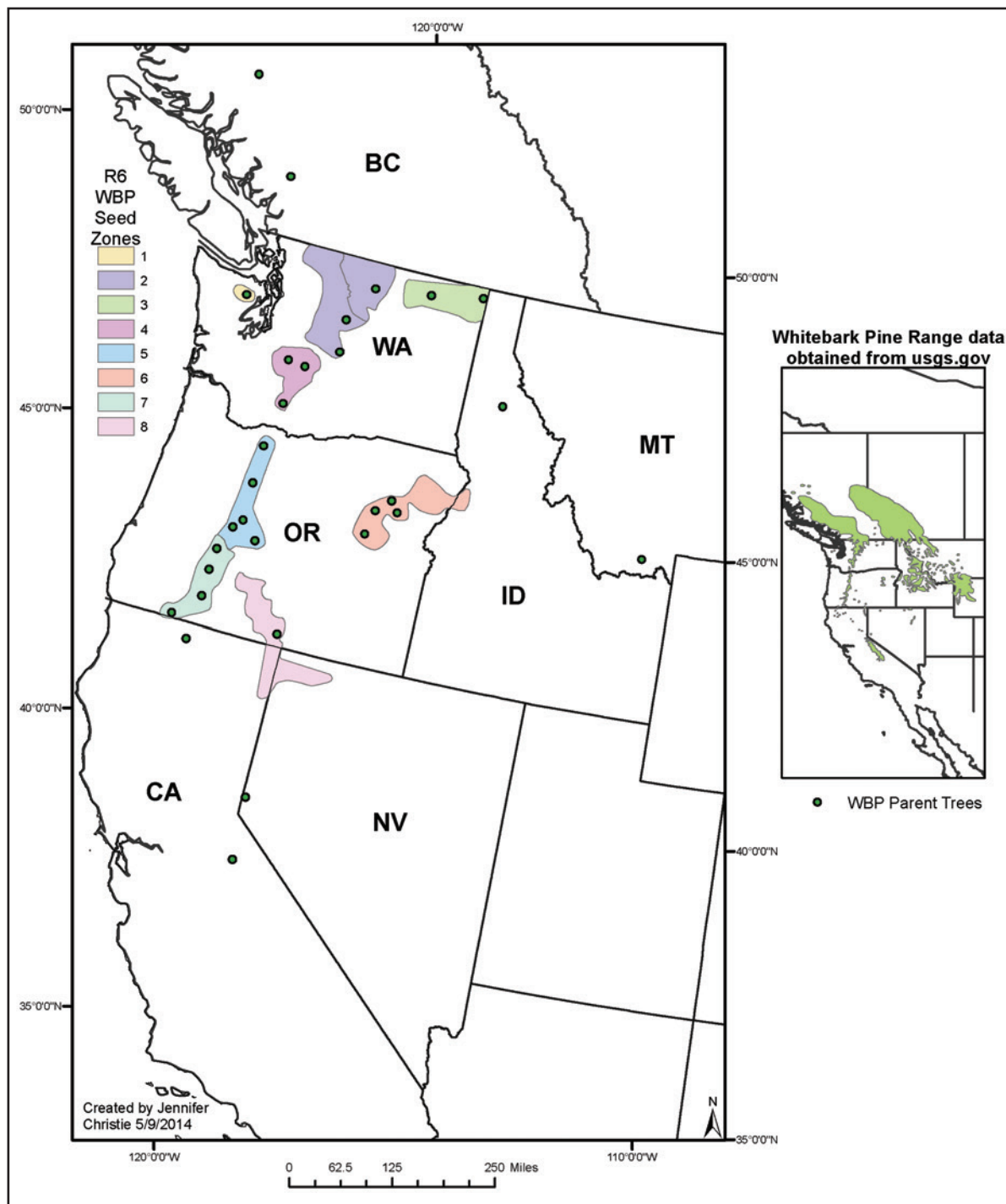


Figure 1—Map of parent tree locations and seed zone designations used in this study.

seedling mortality were made in late July–early August 2009 (inspection 1) and November–December 2009 (inspection 2) for all seedlings, including the sampled ones. Additional assessments of stem infection and mortality were made annually 2010–2013 but were not used in these analyses.

Needle Measurements and Statistical Analyses

Needles were attached to 3-inch × 5-inch (7.6-cm × 12.7-cm) index cards with double-sided tape and photographed with a Dino-Lite digital USB camera (AnMo Electronics Corp., Taipei, Taiwan). Needle

dimensions and stomate densities were measured for 300 sampled seedlings (30 families × 10 seedlings per family) for both the 2008 and 2009 needle cohorts. For each seedling, four photographs were taken from both the abaxial (AB) and adaxial (AD) needle surfaces (eight photos total) at a magnification of 200× (fig. 2a). The abaxial side is the rounded outer surface of a needle, and it typically has two resin channels running alongside the stomate rows (fig. 2b). An individual needle has two adaxial surfaces; these are the inner sides of a needle (fig. 2b). Different needles were photographed for the AB and AD sides, and two to four needles were used for each seedling to obtain the photographs. An approximately 2-mm length of needle was used for measurements; the 2-mm segments were randomly chosen from each needle surface for quantification of stomata and rows of stomata (fig. 2). Needle width was measured in magnified images using the line measure tool in the Dino-Lite software (fig. 2a). Individual seedling means were calculated by averaging these measures for each seedling, and analyses of variance (ANOVAs) and linear regressions of needle attributes were performed on these means (accounting for block differences) using R (R Core Team 2016). Family means were calculated from the mean of block means, and pairwise correlations were performed by using these means and SAS® software (version 9.4, SAS Institute Inc., Cary, North Carolina) with the CORR procedure.

A sequence of analyses was conducted to explore if stomate features mediate the influence of source climate of the seed (maternal) parent on early post-infection rust symptom development. Potential significant predictors and groups of intercorrelated predictors were explored by using 10-fold cross validation techniques with lasso and elasticnet (Friedman et al. 2008) in the glmnet package (Friedman et al. 2010) in R (R Core Team 2016). By using these penalized regression techniques, three sets of family mean stomate data (variables averaged across 2008 and 2009 needles, and those data for each year singly) were independently analyzed. Each dataset was analyzed in a two-part sequence to (1) identify stomate variables that significantly predicted disease development, and (2) identify seed source climate variables that explained variation in the stomate variables identified in step 1. Each step generated linear models to predict disease response from stomate traits and predict stomate traits from

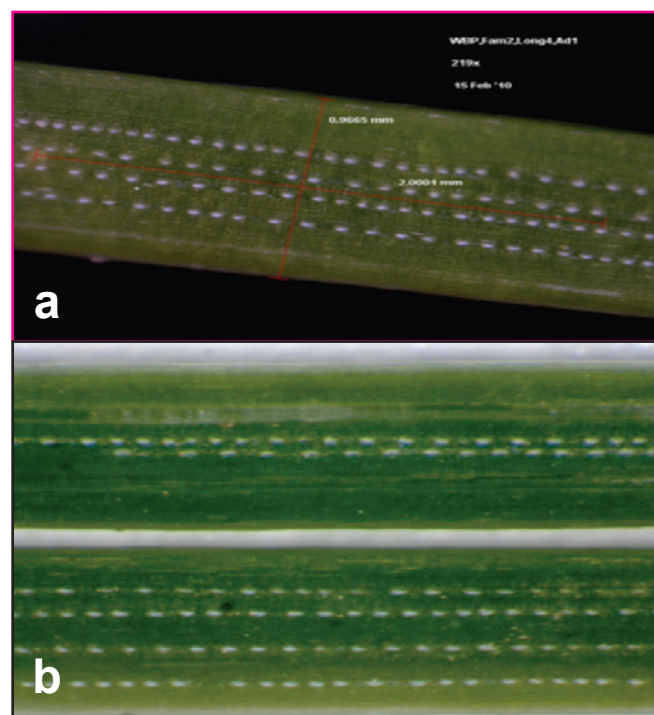


Figure 2—Magnified images of leaf surfaces: (a) width and length measurements shown on photograph of adaxial leaf surface; (b) abaxial (top) and adaxial (bottom) leaf surfaces.

source climatic variables. Normalized (1961–1990) means of derived climate variables (table S1) were estimated for each source location by thin plate spline surfaces (Rehfeldt 2006).

RESULTS

Family (Geographic) Variation in Needle Morphologies

Numbers of Stomata, Stomate Density, Numbers of Rows of Stomata

Families differed significantly for mean number of stomata (nSTOM) on the AD and AB leaf surfaces (ANOVA, $P < 0.0001$ for both sides). Although nSTOM for the 2008 and 2009 cohorts differed significantly (ANOVA, $P < 0.0001$), they were highly correlated with each other. Family mean nSTOM ranged from 46.75 to 73.73 on the AD surface and from 25.25 to 46.58 on the AB surface in the 2008 cohort; means for the 2009 cohort ranged from 70.00 to 98.65 and 39.88 to 66.01 on the AD and AB surfaces, respectively (table 1). The coefficient of variation for nSTOM on the AD surface was 10.0 and 8.3 percent

Table S1—Climate variables and their ranges for seed sources used in this study using the model by Rehfeldt (2006).

Climate variable	Definition	Range among source locations	
		max	min
mat	Mean annual temperature degrees C	5.4	-1.2
map	Mean annual precipitation	3320	497
gsp	Growing season precipitation, April to September	837	188
mtcm	Mean temperature in the coldest month degrees C	-1.2	-11.8
mmin	Mean minimum temperature in the coldest month degrees C	-4.9	-18.1
mtwm	Mean temperature in the warmest month degrees C	14.3	8.8
mmax	Mean maximum temperature in the warmest month degrees C	23	13.6
sday	Julian date of the last freezing date of spring	198	172
fday	Julian date of the first freezing date of autumn	260	222
ffp	Length of the frost-free period	87	22
dd5	Degree-days >5 degrees C (based on mean monthly temperature)	1142	370
gsdd5	Degree-days >5 degrees C accumulating within the frost-free period	622	118
d100	Julian date the sum of degree-days >5 degrees C reaches 100	190	143
dd0	Degree-days <0 degrees C (based on mean monthly temperature)	1776	394
mmindd0	Degree-days <0 degrees C (based on mean minimum monthly temperature)	3450	1162
smrpb	Summer precipitation balance (jul+aug+sep)/(apr+may+jun)	1.03	0.45
smrsprpb	Summer/Spring precipitation balance (jul+aug)/(apr+may)	1.2	0.27
sprp	Spring precipitation (apr+may)	386	89
smrp	Summer precipitation (jul+aug)	168	36
winp	Winter precipitation (nov+dec+jan+feb)	1806	205

for the 2008 and 2009 needle cohorts, and 15.4 and 11.4 percent for AB surfaces in the 2008 and 2009 cohorts, respectively. The total number of stomata on the AD surface of the needles from each of the 2008 and 2009 cohorts was strongly correlated with needle width for that year (table 2), and similar but weaker trends were observed for the AB surface. On average, there were fewer stomata per unit length of row (RDEN) on the AB surface (10.08 and 11.81 for 2008 and 2009, respectively) than on the AD surface (10.84 and 12.33 for 2008 and 2009, respectively) (table 1).

Family mean number of rows of stomata (nROWS) varied from 2.31 to 3.48, and from 1.31 to 2.14 on the AD and AB surfaces, respectively, in the 2008 cohort; family mean nROWS for the 2009 cohort ranged from 2.98 to 3.80 and 1.73 to 2.78 for the AD and AB surfaces, respectively (table 1); there were 1.60 to 1.69 times as many nROWS on the AD side relative to the AB side for the 2009 and 2008 cohorts, respectively. The number of rows on the AD surface for the 2008 and 2009 cohorts was significantly correlated with

NW of both the AD and AB surfaces for the respective cohorts (table 2). There was also a moderate correlation between the nROWS on the AB surface in the 2008 cohort and RDEN on the AD surface of the 2008 cohort ($r = 0.374$, $P = 0.042$). nSTOM was generally highly correlated with nROWS on the corresponding needle surface within the same needle cohort; for example, nSTOM for AD surfaces in the 2009 cohort was highly correlated with nROWS for AD surfaces in the 2009 cohort ($r = 0.879$, $P < 0.0001$).

Stomate density (ADEN) varied from 36.18 stomata mm⁻² (range of family means: 29.30–40.57) to 44.99 (38.77–52.22) on the AD surfaces, and from 17.12 (13.75–22.52) to 22.91 (18.82–29.86) on the AB needle surfaces in the 2008 and 2009 needle cohorts, respectively (table 1). There was a positive correlation between AD and AB ADEN in the 2009 cohort ($r = 0.414$, $P = 0.023$) but not in the 2008 cohort ($r = 0.287$, $P = 0.12$). Values of ADEN were positively correlated between the 2008 and 2009 cohorts for both needle surfaces ($r = 0.543$, $P = 0.002$ and $r = 0.565$,

Table 1—Family means, minimum, and maximum values for needle characteristics and disease assessments.

Trait	2008 Needle cohort										2009 Needle cohort									
	Adaxial					Abaxial					Adaxial					Abaxial				
	Mean	Min	Max	P-val	Mean	Min	Max	P-val	Mean	Min	Max	P-val	Mean	Min	Max	P-val	Mean	Min	Max	P-val
nROWS	2.95	2.31	3.48	0.0034	1.75	1.31	2.14	0.0282	3.35	2.98	3.80	0.0195	2.09	1.73	2.78	0.0091				
nSTOM	63.34	46.75	73.73	<0.0001	35.01	25.25	46.58	0.0002	82.37	70.00	98.65	0.0009	49.05	39.88	66.01	0.0019				
ADEN	36.18	29.30	40.57	0.0094	17.12	13.75	22.52	<0.0001	44.99	38.77	52.22	0.0056	22.91	18.82	29.86	0.0002				
RDEN	10.84	9.97	11.66	0.0042	10.08	9.02	11.39	0.001	12.33	11.34	13.22	<0.0001	11.81	11.00	12.87	<0.0001				
NW	0.877	0.773	0.977	<0.0001	1.019	0.886	1.136	<0.0001	0.917	0.847	1.022	0.0011	1.073	0.978	1.197	0.0129				
HT2	15.88	10.35	21.25	<0.0001																
Spots1	106.1	58.8	154.5	0.0053																
Cank1	2.28	0.1	6.5	<0.0001																
Cank2	8.79	1.1	16.7	<0.0001																

nROWS = number of rows of stomata, nSTOM = total number of stomata in 2-mm view, ADEN = stomata mm², RDEN = (nSTOM/ 2mm)/ nROWS, LDEN = stomata mm⁻¹ of needle, NW = Needle width (mm), HT2 = seedling height at second inspection (cm), Spots1 = number of needle spots attributed to blister rust infection at first inspection after inoculation, Cank1 = number of blister rust cankers at first inspection after inoculation, Cank2 = number of stem symptoms at second inspection after inoculation = number of normal cankers (NC) + number of partial bark reactions (PBR) + number of bark reactions (BR). Spots1, Cank1, and Cank2 P-values are based on natural log transformed variables.

Table 2—Correlation table for needle morphological characteristics related to needle width.

Characteristic	NW_AD_08		NW_AD_09		NW_AB_08		NW_AB_09	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
nROWS_AD_08	0.718	<.0001	0.281	0.133	0.643	0.0001	0.372	0.043
nROWS_AD_09	0.100	0.599	0.568	0.001	0.173	0.361	0.603	0.0004
nROWS_AB_08	0.466	0.009	0.203	0.282	0.423	0.020	0.187	0.324
nROWS_AB_09	-0.251	0.181	0.063	0.740	-0.233	0.216	0.049	0.798
nSTOM_AD_08	0.708	<.0001	0.386	0.035	0.666	<.0001	0.482	0.007
nSTOM_AD_09	0.304	0.102	0.555	0.001	0.347	0.060	0.604	0.0004
nSTOM_AB_08	0.417	0.022	0.207	0.273	0.395	0.031	0.189	0.316
nSTOM_AB_09	-0.100	0.598	0.151	0.424	-0.098	0.608	0.125	0.511

r = Pearson correlation coefficient, *P* = *P*-value, **nROWS** = number of rows of stomata, **nSTOM** = number of stomata, **NW** = Needle width (mm), **AD** = adaxial needle surface, **AB** = abaxial needle surface, **08** = 2008 needle cohort, **09** = 2009 needle cohort.

P = 0.001 for AD and AB sides, respectively). ADEN did not correlate significantly with NW but was correlated with nROWS on the same needle surface within the same cohort. For instance, ADEN on the AD needle surface in the 2009 cohort correlated with nROWS on the AD needle surface in the 2009 cohort (*r* = 0.674, *P* < 0.0001), but not the AD or AB surfaces in the 2008 cohort.

Needle Width

The width of AD and AB surfaces of needles (NW) from both cohorts varied significantly between families across the range of whitebark pines sampled for this analysis (ANOVA): *P* = 0.0011 (AD 2009), 0.0129 (AB 2009), <.0001 (both AD and AB 2008) (table 1). The family means for NW on the AD surfaces averaged over the 2008 and 2009 cohorts was 0.897 mm; the NW for the AB surfaces averaged over the 2008 and 2009 cohorts was 1.046 mm. The coefficient of variation for NW was similar in magnitude within a cohort: 6.4 and 4.6 percent on the AD surfaces and 6.7 and 4.2 percent on the AB surfaces for 2008 and 2009, respectively.

Although the mean NW of the AB side was greater than that of the AD sides by a factor of 1.166 (due to its curvature), there were fewer stomata, as well as fewer rows of stomata on the AB side (1.92 rows AB vs. 3.15 rows AD). nSTOM and nROWS were correlated with NW on the AD and AB surfaces in the

corresponding needle cohorts, except for nSTOM and nROWS for the AB side in the 2009 needle cohort (table 2).

Disease and Climate Correlations

Stomate traits were correlated with disease symptom traits (table 3). Averaged over 2008 and 2009, the number of spots present on the entire seedling at the first inspection (Spots1) was predicted by RDEN on both the AD and AB sides (table 3). Although the 2009 stomate traits showed similar relationships to the yearly averaged data, stomate features of 2008 needles lacked the same level of predictive power (*P* = 0.072 and *P* = 0.0019, for RDEN 2008 and AD 2009, respectively). Cank1 and Cank2 were predicted by nROWS (AD) (negative correlation) for the yearly averaged stomate trait and the 2008 needle cohort, but there were no significant correlations when traits for 2009 needles were analyzed separately. In summary, RDEN (AD), RDEN (AB), and nROWS (AD) predicted disease symptoms traits and were used going forward for the climate variable analyses.

Elasticnet identified three groups of correlated seed source climate variables that correlated with RDEN (AD) and RDEN (AB) from the yearly averaged stomate data (table 4). The climate variables within a group were highly intercorrelated, and univariate analyses and LASSO were used to prioritize the variable with the greatest predictive power in each

Table 3—Correlation table for needle morphological characteristics related to disease symptoms.

Disease symptom	Ave. stomate traits		2008 Stomate traits		2009 Stomate traits	
	Stomate traits that predict disease symptom trait		RDEN(AD)**	RDEN(AD)*	RDEN(AD)***	RDEN(AB)**
Spots1						
	Model		$Y = (27.35)RDEN(AD) - 210.76$		$Y = (29.76)RDEN(AD) - 260.96$	
	P-value	0.010	$Y = (19.78)RDEN(AB) - 110.39$		$Y = (20.30)RDEN(AB) - 133.64$	
		0.031			0.0019	
					0.015	
Cank1	Stomate traits that predict disease symptom trait		nROWS(AD)*	nROWS(AD)**	--	
	Model			$Y = (-2.68)nROWS(AD) + 10.19$		
	P-value			0.011		
Cank2	Stomate traits that predict disease symptom trait		nROWS(AD)**	nROWS(AD)*	--	
	Model		$Y = (-7.82)nROWS(AD) + 33.43$			
	P-value	0.044				

P-value for univariate analysis: * for <0.10, ** for <0.05, *** for <0.01, **** for <0.001

Table 4—Correlation table for needle morphological characteristics related to climate variables. See table S1 for climate variable definitions.

Stomatal trait		Ave. stomate trait	2008 stomate trait	2009 stomate trait
RDEN(AB)	Climatic variables that predict stomate trait	mtwm*, dd5*	--	mtwm**, mmax**
	Model			$Y = (0.19)mtwm + 9.46$
	<i>P</i> -value			0.030
RDEN(AD)	Climatic variables that predict stomate trait	mtwm**, dd5**, gsdd5**	mat*, mtwm**, dd5**, gsdd5**, d100**	mtwm**, dd5*, gsdd5*
	Model	$Y = (0.28)mtwm + (1.51)smrpb + 7.35$	$Y = (0.27)mtwm + (1.30)smrpb + 6.81$	$Y = (0.28)mtwm + (1.72)smrpb + 7.90$
	<i>P</i> -value	0.006	0.008	0.012
nROWS(AD)	Climatic variables that predict stomate trait	sprp**, gsp**	gsp*, sprp**	sprp*
	Model	$Y = (0.0012)sprp + 2.94$	$Y = (0.0014)sprp + 2.71$	
	<i>P</i> -value	0.023	0.048	

P-value for univariate analysis: * for <0.10, ** for <0.05, *** for <0.01, **** for <0.001

group. The first group that correlated with the yearly averaged RDEN (AD) and RDEN (AB) included mean temperature in the warmest month (mtwm), degree-days >5 °C (dd5), and degree-days >5 °C accumulating within the frost-free period (gsdd5), each of which was a significant predictor of RDEN (AD) in univariate analyses, with mtwm providing the best fit. The second group included summer precipitation balance (smrpb) and summer/spring precipitation balance (smrsprpb), which were not significant predictors, but did significantly improve the model fit when combined with mtwm (Akaike's information criterion was used as the criterion). The third group of correlated predictors, Julian date of the last freezing date of spring (sday) and length of the frost-free period (ffp), were not significant singly and did not improve the overall fit when modeled with the previous variables. RDEN (AB) for 2009 was best predicted by mtwm alone with no improvement in fit with additional predictors. Elasticnet identified one group of climate variables, growing season precipitation (gsp) and spring precipitation (sprp), which correlated with the yearly averaged nROWS (AD), providing the best fit

with sprp. nROWS (AD) for 2008 and 2009 singly showed mixed results; gsp and sprp were only weakly correlated with 2009 nROWS (AD), and sprp provided the best univariate model for 2008 nROWS (AD).

DISCUSSION

One of the main objectives of this study was to describe the genetic variation in needle morphology for seedling families from parent trees representing much of the range of *Pinus albicaulis*. Needle traits correlated well between the 2008 and 2009 cohorts, and in most cases, some factors such as needle width were significantly associated with measures of stomate abundance (table 2). Family means for all traits—stomate counts, stomate densities, and needle dimensions—for the 2009 cohort were larger than those of the 2008 cohort (table 1). A study examining within-population genetic variation for the same morphological traits (2009 needle cohort) from this same common garden trial showed similar means and ranges for 22 families from Crater Lake National Park (Sniezko et al., this proceedings, *Genetic Variation*).

Needle morphology may be of interest when considering factors that influence disease infection. Spores of the nonnative blister rust pathogen, *C. ribicola*, are deposited on needle surfaces and typically enter needles through the stomata. Morphological factors, such as stomate density or stomate size, might affect the probability of successful pathogen infection and establishment. In the case of western white pine (*P. monticola*), several factors that contributed to reduced needle lesion frequency included stomate size and shape, as well as needle surface characteristics such as cuticle chemistry (Woo et al. 2001). In the current study disease incidence and severity were represented by number of needle spots and cankers observed in approximately the first year after inoculation. There was a significant positive correlation between RDEN and the number of needle spots at the first inspection after inoculation, suggesting that RDEN may have some influence on the successful entry of the pathogen into foliage, at least for WBP. In an examination of within-population genetic variation, the relationship between RDEN and needle spots was also significant and positive within the Crater Lake National Park population (Sniezko et al., this proceedings, *Genetic Variation*). However, RDEN was not significantly associated with the number of cankers at either inspection, so RDEN does not appear to be related to post-infection disease development.

The number of stomate rows was negatively correlated with the number of cankers that developed on seedlings. This result might be counterintuitive, as one might expect greater numbers of stem symptoms associated with more rows (and in general, greater numbers) of stomata, providing greater potential frequency of infection. However, this study did not include measures of stomate size and activity, which may also help regulate entry of the fungus into the stem or other means of resistance that may be expressed in the needles or at the interface between the short shoot and the stem. Stomate features may only indirectly affect post-infection disease development, and this may be mediated through other associated physiological or biochemical processes.

Another objective of this study was to examine the extent to which geographical or environmental variables affecting the seed (mother) tree in the wild result in stomate differences in a common garden. Climatic

features such as vapor pressure deficit vary along latitudinal and elevational gradients and are expected to influence needle characteristics such as stomate density due to the varying degrees to which trees must regulate water loss (Letts et al. 2009). In a study of needle morphology in *P. flexilis*, a significant decrease in stomate density was observed with increasing elevation, most likely in response to the relatively xeric conditions at higher elevations (Schoettle and Rochelle 2000). Although variables such as elevation and latitude have previously been identified as potential drivers of variation in stomate abundance and needle morphology in conifers, the variation in the abundance and density of stomata across the families in this study could not be explained by any combination of geographic variables. This is not to imply that climatic features do not have a role to play; these same stomate traits may be under adaptive selection. Temperature in the summer at the seed source predicted stomate densities per row, and in some cases this prediction was improved with the addition of a summer precipitation variable. Likewise, spring precipitation at the seed source predicted the number of stomate rows on the adaxial needle surface. This suggests that these stomate traits may be adaptations to both summer temperature and spring and summer precipitation patterns. Temperature appears to correlate with the number of stomata, and spring or summer precipitation with the arrangement of stomata on the needle (i.e., number of stomate rows).

Although these traits may be adaptive in the climates under which they evolved, they may also influence susceptibility to WPBR. If increased summer temperatures under climate change select for genotypes with increased RDEN (AD) or RDEN (AB), they may also increase the infection efficiency of the rust on those seed sources. Likewise, if the spring precipitation decreases, and plants respond with reduced numbers of stomate rows on the adaxial needle surfaces, there may be an associated increase in successful canker development following infection. These results suggest that stomate traits predict early WPBR disease symptoms and that under adaptive selection, seed source climatic factors explain some of the variation in those stomate traits observed under common garden conditions. Consequently, though speculative with this dataset, it appears possible that climate change-driven selection for stomate traits could affect early rust symptom

development. In addition, there have most likely been blister rust-driven selection pressures on some of the populations in this study.

Family variation in needle traits was large across the geographic sources included in this study, but it was also large in a within-population study (Sniezko et al., this proceedings, *Genetic Variation*). Thus, each population may have adaptive capacity in these traits to mitigate some of the impacts of biotic and abiotic influences. Field plantings at Crater Lake National Park of some of the families from the within-population study may provide a better understanding of the relationship of needle traits to long-term survival under a changing climate and the presence of the nonnative blister rust. A better understanding of the tolerance and resistance traits present in natural populations is essential to avoid the collapse of ecologically important five-needle pine ecosystems in western North America. For ecologically important, wild-growing pine species such as WBP, the introduction and maintenance of quantitative disease resistance (Sniezko et al. 2014) in natural ecosystems with plants developed through selective breeding will help ensure the long-term survival of these species (Schoettle and Sniezko 2007). The successful introduction and regeneration of native forest stands with resistant planting stock will depend on a comprehensive knowledge of tree morphological traits and their adaptive significance, their underlying genetic controls, and their roles in influencing pathogen invasion, survival, and proliferation.

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