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RESEARCH ARTICLE

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Disease resistance in whitebark pine and potential for restoration of a threatened species

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Societal impact statement

Forests world-wide are being negatively affected by non-native, invasive pathogens and pests, and some tree species face uncertain futures. To retain these species as components of future forests, the rare genetic resistance that exists needs to be identified and harnessed. The applied tree improvement program for whitebark pine (*Pinus albicaulis*), a threatened (in the United States) and endangered (in Canada) keystone species in many forests in western North America, provides an example of what can be accomplished in a relatively short timeframe. The level and frequency of resistance vary by location, and this information will be used to implement the national restoration plan.

Summary

- Forest trees face serious threats from non-native diseases and pests, often causing high mortality of both the existing trees and regeneration. Developing populations with genetic resistance can help restore forests and retain affected species. Resistance programs have historically focused on species of high economic importance; however, the threats to species of little direct economic value that provide other important ecosystem services are also great.
- We examined the frequency, level, and geographic variation in genetic resistance to white pine blister rust in the threatened *Pinus albicaulis* (whitebark pine), a keystone species in high-elevation ecosystems in western North America. In the two trials reported here, 2-year-old seedling progeny of 225 whitebark pine parent trees were inoculated with two geographic sources of the fungal pathogen *Cronartium ribicola* and evaluated over 5 years for an array of resistance traits. The trials focused primarily on parent trees from the Oregon and Washington populations.
- We found unexpectedly high levels of quantitative resistance in some seedling families and populations, in stark contrast to levels observed in similar resistance programs with other North American white pine species such as *Pinus monticola* and *Pinus lambertiana*.
- The level of resistance found in some whitebark pine populations provides optimism about potential recovery efforts for this species. Restoration efforts are underway by government agencies, tribes, and non-government organizations in

both the United States and Canada. These efforts may help boost support for applied genetic resistance programs in other forest tree species severely affected by non-native pathogens or pests.

KEYWORDS
Cronartium ribicola, endangered species, *Pinus albicaulis*, quantitative resistance, restoration, threatened species, white pine blister rust, whitebark pine

1 | INTRODUCTION

Pinus albicaulis Engelm. (whitebark pine) is a long-lived keystone and foundation conifer that occurs in high-elevation forests throughout the western United States and Canada (Tomback et al., 2022) (Figure 1, inset). It is one of 9 five-needle white pine species in the subgenus *Strobus* present in the United States and Canada. All North American species of five-needle pines are highly susceptible to white pine blister rust (WPBR), caused by the non-native, invasive pathogen *Cronartium ribicola* J.C. Fisch. in Rabh. (Hoff et al., 1980; Tomback & Achuff, 2010). Because of multiple threats, including high mortality from WPBR, predation from mountain pine beetles (*Dendroctonous ponderosae* Hopkins), changes in fire regimes, and the changing climate (Tomback et al., 2022), *P. albicaulis* was listed as endangered in Canada under the Species at Risk Act (SARA) in 2012 (Government of Canada, 2012). In January 2023, *P. albicaulis* was listed as a threatened species in the United States under the Endangered Species Act (ESA) (U.S. Fish & Wildlife Service, 2022). *P. albicaulis* is also listed as endangered on the IUCN Red List (Mahalovich & Stritch, 2013). In the United States, a national plan for the restoration of whitebark pine has been proposed (Tomback & Sprague, 2022). Identifying trees with genetic resistance to WPBR is vital to the success of restoration (Schwandt, 2006) to keep white pine species as functioning elements of both natural and managed forests.

WPBR is one of the major disease epidemics of forest trees in North America (Kinloch, 2003). WPBR has been present in western North America for over 100 years and is now a permanent resident of many white pine ecosystems (Geils et al., 2010; Kinloch, 2003). In the areas of highest hazard, 70%–100% of trees can be infected and killed by WPBR (Kinloch, 2003; Shepherd et al., 2018; Smith et al., 2012; Sniezko et al., 2020). *C. ribicola* is an obligate, biotrophic pathogen that requires both a telial host (most notably *Ribes* species in North America) and an aecial host (white pine species such as *P. albicaulis*). Infection in the pines occurs through the stomata of needles from basidiospores dispersed from the telial host (Geils et al., 2010). In seedling inoculation trials, infection in the form of needle lesions or "spots" is often visible, 3–10 months following inoculation. If infection is not halted in the needles, the fungus will grow into the stem, where one or more cankers may develop (Geils et al., 2010). Both the needles and stems of the pines are potential areas where resistance responses restricting the progress of the pathogen may occur. In the stem, this may be apparent by slower-growing cankers or partial or complete bark reactions (Hoff, 1986, 1992). Fortunately, native white pine species have some genetic resistance (Hoff & McDonald, 1980a; Kinloch et al., 1970; Sniezko et al., 2014; Sniezko & Liu, 2022), but in most cases the underlying inheritance, mechanisms, and frequency of resistance within and among populations are still undetermined.

Initial investigations of *P. albicaulis* provided some optimism concerning the potential to select for WPBR resistance in this species (Hoff et al., 2001). However, only recently have much more extensive WPBR resistance screening trials of seedling families from selected parent trees been undertaken to examine the frequency and level of resistance present in the species with a goal of developing populations of trees to use in restoration (Sniezko et al., 2007, 2008, 2011; Sniezko & Liu, 2022). Natural regeneration and restoration plantings rely on seed from wind-pollinated parent trees. Knowing the types and frequency of resistance within these progenies will allow managers to have a first estimate of the potential level of survival in the presence of the pathogen. However, ultimately, as with *Pinus monticola* Douglas ex D. Don and *Pinus lambertiana* Dougl., the establishment of field trials (Sniezko et al., 2020) and monitoring of restoration plantings will provide validation of the efficacy of seedling inoculation trials to estimate the expected level of survival.

Most detailed examinations to date of WPBR resistance have involved *P. monticola* or *P. lambertiana*. Those studies have identified both qualitative resistance, notably major gene resistance (MGR), and quantitative resistance (QR) (Sniezko et al., 2014, 2020). With MGR, families often segregate into 1:1 Mendelian ratios for stem symptom:stem symptom-free and have a moderate to high survival rate (Kinloch et al., 1970, 1999; Schoettle et al., 2014; Sniezko et al., 2016). In contrast, families with QR display a more continuous distribution of phenotypes. QR might include a gradient from trees with no stem symptoms all the way to trees with many normal cankers (Johnson & Sniezko, 2021). On balance, some trees may develop latent stem symptoms, bark reactions, fewer needle spots, fewer stem symptoms, or a combination of these phenotypes, resulting in improved overall survival relative to the most susceptible families (Johnson & Sniezko, 2021; Sniezko et al., 2008, 2014, 2020). Virulent strains of the pathogen to MGR have already been documented in two white pine species (Kinloch et al., 2004), but QR is expected to be more durable and thus important for restoration (Johnson & Sniezko, 2021; Sniezko et al., 2020; Sniezko & Liu, 2021).

The primary objective of this study was to provide the first extensive assay of genetic variation in *P. albicaulis* in resistance to WPBR. We used two screening trials, including progeny of 225 *P. albicaulis* parent trees; each trial was inoculated with a different geographic

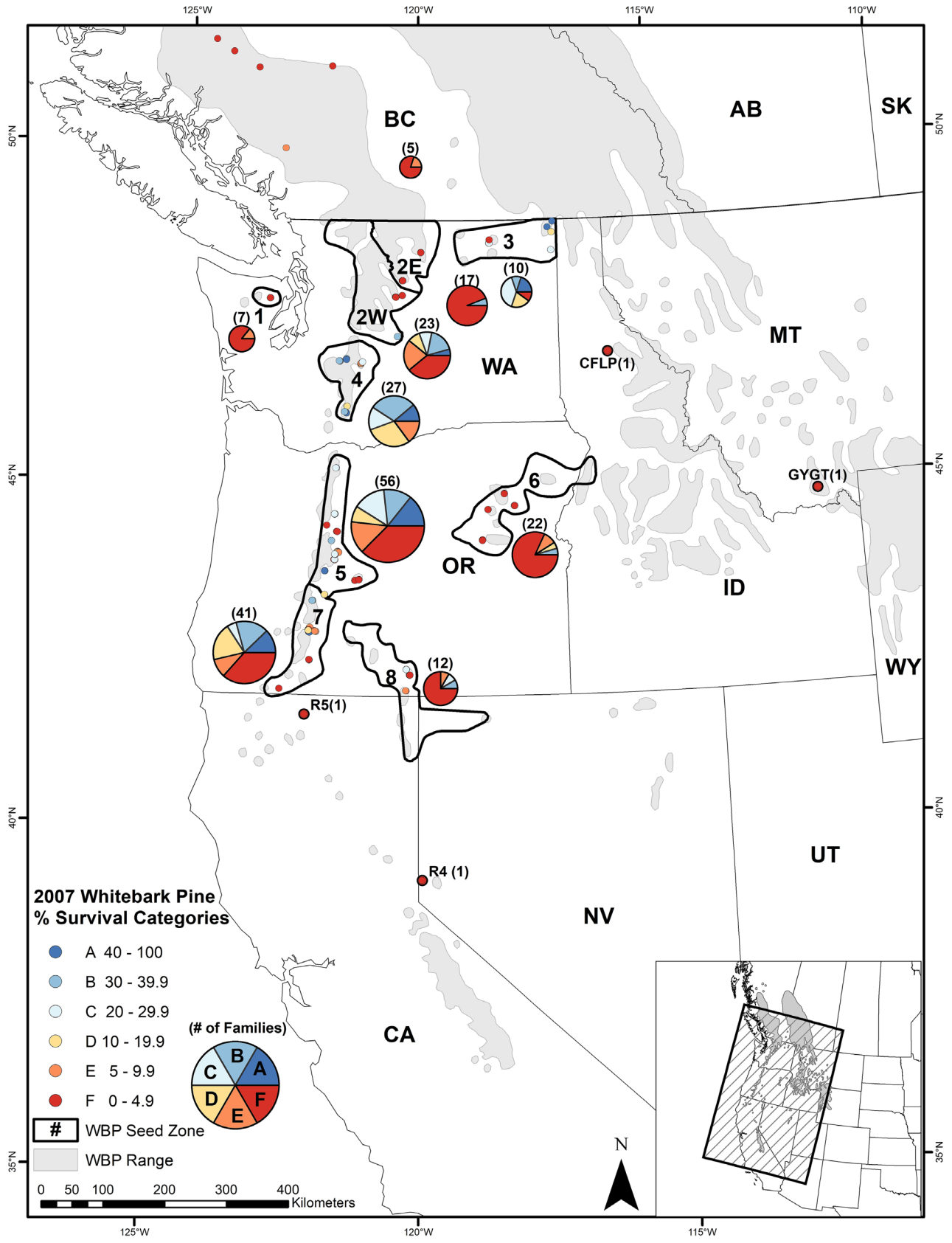

FIGURE 1 Geographic variation in genetic resistance in 225 whitebark pine (WBP) seedling families inoculated with the white pine blister rust pathogen (*Cronartium ribicola*). Each piechart shows the frequency and level of survival (averaged over both trials), 64 months post-inoculation, of whitebark pine seedling families in the nine seed zones designated for Oregon and Washington and for several families from British Columbia, Idaho/Montana, and California/Nevada. The number of seedling families tested for each area is indicated in parentheses.

source of the pathogen (Table 1) to characterize the various phenotypic components of resistance and to provide an initial look at geographic variation in resistance, quantifying the range and level of resistance among families and seed zones and providing guidance to aid restoration of the species.

2 | MATERIAL AND METHODS

2.1 | Seedlots (genetic composition)

Wind-pollinated half-sibling progeny of *P. albicaulis* from seed collections of 225 parent trees (Tables S1 and S2) were included. Of those, 215 families were from the nine seed zones in Oregon (OR) and Washington (WA) (Shoal et al., 2008), with 7–56 families per seed zone. The other 10 families included three from California and Nevada, two from Idaho (ID) and Montana (MT), and five from British Columbia (BC) (Figure 1, Table S2). The parent trees were cone-bearing individuals, generally free of WPBR symptoms, located in areas with varying levels of natural WPBR infection. Seed collections were made between 1995 and 2006. The resistance of 16 of the OR and WA families had been characterized in earlier trials, and they were included as checklots: 10 resistant (R) and six susceptible (S). The two seedlots from ID and MT had been previously tested in another regional program and putatively showed resistance (Mahalovich et al., 2006; Sniezko et al., 2011).

2.2 | Seedling propagation

Seed was stratified in fall 2006, germinated in spring 2007, and the germinated seed was sown in Ray Leach "cone-tainers" (164 cm^3; Stuewe and Sons, Tangent, OR). Seedlings were grown in family blocks for 2 years (2007 and 2008 growing seasons) in an unheated greenhouse at the USDA Forest Service's Dorena Genetic Resource Center (DGRC), near Cottage Grove, OR.

2.3 | Experimental design

Prior to inoculation, the seedling families were placed into a randomized complete block design with six blocks of up to 10 seedlings per family per block. For inoculation, the six blocks were divided into two equal sets (Trial 1 [T1] and Trial 2 [T2]) of three blocks and inoculated approximately 3 weeks apart. Both trials averaged 22.4 seedlings for each family, with the number of seedlings varying from 7 to 30 per family per trial (Table S2). The variation in seedling numbers was due to variable germination among the seedlots. After inoculation, seedlings were placed outdoors to harden off, transplanted into wooden boxes in mid-November 2008, and retained outdoors in the randomized complete block design. For each of the two trials, there were three blocks, with each block consisting of 20 boxes. Each box typically accommodated up to 12 family row plots with up to 10 seedlings per family (Figure 2a,b). The two trials were located adjacent to each other (Figure 2a).

2.4 | WPBR inoculation

Inoculation of seedlings with WPBR followed standard DGRC protocols (Kegley & Sniezko, 2004). Briefly, seedlings were brought into the inoculation fog chamber and held for 2 days at 16.7°C and 100% relative humidity. Infected leaves from *Ribes* species (Table 1), the primary alternate host for *C. ribicola*, were placed telial side down on wire screens above the seedlings. The *Ribes* leaves remained above the seedlings for approximately 12–15 h, during which basidiospore drop occurred. Basidiospore density was monitored throughout the process using a series of microscope slides placed adjacent to the seedlings throughout the inoculation chamber. For both trials, the *Ribes* leaves were removed from each frame when monitoring indicated that a basidiospore density of approximately 3000 spores/cm^2 was reached. However, due to the rapidity of spore drop during the peak period and the time limitations of monitoring the many slides, the average spore density of T1 was 1.79 times greater than that of T2 (Table 1).

TABLE 1 White pine blister rust inoculation details for Trial 1 and Trial 2 of whitebark pine. Dates relevant to inoculation and differences between inoculum and source are reported.

Inoculation parameter	Trial 1	Trial 2
Trees moved into chamber	15-Sep-08	6-Oct-08
Total number of seedlings	5030	5056
Inoculum sources	Western Oregon, *vcr2*—Umpqua and Willamette National Forests and Dorena GRC Ribes Garden	Eastern Oregon, *AVCr2*—Malheur, Ochoco, Umatilla, and Wallowa-Whitman National Forests
Ribes species	*Ribes bracteosum*, *Ribes nigrum*, *Ribes hudsonianum* var. *petiolare*	*R. hudsonianum* var. *petiolare*
Ribes leaves placed on screens	17-Sep-08	9-Oct-08
Inoculum density	6013 ± 1833 SE	3359 ± 585 SE
Basidiospore germination (%)	99.7	99.8

FIGURE 2 White pine blister rust resistance trials (Trial 1 [T1] and Trial 2 [T2]) of *Pinus albicaulis*, (a) overview of trials 24 months post-inoculation (mpi), (b) contrasting survival of 12 families (in 10 tree row plots) in T2, (c) seedling of susceptible family "231" in T1 with 350 needle spots and incipient cankers (d) partial needle shed, 11 mpi for seedling in resistant family "108" in T2; seedling is canker-free throughout the trial, (e) many cankers on a seedling of susceptible family "193" in T1; tree was dead by 3rd assessment, (f) contrast of heavily cankered and canker-free seedlings in resistant family "160" in T1, (g) bark reaction on seedling of resistant family 157 in T2, (h) tree of resistant family "237" in T2, surviving approximately 48 mpi despite several large stem infections, (i) cones being collected for resistant family "14" from parent tree 011081 on Deschutes National Forest; this is also the parent tree being used to sequence the genome of whitebark pine. (Photo credits: R. Sniezko (a–h), C. Jensen (i)).

After all of the *Ribes* leaves were removed, the seedlings remained in the inoculation chamber for approximately 48 h at 20°C and 100% relative humidity to facilitate basidiospore germination and subsequent infection of the needles. Different geographic sources of *C. ribicola* were used for each of the two trials (Table 1); T1 used western OR sources known to include the virulent *vcr2* pathotype (virulent against resistance mediated by the *Cr2* gene in western white pine; Kinloch et al., 2004), and T2 used eastern OR sources outside the known range of *vcr2* (Table 1). T2 was inoculated 3 weeks after T1.

2.5 | Tree assessments

WPBR infection and survival were assessed periodically for 5 years (Table S3). In late spring/summer 2009, approximately 10 months post inoculation (mpi), total 2nd year and 3rd year height (from ground level to terminal bud), number of needle lesions ("spots") on all secondary needles, number of stem symptoms, and survival were evaluated for each seedling (Table S1). Family and seed source variation in height has been reported elsewhere (Hamlin et al., 2011), but is used here to examine the correlation with WPBR-related traits.

In late fall 2009 (approximately 15 mpi), the presence/absence of needle lesions (an indicator of needle shed or latent spot development), the number of stem symptoms by type (normal, partial bark reaction, complete bark reaction), seedling vigor, severity of infection, and survival were evaluated for each seedling (Tables S1 and S3). Severity of WPBR infection was quantified visually on a 0–9 scale, where 0 = no stem symptoms, 1–4 = one or more stem symptoms with relatively minor cumulative impact (encircling <100% stem diameter), 5–8 = one or more cankers encircling 100% of the stem and correspondingly greater vertical growth for categories 6–8, with seedlings assessed as "8" having nearly the entire seedling cankered, and 9 = dead from WPBR.

Assessments in subsequent years were similar to those at 15 mpi, except the presence of needle lesions was not assessed (Table S3). Because of the extensive merging of stem symptoms and the high mortality of seedlings, the counts of stem symptoms after the fall 2009 assessment were used primarily to discern whether latent infection occurred on previously canker-free seedlings and to validate seedling data. The few seedlings dead from non-rust-related causes, which showed no signs of stem infections, were also recorded at each assessment. Generally, the cause of the non-rust mortality was

unknown, but some of those seedlings were atypically small at the trials' onset.

In the later years of the trials, newer growth was infected by *Sirococcus conigenus* (Josh Bronson, per. Comm.), causing some dieback of stems but having little or no effect on overall seedling health or survival. There were some new WPBR stem symptoms recorded after the third assessment; validation of most of those indicated that they were on newer growth (the tissue was not present at the time of inoculation) and, putatively, all from *S. conigenus* and not WPBR.

Calculated traits included the percentage of trees in each seedling family (1) with needle spots (first two assessments), (2) with stem symptoms at all assessments, (3) with early stem symptoms (ratio of SS 15 mpi and SS 40 mpi), (4) with complete, partial, or either type of bark reactions, (5) alive with stem symptoms at 40 and 64 mpi, and (6) alive at each assessment (survival). Because some of the resistance traits are sequential, for example, a tree with no stem symptoms cannot have a bark reaction, the number of seedlings used in calculations varied by trait and family (Table S4).

2.6 | Statistical analysis of phenotypes

2.6.1 | Geographic variation in resistance

Because the objective of the trials was to assess overall genetic resistance and variability in survival among families and seed zones, the first set of analyses for measured phenotypes was carried out on the family block means (Table S4). Differences among trials, families within trials, and seed zones were assessed using analyses of variance (ANOVAs). Significance was assessed at 95% confidence, and variance components and percent of variance were estimated in R (RCoreTeam, 2022) using the variance component analysis (VCA; Schuetzenmeister & Dufey, 2022) and linear mixed effects 4 (lme4; Bates et al., 2015) packages.

2.6.2 | Correlations

Pearson's product-moment correlation between family means ($n = 225$) for traits was compared between the two trials. Correlations were also calculated between all traits within each trial. Significance was assessed at 95% confidence.

2.6.3 | Heritabilities

A second objective of the study was to identify the WPBR resistance phenotypes that contributed to survival and estimate their heritabilities. Data were filtered to remove individual trees that died from causes other than rust and then were assessed using either a linear mixed-effects model (LMM) fit by restricted maximum likelihood (REML) on numeric data or a generalized linear mixed-effects models (GLMM) fit by maximum likelihood, using a binomial distribution and logit link for binary data (e.g., rust survival). Trial, block within trial, and box within block within trial were considered fixed effects. Seed source (population), seed zones, and families were considered random factors. For GLMM, residual variance was estimated following Nakagawa and Schielzeth (2010): (Table 1) (Equation 1). Variance components were extracted from the models, and narrow sense heritabilities h^2 (Equation 2) and SE (Equation 3) were estimated for selected traits following Lynch and Walsh (2018). Seed zone h^2 estimates included trial and block within trial as fixed effects and families as random effects.

$$\sigma^2_{residual} = \omega * \left(\frac{\pi^2}{3}\right), where\ \omega = the\ model\ dispersion\ parameter\ 3.28. \quad (1)$$

$$h^2 = \frac{3\sigma^2_{sz}}{\sigma^2_{sz} + \sigma^2_{residual}}, where\ \sigma^2_{sz}\ is\ either\ seed\ zone\ or\ family. \quad (2)$$

$$SE = \sqrt{\frac{6 * \sigma^2_{sz} * \sigma_{residual}}{\sigma_{sz} + \sigma_{residual}}}, where\ \sigma^2_{sz}\ is\ either\ seed\ zone\ or\ family. \quad (3)$$

2.6.4 | Prediction of rust survival

To assess the overall association between observed phenotypes and blister rust survival (binary response variable), GLMM were fit by maximum likelihood using a binomial distribution and logit link. The full model included the fixed effects of height pre-inoculation (0 mpi), number of cankers at 15 mpi, number of all bark reactions at 64 mpi, trial, block within trial, and box within block within trial. Subsequent reduced models dropped one or more of the fixed effects. Seed source (population) and families were considered random effects. Seven models were compared, and the model with the lowest Akaike information criterion (AIC) was selected. Fixed effects were assessed for significance at 95% confidence.

2.6.5 | Test for major gene resistance

Although MGR has not been previously documented in *P. albicaulis*, we tested for phenotypic segregation patterns suggestive of this type of resistance. The occurrence of stem symptoms between inoculation and 64 mpi was aggregated into a binary measure of the phenotype. The Mendelian segregation ratios 1:1 (Rr × rr) and 3:1 (Rr × Rr) were used to test the hypotheses that each family did not differ significantly from a probability of .5 and .75 using an exact binomial test. Seedlings that died from causes other than rust were excluded from these analyses. Parent trees whose progeny failed to differ significantly at 95% confidence from expected ratios may be heterozygous for an MGR allele and thus candidates for MGR and additional screening. All statistical analyses were conducted using the R statistical program (RCoreTeam, 2022) (Code S1).

3 | RESULTS

We found significant differences in means between trials, seed zones, and families for most traits (Table 2). Overall, T1 showed a higher impact from inoculation. The family mean correlations between the same traits in the two trials were generally significant and moderate to high (Table 3). Mortality from non-rust causes was low: 1.4% for T1 and 1.1% for T2.

3.1 | Needle traits

Inoculation of both trials was successful (Figure 2). Needle spots are the first obvious signs of infection (Figure 2c). At 10 mpi, 99.9% of seedlings had needle spots, and by 15 mpi, the percentage of seedlings with needle spots had dropped only slightly (Table 4). Although "needle shed" was observed (Figure 2d), it was generally incomplete; 98.4% of seedlings still had needle spots at second assessment, indicating that this trait was not very effective in preventing stem infection in these two trials; only 21 of the seedlings that completely shed their infected needles showed no stem symptoms at 40 mpi, and only two of those seedlings originally had more than five needle spots.

On an individual seedling basis, the number of needle spots ranged from 0 to 600; at 10 mpi, only nine seedlings had no needle spots, and 20 seedlings had 450 or more needle spots. Families averaged 126.4 (T1) and 71.3 (T2) needle spots, and there was a 9-fold (T1) and a 16-fold (T2) range in family means (Tables 4 and S2). T1 averaged 1.77 times more needle spots than T2, which was similar to the difference in spore density (1.79) at inoculation for the two trials. There was little difference in the mean number of spots between the six susceptible and 10 resistant checklots (previously tested families), but the mean number of spots for the top nine surviving families in T1 was less than the trial mean in both T1 and T2 (Table 5).

3.2 | Stem symptoms

Approximately half of the seedlings showed stem symptoms at 10 mpi, with family means varying from 0% to 100% (Table S2). Only nine families had no stem symptoms at this stage (two in T1, seven in T2), while only three families (all in T1) had 100% stem symptoms. The percentage of seedlings with stem symptoms continued to increase over time and varied by family (Table 4, Figure 3a). By the final assessment (64 mpi), the trials averaged 93.7% and 89.3% stem symptoms for T1 and T2, respectively (Table 4). Even at the final assessment, there was notable variation among families in percent stem symptoms (Figure 3a, Table S2). At the final assessment, 104 (T1) and 131 (T2) of the 225 families had less than 100% stem symptoms, and 86 common families had 100% stem symptoms in both trials.

The six susceptible checklots showed a very high percentage of seedlings with stem symptoms at 15 mpi, while the incidence for the 10 resistant checklots and the top nine surviving T1 families was much lower (Table 5, Figure 3b). Although the incidence of stem symptoms increased over time in the resistant families, the means were still lower than the susceptible checklots at 64 mpi (Figure 3b, Table S2). On a seed zone basis, four of the 12 seed zones or regional areas (Seed Zones 4, 5, and 7 in the Cascades of OR and WA and Seed Zone 3 in eastern WA) averaged 75% or less for stem symptoms at 15 mpi in both trials (Table 6). However, infection incidence in families from these four areas increased, notably by 64 mpi (Table 6).

Seedlings varied widely for number of stem symptoms. On an individual seedling basis, the number of stem symptoms at 10 mpi varied from 0 to 30, and from 0 to 85 at 15 mpi (Table S1). Families averaged 2.3 (T1) and 1.5 (T2) stem symptoms per tree at 10 mpi, increasing to 8.9 (T1) and 7.6 (T2) at 15 mpi. Families varied widely for the mean number of stem symptoms at 15 mpi in both trials (Tables 4 and S2).

3.2.1 | Early stem symptoms incidence

Early rust infection, quantified by the number of stem symptoms per tree and the percentage of trees with stem symptoms at 15 mpi, can provide a distinction between the most susceptible and more resistant families. T1 (81.6%) averaged slightly higher percent early stem symptoms than T2 (77.3%) ($F = 4.635$, $DF = 1448$, $p = .032$) (Table 4). Over 90% of the seedlings in the six susceptible checklots showed stem symptoms early, approximately double that of either the resistant checklots or the nine highest surviving families from T1 (Table 5). Seed Zones 3, 4, 5, and 7 showed the lowest frequency of seedlings with early stem symptoms (Table 6).

3.2.2 | Bark reactions

Stem symptoms can be either normal cankers, complete bark reactions, or partial bark reactions, and a seedling with multiple stem symptoms may have one or more of these types. Overall, only a relatively low percentage of seedlings displayed any type of bark reaction (Table 4), but nine families had >30% of the seedlings with some type of bark reaction in both trials (Table S2). The six susceptible checklots had little or no bark reaction, while the 10 resistant checklots and the nine highest surviving families in T1 averaged 18.3%–38.1% in the two trials (Table 5). Seed Zones 3, 4, and 5 were particularly notable for bark reactions (Table 6).

3.2.3 | Stem symptom alive

Not all seedlings with stem symptoms died. The percentage of seedlings with stem symptoms that were alive at 64 mpi in the two trials was 6.3 (T1) and 11.7 (T2). The percent of seedlings alive with stem symptoms includes trees with small complete bark reactions (Figure 2g) as well as some trees surviving with stem infections that encircled the bole (Figure 2h), which may be active or inactive. Family "61" was one of the most notable over both trials, with six of the

TABLE 2 Whitebark pine infected with white pine blister rust was scored for several resistant phenotypes in two artificial inoculation trials. The differences between trials, families within trials, and seed zones were assessed using ANOVA and significance was assessed at 95% confidence, and variance components and percent of total variance were estimated. #Spots1 = mean number of spots at 10 months post inoculation (mpi), %Spots1 = percent of trees with spots at 10 mpi, #SS1 = number of stem symptoms per tree at 10 mpi, #SS2 = number of stem symptoms per tree at 15 mpi, %SS2 = percent of trees with stem symptoms at 15 mpi, %SS6 = percent of trees with stem symptoms at 64 mpi, %BRall = percent of trees with partial bark reactions + complete bark reactions at 64 mpi, %RSurv6 = percent of infected trees surviving at 64 mpi. Degrees of freedom (DF), sum of squares (SS), mean square (MS), variance component (VC), percent of total variation (%Total), and standard deviation (SD) are reported.

	Trial							Family within trial		
Trait	DF	SS	MS	VC	%Total	SD	*p*	DF	SS	MS
#Spots1	1.00	1022594.05	1022594.05	1511.27	37.80	38.88	.033	448.00	1367995.33	3053.56
%Spots1	1.00	0.14	0.14	0.00	0.00	0.00	.834	448.00	901.20	2.01
#SS1	1.00	242.36	0.35	4.53	0.59	15.93	.012	448.00	7299.79	16.29
#SS2	1.00	70.73	70.73	0.09	1.17	0.31	.003	448.00	9129.37	20.38
%SS2	1.00	13193.12	13193.12	18.37	2.26	4.29	.005	448.00	816115.66	1821.69
%SS6	1.00	6430.18	6430.18	9.18	3.81	3.03	.032	448.00	187363.65	418.22
$\%BR_{all}$	1.00	5191.84	5191.84	7.21	2.10	2.69	.251	448.00	252749.27	564.17
%RSurv6	1.00	20273.71	20273.71	29.39	6.33	5.42	.007	448.00	419618.41	936.65

TABLE 2 (Continued)

	Family within trial				Seed Zone						
Trait	VC	%Total	SD	*p*	DF	SS	MS	VC	%Total	SD	*p*
#Spots1	282.76	7.07	16.82	<.001	11.00	7177.01	9743.36	62.22	1.91	7.89	<.001
%Spots1	0.00	0.00	0.00	.558	11.00	71.80	6.53	0.04	2.12	0.21	<.001
#SS1	4.47	58.24	2.12	<.001	11.00	1783.49	162.14	1.48	19.18	1.22	<.001
#SS2	6.25	78.67	2.50	<.001	11.00	2057.46	187.04	1.71	21.06	1.31	<.001
%SS2	512.06	62.87	22.63	<.001	11.00	219464.57	19951.32	183.28	22.05	13.54	<.001
%SS6	93.13	38.66	9.65	<.001	11.00	24743.76	2249.43	19.27	8.06	4.39	<.001
$\%BR_{all}$	114.07	33.17	10.68	<.001	11.00	45338.59	4121.69	36.30	10.51	6.02	<.001
%RSurv6	250.65	54.02	15.83	<.001	11.00	86188.33	7835.30	70.70	15.39	8.41	<.001

TABLE 3 Whitebark pine infected with white pine blister rust was scored for several resistant phenotypes in two artificial inoculation trials. Pearson's product-moment correlations were used to compare family means ($n = 225$) of comparable traits between Trial 1 and Trial 2. Mean 2nd year height (HT2yr), mean 3rd year height (HT3yr), mean number of spots at inspection one (%Spot1, 10 mpi), percent of stem symptoms at inspection two (%SS2, 15 mpi), percent of stem symptoms at inspection six (%SS6, 64 mpi), percent of seedlings with early stem symptoms (% ESS, 15 mpi), total number of stem symptoms at inspection two (#SS2, 15 mpi), percent of seedlings with bark reactions or partial bark reactions ($\%BR_{all}$), and percent rust survival at inspection six (%RSurv6, 64 mpi).

Trait	*r*	95% CI lower	95% CI upper	*p*
HT2yr	.71	.646	.775	<.0001
HT3yr	.74	.673	.792	<.0001
%Spot1	.45	.337	.547	<.0001
#SS2	.65	.563	.716	<.0001
%SS2	.84	.791	.871	<.0001
%SS6	.66	.578	.727	<.0001
$\%ESS_{2\_4}$	.75	.691	.805	<.0001
$\%BR_{all}$	.72	.65	.777	<.0001
%RSurv6	.77	.705	.815	<.0001

TABLE 4 Whitebark pine infected with white pine blister rust was scored for several resistant phenotypes in two artificial inoculation trials. Overall trait mean, minimum and maximum for 225 families in Trials 1 and 2. HT2yr (cm) = height at 0 months post inoculation (mpi), HT3yr (cm) = height at 10 mpi, #Spot1 = mean number of spots at 10 mpi, %Spot1 = mean percent of seedlings with spots within families at 10 mpi, % Spot2 = mean percent of seedlings with spots within families at 15 mpi, #SS1 = mean number of stem symptoms at 10 mpi, #SS2 = mean number of stem symptoms at 15 mpi, %SS2 = mean percent of seedlings with stem symptoms at 15 mpi, %SS6 = mean percent of seedlings with stem symptoms at 64 mpi, $\%ESS_{2\_4}$ = mean percent of seedlings with early stem symptoms (15 mpi) relative to incidence at 40 mpi (SS2/SS4), % SSAL6 = mean percent of seedlings alive with stem symptoms at 64 mpi, %BRc = mean percent of seedlings with complete bark reactions at 64 mpi, %BRp = mean percent of seedlings with partial bark reactions at 64 mpi, %BRall = mean percent of seedlings with BRc + BRp at 64 mpi, % RSurv3 = mean percent of seedlings surviving rust at 27 mpi, %RSurv6 = mean percent of seedlings surviving rust at 64 mpi.

	Trial 1			Trial 2		
Trait	Mean	Minimum	Maximum	Mean	Minimum	Maximum
HT2yr (cm)	10.4	5.4	16	9.9	4.6	15.7
HT3yr (cm)	14.7	7.1	21.5	14.6	5.3	22.5
#Spots1	126.4	25.8	247.4	71.3	9.3	149.9
%Spots1	99.9	88.9	100	99.9	93.3	100
%Spots2	98.4	76.7	100	98.7	83.3	100
#SS1	2.3	0	9.6	1.5	0	6.5
#SS2	8.9	0	19.9	7.6	0.3	23.3
%SS2	77.8	0	100	71.5	10	100
%SS6	93.7	61.1	100	89.3	24.4	100
$\%ESS_{2\_4}$	81.6	0	100	77.3	13.2	100
%SSAL6	5.3	0	50	10.2	0	63.3
$\%BR_c$	1.6	0	27.8	3.5	0	46.7
%BRp	7.9	0	50	11.8	0	80.4
$\%BR_{all}$	8.1	0	50	12.1	0	80.4
%RSurv3	34.9	0	90.5	28.5	0	86.7
%RSurv6	10.2	0	57.1	17.9	0	82.2

eight surviving trees in T1 having stem symptoms and eight of the 10 surviving trees in T2 having stem symptoms, and in most cases, the severity of infection was low, with infections not completely encircling the circumference of the bole. The six susceptible checklots averaged less than 1% survival with stem symptoms, while the 10 resistant checklots and the nine highest surviving families from T1 averaged 13.7%–29.4% (Table 5). In T1, the 10 resistant checklots showed a notably lower percent of seedlings alive with stem symptoms than the nine high-surviving families (Table 5). Seed Zones 3, 4, and 5 had the highest percent of seedlings living with stem symptoms,

TABLE 5 Whitebark pine seedling families (225) infected with the white pine blister rust pathogen (*Cronartium ribicola*) were scored for several resistant phenotypes in two artificial inoculation trials. Comparison of overall means of six susceptible (S) checklots, 10 resistant (R) checklot families, the nine highest surviving families in Trial 1 (all with survival >40%), and overall trial mean for 2nd year height (HT2yr), number of spots per tree (#Spots1), number of stem symptoms at 15 months post-inoculation (#SS2), percent of trees with stem symptoms at 15 months post-inoculation (%SS2), percent of trees with stem symptoms at 64 mpi (%SS6), percent of trees alive with SS 64 mpi (%SSAL6), percent survival at 64 mpi (%RSurv6), percent of trees with early stem symptoms (% ESS_{2_4}), and percent of trees with bark reactions (complete or partial, $\%BR_{all}$) in both Trial 1 (T1) and Trial 2 (T2).

	#families	HT2yr T1	HT2yr T2	#Spots1 T1	#Spots1 T2	#SS2 T1	#SS2 T2	%SS2 T1	%SS2 T2
S checklots	6	9.5	9	127.8	68.6	11.5	10.5	96.22	88.85
R checklots	10	10.9	10.4	132.5	70.2	4.1	3.3	49.59	32.28
Top 9	9	10.6	9.3	106.1	48.8	4.2	2.9	39.6	33.4
Mean	225	10.4	9.9	126.4	71.3	8.9	7.6	77.8	71.5

TABLE 5 (Continued)

	%SS6 T1	%SS6 T2	%SSAL6 T1	%SSAL6 T2	%RSurv6 T1	%RSurv6 T2	$\%ESS_{2\_4}$ T1	$\%ESS_{2\_4}$ T2	$\%BR_{all}$ T1	$\%BR_{all}$ T2
S checklots	99.45	96.1	0.7	0.7	1.25	4.6	96.8	91.3	0	0.7
R checklots	92.39	77.13	13.7	32.5	19.91	46.01	54.8	41.9	18.3	38.1
Top 9	73.2	65.8	29.4	36.3	47.2	53.3	49.1	48.4	29.9	36.6
Mean	93.7	89.3	6.3	11.7	10.2	17.9	81.6	77.3	9	13.4

while the families from the ID/MT and California/Nevada sources had no trees alive with stem symptoms in either trial (Table 6).

3.3 | Rust survival

Survival in both trials was >99% at 15 mpi, but by 27 mpi, it had decreased to 34.9% (T1) and 28.5% (T2); families varied widely for survival (Figures 2a,b, 3c,d, and 4). Though T1 had a higher earlier incidence of stem symptoms and averaged more stem symptoms per tree, it also had higher earlier survival than T2. However, by 64 mpi, survival in T1 (10.2%) had fallen below that of T2 (17.9%) (Table 4, Figure 3c). At 27 mpi, 15 families in T1 and 39 families in T2 had no survival (three families in common) (Table S2). By 64 mpi, 93 (T1) and 73 (T2) of the 225 families in each trial had no survival. There was a relatively large and continuous range in survival among the remaining families, with a maximum of 57.1% (T1) and 82.2% (T2) in the two trials (Figures 3c and 4). In some cases, families with moderate survival in T2 had notably lower survival in T1 (Figure 4). None of the families with 100% stem symptoms at 15 mpi had more than 4.2% survival at 64 mpi. Only 63 of the seedlings with stem symptoms at 15 mpi were among the 1387 survivors at 64 mpi.

Overall, the resistant checklots had much lower survival in T1 than in T2 (Table 5). Although the top nine families in T1 for survival at 64 mpi were comparable to the 10 resistant checklots for percentage of trees with early stem symptoms in both trials, they were notably lower for stem symptom percentage at 64 mpi and much higher for final survival in T1 (Table 5). The top nine surviving families in T1 showed more than 40% survival (41.7–57.1), and these same families had 30.4%–81.1% survival in T2 (Figures 3d and 4; Table S2).

Only 30% of the surviving trees were stem symptom-free. The 1387 surviving trees at 6th assessment (64 mpi) fell into several categories: (1) no stem symptoms throughout the trial (415), (2) bark reactions with severity <5 (645), and (3) stem infections, severity 5–8, where the infections encircle the bole (327). Families with higher survival often included both seedlings with no stem symptoms as well as seedlings with bark reactions. However, some families with 100% stem symptoms also had >0% survival. Thirty-one families from T1 with 100% stem symptoms had survival >0%, and 23 of these families had bark reactions (ranging from 3.3% to 50%). One example is Family "181" from Seed Zone 3, which had 20.8% survival and 50% bark reaction; in this case, most of the surviving 12 trees had cankers that had encircled the tree rather than complete bark reactions. Although T1 had lower survival than T2, the percentage of trees that were SS-free, low- and moderate-severity infections, and recent dead were similar.

3.4 | Family mean correlations within/between traits

Many of the family mean correlations between traits within a trial were significant in both trials (Figure S1, Table S5), and most of the

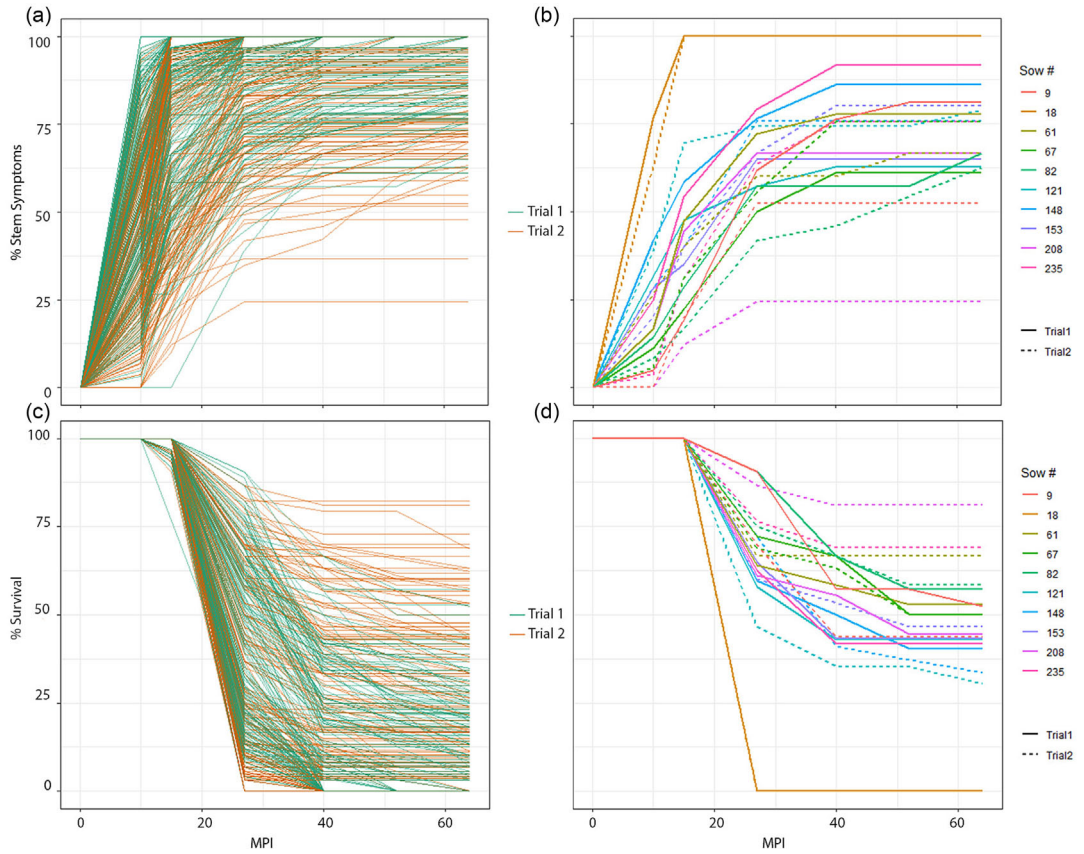

FIGURE 3 Results from two white pine blister rust resistance inoculation seedling trials for whitebark pine (*Pinus albicaulis*). Time trends for (a) percent of trees with stem symptoms for all 225 families (green lines Trial 1 and orange lines Trial 2), (b) percentage of trees with stem symptoms in the top nine surviving families from Trial 1: Sow#, 9, 61, 67, 82, 121, 148, 153, 208, 235 and high susceptible family "18," (c) percent of surviving trees for all 225 families, and (d) percent survival of the top nine surviving families from Trial 1 and high susceptible family "18". Months past inoculation (MPI) is shown on the *x*-axis.

traits were significantly correlated between trials (Tables 3, S6, and S7). There was a significant moderate correlation between seedling height at time of inoculation (0 mpi) and number of needle spots in both trials ($r = .44$ and .38 for T2 and T1, respectively). Larger seedlings may have more needles for infection. Pre-inoculation height also had a similar level of correlation with the number of stem symptoms (Tables S6 and S7). Part of this relationship may be due to the difficulty in assessing the shorter seedlings and families for the number of stem symptoms (small stem area and more early merging of cankers). Although height was also significantly correlated with several other traits, the correlations were low, and pre-inoculation height was not significantly correlated with survival in either trial (Tables S6 and S7).

There was a moderate correlation between the number of needle spots at 10 mpi and the number of stem symptoms at 15 mpi ($r = .47$, $p < .01$ for T1 and $r = .46$, $p < .01$ for T2). However, the number of needle spots was only weakly correlated with most other traits, including survival (Tables S6 and S7). The number of needle spots in these two trials was much higher than in previous trials (Sniezko et al., 2007), and the correlation of this trait with other traits may differ in trials with a lower number of needle spots.

There was a relatively strong and significant negative correlation between the percentage of trees with early stem symptoms (15 mpi) and both survival at 64 mpi (-0.83, $p < .01$ for T1 and -0.89, $p < .01$ for T2) and the percentage of seedlings with bark reactions ($\%BR_{all}$, $r = -.73$, $p < .01$ for T1 and $r = -.80$, $p < .01$ for T2). There was a relatively high and significant correlation of percent stem symptoms at 15 mpi with percent stem symptoms at 64 mpi ($r = .70$, $p < .01$ for T1 and $r = .68$, $p < .01$ for T2), and a moderately high significant negative correlation between percent stem symptoms and percent rust survival at 64 mpi ($r = -.82$, $p < .01$ for T1 and $r = -.87$, $p < .01$ for T2). The higher surviving families tended to have both a lower percentage of seedlings with early stem symptoms as well as a higher percentage of trees with bark reactions (Figure 5). The data suggest that the percentage of seedlings in a family with early stem symptoms may be a good indicator in identifying the most susceptible families very early.

The family mean number of stem symptoms per seedling at 15 mpi was moderately to highly significantly positively correlated with the percentage of seedlings with stem symptoms at all assessment periods and showed a moderate negative correlation with survival at the various assessments and with bark reactions (64 mpi) (Tables S6 and S7). The latter correlation indicates the tendency for families with fewer stem symptoms per seedling to also have a higher percent bark reaction.

TABLE 6 Whitebark pine seedling families (225) were infected with the white pine blister rust pathogen (*Cronartium ribicola*) were scored for several resistant phenotypes in two artificial inoculation trials. Comparison of nine seed zones (in Oregon and Washington) and three other geographic areas (British Columbia, Idaho/Montana, California/Nevada), and overall trial mean for number of spots per tree at 10 mpi (#Spots1), number of stem symptoms at 15 mpi (#SS2), and percent of trees with stem symptoms at 15 mpi (%SS2), percent of trees with stem symptoms at 64 mpi (%SS6), percent of trees with early stem symptoms (%ESS), percent of trees with bark reactions (%BR_{all}), percent of trees alive with SS at 64 mpi (%SSAL6), and percent survival at 64 mpi (% RSurv6), in both Trial 1 (T1) and Trial 2 (T2). See Figure 1 for the location of seed zones.

Seed Zone	Number of families	HT2yr T1	HT2yr T2	#Spots1 T1	#Spots1 T2	#SS2 T1	#SS2 T2	%SS2 T1	%SS2 T2
1	7	12.9	12.9	146.3	81.0	14.4	11.0	96.7	93.3
2E	17	11.2	11.2	139.0	91.3	12.8	13.3	94.5	94.3
2 W	23	10.4	10.4	118.4	67.9	9.2	8.5	82.1	74.9
3	10	9.3	9.3	133.0	60.6	4.8	3.4	51.4	42.2
4	27	11.6	11.6	135.2	72.8	6.5	5.9	61.7	54.5
5	56	10.5	10.5	123.7	76.8	8.7	7.4	75.0	66.4
6	22	9.0	9.0	118.0	63.2	9.2	7.5	87.5	90.0
7	41	9.6	9.6	113.9	60.4	7.3	6.1	73.6	63.6
8	12	9.9	9.9	140.3	84.2	10.8	8.4	88.9	84.1
BC	5	11.0	11.0	156.3	60.1	14.6	10.7	95.3	85.7
ID/MT	2	12.5	12.5	165.1	60.2	10.3	12.6	92.4	97.9
CA/NV	3	9.8	9.8	118.5	65.5	11.7	6.9	92.6	94.8
Mean	225	10.4	9.9	126.4	71.3	8.9	7.6	77.8	71.5

TABLE 6 (Continued)

Seed Zone	%SS6 T1	%SS6 T2	%SSAL6 T1	%SSAL6 T2	%RSurv6 T1	%RSurv6 T2	$\%ESS_{2\_4}$ T1	$\%ESS_{2\_4}$ T2	$\%BR_{all}$ T1	$\%BR_{all}$ T2
1	99.2	100.0	0.0	1.7	0.8	1.7	97.3	93.3	0.0	1.7
2E	98.0	97.3	1.7	2.9	2.3	4.4	95.5	95.9	2.6	2.8
2 W	94.7	91.1	5.9	10.8	10.0	15.9	86.2	79.3	6.1	11.1
3	88.6	85.4	14.5	24.5	19.1	33.4	56.8	49.6	21.8	23.5
4	89.2	81.2	12.3	23.0	19.4	33.5	67.6	64.3	16.2	24.7
5	93.9	86.3	8.0	14.5	11.9	21.9	78.7	73.5	12.6	17.8
6	95.8	97.0	1.6	0.7	4.2	2.9	90.1	92.9	2.9	3.0
7	91.4	85.5	6.2	13.8	11.4	23.1	78.5	71.5	8.4	16.2
8	95.8	96.1	3.1	4.4	5.8	7.2	91.9	86.3	4.6	5.3
BC	98.7	95.6	0.0	1.8	1.3	4.0	98.3	90.1	0.0	1.8
ID/MT	100.0	100.0	0.0	0.0	0.0	0.0	92.4	97.9	3.7	0.0
CA/NV	96.3	100.0	0.0	0.0	0.0	0.0	100.0	94.8	2.8	1.1
Mean	93.7	89.3	6.3	11.7	10.2	17.9	81.6	77.3	9.0	13.4

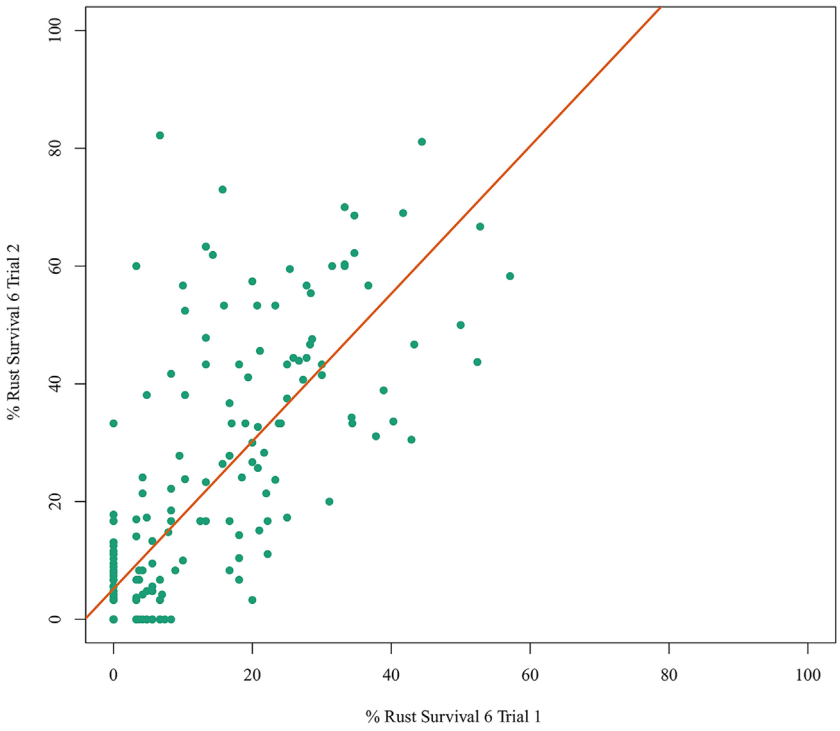

FIGURE 4 Comparison of survival of 225 whitebark pine (*Pinus albicaulis*) seedling families at 64 months post-inoculation in two inoculation trials to examine the range of genetic resistance to white pine blister rust. Adjusted $R^2 = 0.584$, $F = 315.5$, DF = 1 and 223, $p < .001$.

3.5 | Geographic variation in resistance

There was a wide range of variation in stem infection and survival within and among many of the nine OR and WA seed zones in the Pacific Northwest (PNW) region and three other geographic areas (Figures 1 and 6). Within the PNW region, several seed zones showed very low levels of survival, as did the families from ID/MT, California/Nevada, and BC. Most of the areas with low survival were represented by relatively few families, so testing of more families will be needed to ascertain the level of resistance in those areas. However, Seed Zones 3, 4, 5, and 7 had an average survival greater than the mean in both trials (Table 6), indicating a higher frequency of resistance in those areas. Survival was particularly notable in Seed Zones 3 and 4, representing northeastern-most WA (Colville National Forest) and southern WA Cascades populations (Table 6, Figures 1 and 6).

3.6 | Heritability

Narrow sense heritability estimates for rust survival, both overall and within seed zones, were moderate (Table 7). About 45% of the phenotypic variation for rust survival was attributable to variation in family genotypes across populations and seed zones. Survival is a composite trait that represents the most important component of blister rust resistance, but it is also a complex polygenic trait that is challenging to untangle. Height pre-inoculation (0 mpi) had moderately high heritability (0.682± 0.490), while bark reactions at 64 mpi had low overall heritability (0.106 ± 0.086) (Table 7). Seed zone h^2 was only marginally higher than those found within populations. Between seed zones, h^2 varied more widely (Table 8), with Seed Zone 7 having the largest percent of phenotypic variation in rust survival explained by variation in family genotypes ($h^2 = 0.705 \pm 0.503$). The estimated h^2 for selected traits should be interpreted with care, as these values represent assessments at a single site and point in time, as well as different numbers of families represented per seed zone.

3.7 | Prediction of rust survival

Rust survival at 64 mpi was best characterized by height at 0 mpi, number of cankers at 15 mpi, and total bark reactions at 64 mpi. The full model had the lowest AIC (Table 9) compared with the six reduced models. The second-best model had only a marginally higher AIC when pre-inoculation height was removed.

3.8 | Segregation ratios

At 64 mpi, seven families failed to reject a ratio of 1:1 (stem symptom:stem symptom-free) in both trials ($p > .05$) (Table S8). These findings suggest that parent trees could potentially possess MGR, although alternatively, it could be high levels of QR. Ten families in T1 and 26 families in T2 were segregated 1:1 (stem symptom:stem symptom-free).

4 | DISCUSSION

The two *P. albicaulis* seedling inoculation trials provide the first detailed examination of the levels of genetic resistance to WPBR in

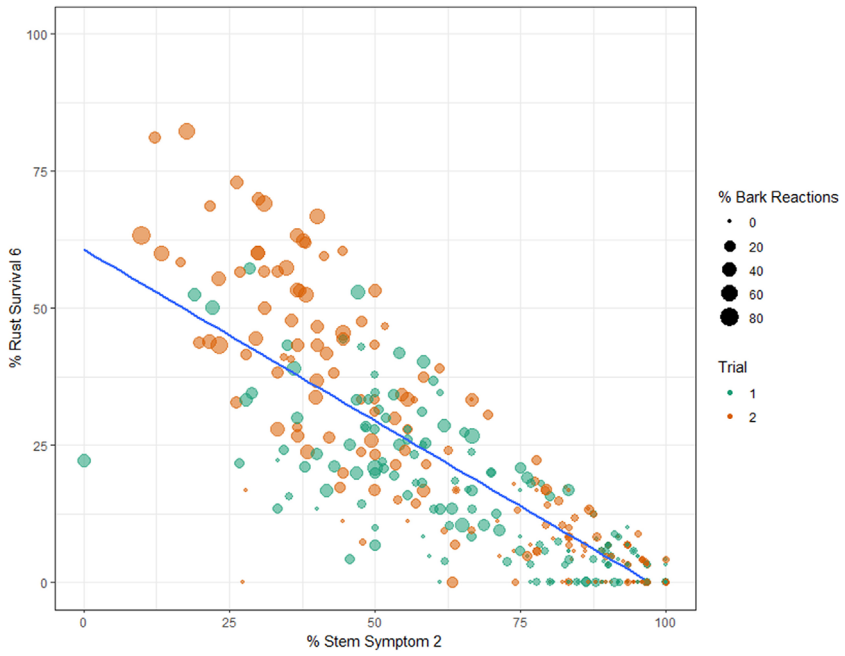

FIGURE 5 Whitebark pine seedling families were infected with *Cronartium ribicola*, which causes white pine blister rust and were scored for phenotypes associated with blister rust survival in two artificial inoculation trials (green points Trial 1 and orange points Trial 2). Family variation in survival percentage related to percentage of trees with stem symptoms at 15 months post-inoculation (%SS2) and percentage of trees with bark reactions at 64 months post-inoculation. Points are scaled to percent bark reactions.

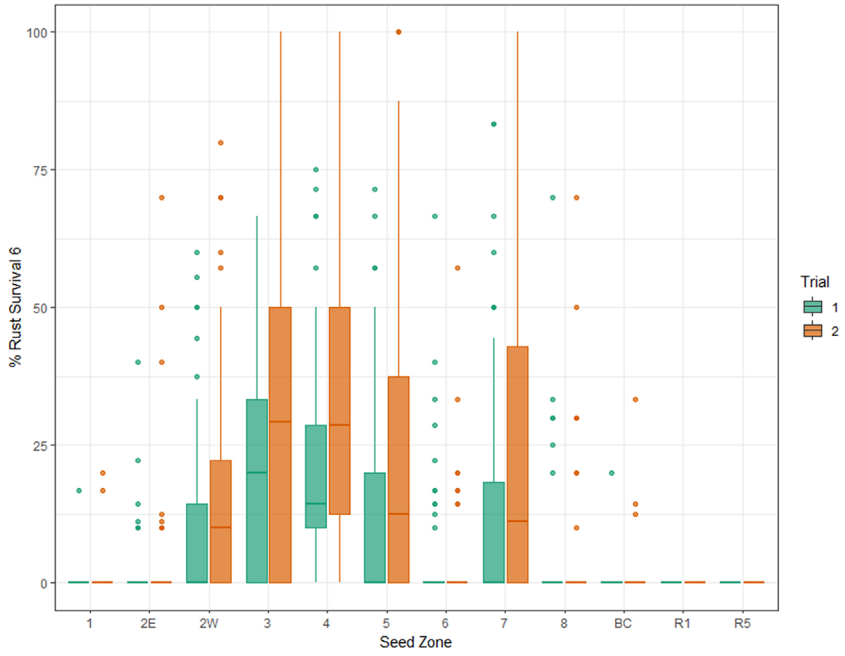

FIGURE 6 Whitebark pine seedling families were infected with *Cronartium ribicola*, which causes white pine blister rust, in two artificial inoculation trials. Family variation for survival 64 months post-inoculation, was compared within and among progeny of wind-pollinated parent trees from nine Oregon and Washington seed zones and three other regions. The number of families in each zone is 7, 17, 23, 10, 27, 56, 22, 41, 12, 5, 2, and 3 for the 11 groups (from left to right, respectively). Overall survival averaged 10.2 and 17.9 in Trials 1 and 2, respectively.

the species. Results from these trials provide initial insight on what resistant phenotypes are observed, as well as into how the frequency and level of resistance vary geographically between seed zones in the PNW portion of the species range; this resistance is of immediate utility in restoration efforts without advanced breeding.

Seedling families varied widely in survival. Surviving trees included those with no apparent stem symptoms, trees with complete bark reactions or low-severity infections, and trees with stem symptoms (one or more on a tree) that had grown substantially, encircling the trees but not killing them and often showing little or no activity in later years. In many of the families with the highest survival, all three phenotypes were represented and were suggested by the best fit mixed model. This is a pattern, along with the tendency of resistant families to have delayed stem symptom appearance, also seen in WPBR resistance testing of western white pine (Sniezko et al., 2014) and southwestern white pine (Johnson & Sniezko, 2021). Incorporating all of these components of resistance into the restoration populations will likely increase the potential for durable resistance.

The data suggest that resistance in *P. albicaulis* is likely QR rather than MGR. QR has been documented in three of the four species in which MGR has also been documented (Johnson & Sniezko, 2021; Kinloch et al., 2012; Sniezko et al., 2008, 2014, 2020), and further

TABLE 7 Whitebark pine seedling families infected with the white pine blister rust pathogen (*Cronartium ribicola*) were scored for several resistant phenotypes in two artificial inoculation trials. Overall narrow sense heritabilities (h^2) for selected traits predicted from linear mixed-effects models fit by REML for Ht, NC2, BRx6, or generalized linear mixed-effects models fit by ML for Survival, SS2, and SS6. Residual variance for generalized linear mixed models set to $\sigma^2_{residual} = \omega * \left(\frac{\pi^2}{3}\right)$, *where* ω = *the model dispersion parameter* 3.28. h^2 = narrow sense heritability calculated as $\frac{3\sigma^2_{sz}}{\sigma^2_{sz}+\sigma^2_{residual}}$ for selected traits. Traits include survival = survival of infected seedlings (had spots or stem symptoms) at 64 months post inoculation (mpi), Ht = height pre-inoculation at 0 mpi, NC2 = number of cankers at 15 mpi, SS2 = stem symptoms present (0/1) at 15 mpi, BRx6 = all bark reactions at 64 mpi, and SS6 = stem symptoms present (0/1) at 64 mpi. SE± = standard error for h^2.

	Variance				
Trait	**Pop:Family**	**Pop**	**Residual**	h^2	**SE±**
Survival	2.040	3.419	10.791	0.477	0.357
Ht	5.848	2.658	19.866	0.682	0.490
NC2	10.481	6.569	58.689	0.455	0.342
SS2	1.801	1.980	10.791	0.429	0.324
SS6	1.637	1.204	10.791	0.395	0.301
BRx6	0.021	0.019	0.568	0.107	0.086
	Seed.Zone:Family	Seed.Zone	Residual		
Survival	2.215	2.362	10.791	0.511	0.380
Ht	5.803	3.017	19.866	0.678	0.487
NC2	10.984	6.495	58.696	0.473	0.354
SS2	1.916	1.392	10.791	0.452	0.340
SS6	1.761	1.024	10.791	0.421	0.319
BRx6	0.027	0.007	0.569	0.134	0.107

investigations in *Pinus flexilis* are underway at the DGRC. The level of QR in the progeny of most of the field selections in species of white pine is low, with most seedling families showing less than 10% survival (Kegley & Sniezko, 2004; Sniezko et al., 2008, 2020). However, there is a very low frequency of families from field selections in *P. monticola*, *P. lambertiana*, and *Pinus strobiformis*, where higher levels of survival have been noted (Johnson & Sniezko, 2021; Kegley & Sniezko, 2004; Kinloch et al., 2012; Sniezko et al., 2008, 2014). By contrast, in the two trials reported here, the frequency of *P. albicaulis* families with low to moderate levels of surviving seedlings is much higher, and the distribution of survival among families appears to be relatively continuous from none to moderate (>40%), further suggesting QR (Figure 4). Additional support in favor of QR over MGR is provided by the higher stem infection percentage and lower survival of the highest resistant families in the trial inoculated with the higher spore density (T1), in contrast with species such as *P. monticola* and *P. lambertiana*, in which there is little or no differential impact of higher inoculum densities on the percentage of trees with stem symptoms in MGR families. Note that the source of rust used in T1 has specific virulence to MGR in western white pine (*vcr2*), but results from an earlier trial suggested that this source of rust had little or no differential effect on *P. albicaulis* resistance compared with the *Avcr2* source (Sniezko et al., 2008).

The two trials showed a wide range of survival among families. Overall, the results indicated that resistance is apparent relatively early after inoculation and that it varies among and within families. Families differed in traits such as the number of needle spots, the number of stem symptoms, the frequency of stem symptoms, the incidence of bark reactions, and the frequency of early stem symptoms. Similar variation has also been observed in *P. monticola* and *P. lambertiana*, although at mostly lower levels (Hoff & McDonald, 1980a, 1980b; Kegley & Sniezko, 2004; Kinloch et al., 2008; Sniezko et al., 2008, 2014, 2020). On a family basis, the frequency of early stem symptoms appears to provide a means to separate the most susceptible families from those with higher levels of resistance, including final survival, and is currently being used in the PNW region to provide land managers with an early listing of parent trees from which to collect seed for restoration.

The difference in the timing of the appearance of stem symptoms in the most susceptible seedlings (10 and 15 mpi) versus those in the most resistant seedlings (27 mpi or later) of *P. albicaulis* suggests that the growth of the fungus is being slowed in the more resistant seedlings. In earlier investigations of *P. monticola*, both "slow fungus growth in secondary needles" and "slow fungus growth in stem" were noted (Hoff & McDonald, 1980a; Sniezko et al., 2014). Bark reactions were also an important component of resistance identified in *P. monticola* (Hoff, 1986) and *P. strobiformis* (Johnson & Sniezko, 2021).

Seedling inoculation trials have limitations, but the family variation in survival in these two trials suggests moderate levels and frequency of resistance to WPBR exist in *P. albicaulis* and that it varies by population. Extensive further testing completed or currently underway provides additional support to the geographic trends observed in resistance, at least in the PNW portion of the range of *P. albicaulis* (Sniezko & Liu, 2022).

Seedling assays for resistance can be an invaluable aid to assessing the relative level of resistance of hundreds or thousands of

TABLE 8 Whitebark pine seedling families (225) were infected with the white pine blister rust pathogen (*Cronartium ribicola*) were scored for several resistant phenotypes in two artificial inoculation trials. Narrow sense heritabilities (h^2) for selected traits predicted from linear mixed effects models fit by REML for height, number of cankers at 15 months post inoculation (mpi), number of bark reactions (all types) at 64 mpi or generalized linear mixed-effects models fit by ML for binary traits: rust survival (64 mpi), stem symptoms at 15 mpi, stem symptoms at 64 mpi. Residual variance for generalized linear mixed models set to $\sigma^2_{residual} = \omega * \left(\frac{\pi^2}{3}\right) = 10.791, where\ \omega = the\ model\ dispersion\ parameter\ 3.28$. $n_{families}$ = the number of families tested from each seed zone. n_{rsurv} = the number of individuals surviving at 64 mpi. h^2 = narrow sense heritability calculated as $\frac{3\sigma^2_{sz}}{\sigma^2_{sz}+\sigma^2_{residual}}$ for selected traits. Traits include rust survival (infected trees) at 64 mpi, height pre-inoculation at 0 mpi, number of cankers at 15 mpi, stem symptoms present (0/1) at 15 mpi, all bark reactions at 64 mpi, and stem symptoms present (0/1) at 64 mpi. SE± = standard error for h^2.

		Rust survival (64 mpi)			Height (0 mpi)		Number of cankers (15 mpi)		Stem symptoms (15 mpi)		Bark reaction$_{all}$ (64 mpi)		Stem symptoms (64 mpi)	
Seed Zone	$n_{families}$	n_{rsurv}	$^*h^2_{rsurv}$	SE±	h^2_{ht}	SE±	h^2_{nc2}	SE±	$^*h^2_{SS2}$	SE±	h^2_{brx6}	SE±	h^2_{SS6}	SE±
1	7	3	0.352	0.270	0.412	0.313	0.263	0.205	0.184	0.145	0.011	0.009	0.000	0.000
3	10	87	0.142	0.113	0.839	0.582	0.136	0.108	0.088	0.071	0.376	0.287	0.241	0.189
4	27	334	0.172	0.136	0.401	0.305	0.467	0.350	0.216	0.169	0.163	0.130	0.166	0.132
5	56	421	0.569	0.418	0.608	0.444	0.437	0.330	0.553	0.408	0.346	0.266	0.474	0.355
6	22	29	0.609	0.444	0.576	0.423	0.202	0.160	0.329	0.254	0.222	0.174	0.814	0.568
7	41	297	0.705	0.503	0.859	0.593	0.607	0.443	0.564	0.415	0.521	0.387	0.565	0.416
8	12	41	1.000	0.714	1.000	0.722	0.415	0.315	0.691	0.495	0.353	0.270	0.705	0.504
2E	17	33	0.481	0.359	0.299	0.231	0.371	0.284	0.401	0.305	0.366	0.280	0.715	0.509
2 W	23	138	0.432	0.326	0.831	0.577	0.391	0.298	0.385	0.293	0.341	0.262	0.347	0.267
BC	5	4	0.000	0.000	0.679	0.487	0.507	0.378	0.750	0.530	0.000	0.000	0.003	0.002
ID/MT	2	0	0.000	0.000	1.000	0.734	0.143	0.114	0.359	0.276	0.018	0.015	0.359	0.276
CA/NV	3	0	0.000	0.000	0.171	0.136	0.532	0.394	0.000	0.000	0.000	0.000	0.000	0.000
Total	225	1387												

TABLE 9 Whitebark pine seedling families infected with the white pine blister rust pathogen (*Cronartium ribicola*) were scored for several resistant phenotypes in two artificial inoculation trials. Generalized linear mixed-effects models (GLMM), fit by maximum likelihood, using a binomial distribution and logit link, to assess rust survival at 64 mpi (Surv). The full model included the fixed effects of height pre-inoculation (0 mpi, Ht), number of cankers at 15 mpi (NC2), number of all bark reactions at 64 mpi (BRx6), trial, block within trial, and box within block within trial. Subsequent reduced models dropped one or more of the fixed effects. Seed source (population), and families were considered random effects. Seven models were compared and the model with the lowest AIC was selected. Fixed effects were assessed for significance at 95% confidence.

Model	AIC	BIC	logLik	Deviance	DF residual
Surv = Ht + NC2 + BRx6	3631.2	4532.2	−1690.6	3381.2	9854
Surv = NC2 + BRx6	3631.8	4525.6	−1691.9	3383.8	9858
Surv = Ht + NC2	3807.4	4701.2	−1779.7	3559.4	9855
Surv = NC2	3823.7	4710.3	−1788.8	3577.7	9859
Surv = Ht + BRx6	5457.7	6351.5	−2604.8	5209.7	9855
Surv = BRx6	5489.8	6376.4	−2621.9	5243.8	9859
Surv = Ht	6316.8	7203.4	−3035.4	6070.8	9856

parent trees, but they need field validation. Several field trials are underway at both high and low elevation sites (Cartwright et al., 2022) (Figure 7). However, it will likely be a decade or more before levels of infection are high enough in the field trials to fully evaluate the durability and stability of resistance (Sniezko et al., 2020). Ongoing field trials may also help clarify to what extent traits such as the number of needle spots or the number of stem symptoms observed in seedling trials are effective in the field.

Because of the long-lived nature of tree species such as *P. albicaulis*, any resistance will need to be durable (Sniezko & Liu, 2021). The durability of WPBR resistance will have to be assessed over time in the field. However, some of these families are from areas where the parent trees have been exposed to WPBR for more than 50 years and survived (Rochefort et al., 2018), and this, along with current field data for QR families in *P. monticola* (Sniezko et al., 2020), makes us cautiously optimistic about the durability of the resistance.

FIGURE 7 Combination restoration planting and genetic trial of whitebark pine (*Pinus albicaulis)* at Crater Lake National Park, Oregon, planted in 2009. Seedlings from the same families planted had been previously tested for genetic resistance to white pine blister rust in inoculation trials at Dorena Genetic Resource Center, Oregon, and the information used to rate the parent trees of the half-sib families. Seed from the most resistant parent trees will be used for future restoration efforts. Trials such as these will be used for operational restoration of the species and will also supply information to field validate rust resistance at high-elevation sites and to monitor the durability of resistance. Each seedling is tagged with seed parent identity, and both susceptible and resistant families from the trials reported in this paper have been planted. This planting is in an area visible to hundreds or thousands of visitors a year (Rim Village), so it can also serve to provide conservation education. (Photo credit: R. Sniezko).

Stability of resistance in different environments and under changing climates is also important. Inoculation trials of this high-elevation species typically occur at low elevations under widely different conditions of seedling growth and temperature than in native ecosystems. However, current data from *P. monticola* generally provide support for the stability of resistance, with some possible exceptions that may caution against long-distance movement of seed sources (Sniezko et al., 2020).

4.1 | Variation between trials

The main focus of these trials was to provide the first detailed documentation of the various phenotypes of rust resistance, along with their frequency, the level of resistance (notably survival) that is present in progeny, and any geographic pattern in resistance. The inclusion of two trials here strengthens the results. Although the frequency of survival differed somewhat between the two trials, common families with moderate levels of survival did occur in both trials, and family means for the same traits were significantly correlated for most traits. Three factors possibly influenced the differences in the resistance between the two trials: (1) the higher spore density (1.79 times greater) in T1, (2) inoculating T1 3 weeks earlier than T2, and (3) the potential difference in aggressiveness or virulence of the different geographic sources of rust used (*Avcr2* vs. *vcr2*). In a previous trial, we noted that increased spore density resulted in an increased number of needle spots as well as the number of early stem symptoms, with the increase in the number of spots being of larger magnitude and survival being slightly higher under the lower inoculum level (Sniezko & Liu, 2022). The inoculation of T1 3 weeks earlier than T2 potentially provided the fungus with a slightly longer growing season before winter, as well as seedlings that were more actively growing before the fall dormant period. The source of rust used in T1 was from areas with known virulence to the *Cr2* major gene in *P. monticola* (Kinloch et al., 2004), but little is known about how it might affect resistance in *P. albicaulis*. However, a smaller, earlier trial suggested that the virulent source of the rust had little or no differential impact on *P. albicaulis* relative to avirulent sources (Sniezko et al., 2007, 2008; Sniezko & Liu, 2022). We suspect that the major cause of the survival difference is the higher spore density in T1. However, we cannot rule out that the earlier inoculation timing, the growth stage of the seedling, or different sources of inoculum also had an impact. It is also conceivable that a combination of these factors was responsible for the differences. In spite of these confounded factors, the primary objective of the study was accomplished, documenting that there are families with moderate levels of resistance and that they are common to both trials.

4.2 | Geographic pattern of resistance

Seed zones varied greatly in overall level of resistance and the heritability of resistance traits, but most of them had at least some families with notable levels of resistance. Of the nine seed zones in OR and

WA, only the area encompassing the Olympic Peninsula in WA (Seed Zone 1) had no families with survival greater than the overall mean. In another study, Seed Zone 1 was noted as having the lowest genetic diversity of the OR and WA zones (Liu et al., 2016), and its low level of resistance may reflect its relative isolation from other populations and low overall genetic diversity; it also had the fewest families for the OR and WA zones. Seed Zones 3 and 4 had the highest overall survival and frequency of resistance (Table 6, Figure 1). The top nine surviving families in T1 were distributed across five seed zones, which makes us cautiously optimistic that testing of more parent trees will identify additional selections from most areas with moderate levels of genetic resistance to WPBR for use in restoration. The trials summarized here represent one test year and 215 seedling families from OR and WA, but resistance screening has continued, and the results from testing over 1220 parent trees show a similar geographic pattern of resistance (Sniezko & Liu, 2022).

The variation in frequency of resistance and heritability of resistance traits among the seed zones in the PNW may be due to several factors, including some natural selection in areas with a higher incidence of rust infection and mortality (Hoff et al., 2001). However, the overall infection level in the PNW portion of the range can vary from very low to moderately high, with a few localized areas having very high infection and mortality (Aubry et al., 2008). A more detailed assessment of WPBR incidence in the selected areas is needed to more clearly denote whether natural selection is the primary factor in the different levels of resistance between seed zones. The few families (a total of 10) tested from BC, ID, MT, and California were generally highly susceptible. However, subsequent testing of additional families from BC (Murray & Strong, 2021), some at both DGRC and in BC, has identified a number of resistant families. The two families from ID/MT showed some resistance when tested elsewhere (Mahalovich et al., 2006; Sniezko et al., 2011) but showed little or no resistance in our trials. We suspect the discrepancy may be due to the more robust inoculation present in our trials, but full validation will await examination of field performance under conditions of varying pathogen pressure.

4.3 | Deploying WPBR resistance

In the United States, a national *P. albicaulis* restoration plan is being prepared (National Whitebark Pine Restoration Plan—Whitebark Pine Ecosystem Foundation) (Tomback & Sprague, 2022), and the availability of seedlots with resistance to WPBR will be key to its success (Tomback et al., 2022). For the next several decades, seed for restoration will come from parent trees in the field, generally from climbing the trees (Figure 2i). Cones are being collected from trees highly rated for rust resistance. Good seed crops in *P. albicaulis* are sporadic, and efforts to collect large seed crops across many parent trees in good seed years will need to continue. Seed orchards are also being established in the United States and Canada by grafting either parent trees or resistant seedling offspring from WPBR resistance tests. Seed from the orchards will be expected to have an even higher level of resistance, and perhaps a greater mix of resistance because parent trees from throughout a seed zone will be brought together to intermate. However, seed orchards will likely take several decades to produce much seed.

In the PNW region, limited field trials and restoration plantings of *P. albicaulis* have been underway since 2009 (Figure 7). Survival in the field will depend upon the WPBR hazards in each area and the cohort of parent trees from which seed is collected. The most susceptible families may have little or no survival on moderate to higher hazard sites, while the survival of the most resistant open-pollinated families may be expected to be moderate. However, on a seed zone basis, the highest levels of survival averaged only 19%–33%. Thus, even for seedlings from the best resistant parent trees or in those seed zones, extra seedlings will need to be planted initially to account for expected mortality from WPBR, particularly on the sites of the highest rust hazard. In the field, the trees will be exposed to the pathogen in different environments (high elevation) and rust populations, as well as over time, encompassing somewhat different physiological stages of the trees during the year as well as across years.

Depending on the frequency of resistance and the number of mature trees in each location, there are several strategies that might facilitate the recovery of *P. albicaulis* forest ecosystems, including (1) activities to promote natural regeneration in areas where the frequency and level of resistance is relatively abundant and where there are sufficient trees remaining to inter-mate, retain genetic diversity, and retain the presence of the Clark's Nutcracker (*Nucifraga columbiana*), the primary dispersal agent for *P. albicaulis* seed (Tomback, 2001), (2) collection of seed from resistant parent trees during the large cone crop years; however, because good cone crops can be very irregular in many areas, it is imperative to facilitate a strong collection effort in the years with abundant cones; this seed can then be used immediately for restoration or potentially stored for a decade or more (Sniezko et al., 2017), (3) development of seed orchards by grafting resistant parent trees and/or their resistant progeny; however, there will likely be at least a 15–20 years or longer delay between grafting and when trees will be large enough and/or mature enough to produce moderate to large cone crops, and (4) some combination of all three activities.

Parent trees in the field are a good potential source of resistant seed, but environmental and biological factors, such as wildfire and predation by mountain pine beetles, have already killed perhaps 20% of resistant trees in some areas. Thus, orchards or clone banks will be needed to provide a backup to preserve resistant genotypes. Seed orchards have been established in some areas and are planned for other parts of the *P. albicaulis* range in both the United States and Canada.

It will be difficult and expensive to restore all the areas, so strategic planning efforts that utilize a combination of planting and natural regeneration, such as restoration islands or "applied nucleation" (Corbin & Holl, 2012; Sniezko & Koch, 2017), could help focus resources to establish areas that will serve as future seed sources for natural regeneration to aid species recovery. In the PNW region, Crater Lake National Park and Deschutes National Forest are two areas where the earliest restoration plantings were established and

serve as models for the subsequent plantings. In both of those areas, the identity of each seedling's parent is being retained in some plantings, and those plantings act as informal common garden trials to examine the efficacy of resistance and its durability, as well as the adaption of the different families to environments affected by climate change. A national strategy involving many groups is underway to develop core areas throughout the United States range of *P. albicaulis* to emphasize restoration (Tomback & Sprague, 2022). Although *P. albicaulis* was recently listed in the United States as "threatened" under ESA, the finding of genetic resistance to the rust and the restoration efforts underway or planned by many groups may make this one of the first species to be successfully restored in the presence of a non-native disease. The resistance program and restoration effort will serve as an example to other tree species severely affected by non-native pathogens or pests.

AUTHOR CONTRIBUTIONS

Conceptualization: Richard A. Sniezko and Angelia Kegley. *Methodology*: Richard A. Sniezko, Jeremy S. Johnson, Angelia Kegley, and Robert Danchok. *Investigation*: Richard A. Sniezko, Jeremy S. Johnson, Angelia Kegley, and Robert Danchok. *Project administration*: Richard A. Sniezko. *Writing—original draft*: Richard A. Sniezko and Jeremy S. Johnson. *Writing—review and editing*: Richard A. Sniezko, Jeremy S. Johnson, and Angelia Kegley.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

All data and materials are available in the main text or supplementary materials.

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REFERENCES

Aubry, C., Goheen, D. G., Shoal, R., Ohlson, T., Lorenz, T., Bower, A. D., Mehmel, C., & Sniezko, R. A. (2008). *Whitebark pine restoration strategy for the Pacific Northwest 2009–2013*. U.S. Department of Agriculture, Forest Service, Pacific Northwest Region. 212p.

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Cartwright, C., Sniezko, R. A., Murray, M., & Reid, I. (2022). Whitebark pine genecology field trials in British Columbia: Age 5 results. *Forest Ecology and Management*, *521*, 120419. https://doi.org/10.1016/j.foreco.2022.120419

Corbin, J. D., & Holl, K. D. (2012). Applied nucleation as a forest restoration strategy. *Forest Ecology and Management*, *265*, 37–46. https://doi.org/10.1016/j.foreco.2011.10.013

Geils, B. W., Hummer, K. E., & Hunt, R. S. (2010). White pines, ribes, and blister rust: A review and synthesis. *Forest Pathology*, *40*(3–4), 147–185. https://doi.org/10.1111/j.1439-0329.2010.00654.x

Government of Canada. (2012). Order amending Schedule 1 to the Species at Risk Act. *Canada Gazette Part II*, *146*(14), SOR/2012-2113. Retrieved September 11, 2023, from https://species-registry.canada.ca/index-en.html#/species/1086-748

Hamlin, J., Kegley, A., & Sniezko, R. A. (2011). Genetic variation of whitebark pine (*Pinus albicaulis*) provenances and families from Oregon and Washington in juvenile height growth and needle color. In R. E. Keane, T. D., M. P. Murray, & C. M. Smith (Eds.), *The future of high-elevation, five-needle white pines in Western North America: Proceedings of the High Five Symposium* (pp. 133–139). U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

Hoff, R. J. (1986). *Inheritance of the bark reaction mechanism in* Pinus monticola *infected by* Cronartium ribicola (Vol. 361). USDA Forest Service, Intermountain Research Station, Research Note INT-.

Hoff, R. J. (1992). *How to recognize blister rust infection on whitebark pine*. USDA Forest Service Intermountain Research Station Research Note INT-406. Retrieved September 11, 2023, from https://www.fs.usda.gov/rm/pubs_int/int_rn406.pdf

Hoff, R. J., Bingham, R. T., & McDonald, G. I. (1980). Relative blister rust resistance of white pines. *European Journal of Forest Pathology*, *10*(5), 307–316. https://doi.org/10.1111/j.1439-0329.1980.tb00042.x

Hoff, R. J., Ferguson, D., McDonald, G. I., & Keane, R. E. (2001). Strategies for managing whitebark pine in the presence of white pine blister rust. In D. Tomback, S. F. Arno, & R. E. Keane (Eds.), *Whitebark pine communities: Ecology and restoration* (pp. 346–366). Island Press.

Hoff, R. J., & McDonald, G. (1980a). *Improving rust-resistant strains of inland western white pines*. USDA For. Serv. Res. Pap. INT-245, 13 p. Intermt. For. and Range Exp. Stn. https://doi.org/10.5962/bhl.title.68980

Hoff, R. J., & McDonald, G. (1980b). Resistance to *Cronartium ribicola* in *Pinus monticola*: Reduced needle-spot frequency. *Canadian Journal of Botany*, *58*(5), 574–577. https://doi.org/10.1139/b80-071

Johnson, J. S., & Sniezko, R. A. (2021). Quantitative disease resistance to white pine blister rust at southwestern white pine's (*Pinus strobiformis*) northern range. *Frontiers in Forests and Global Change*, *4*(169), 765871. https://doi.org/10.3389/ffgc.2021.765871

Kegley, A. J., & Sniezko, R. A. (2004). Variation in blister rust resistance among 226 *Pinus monticola* and 217 *P. lambertiana* seedling families in the Pacific Northwest. In R. A. Sniezko, S. Samman, S. E. Schlarbaum, & H. B. Kriebel (Eds.), *Breeding and genetic resources of five needle pines: Genetics, breeding and adaptability, Proceedings of the IUFRO 2.02.15 Working Party Conference*. USDA Forest Service. Rocky Mountain Research Station RMPS-P-32. Fort Collins, CO. 209-225.

Kinloch, B. B. (2003). White pine blister rust in North America: Past and prognosis. *Phytopathology*, *93*(8), 1044–1047. https://doi.org/10.1094/PHYTO.2003.93.8.1044

Kinloch, B. B., Burton, D., Davis, D. A., Westfall, R. D., Dunlap, J., & Vogler, D. R. (2012). Strong partial resistance to white pine blister rust in sugar pine. In R. A. Sniezko, A. D. Yanchuk, J. T. Kliejunas, K. M. Palmieri, J. M. Alexander, & S. J. Frankel (Eds.), *Proceedings of the fourth international workshop on the genetics of host-parasite interactions in forestry: Disease and insect resistance in forest trees* (pp. 80–91). Pacific

Southwest Research Station, Forest Service, U.S. Department of Agriculture.
Kinloch, B. B., Davis, D. A., & Burton, D. (2008). Resistance and virulence interactions between two white pine species and blister rust in a 30-year field trial. *Tree Genetics & Genomes*, *4*(1), 65–74. https://doi.org/10.1007/s11295-007-0088-y
Kinloch, B. B., Parks, G. K., & Fowler, C. W. (1970). White pine blister rust: Simply inherited resistance in sugar pine. *Science*, *167*(3915), 193–195. https://doi.org/10.1126/science.167.3915.193
Kinloch, B. B., Sniezko, R. A., Barnes, G. D., & Greathouse, T. E. (1999). A major gene for resistance to white pine blister rust in western white pine from the western Cascade range. *Phytopathology*, *89*(10), 861–867. https://doi.org/10.1094/phyto.1999.89.10.861
Kinloch, B. B., Sniezko, R. A., & Dupper, G. E. (2004). Virulence gene distribution and dynamics of the white pine blister rust pathogen in western North America. *Phytopathology*, *94*, 751–758. https://doi.org/10.1094/PHYTO.2004.94.7.751
Liu, J.-J., Sniezko, R., Murray, M., Wang, N., Chen, H., Zamany, A., Sturrock, R. N., Savin, D., & Kegley, A. (2016). Genetic diversity and population structure of whitebark pine (*Pinus albicaulis* Engelm.) in western North America. *PLoS ONE*, *11*(12), e0167986. https://doi.org/10.1371/journal.pone.0167986
Lynch, M., & Walsh, B. (2018). *Genetics and analysis of quantitative traits*. Sinauer.
Mahalovich, M. F., Burr, K. E., & Foushee, D. L. (2006). Whitebark pine germination, rust resistance, and cold hardiness among seed sources in the Inland Northwest: Planting strategies for restoration. In L. E. Riley, R. K. Dumoroese, & T. D. Landis (Eds.), *National Proceedings: Forest and Conservation Nursery Associations—2005. Proceedings RMRS-P-43* (pp. 91–101). U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.
Mahalovich M.F., Stritch L. (2013). *Pinus albicaulis*. The IUCN Red List of Threatened Species: e.T39049A2885918. International Union for Conservation of Nature (IUCN). Retrieved September 11, 2023, from https://doi.org/10.2305/IUCN.UK.2013-1.RLTS.T39049A2885918.en
Murray, M. P., & Strong, W. (2021). Disease screening for endangered whitebark pine ecosystem recovery in Canada. *Journal of Ecosystems and Management*, *21*(1), 1–7. https://doi.org/10.22230/jem.2021v21n1a609
Nakagawa, S., & Schielzeth, H. (2010). Repeatability for Gaussian and non-Gaussian data: A practical guide for biologists. *Biological Reviews*, *85*(4), 935–956. https://doi.org/10.1111/j.1469-185X.2010.00141.x
RCoreTeam. (2022). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.
Rochefort, R. M., Howlin, S., Jeroue, L., Boetsch, J. R., & Grace, L. P. (2018). Whitebark pine in the northern Cascades: Tracking the effects of blister rust on population health in North Cascades National Park Service Complex and Mount Rainier National Park. *Forests*, *9*(5), 244. https://doi.org/10.3390/f9050244
Schoettle, A. W., Sniezko, R. A., Kegley, A., & Burns, K. S. (2014). White pine blister rust resistance in limber pine: Evidence for a major gene. *Phytopathology*, *104*(2), 163–173. https://doi.org/10.1094/PHYTO-04-13-0092-R
Schuetzenmeister A., Dufey F. (2022). *VCA: Variance component analysis*. R package version 1.4.5. https://CRAN.R-projects.org/package=VCA
Schwandt, J. W. (2006). *Whitebark pine in peril: A case for restoration*. Report R1–06-28. U.S. Department of Agriculture, Forest Service, Northern Region, Forest Health Protection.
Shepherd, B., Jones, B., Sissons, R., Cochrane, J., Park, J., Smith, C. M., & Stafl, N. (2018). Ten years of monitoring illustrates a cascade of effects of white pine blister rust and focuses whitebark pine restoration in the Canadian Rocky and Columbia Mountains. *Forests*, *9*(3), 138. https://doi.org/10.3390/f9030138
Shoal, R., Aubry, C., & Ohlson, T. (2008). *Land managers guide to whitebark pine restoration in the Pacific Northwest 2009–2013*. U.S. Department of Agriculture, Forest Service, Pacific Northwest Region. 37p.
Smith, C. M., Shepherd, B., Gillies, C., & Stuart-Smith, J. (2012). Changes in blister rust infection and mortality in whitebark pine over time. *Canadian Journal of Forest Research*, *43*(1), 90–96. https://doi.org/10.1139/cjfr-2012-0127
Sniezko, R. A., Danchok, R., Savin, D. P., Liu, J.-J., & Kegley, A. (2016). Genetic resistance to white pine blister rust in limber pine (*Pinus flexilis*): Major gene resistance in a northern population. *Canadian Journal of Forest Research*, *46*(9), 1173–1178. https://doi.org/10.1139/cjfr-2016-0128
Sniezko, R. A., Johnson, J. S., & Savin, D. P. (2020). Assessing the durability, stability, and usability of genetic resistance to a non-native fungal pathogen in two pine species. *Plants, People, Planet*, *2*(1), 57–68. https://doi.org/10.1002/ppp3.49
Sniezko, R. A., Kegley, A., & Danchok, R. (2008). White pine blister rust resistance in North American, Asian and European species—Results from artificial inoculation trials in Oregon. *Annals of Forest Research*, *51*, 53–66.
Sniezko, R. A., Kegley, A., & Savin, D. P. (2017). Ex situ genetic conservation potential of seeds of two high elevation white pines. *New Forests*, *48*(2), 245–261. https://doi.org/10.1007/s11056-017-9579-3
Sniezko, R. A., Kegley, A. J., Danchok, R., & Long, S. (2007). Variation in resistance to white pine blister rust among 43 whitebark pine families from Oregon and Washington—Early results and implications for conservation. In E. M. Goheen & R. A. Sniezko (Eds.), *Whitebark pine: A Pacific Coast perspective* (pp. 82–97). U.S. Department of Agriculture, Forest Service, Pacific Northwest Region.
Sniezko, R. A., & Koch, J. (2017). Breeding trees resistant to insects and diseases: Putting theory into application. *Biological Invasions*, *19*(11), 3377–3400. https://doi.org/10.1007/s10530-017-1482-5
Sniezko, R. A., & Liu, J.-J. (2021). Prospects for developing durable resistance in populations of forest trees. *New Forests*, *54*, 751–767. https://doi.org/10.1007/s11056-021-09898-3
Sniezko, R. A., & Liu, J.-J. (2022). Genetic resistance to white pine blister rust, restoration options, and potential use of biotechnology. *Forest Ecology and Management*, *520*, 120168. https://doi.org/10.1016/j.foreco.2022.120168
Sniezko, R. A., Mahalovich, M. F., Schoettle, A. W., & Vogler, D. R. (2011). Past and current investigations of the genetic resistance to *Cronartium ribicola* in high-elevation five-needle pines. In R. E. Keane, D. F. Tomback, M. P. Murray, & C. M. Smith (Eds.), *The future of high-elevation, five-needle white pines in western North America. Proc High Five Symp RMRS-P-63* (pp. 246–264). USDA Forest Service Rocky Mountain research Station.
Sniezko, R. A., Smith, J., Liu, J.-J., & Hamelin, R. (2014). Genetic resistance to fusiform rust in southern pines and white pine blister rust in white pines—A contrasting tale of two rust pathosystems—Current status and future prospects. *Forests*, *5*(9), 2050–2083. https://doi.org/10.3390/f5092050
Tomback, D. F. (2001). Clark's nutcracker: Agent of regeneration. In D. F. Tomback, S. F. Arno, & R. E. Keane (Eds.), *Whitebark pine communities: Ecology and restoration* (pp. 89–104). Island Press.
Tomback, D. F., & Achuff, P. (2010). Blister rust and western forest biodiversity: Ecology, values and outlook for white pines. *Forest Pathology*, *40*(3–4), 186–225. https://doi.org/10.1111/j.1439-0329.2010.00655.x
Tomback, D. F., Keane, R. E., Schoettle, A. W., Sniezko, R. A., Jenkins, M. B., Nelson, C. R., Bower, A. D., DeMastus, C. R., Guiberson, E., Krakowski, J., Murray, M. P., Pansing, E. R., & Shamhart, J. (2022). Tamm review: Current and recommended management practices for the restoration of whitebark pine (*Pinus albicaulis* Engelm.), a threatened high-elevation Western North

American forest tree. *Forest Ecology and Management*, *522*, 119929. https://doi.org/10.1016/j.foreco.2021.119929

Tomback, D. F., & Sprague, E. (2022). The national whitebark pine restoration plan: Restoration model for the high elevation five-needle white pines. *Forest Ecology and Management*, *521*, 120204. https://doi.org/10.1016/j.foreco.2022.120204

U.S. Fish and Wildlife Service. (2022). Endangered and threatened wildlife and plants; Threatened species status with section 4(d) rule for whitebark pine (*Pinus albicaulis*). *Federal Register*, *87*(240), 76882–76917. Retrieved September 11, 2023, from https://www.federalregister.gov/documents/2022/12/15/2022-27087/endangered-and-threatened-wildlife-and-plants-threatened-species-status-with-section-4d-rule-for

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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