Forest Ecology and Management 520 (2022) 120168

Contents lists available at ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco

Genetic resistance to white pine blister rust, restoration options, and potential use of biotechnology

Richard A. Sniezko[a,*], Jun-Jun Liu[b]

[a] USDA Forest Service, Dorena Genetic Resource Center, 34963 Shoreview Drive, Cottage Grove, OR 97424, USA
[b] Canadian Forest Service, Natural Resources Canada, 506 West Burnside Road, Victoria, BC V8Z 1M5, Canada

ARTICLE INFO

Keywords:
Genetic resistance
Durability
Stability
Usability
Restoration
White pine blister rust
Biotechnology
Cronartium ribicola

ABSTRACT

All nine five-needle white pine species (genus *Pinus*, subgenus *Strobus*, subsections *Strobus* and *Balfourianae)* native to the U.S. and Canada are highly susceptible to white pine blister rust (WPBR), caused by the non-native fungal pathogen *Cronartium ribicola*. WPBR is present within the geographic range of eight of the nine species in the U.S. including the four species also present in Canada, but has not yet been documented in Mexico. Genetic resistance to WPBR has been documented in eight of the white pine species present in the U.S., with extensive work on foxtail pine (*Pinus balfouriana*) just recently started. The development of populations of trees with durable genetic resistance, while also retaining genetic diversity and adaptability, is seen as a fundamental step in restoring white pine species. Major gene resistance (MGR) has been documented in four species, and quantitative resistance (QR) is likely present in all species, but at levels ranging from very low to moderately high. Restoration using seed from WPBR resistant parent trees has been underway for several decades for western white pine (*P. monticola*), sugar pine (*P. lambertiana)*, and eastern white pine (*P. strobus*), and has begun more recently for whitebark pine (*P. albicaulis*) and limber pine (*P. flexilis*). For many of these white pine species, locating additional resistant parents and acquiring more seed will be needed over the ensuing decades. The previous efforts in developing populations of trees with genetic resistance to WPBR has used conventional tree improvement techniques of tree selection and seedling inoculation trials. However, in the future with the continued development of omics resources and tools in white pines, biotechnology has the potential to aid resistance programs. Candidate genes have been identified for host MGR, QR, and disease susceptibility (S) to WPBR, as well as for *C. ribicola* effectors. Marker-assisted selection (MAS) tools developed from MGR-linked genes would be useful to combine MGR and QR, which should improve the potential durability of resistance. Integration of breeding programs with omics information and tools can help pave a road towards improvement of WPBR resistance through biotechnological approaches such as MAS, and genomic selection (GS), or potentially helping to incorporate unique resistance not currently found in North American five-needle white pines. In the near future, these tools could potentially aid in the initial search for candidate trees which would increase the efficiency of developing WPBR resistant populations, as well as defining the extent and distribution of adaptive genetic variation in the species, which will aid in planning restoration efforts.

1. Introduction

Nine five-needle white pine species, (genus *Pinus*, subgenus *Strobus*, subsections *Strobus* and subsections *Strobus* and *Balfourianae*), are native to the U.S, with four of them also native to Canada. These species are long-lived conifers covering a wide geographic range and provide an array of ecosystem services (Tomback and Achuff, 2010). However, all nine of these species are highly susceptible to white pine blister rust (WPBR), caused by the non-native, invasive fungal pathogen *Cronartium ribicola*, and WPBR is present in the geographic range of all but Great Basin bristlecone pine (*P. longaeva*) (Hoff et al., 1980; Stephan, 2004; Kinloch, 2003; Tomback and Achuff, 2010). The infection and mortality from WPBR in some populations can exceed 95 percent. In the three species of highest economic value, western white pine (*Pinus monticola*), sugar pine (*P. lambertiana*) and eastern white pine (*P. strobus*), the high susceptibility led to extensive research and management activities to try to mitigate the impacts of the disease (Maloy, 1997; Geils et al., 2010), including investigations on genetic resistance to WPBR (Hoff and

---

* Corresponding author.
E-mail addresses: richard.sniezko@usda.gov (R.A. Sniezko), jun-jun.liu@nrcan-rncan.gc.ca (J.-J. Liu).

https://doi.org/10.1016/j.foreco.2022.120168
Received 9 December 2021; Received in revised form 3 March 2022; Accepted 13 March 2022

McDonald, 1980; Bingham, 1983; King et al., 2010; Sniezko et al., 2014, 2020).

The research on genetic resistance to WPBR led to the development of resistance breeding programs for the three species of highest economic value (King and Hunt, 2004; Kriebel, 2004; McDonald et al., 2004), and these continue today. More recently, investigations into genetic resistance have begun in the six high elevation five-needle white pine species: whitebark pine (*P. albicaulis*), limber pine (*P. flexilis*), southwestern white pine (*P. strobiformis*), Great Basin bristlecone pine, Rocky Mountain bristlecone pine (*P. aristata*), and foxtail pine (*P. balfouriana*) (Sniezko et al., 2011; Schoettle et al., 2018b; Johnson and Sniezko, 2021), and these species are the primary focus here. A consensus has developed that finding and using genetic resistance within these species will be a key determinant to future successful restoration efforts with North American five-needle white pines (Samman et al., 2003; Schwandt, 2006; Aubry et al., 2008; Tomback et al., 2022, this issue), which strengthens support to continue to examine resistance in these species.

In the Pacific Northwest, the USDA Forest Service's Dorena Genetic Resource Center (DGRC), established in 1966, has had as one of its primary missions the development of resistant populations of five-needle white pine species for reforestation and restoration. Although there are several WPBR resistance programs in North America (King and Hunt, 2004; McDonald et al., 2004; Murray and Strong, 2021; Sniezko et al., 2011), DGRC has the most extensive experience and has evaluated all nine species of these white pines, to varying degrees (Kegley and Sniezko, 2004; Sniezko et al., 2008, 2011, 2014, 2020; Johnson and Sniezko, 2021), while other programs focus primarily on one or two white pine species. Thus, the trials at DGRC, all done under similar protocols and environment, provide a basis to note not only components of resistance within a species, but also to make comparisons among species of the type, level, and frequency of resistance that updates earlier work from others that used more limited sampling (Hoff et al., 1980; Stephan, 2004). Table 1 summarizes some aspects of results of resistance evaluations for the nine species native to the U.S. and three Eurasian species (*P. sibirica, P. armandii*, and *P. peuce*) tested at DGRC, including number of tested parent trees from forest selections, confirmation of any major gene resistance (MGR), and level of survival from quantitative resistance (QR) of open-pollinated half-sib seedling families.

Table 1

White pine blister rust resistance summary from testing at Dorena Genetic Resource Center (MGR = major gene resistance, QR = quantitative resistance).

Species	Number families tested	MGR (Gene)	QR – overall survival of families (%) [c]	QR survival % (family range)	Relative QR Rank
P. lambertiana	>4000	Yes (*Cr1*)	<5	0 to ~ 10	5
P. monticola	>4000	Yes (*Cr2*)	<10	0 to ~ 20 [d]	4
P. albicaulis	>1500	No	<20	0 to ~ 90	1
P. strobiformis	>400	Yes (*Cr3*)	<10	0 to ~ 85	3
P. flexilis	>200	Yes (*Cr4*)	<5	0 to 10	5
P. balfouriana	>150	No	<1	0 to 5	8
P. aristata	~170	No	<60	n.a.	1
P. longaeva	~30	No	<35	n.a.	n.a.
P. strobus	18	No	<5	0 to 10	5
P. sibirica	2	n.a.	98[b]	95 to 100	–
P. armandii	2 bulked lots	Yes	100 [a,b]	–	–
P. peuce	1 bulked lot	n.a.	68.9 [b]	–	–

[a] 3.3% and 9.5% of seedlings had stem symptoms, but survived.

[b] The contribution of QR versus MGR is unknown for the Eurasian species.

[c] Overall survival means are generally for selections in stands with low or unknown level of white pine blister rust infection.

[d] Very few (<5) families out of > 4000 parent trees tested showed higher survival. n.a. Not available.

Classical selective breeding has been the source of developing resistance to date. The main method used for WPBR resistance evaluation has been the inoculation trials of seedling families of parent trees selected in natural stands. These progeny trials are used to evaluate the resistance of parent trees and the trials can take five years or more (Kegley and Sniezko, 2004; Sniezko et al., 2011; Johnson and Sniezko, 2021). The overall process includes selecting parent trees in the field, collecting cones, seed extraction from the cones, growing seedlings from one to three years before inoculation with *C. ribicola*, and subsequently evaluating the seedlings for three to five years for a range of resistance traits (Sniezko et al., 2014; Johnson and Sniezko, 2021).

A significant amount of work on evaluating genetic resistance to WPBR in this group of high elevation white pine species has been underway since an earlier summary was compiled a decade ago (Sniezko et al., 2011). In this paper, we discuss some of the results of resistance testing and comparisons among species for resistance, as well as a summary of the genomics related work undertaken to-date that will help increase understanding of resistance and may provide useful tools for their evaluation in the future. We also provide some thoughts on restoration based on what is known about WPBR resistance in the different species, and briefly discuss potential use of biotechnology to increase the efficacy of developing populations of white pines with genetic resistance.

2. Lessons from western white pine and sugar pine - setting the stage for evaluating resistance in the high elevation white pine species

In forest trees, if resistance is going to be useful, one needs some basic information on the frequency of resistance, the level of resistance, the geographic distribution of resistance, and the type of resistance, as well as whether the resistance is likely to be durable (effective under potential evolutionary changes in the virulence or aggressiveness of the rust pathogen) and stable over varying environmental conditions, including a changing climate.

The long-term programs with western white pine and sugar pine provide some guidance for examining resistance in the six high elevation species and on the potential utility and limitations of resistance. For both species, seedling families of>4000 parent trees have been tested for WPBR resistance over five decades at DGRC. The work with these species has documented the presence of both MGR, conditioned by a single dominant R gene, *Cr1* in sugar pine and *Cr2* in western white pine, as well as QR (Kinloch et al., 1999; Kinloch and Dupper, 2002; Sniezko et al., 2014; Weiss et al., 2020). The work with these two species has also noted that MGR appears to be less durable than QR due to presence of virulent pathotypes (Kinloch et al., 2004, 2008; Sniezko et al., 2020).

Resistance in both sugar pine and western white pine is relatively rare (Kinloch et al., 2003, 2018; Sniezko et al., 2008; King et al., 2018). In general, the level of QR in western white pine is greater than that in sugar pine (Kinloch et al., 2008; Kegley and Sniezko, 2004; Sniezko et al., 2008, 2020), but in both species, open-pollinated half-sib seedling families from field selections generally show<10 percent survival (Table 1, Kegley and Sniezko, 2004; Sniezko et al., 2008). Breeding to increase the level of QR is underway and shows great promise (Kinloch et al., 2012; Sniezko et al., 2014). Seed orchards of both species have been established and are supplying seed for reforestation and restoration, but future orchards using selections from the ongoing breeding work will provide higher levels of resistance.

For families with MGR, 50 percent or more of seedlings may be canker-free in absence of a virulent pathotype of rust. However, for western white pine *Cr2*, a virulent *vcr2* pathotype of the rust is present in much of western Oregon, rendering MGR ineffective in those areas (Kinloch et al., 2004; Sniezko et al., 2020). For sugar pine *Cr1*, the virulent *vcr1* pathotype has been documented only in a few locations in California (Kinloch et al., 2004). By contrast, the families with highest level of survival from QR may have a combination of some canker-free seedlings and some seedlings with stem symptoms, and often many of these are small bark reactions or somewhat larger inactive cankers (Hoff and McDonald, 1980; Bingham, 1983; Kinloch et al., 2012; Sniezko et al., 2014). In the QR families, the progress of the rust appears to be slowed, with some seedlings showing stem symptoms later than the susceptible control and having fewer stem symptoms (Hoff and McDonald, 1980; Kegley and Sniezko, 2004; Sniezko et al., 2014).

Similar to both sugar pine and western white pine, eastern white pine appears to have only a low level of QR (Table 1) (Hoff et al., 1980; Stephan, 2004). However, for eastern white pine, there was a relatively high percentage of partial bark reaction in the tests at DGRC (Sniezko et al., 2008), which could suggest that field resistance could be higher. In an early international test series eastern white pine from the U.S. was also shown to be very susceptible, although seedlots collected from the species grown in Germany showed much more resistance than those from the U.S. (Stephan, 2004), likely from natural selection in presence of the rust. More recent work on eastern white pine elsewhere provides somewhat more encouraging news on resistance (Pike et al., 2018).

3. Resistance testing of high elevation white pine species at DGRC

Testing at DGRC for WPBR resistance in the six high elevation species is relatively recent, beginning in 2001 with southwestern white pine and 2002 with whitebark pine. In many cases, detailed summary of test results is still pending. However, because all testing has been at DGRC under similar protocols, there is a unique opportunity to begin to compare the type and level of resistance found among the species (Table 1), which expands on earlier reports by others (Hoff et al., 1980; Stephan, 2004). The whitebark pine trials have involved primarily parent trees selected in the Pacific Northwest U.S. (Oregon and

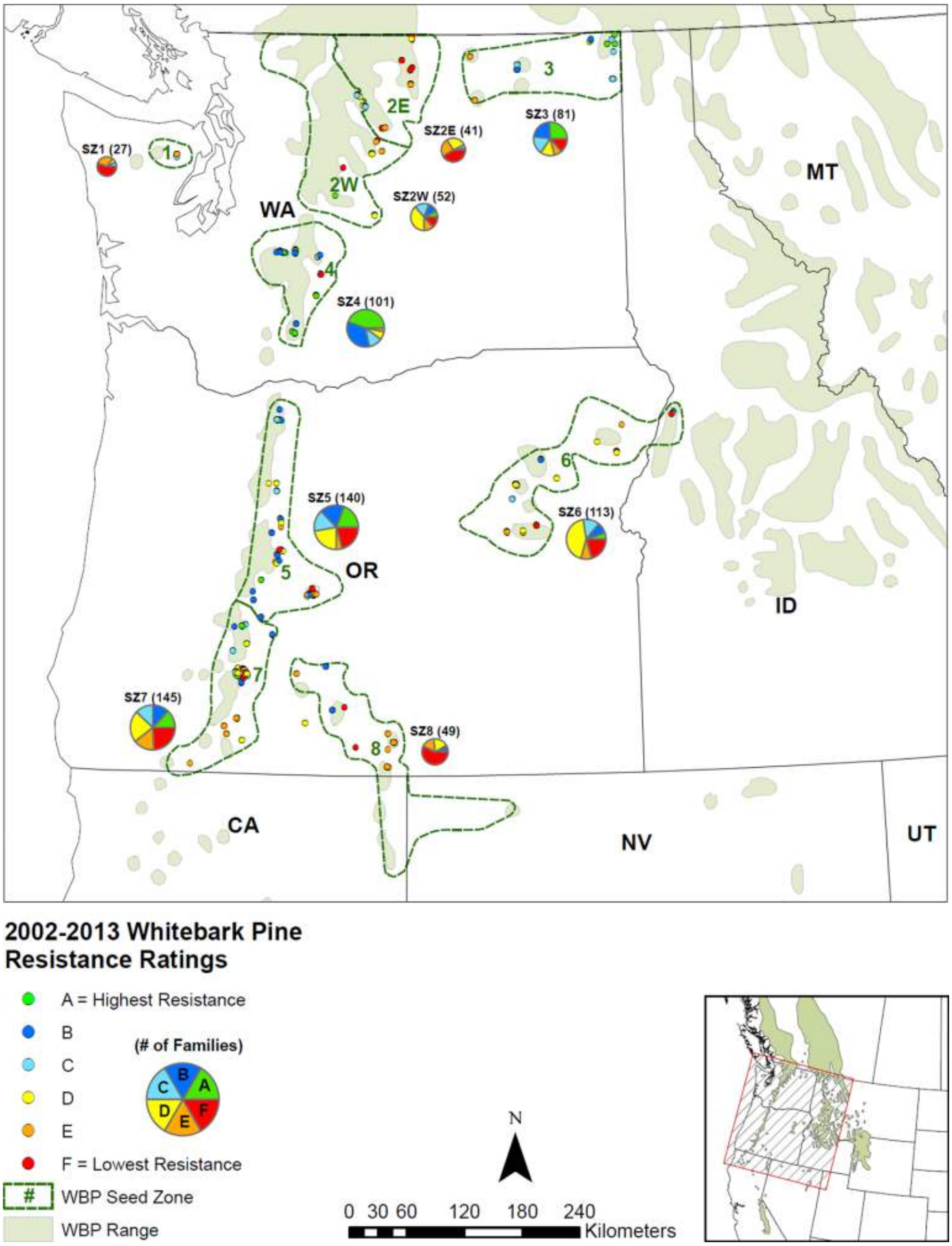

Fig. 1. Geographic variation in white pine blister rust resistance for the first 749 whitebark pine (*Pinus albicaulis*) parent trees that were evaluated in seedling inoculation trials at Dorena Genetic Resistance Center. Trials were sown from 2002 to 2013, and parents tested represent all nine seed zones in Oregon and Washington. These interim ratings reflect primarily the percentage of seedlings within each family that have stem symptoms ~ 1-year post-inoculation, one measure of resistance, and percent survival for the two earliest trials. Each parent is assigned a A to F letter grade, with A indicating the highest level of resistance in a trial (often < 50 percent of seedlings cankered) and F generally indicating 95–100 percent of seedlings cankered.

Washington), while the other species have involved partners from other areas in the western U.S., Mexico, and Canada.

3.1. Whitebark pine

Whitebark pine is the most extensively tested species of the high elevation white pines with several programs underway in the U.S. and Canada (Murray and Strong, 2021; Sniezko et al., 2011). In the DGRC program (Table 1), seedling families of>1500 parent trees have been included in testing at DGRC since a 2004 inoculation of seedlings sown in 2002. Trials usually include ~ 120 seedling families and trials have been sown in 2002, 2004, 2005, 2007, and in almost every year since 2011. The seedling families tested come from parent trees in all nine seed zones designated for this species in Oregon and Washington (Fig. 1) (Aubry et al., 2008). Several susceptible and resistant families, documented in earlier trials, are included as controls in newer trials.

Early results of some of earlier trials indicate that although whitebark pine is very susceptible, there is a very wide range in resistance among seedling families and geographic areas (Figs. 1, 2, 3, 4) (Sniezko et al., 2007, 2008, 2018; Kegley et al., 2012). Most seedling families have <10% survival in testing, but the most resistant families have 30 to 90 percent survival (Figs. 2, 3, 4; unpublished data), and the frequency of resistant families can vary dramatically by geographic area (Fig. 1). At this stage QR is documented, but MGR has not yet been documented in whitebark pine.

Resistance can encompass an array of traits and effectiveness, including fewer needle spots, fewer stem symptoms, later developing stem symptoms, bark reactions, stem-symptom-free seedlings, and higher survival (Sniezko et al., 2007, 2011; Kegley et al., 2012). The percentage of seedlings with early stem symptoms varies widely by family and by geographic area (Fig. 1) (Sniezko et al., 2018).

One question that arises is whether the genetic variation in the rust can influence results. In an early trial at DGRC two geographic sources of rust were used, one from western Oregon with *vcr2* rust source and the second from eastern Oregon with *AVCr2* rust source. The source of rust used had little impact on relative ranking of families for resistance, but further trials will be needed to discern whether levels of resistance can be impacted by different races of the pathogen (Fig. 3) (Sniezko et al., 2007, unpublished). Also notable in this trial is the relatively low level of resistance of a bulked seedlot from Shoshone National Forest in testing at DGRC (Fig. 3). The Shoshone NF seedlot was widely used in some early sowings for restoration in the Northern Rockies (Mahalovich et al., 2006). There have been relatively few common seedlots tested between different programs, but in two separate trials of seedlots tested at both DGRC and in British Columbia, the resistance results are similar, suggesting little or no impact of different source of rust or environmental condition (Reid, 2020; Sniezko unpublished data). In one case, the seedlings of 81 families tested at DGRC were planted in British Columbia in a nursery setting and inoculation occurred from natural dissemination of rust spores from *Ribes* planted near the trial (Reid, 2020), while the other trial, involving 10 families, an inoculation system similar to that of DGRC was used (Murray and Strong, 2021).

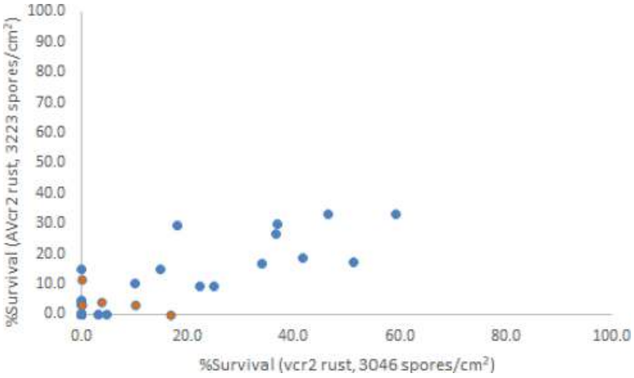

Fig. 3. Variation in whitebark pine survival (5 years post-inoculation) for seedlots inoculated with populations of *Cronartium ribicola*. A total of 43 seedling families and a bulked seedlot from Shoshone NF (Shoshone NF = orange, and there are 5 reps of this lot in each block). Overall survival averaged 9.1% (using *vcr2* rust source, western Oregon) and 6.3% (*AVCr2* rust source). See Sniezko et al., 2007 for trial background and survival two years post-inoculation.

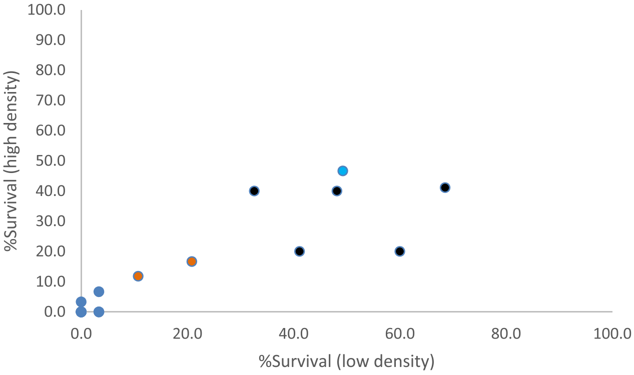

Fig. 4. Impacts of *Cronartium ribicola* inoculum density on variation in whitebark pine survival four years post-inoculation. A total of 18 seedling families and seedlings from two nursery sources of a Shoshone NF bulked lot are included. Inoculum densities: Low density = 1017 spores/cm^2; High density = 4968 spores/cm^2. Seedlots: Shoshone NF bulked lot = orange; Colville NF families = black; Mount Rainier NP family = light blue; Deschutes & Fremont NF families = dark blue).

Fig. 2. Large variation in survival of whitebark pine seedling families in SY2016 trial following inoculation with white pine blister rust. Families are planted in 10-tree row plots.

Another question that arises is whether a large increase in inoculum density can have a negative impact on survival. In a small trial of inoculum density sown in 2002, an increase in inoculum density increased the number of needle spots (first symptoms of infection), the number of stem symptoms, and the percentage of trees with stem symptoms, but decreased the percentage of trees alive with stem symptoms and the percent survival (Table 2), although the differences were not statistically significant (unpublished data). And even at the high inoculum density, the top five families had survival ranging from 20 to 46 percent (Fig. 4). A follow-up study is currently underway at DGRC to examine the susceptibility of both resistant and susceptible seedling families under a much wider range of inoculum density.

Table 2
Impact of inoculum density on resistance related traits. Seedling trial of whitebark pine was inoculated with white pine blister rust in 2004 (following similar protocols to Sniezko et al., 2007). Eighteen families and two groups of seedlings from a Shoshone NF bulked seedlot (sown at different nurseries) are included.

	Inoculum Density	
	Low (1017 spores/cm^2)	High (4968 spores/cm^2)
% Infection	100	100
% Stem Symptoms (SS)	88.0	93.0
% SS Alive	11.6	9.2
Rust Survival %	17.1	12.3
# Needle Spots	7.1	21.9
# Stem Symptoms2	8.0	15.6

3.2. Southwestern white pine

Seedling families from over 400 parent trees have been included in seedling inoculation trials since 2001, with the majority of families in tests beginning in 2014 (Fig. 5). The recent tests, still in progress, include nearly the entire range of the species, both its U.S. distribution as well as much of its large range in Mexico. Both MGR and QR have been documented in this species (Kinloch and Dupper, 2002; Sniezko et al., 2008; Johnson and Sniezko, 2021).

A summary of the 2009 trial, involving 40 half-sib families from parent trees selection from three National Forests in New Mexico, provides some information on both the level and frequency of QR. Seedling families in this trial varied widely in level of QR, with survival ranging from 0 to > 84%. Ten percent of the parents selected from the two stands with little WPBR (essentially random selections) showed moderate levels of QR (Johnson and Sniezko, 2021). In contrast, nearly all the seedling families from canker-free parent trees in the highly infected Bradford Canyon stand (~90 percent of stand infected) from the Lincoln National Forest showed moderate levels of QR (and averaged 40% survival versus < 10% survival of the random selections from the other two National Forests), but the level varied by family (Johnson and Sniezko, 2021). As suspected, there is higher efficiency of making selections for testing from stands with high infection levels, if available; a similar result was also previously shown in whitebark pine (Hoff et al., 2001). Nearly 71 percent of the surviving trees had stem symptoms but were bark reactions or inactive cankers (Johnson and Sniezko, 2021). Southwestern white pine has more resistance than seen in sugar pine and limber pine, which was also found to be the case in the early IUFRO trials (Hoff et al., 1980; Stephan, 2004). The level of survival from QR in the best families is among the highest reported to date in any North American white pine species. Seed from the parent trees with the highest QR level or with MGR could be immediately useful for reforestation, without further breeding.

It is also notable that both MGR and QR have been documented in the Bradford Canyon stand on the Lincoln National Forest where MGR was first documented and thus some natural pyramiding of the two types of resistance is likely (Johnson and Sniezko, 2021; Sniezko et al., 2008). Summaries of the larger more recent trials are beginning and should provide more information on the both the frequency and geographic variation of both MGR and QR for this species. At this stage, there is no evidence that virulent pathotypes to MGR have arisen in the range of this species. The first field trials to monitor resistance over time have been established in New Mexico (Kristin Waring, personal communication) and a smaller one in Oregon (Fig. 5d).

3.3. Limber pine

Testing of limber pine resistance at DGRC began in 2003 with seedlings from a bulked seedlot (Jacobi et al., 2018) and has since expanded to include hundreds of seedling families from populations in the U.S. and Canada (Fig. 6). Most of the parent trees tested from U.S. sources are from areas with little or no WPBR, while most of the Canadian parent trees are from areas with very high levels of blister rust.

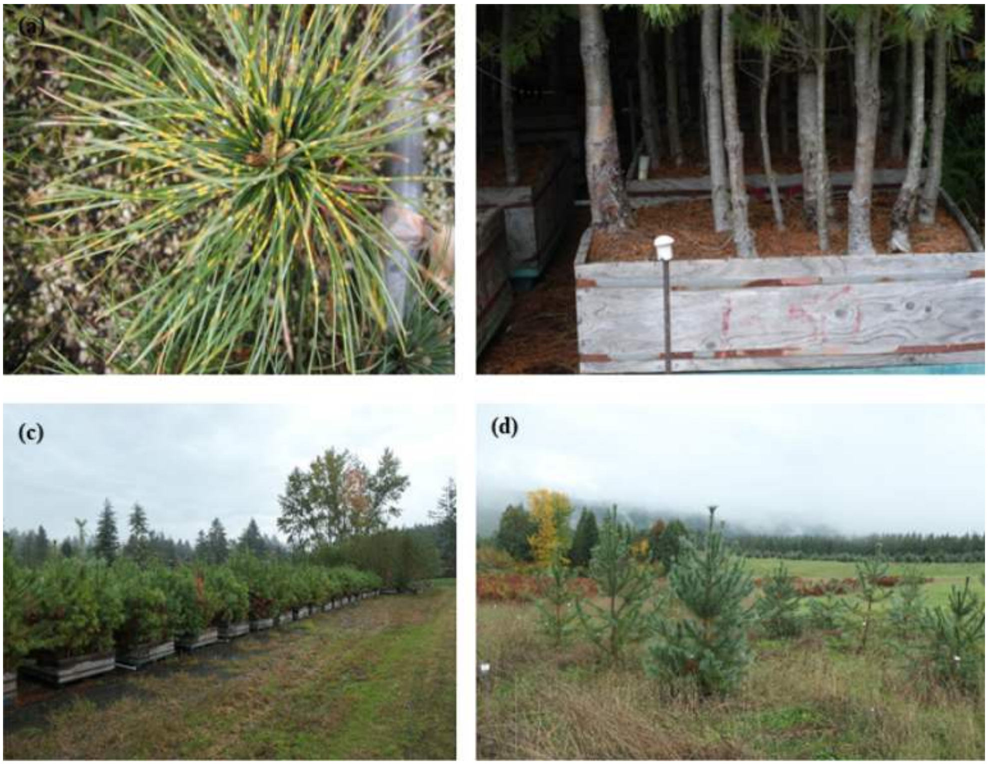

Fig. 5. Southwestern white pine (*P. strobiformis*) white pine blister rust resistance trials at DGRC. (a) Hundreds of needle spots on a seedling in sow year 2009 test, (b) resistant survivors in the 2009 test with some seedlings canker-free, some with bark reactions or healed cankers, and a few with active cankers, (c) Sow year 2017 trial in 2021, three years post-inoculation, with heavy mortality expected in the next year from rust, (d) small field trial in Oregon at Tyrrell Seed Orchard.

Fig. 6. Limber pine (*P. flexilis*) white pine blister rust resistance trials at DGRC. (a) Prairie Bluff#2, a MGR family, with high survival in 2014 trial, (b) heavily cankered seedling in 2014 trial, (c) 2019 trial in fall 2021, (d) cankered seedling in fall 2021 in a trial inoculated in early fall 2020.

MGR has been documented in limber pine in both U.S. and Canadian populations (Schoettle et al., 2014; Sniezko et al., 2016). In two small trials initiated in 2014, that included parent tree selections from Oregon and Alberta, all seedling families were very susceptible, except one family from Alberta, from parent Prairie Bluff #2 (PB2) which was documented as having MGR (Fig. 6). A second family in those trials, PB3, had a few canker-free seedlings, likely the result of pollination with PB2 or another parent with MGR (Sniezko et al., 2016, Sniezko, unpublished). A preliminary summary of a test of 74 limber pine families from the southern Rockies documented only a very low level of QR in non-MGR families (Schoettle et al., 2018a). The very low level of QR documented to this stage in limber pine appears to be similar to the low levels in sugar pine, and among the lowest of species tested (Table 1). This low level of resistance was also noted in the early IUFRO trials (Hoff et al., 1980; Stephan, 2004). The more recent trials of > 180 families from canker-free parent trees in heavily infected stands from Alberta should provide more information on the potential upper level of QR and its frequency, at least for northern populations (Fig. 6) (Sniezko et al., 2022a).

No virulent pathotypes for MGR in limber pine have currently been confirmed, but investigations on one potential source in Alberta are underway (unpublished data). In addition, some of the MGR seedling survivors from the 2014 test have been planted in Alberta and will serve as sentinels to see if a pathotype virulent to MGR is present or arises in the future. The MGR parent trees themselves will also serve as sentinels. Restoration plantings have begun in Alberta (Krakowski et al., 2022).

Currently, seeds from MGR parent trees are the best source of resistance to use for restoration in areas of concern. The long-term durability of MGR is uncertain, but it may continue to have some utility even if virulence develops (Sniezko and Liu, 2021). Breeding of parent trees with QR or the selections from the most resistant seedlings surviving in resistance trials can be used to increase resistance, but this will take significant time and resources.

3.4. *Foxtail pine*

Testing of foxtail pine began at DGRC in 2014 (Fig. 7). In this trial of 21 seedling families, 100 percent of the 705 seedlings were cankered and only two seedlings survived (unpublished data). Concurrently with the foxtail pine trial, there were seedling trials of limber pine, whitebark pine, southwestern white pine and western white pine, all of which showed higher survival than foxtail pine (unpublished results). Four of the species in the 2014 trials, including foxtail pine, were inoculated together, with one half of the seedlings inoculated in one week and the remaining seedlings for each species inoculated in the following week. The western white pine was inoculated a week earlier than the first of the two trials that included foxtail pine. The results from the 2014 trial suggests that foxtail pine may be the most susceptible of all the nine species native to the U.S. (Table 1). This result concurs with results of one of the two previous IUFRO trials, but the trial in Germany indicated

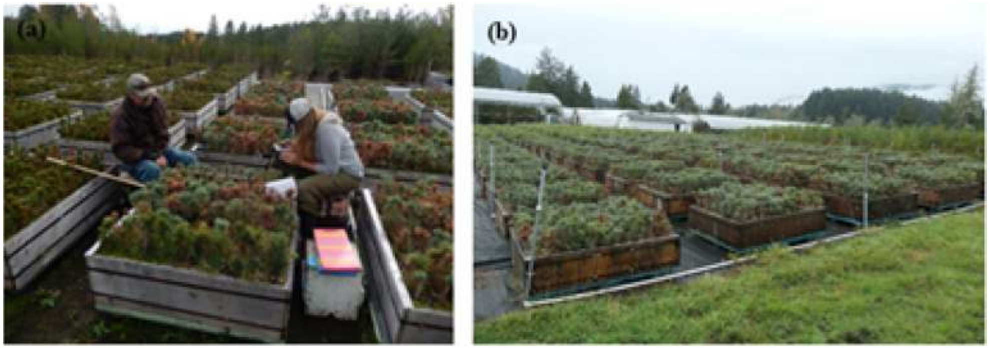

Fig. 7. Foxtail pine white pine blister rust resistance trials. (a) Assessing 2014 trial one year after inoculation, (b) 2019 trial one year after inoculation with mortality just beginning.

that some variation was present among the four sources tested (Hoff et al., 1980; Stephan, 1986, 2004).

Additional trials are underway to search for resistance to WPBR in foxtail pine (Sniezko et al., 2022b). A trial initiated in 2018 is examining whether resistance can be detected with a lower spore density during inoculation. A larger trial initiated in 2019 is testing a range-wide collection of half-sib families from 150 parent trees (Fig. 7). Over the next three years, these trials will provide information on both the frequency and level of resistance in this species as well as whether it varies between the two subspecies of foxtail pine. Concurrently, as part of a collaborative effort, a subset of the families in testing at DGRC are also in tests in Placerville, California (John Gleason, personal communication) and will provide resistance data from inoculation under different environmental conditions. Two small field trials have also been established with a subset of the families that are also in the inoculation trials and over the next decade results will help validate the results of the seedling inoculation trials.

3.5. Rocky Mountain bristlecone pine and Great Basin bristlecone pine

Testing of Rocky Mountain bristlecone pine for resistance at DGRC began in 2003 with seedlings from a bulked seedlot. In the 2003 trial three inoculum densities were used to infect seedlings, and results indicated that the level of resistance (survival) decreased as the level of inoculum increased, from 42 to 23 to 13% (Jacobi et al., 2018). This was in contrast to limber pine, also in the 2003 trial, where a higher inoculum density did not result in higher mortality. The resistance in limber pine was likely due to MGR, which would not be expected to be affected by inoculum density, while the resistance in bristlecone pine is likely QR and shows a substantial increase in mortality with increasing inoculum density. Even at the high inoculum level, survival was 13% in this seedlot, which would still rank it among the most resistant of the North American species.

Small early studies by others indicated that Rocky Mountain bristlecone might have the highest level of resistance among the North American white pine species (Hoff et al., 1980; Stephan, 2004), although some of the seedlots tested may have been Great Basin bristlecone pine since no taxonomic division had been made at that time and some seed collections were made in the range of what is now Great Basin bristlecone pine. A more complete summary of other trials with Rocky Mountain bristlecone pine at DGRC is pending, but initial observations suggest that resistant phenotypes similar to those found in other species are present and that the resistance varies by population, with survival of families varying from 0 to 92 percent (Table 1, Fig. 8) (Schoettle et al., 2012, 2018b). The resistance levels in inoculation trials of this species are encouraging, however results from the Jacobi et al. (2018) trial suggest that more information on resistance under high spore densities and from field sites with high disease pressure will be needed to more fully understand the efficacy of resistance in this species. This species is notable for the presence of resin dots on its foliage (Fig. 8c), but its impact on resistance to WPBR is currently uncertain. Only a small number of seedlots from Great Basin bristlecone have been tested and results are pending, but it appears to have family variation in resistance (unpublished data).

Fig. 8. Rocky mountain bristlecone pine (*P. aristata*) (a) White pine blister rust inoculation trial, (b) bark reaction on inoculated seedling, (c) abundant ‘resin dots’ on foliage that can be common on this species.

4. Field trials to validate WPBR resistance

Seedling inoculation trials provide a relatively efficient method for testing progeny of many parent trees in a relatively short period of time for WPBR resistance. However, the inoculation trials are on young, well-watered and well-fertilized seedlings and at a low elevation environment. As with western white pine and sugar pine, confirmation of the efficacy of resistance in these trials will be needed and field trials underway for some of the species will serve this purpose (Kinloch et al., 2008; Sniezko et al., 2020). The field trials also serve to monitor for durability and stability of MGR and QR (Sniezko et al., 2020; Sniezko and Liu, 2021).

The most extensive genetic field trials for the six high elevation species discussed here are established for whitebark pine. In the Pacific Northwest, trials with seedling families from both susceptible and resistant parent trees have been established. At Crater Lake National Park, six restoration plantings have been established since 2009, and in these trials, seedlings have been individually tagged. Assessments over time as rust infection becomes common will permit comparison of field results with seedling screening results (Fig. 9). However, infection is still very low (<10 percent) and it can take a decade or more to reach moderate or high infection levels even for the susceptible control family. A number of other trials have also been established in the Pacific Northwest including a provenance trial series, that includes 81 seedlots from the screening program at DGRC (Cartwright, 2018; Cartwright et al., 2022, this issue); and three field trials in eastern Washington (Omdal et al., 2018). Results from long-running field trials of western white pine and sugar pine suggest that the seedling screening will provide resistance that is effective under field conditions (Kinloch et al., 2008; Sniezko et al., 2020).

The parent trees of each of the species rated for resistance in seedling trials can also be useful for documenting the efficacy of the screening trials as well as for monitoring durability of resistance. For example, for whitebark pine, >1500 parent trees have been tested for resistance at DGRC and monitoring both those rated as resistant or susceptible can provide information. The susceptible parent trees help monitor for any increase rust infection in an area, while monitoring the resistant parent trees will provide documentation to help verify results of seedlings trials as well as monitoring for durability, and for stability of WPBR resistance under a changing climate. For example, cankers noted on one putative MGR limber pine parent in Alberta, Canada suggest a genetic variant with virulence to limber pine MGR may have arisen.

5. Omics advances toward biotechnological improvement of resistance to WPBR

Traditional tree improvement involving selection and seedling testing can be effective, but advancing technologies may permit more efficient development of resistant populations or new paths to adding resistance genes if the efforts from traditional tree improvement are insufficient. Integration of advanced omics resources (genomics, transcriptomics, proteomics, metabolomics, phenomics) and tools into traditional breeding practices has great potential to develop novel resistance breeding strategies. With application of next generation sequencing (NGS) and high throughput genotyping technologies, omics researchers of white pines have opened an avenue for biotechnological improvement of host genetic resistance to WPBR. MGRs (*Cr1*-*Cr4*) have been documented in four white pine species while QR genotypes have been documented in eight of nine white pine species in resistance programs using seedling inoculation trials (Table 1). Identification of resistance (R) genes and elucidation of molecular defense mechanisms are key steps toward development of biotechnological strategies and tools, such as those for marker-assisted selection (MAS), genomic selection (GS), and introgression of novel R genes.

If the pathogenic threat to a species is very high and it has little or no genetic resistance then biotechnology offers some options that can be considered (National Academies of Science, Engineering and Medicine, 2019). For a species like foxtail pine where resistance may be very rare, either genetic engineering or genome editing is promising for introgression of novel R genes, but requires a selection of R candidate targets based on discovery of novel genes from omics studies. Discovery of candidate genes for MGR, QR, and susceptibility (S) to WPBR depends on the dissection of genomic architectures underlying host disease resistance in white pines, which is achievable through various omics approaches, including genomics, metagenomics, transcriptomics, proteomics, and phenomics (Sniezko et al., 2014). Here we give an overview on omics-related research of white pine species reported in the past decade .

Genomic resources, including seedling families/populations for genetic mapping, genome sequences, transcriptome dynamic profiles, datasets of genome-wide DNA variations (mainly single nucleotide polymorphisms-SNP), genetic maps, and candidates for MGR, QR and S genes, have been developed in several white pine species through various omics approaches in the past decade. Availability and continuous accumulation of these resources and tools will facilitate breeding of WPBR resistance by identification of candidate genes and development of MAS and genome selection tools. In addition, if durable resistance was lacking, genetic modification through engineering or genomic editing may be an option, with due thought to the various considerations (National Academy of Sciences, Engineering and Medicine, 2019).

5.1. Sequences of whole genomes and transcriptomes enabled better understanding WPBR resistance

Whole genome sequencing (WGS) using various NGS-based technologies has led to availability of the sugar pine genome sequence, which was followed by gene prediction and functional annotation across the whole genome, highlighting the genomic architectures and features with putative involvement in disease resistance (Stevens et al., 2016). Two high elevation white species, whitebark pine and Great Basin bristlecone pine, are subject to WGS (Dave Neale, personal communication). Availability of genome sequence drafts of several white pine

Fig. 9. Whitebark pine (*P. albicaulis*) 2009 restoration planting and genetic trial at Crater Lake National Park. (a) Trees in summer 2020, (b) branch of one tree infected with white pine blister rust in summer 2020.

species in coming years will allow comparison of the whole genomes of closely related white pine species, helping elucidate distinct genomic components and interaction networks for genetic resistance with variable levels among them.

Transcriptome analysis started in sugar pine by Sanger-sequencing (Lorenz et al., 2012). More recently, transcriptomes were profiled by RNA-seq in at least six of nine native North American while pines. They included sugar pine (Gonzalez-Ibeas et al., 2016), western white pine (Liu et al., 2013b), eastern white pine (Hwang et al., 2021), whitebark pine (Liu et al., 2016b), limber pine (Liu et al., 2016a), and southwestern white pine (Jin et al., 2021). Comparative transcriptome profiling identified defense-related genes with transcript expression regulated in defense response, or with DNA variations between different genotypes of the same species (Liu et al., 2013b, 2016b, 2017b), as well as among different species of white pines (Baker et al., 2018), shedding light on molecular white pine-blister rust interactions.

5.2. SNP datasets applied for genetic mapping, genetic diversity analysis, and landscape genomics

SNPs were first detected by Sanger sequencing of PCR fragments, and used for construction of genetic maps in sugar pine (Jermstad et al., 2011) and association mapping of western white pine QR-related traits (Liu et al., 2011). Later, genome-wide SNPs of functional genes were detected by RNA-seq through comparative transcriptomic studies between different genotypes (Liu et al., 2014). Using SNP markers within functional genes, genetic maps were constructed for *Cr2*, *Cr3*, and *Cr4* (Liu et al., 2016a, 2017a, 2021d), as well as for analysis of whitebark pine genetic diversity with linkage to QR-related traits (Liu et al., 2016b).

Other NGS-based approaches were also used for SNP genotyping in white pine populations by reducing the complexity of white pine genomes. For example, exome-sequencing was used for landscape genomics studies in whitebark pine (Syring et al., 2016) and construction of high density genetic maps in limber pine (Liu et al., 2019). Double digest restriction-site associated DNA sequencing (ddRADseq) was used to detect genome-wide SNPs for constructing foxtail pine genetic maps (Friedline et al., 2015) and for landscape genomics studies in limber pine and southwestern white pine (Menon et al., 2018).

As large sets of SNPs were in-silico mined in different white pine species by various NGS approaches and a part of them was validated, in the future, SNP arrays with multiple white pine species sources can be developed and shared for different work objectives. Ultimately, these SNP resources and genetic maps constructed with SNPs will enhance white pine breeding through their continuous applications to understand genomic basis for WPBR resistance, as well as the landscape-scale patterns of genetic variation and potential responses to climate change.

5.3. MGR and QR candidate genes identified for biotechnological improvement of WPBR resistance

MGR candidate genes for *Cr1*-*Cr4* were determined by comparative genetic mapping, with their successful localization on *Pinus* consensus linkage groups (LGs) in four white pine species, including two economic species (sugar pine *Cr1* and western white pine *Cr2*) and two high elevation species (southwestern white pine *Cr3* and limber pine *Cr4*). *Cr1* and *Cr2* were anchored on the Pinus consensus LG-2 and LG-1 (Jermstad et al., 2011; Liu et al., 2017a), respectively. *Cr3* and *Cr4* appear to be the same orthologous R locus and anchored on LG-8 (Liu et al., 2016a, 2021d). Plant MGRs are typically mediated by nucleotide-binding site leucine-rich repeat (NLR) proteins. A large number of NLR genes were uncovered and mapped through Sanger-sequencing, WGS, RNA-seq, targeted gene sequencing, and exome-seq-based genetic mapping in various white pine species (Liu and Ekramoddoullah, 2003, 2007, Liu et al., 2019, 2021c; Weiss et al., 2020). WGS and genetic mapping determined NLR genes as positional candidates of *Cr1*-*Cr4* (Stevens et al., 2016; Liu et al., 2020b, 2021c, 2021d).

These positional NLR genes of *Crs* provide candidates for functional verification. All available evidence demonstrated that *Cr1*, *Cr2*, and *Cr3*/*Cr4* might have independently evolved with convergent resistance to WPBR, probably without evolutionary arms races with *C. ribicola* at all because it was introduced to North America only about 100 years ago. Low frequency of all four *Crs* across the species' landscapes also suggests they may have some fitness cost as that observed for most of plant R genes (Barabaschi et al., 2020). Corresponding orthologous loci may be present in other white pine species but only with susceptible alleles (*cr1*-*cr4*). Thus, genomic manipulation of corresponding orthologous *cr*-alleles provides another potential avenue to enable introgression of all *Crs* genes into one white pine species by biotechnological manipulation of the corresponding *cr*-alleles.

QR candidate genes were identified by genetic mapping, association studies and investigations of molecular WP-BR interactions. Association and QTL mapping detected several NLR and defense-related genes with genetic contribution to QR against WPBR in sugar pine (Vázquez-Lobo et al., 2017; Weiss et al., 2020) and western white pine (Liu et al., 2011, 2013a, 2013c). Of those defense-responsive genes, several of them, such as those encoding anti-microbial protein and pathogenesis-related proteins, were confirmed to be real QR genes with functional contribution to resistance against *C. ribicola* and other fungal pathogens (Zamany et al., 2011; Liu et al., 2021a). Over-expression of white pine anti-microbial genes has been demonstrated to enhance disease resistance to fungal pathogens in transgenic plants (Verma et al., 2012). Following successful verification of candidate genes' function, candidate genes can be over-expressed to get a desired resistant phenotype in white pines by biotechnological engineering.

Whitebark pine genes involved in induced systemic resistance (ISR) were detected by exogenous jasmonate treatment followed by RNA-seq, revealing over 50% of *C. ribicola*-responsive genes overlapped with ISR-related genes by Blast analysis (Liu et al., 2017b), suggesting that methyl jasmonate (MeJA) may be a promising tool for WPBR management in whitebark pine. Consistently, non-synonymous SNPs of defense-responsive genes clearly distinguished whitebark pine populations into distinct genotypic groups associated significantly with phenotypes as related to QR to *C. ribicola* (Liu et al., 2016b), suggesting that populations differ in their resistance (Table 1).

A recent study shows the EDS1–PAD4–ADR1 node is a convergence point linking both pattern-triggered immunity (PTI) and effector-triggered immunity (ETI) in defense signaling cascades for conferring pathogen immunity (Pruitt et al., 2021). The crosstalk between PTI-mediated QR and ETI-mediated MGR is essential to confer effective disease resistance. Once this crosstalk is confirmed between MGR and QR to WPBR in white pines, engineering plant immune networks (Ngou et al., 2022) will provide another biotechnological strategy in white pine breeding for robust and durable protection against diverse pathogens/pests, including *C. ribicola*.

5.4. MAS tools developed for selecting, predicting, and pyramiding R genes

MAS tools for MGR selections of *Cr2* and *Cr3*/*Cr4* were developed as TaqMan arrays or similar genotyping platforms using their NLR positional candidates (Liu et al., 2019, 2020a, 2020b, 2021d). These developed MAS tools were effective for selection of R genes in the progeny of MGR segregating families in western white pine, limber pine, and southwestern white pine (Liu et al., 2020a, 2021d). The MAS prediction accuracy was also evaluated by comparing the genotyping data from the SNP assays with the phenotype data from rust inoculation in the breeding programs, and it proved that the MAS tool was also very effective for *Cr2*-prediction in the BC wild stands prior to their seed collection and progeny testing (Liu et al., 2020b). Because MGR frequencies are low across the species' landscapes, wide application of these MAS tools will increase the efficiency of making selections of wild parental trees to include in building resistant populations.

DNA markers tightly linked to QTLs are very useful to develop MAS tools for selection of complex quantitative traits in crop breeding. Association study (especially genome-wide association study-GWAS) and genomic selection have become available for identifying QTLs without controlled cross-pollination. GWAS identified four QTLs and multiple SNPs associated with sugar pine QR to WPBR with effect sizes ranging from 6.2% to 14.5% (Vázquez-Lobo et al., 2017; Weiss et al., 2020). In western white pine, DNA and isoform variations of PR10 proteins (PmPR10-2 and PmPR10-3), anti-microbial protein (PmAMP1), and chitinase (PmCh4B) were associated with QR to WPBR in seed families from BC, WA and OR with similar effect sizes (5% to 22 %) by Sanger-sequencing and proteomics studies (Liu et al., 2005, 2011, 2021a). Small effect sizes of individual QR-associated markers suggest many genes may contribute to QR in the WPBR pathosystems, probably with complex environmental interactions. Of the high elevation white pines, whitebark pine QR genotypes were distinguishable from susceptible genotypes by using SNPs of a set of genes with putative functions in disease resistance and defense responses (Liu et al., 2016b). More comprehensive understanding of genetic variations within a white pine species across its geographical distribution is required to help guide restoration efforts using resistant populations. Genotyping arrays containing a complete set of targeted genes, or genomic selection would be more powerful for QR selection, but it still awaits development and verification in white pine breeding programs.

Development of durable and stable resistance is a key objective in white pine breeding programs and could be strengthened by pyramiding MGR and QR, or introgression of novel R genes (Sniezko and Liu, 2021). MGR and QR parent trees have been selected in breeding programs (Table 1), and controlled pollinations between them will generate progeny with genes of both MGR and QR. Because MGR-associated SNP alleles were absent or near absent in the QR seedling families (Liu et al., 2020b, 2021c), application of these well-developed MAS tools will be effective to identify MGR genotypes when both QR and MGR genotypes are pyramided in the seed orchards. Pyramiding multiple R genes should prove useful in future white pine breeding programs for improving the durability of resistance against a broad rust pathotypes.

Current understanding of gene variations and their functions underlying developments of QR-related traits is still very limited in white pines. In particular, QTLs still await characterization in high elevation white pines although some seedling families are identified with moderate to high QR levels in inoculation trials. QR candidate genes identified in whitebark pine, western white pine, and sugar pine provide genomic resources for potential applications in other high elevation white pine species where MGR is not available. Further dissecting the inheritance and genomic QR to WPBR, especially in Rocky Mountain bristlecone pine, which may have the highest QR level among all nine North American white pine species, will help understand more about potential resistance durability.

5.5. Rust effectors and white pine S candidate genes targeted for broad-spectrum WPBR resistance

In recent years, S genes have attracted attention because loss of S functions confers durable resistance to a wide range of pests/pathogens in crop breeding (Gorash et al., 2021). In addition to MGR and QR candidates, identification of S candidate genes provides genomic targets for future genetic engineering and genome editing using technologies like clustered regulatory interspaced short palindromic repeat/CRISPR associated protein9 (CRISPR/Cas9) (Komor et al., 2017), which could facilitate development and introgression of novel R genotypes in the seed orchards of white pines. High susceptibility of all nine native white pine species to WPBR indicates presence of host S genes as targeted by *C. ribicola* effectors, an interaction essential for successful rust infection, followed by canker development. However, the molecular basis for S-gene conferred broad-spectrum resistance is still poorly understood in plant breeding, especially in tree breeding. It is known that plant S genes are targeted by pathogenic effectors in well-characterized plant pathosystems. However, S-genes interacting with pathogenic Avr genes have not been found in any conifer species yet. *C. ribicola Avcr2* and *vcr2* transcriptomes were de novo assembled and a core set of pathogenic effectors were identified (Liu et al., 2015, 2021b). GWAS has identified candidate genes of rust *vcr2* and *Avcr2* effectors (Liu et al., 2021b). One of the *C. ribicola* candidate effectors was demonstrated with function affecting plant resistance (Ma et al., 2019). With the advancement of effectoromics in biotrophic rust fungi (Lorrain et al., 2019), those *C. ribicola Avcr* effectors can be used as probes to identify S genes while *vcr* effectors can be used to search for cognate novel R proteins against WPBR from wild stands of white pines.

A natural S mutant has not been reported in any conifer species yet. A forward genetics approach to screen resistant traits with recessive inheritance (loss of susceptibility) may be too challenging in breeding of white pines. As the first step to identify host S genes in the WPBR pathosystems, proteomes and transcriptomes were profiled during compatible interaction (*cr2*/*cr2* vs. *Avcr*) of western white pine with blister rust (Zamany et al., 2012, Liu et al., 2013b). An array of differentially expressed genes (DEGs) were identified with annotated functions as S candidates, including pattern recognition receptors (PRRs) of the receptor-like protein kinase (RLK) and NLR gene families for perceiving molecules associated with pathogens and down-stream components of the signaling pathways. Because a list of S genes was well characterized in other plants (van Schie and Takken 2014), reverse genetic analysis is another promising approach to identify putative S genes in white pines by mining orthologs of those well-known S genes from genome and transcriptomes of white pines.

Modification of S genes is considered as a novel approach to deliver durable and broad-spectrum disease resistance (Engelhardt et al., 2018). With adaptation of genome-editing technologies, broad-spectrum resistance to WPBR can be attained by silencing S genes once they are identified with aide of *C. ribicola* effectors documented so far. Current progress and further advances of this type of genomic research on white pines and rust fungus will pave the way forward to biotechnological development of superior genotypes with enhanced resistance with higher likelihood of durability against a much broader spectrum of *C. ribicola* pathotypes.

In reality, due to the complexity of conifer genomes, the cost and time of fully developing the genomic resources and the non-commercial use of these high elevation white pine species, traditional selective breeding is likely to continue to be the predominant path forward. However, as we increase our understanding of resistance through continued development of genomic resources, there is potential to increase the efficiency of these traditional selective breeding programs, as well as developing new strategies with respect to public perceptions of biotechnology if societal concerns about the fate of these species increases.

6. Utilization of resistance in restoration

The presence or absence of MGR and the level of QR varies among white pine species (Table 1), and thus the utility of resistance for immediate use from the different white pine species varies. Foxtail pine currently has little documented resistance; limber pine has MGR, but at this stage, only very low levels of QR documented; the other four high elevation species appear to have moderate to high QR in at least some populations or families. Southwestern white pine also has MGR in addition to QR.

Whitebark pine has been much more extensively tested than the other high elevation white pine species (Table 1), and more testing of the other species would provide a clearer picture of the frequency and levels of resistance available. The level of inoculum density may have a fairly large impact on QR for Rocky Mountain bristlecone pine, but more work is needed in all the high elevation white pines to see if this is common across species and to clarify its efficacy on sites of highest rust hazard. At this stage, investigations are still underway on whether MGR in limber pine and southwestern white pine are due to the same gene or different R genes (Liu et al., 2021d).

Since no substantial breeding work is anticipated for the high-elevation species, at least in the near future, restoration efforts will have to take into account current levels of resistance, number of resistant parental trees identified, and the quantity of seed that is available or can be collected from resistant parent trees. Current investigations are examining whether a pathotype of *C. ribicola* virulent to MGR in limber pine is present in at least one area of Alberta. If so, this could limit the future use of MGR in limber pine, and potentially in southwestern white pine. However, for much of the range of limber pine and southwestern white pine no virulence is currently documented and MGR could be useful at this stage and perhaps in many locations for the foreseeable future (Sniezko and Liu 2021). However, all plantings, and in particular those with only MGR should be monitored over time for potential the development of virulence to MGR and the subsequent loss of utility of MGR.

Whitebark pine and limber pine are under the highest current threat from WPBR (Goeking and Windmuller-Campione 2021, Tomback and Achuff 2010) and most restoration efforts in the next decade will be with these species. Fortunately, there are moderate levels of resistance documented in at least some populations for both species, although it is QR for whitebark pine and mostly MGR for limber pine. The whitebark pine resistance program in the Pacific Northwest has benefited from cooperation among many interested groups and individuals and serves as a model for other efforts with other species (Sniezko et al., 2022c). Additional field selections and subsequent resistance screening is needed for all species to provide enough parent trees to help ensure wide genetic diversity in restoration plantings and to retain adaptability in the different seed zones. This is one area where advances in omics could play an important role –providing a faster and less expensive method to identify resistant parent trees (Liu et al 2020b). However, the new technologies will complement, rather than replace traditional tree improvement. For the foreseeable future, the traditional seedling screening will be needed to confirm resistance of parent trees, but advances in omics could facilitate more efficient selection of candidate parent trees for testing via application of MAS and/or GS tools, particularly in stands that currently have low to moderate WPBR infection with little natural selection. Many hundreds or even thousands of parent trees have to be evaluated for resistance to find enough parents with resistance to ensure that both genetic diversity and resistance are well balanced in restoration populations. Such testing with current traditional methods can take one or more decades and is very expensive. If MAS or GS technology becomes readily available and reliable, ten-fold or more candidate trees might be able to have initial evaluation without waiting for a cone crop for each tree, greatly increasing the selection intensity, shortening the time, and saving resources needed since only the most promising parent trees will have seedlings evaluated in traditional screening trials.

The seed for restoration is potentially available from several sources. Where there is sufficient frequency and level of resistance, particularly QR, activities that facilitate natural regeneration, such as removing competing species, can be used. This may be feasible for whitebark pine in portions of the Cascade Range of Oregon and Washington where the frequency and level of WPBR resistance is relatively high (Fig. 1). In other cases, collections of seed from documented resistant parent trees will be used, and this has been ongoing on a relatively modest scale for more than a decade. To a certain extent the periodicity of large seed crops in the surviving trees of a species like whitebark pine may constrain natural regeneration or restoration efforts. Therefore, more concerted efforts than in the past will be needed for collecting large quantities of seed in a bumper cone year, which will be much more efficient than trying to collect in many years of mediocre cone crops.

A longer-term strategy is the development of seed orchards, which has the advantages of producing both higher levels of resistance as well as management to facilitate cone crops, and protection of parent trees. However, although seed orchards have been started for some species, notably whitebark pine and limber pine, it will likely be a decade or much longer before the grafted trees are large enough to produce significant quantities of resistant seed. With this constraint in mind, there is excellent potential over the next 10 to 20 years for restoration of some species using seed collected from parent trees. With concerted effort much of the needed restoration in whitebark pine might be completed before seed orchards are productive. However, the seed orchards will serve the important role of a long-term backup of the resistant parents.

There likely will not be enough resistant seed or other resources to restore all the whitebark pine or limber pine lost to WPBR. Therefore, strategic ‘nucleation plantings’ (Corbin and Holl 2012) across the range of the species will be needed to begin the process. If the resistance is effective and durable, then as the trees in these plantings become mature, natural seed dispersal and regeneration can help increase the frequency of the species. In the U.S., a national whitebark pine restoration strategy is being devised and a key component is designation of core areas for restoration (Whitebark Pine Ecosystem Foundation, 2018). Seed from WPBR-resistant parent trees will be a key component of the restoration plan.

7. Future prospects and challenges of developing and using durable resistance to WPBR

Whether resistant seed from natural stands or seed orchards is utilized, the dynamics of climate change presents another consideration, namely whether some form of assisted migration, notably assisted population migration should be incorporated. Using genomic resources to map the current patterns of genetic variation over the landscape will allow more comprehensive understanding of genetic variation in each species and provide information to help guide movement of seedlots into other areas to help maximize the potential for species survival into the future. Currently, at least in the U.S. part of the range, this consideration has not been well evaluated and discussions on what seed sources to use for where in an effort like the national whitebark pine restoration plan is needed. Available genomic resources through open-source genomics such as TreeGene (Falk et al., 2019), allow for a survey of standing genetic variations across landscape using landscape genomics approaches. Gene variations across the whole genome are the breeding source where elite genotypes for genetic resistance and other adaptive traits can be selected. It is critical for a breeding program to maintain high genetic diversity in the seedlots coming from the seed orchards or parent tree collections. The high genetic diversity will provide the species the best opportunity to evolve in the face of future biotic and abiotic challenges, including a changing climate. Using genome-wide SNPs, landscape genomics studies can help evaluate adaptive genomic components for their linkage with environmental heterogeneity among natural stands.

Although great progress in development of white pine genomic resources has been achieved in recent years, challenges remain to be solved before their direct application in biotechnological improvement of resistance to WPBR in white pines. The current understanding of genes contributing to QR is very limited, hampering the selection of the low frequency QR genotypes that would hold the most promise for the development of stable and durable resistance to WPBR.

Eurasian white pines have high levels of resistance, likely due to MGR and/or QR due to long history of co-evolution with native *C. ribicola* (Table 1, Hoff et al., 1980; Sniezko et al., 2008; Zhang et al., 2010), but R genes have not been characterized in the Eurasian white pines yet. Understanding the nature of resistance genes in the Eurasian species, which generally appear to have durable resistance to WPBR, may provide insights on whether any of the same orthologous genes are present in North American species or what combination of genes may foster durability. If needed, introgression of the uncharacterized R genes from Eurasian white pines into North American ones could be undertaken, but it takes many cycles of backcrossing in the conventional breeding (Lu and Derbowka, 2009). In addition, the complexity of the very large white pine genomes makes it a slow and difficult task for dissection of QTLs to reveal their interactions with *C. ribicola* and environmental factors. Although several genes have been documented for their genetic and functional contribution to QR-related traits, their phenotypic effects were usually small. Searching for many more QTLs or those with phenotypic effects at moderate resistance levels is necessary for effective use of QR genotypes in sustainable management of WPBR in white pines. In addition, phenomic technologies using high throughput phenotyping for WPBR resistance, such as fourier-transform infrared (FT-IR) spectroscopy and hyperspectral imaging (Villari et al., 2017), may have great potential to increase the efficiency of locating resistant genotypes, but unfortunately these technologies are still at initial development stages in white pines. Their applications with high phenotypic resolution will help association mapping of those elite QR seedling families identified in breeding programs, enabling tree breeders to determine the usefulness of these seedling families in the next breeding cycle.

Another need is for the candidate genes identified so far need to be verified for their functions in either MGR or QR. In addition, although loss of S gene functions is proposed as a novel breeding strategy for developing durable and broad-spectrum resistance, more research is needed before it can be used. Genome editing and other biotechnological manipulation technologies hold great promise to speed up breeding in forestry, but it is challenging to establish a robust experimental system for gene functional analysis in a non-model tree species. Successful transformation of novel genes depends on specific genetic backgrounds among varieties of a plant species. It would be a concerted and long-term effort to provide a comprehensive understanding of genetic diversity and its dynamic shift over environmental factors in populations of both host and pathogen.

We are lacking information on the effects of selected genotypes in the variable environments. It appears that temperature, drought, and other environmental factors have significant impacts on the presence of the pathogen and disease development (Velásquez et al., 2018; Dudney et al., 2021). Environmental factors have to be integrated when durable and stable resistance is sought in the breeding. Even if elite genotypes are selected in white pines by MAS tools and GS models, or modified by genetic engineering, it will take many years for confirmation of their durability and stability for resistance to WPBR, especially in field trials. A long-term sustained effort coordinated among white pine research and management communities is important in realizing the potentials of modern biotechnology in white pine resistance breeding.

8. Summary

White pine blister rust will continue to infect and kill white pines in North America, and the development and use of populations with genetic resistance will be essential for successful restoration and maintenance of these species in high elevation forests. However, the frequency, and level of resistance differs greatly by species and this information provides baseline data to land managers and the public to help manage restoration of affected species. The resistance breeding programs for each species will also need to ensure that genetic diversity is maintained in restoration populations to give the species an opportunity to evolve and persist under a changing climate.

Hundreds of additional candidate trees are still being evaluated for resistance to increase the number of resistant whitebark pine and limber pine parent trees used for restoration. For the other high elevation species, the resistance work is currently in a more exploratory phase to document resistance. Updated data on the incidence of rust present now and into the future in populations of the other high elevation white pine species will be needed to provide land managers information on which populations of those species may need greater effort in developing and using WPBR resistant seed in restoration.

Restoration using WPBR resistant seedlots is underway in whitebark pine and limber pine, the two species currently most impacted by the rust. Restoration in the other high-elevation white pine species will be contingent upon managers evaluating the need, as well as additional screening to identify more resistant parents. Both whitebark pine and limber pine are generally non-economic species but recognized for their ecological importance. The implementation of restoration in these two species has the potential to provide a guide to use for other species heavily impacted by biotic or abiotic agents using tree improvement programs to harness the genetic variation and develop populations suitable for use. The frequency and levels of QR in at least some populations of whitebark pine make us cautiously optimistic of the success of restoration, at least from a WPBR-resistance standpoint. However, further information and thought on assisted migration needed due to climate change will likely also be needed to provide the WPBR-resistant seedlots the best opportunity for long-term utility.

The use of biotechnology tools has been limited to date. However, tools to accelerate the confirmation of resistant parent trees have the potential to greatly increase the efficiency of selecting resistant parent trees, and speeding up resistance programs, and this may be their most significant contribution in the next few decades. However, significantly more investment will be needed to develop these tools. They also will provide background on the genes controlling resistance in each species.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This paper resulted from the invitation for a keynote address at the 'The H5II Conference: The Second Conference on the Research and Management of High Elevation Five Needle Pines' that was presented in October 2021. We thank the conference organizers for the invitation to present at the conference and contribute to this special journal issue. We also thank Bob Danchok, Emily Boes, Angelia Kegley, Douglas Savin and many other co-workers (past and present) at Dorena GRC for their help with resistance trials, and Kristin Silva for producing the map used in this paper. We also thank our many cooperators, in seedling inoculation trials including Anna Schoettle (Rocky Mountain bristlecone pine, Great Basin bristlecone pine, and limber pine), Jennifer Hooke (whitebark pine), and Jodie Krakowski, Robert Sissons, and Genoa Alger (limber pine from Alberta). We gratefully acknowledge funding from the USDA Forest Service PNW Region Genetic Resource and Forest Health Protection programs. We thank Ron Mastrogiuseppe for the impetus to examine resistance in foxtail pine, the USFS Region 5 Genetics program for providing seed of this species, and Jim Hamlin for his review of an earlier version on this paper and earlier inputs related to the resistance program. We acknowledge funding from both the USDA Forest Service and Canadian Forest Service for the long-term programs and efforts summarized here. We thank the anonymous reviewers for their comments and suggested edits on an earlier version that helped improve this final version of this paper.

References

Aubry, C., Goheen, D., Shoal, R., Ohlson, T., Lorenz, T., Bower, A., Mehmel, C., Sniezko, R.A., 2008. Whitebark pine restoration strategy for the Pacific Northwest 2009-2013.

Baker, E.A.G., Wegrzyn, J.L., Sezen, U.U., Falk, T., Maloney, P.E., Vogler, D.R., Delfino-Mix, A., Jensen, C., Mitton, J., Wright, J., Knaus, B., Rai, H., Cronn, R., Gonzalez-Ibeas, D., Vasquez-Gross, H.A., Famula, R.A., Liu, J.-J., Kueppers, L.M., Neale, D.B., 2018. Comparative transcriptomics among four white pine species. G3 (Bethesda) 8 (5), 1461–1474.

Barabaschi, D., Tondelli, A., Valè, G., Cattivelli, L., 2020. Fitness cost shapes differential evolutionary dynamics of disease resistance genes in cultivated and wild plants. Mol. Plant 13 (10), 1352–1354.

Bingham, R.T., 1983. Blister rust resistant western white pine for the Inland Empire: the story of the first 25 years of the research and development program. Gen. Tech. Rep. INT-146 Ogden, UT, U.S. Department of Agriculture, Forest Service. Intermountain Forest and Range Experiment Station, 1983. 45 p.

Cartwright, C., 2018. Whitebark pine (*Pinus albicaulis*) field screening for blister rust resistance in British Columbia: Germination results. In: Schoettle, A.W., Sniezko, R. A., Kliejunas, J.T., (Eds.) Proceedings of the IUFRO joint conference: Genetics of five-needle pines, rusts of forest trees, and Strobusphere; 2014 June 15-20; Fort Collins, CO. Proc. RMRS-P-76. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. pp. 81-87.

Cartwright, C., Sniezko, R.A., Murray, M., Reid, I., 2022. Whitebark pine genecology field trials in British Columbia: Age 5 results. For. Ecol. Manage. https://doi.org/10.1016/j.foreco.2022.120419. In this issue.

Corbin, J.D., Holl, K.D., 2012. Applied nucleation as a forest restoration strategy. For. Ecol. Manage. 265, 37–46. https://doi.org/10.1016/j.foreco.2011.10.013.

Dudney, J., Willing, C.E., Das, A.J., Latimer, A.M., Nesmith, J.C.B., Battles, J.J., 2021. Nonlinear shifts in infectious rust disease due to climate change. Nat. Commun. 12, 5326.

Engelhardt, S., Stam, R., Hückelhoven, R., 2018. Good riddance? Breaking disease susceptibility in the era of new breeding technologies. Agronomy 8, 114.

Falk, T., Herndon, N., Grau, E., Buehler, S., Richter, P., Zaman, S., Baker, E.M., Ramnath, R., Ficklin, S., Staton, M., Feltus, F.A., Jung, S., Main, D., Wegrzyn, J.L., 2019. Growing and cultivating the forest genomics database. TreeGenes. Database (Oxford) baz043.

Friedline, C.J., Lind, B.M., Hobson, E.M., Harwood, D.E., Mix, A.D., Maloney, P.E., Eckert, A.J., 2015. The genetic architecture of local adaptation I: the genomic landscape of foxtail pine (*Pinus balfouriana* Grev. & Balf.) as revealed from a high-density linkage map. Tree Genet. Genomes 11, 49.

Geils, B.W., Hummer, K.E., Hunt, R.S., 2010. White pines, Ribes, and blister rust: a review and synthesis. Forest Pathol. 40, 147–185.

Goeking, S.A., Windmuller-Campione, M.A., 2021. Comparative species assessments of five-needle pines throughout the western United States. For. Ecol. Manage. 496, 119438.

Gonzalez-Ibeas, D., Martinez-Garcia, P.J., Famula, R.A., Delfino-Mix, A., Stevens, K.A., Loopstra, C.A., Langley, C.H., Neale, D.B., Wegrzyn, J.L., 2016. Assessing the gene content of the megagenome: Sugar pine (Pinus lambertiana). G3 (Bethesda) 6 (12), 3787–3802.

Gorash, A., Armonienė, R., Kazan, K., 2021. Can effectoromics and loss-of-susceptibility be exploited for improving Fusarium head blight resistance in wheat? Crop J. 9, 1–16.

Hoff, R.J., McDonald, G.I., 1980. Improving rust-resistant strains of inland western white pine. USDA For. Serv. Res. Pap. INT-245. 13 p.

Hoff, R., Bingham, R.T., McDonald, G.I., 1980. Relative blister rust resistance of white pines. Eur. J. For. Pathol. 10 (5), 307–316.

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: Tomback, D., Arno, S.F., Keane, R.E. (Eds.), Whitebark Pine Communities: Ecology and Restoration. Island Press, Washington, pp. 346–366.

Hwang, H.S., Han, J.Y., Choi, Y.E., 2021. Enhanced accumulation of pinosylvin stilbenes and related gene expression in *Pinus strobus* after infection of pine wood nematode. Tree Physiol. 41 (10), 1972–1987.

Jacobi, W.R., Kearns, H.S.J., Kegley, A., Savin, D.P., Danchok, R., Sniezko, R.A., 2018. Comparative look at rust infection and resistance in limber pine (*Pinus flexilis*) and Rocky Mountain bristlecone pine (P. aristata) following artificial inoculation at three inoculum densities. In: Schoettle, A.W., Sniezko, R.A., Kliejunas, J.T. (Eds.) Proceedings of the IUFRO joint conference: Genetics of five-needle pines, rusts of forest trees, and Strobusphere, 2014 June 15-20, Fort Collins, CO. Proc. RMRS-P-76. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. pp. 151-157.

Jermstad, K.D., Eckert, A.J., Wegrzyn, J.L., Delfino-Mix, A., Davis, D.A., Burton, D.C., Neale, D.B., 2011. Comparative mapping in *Pinus*: sugar pine (*Pinus lambertiana* Dougl.) and loblolly pine (*Pinus taeda* L.). Tree Genet Genomes 7, 457–468.

Jin, W.T., Gernandt, D.S., Wehenkel, C., Xia, X.M., Wei, X.X., Wang, X.Q., 2021. Phylogenomic and ecological analyses reveal the spatiotemporal evolution of global pines. Proc. Natl. Acad. Sci. USA 118 (20), e2022302118.

Johnson, J.S., Sniezko, R.A., 2021. Quantitative disease resistance to white pine blister rust at southwestern white pine's (*Pinus strobiformis*) Northern Range. Front. For. Glob. Change 4, 765871. https://doi.org/10.3389/ffgc.2021.765871.

Kegley, A., Sniezko, R.A., 2004. Variation in blister rust resistance among 226 *Pinus monticola* and 217 *P. lambertiana* seedling families in the Pacific Northwest. In Breeding and genetic resources of five-needle pines: growth, adaptability, and pest resistance; 2001 July 23-27; Medford, OR, USA. Edited by Sniezko, R,A., Samman, S., Schlarbaum, S,E., and Kriebel, H.B., U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fort Collins, CO. pp. 209–226.

Kegley, A., Sniezko, R.A., Danchok, R., Savin, D.P., 2012. Blister rust resistance among 19 families of whitebark pine, *Pinus albicaulis*, from Oregon and Washington – early results from an artificial inoculation trial. In: Sniezko, R.A., Yanchuk, A.D., Kliejunas, J.T., Palmieri, K.M., Alexander, J.M., Frankel, S.J., tech. coords. (Eds.) Proceedings of the fourth international workshop on the genetics of host-parasite interactions in forestry: Disease and insect resistance in forest trees. Gen. Tech. Rep. PSW-GTR-240. Albany, CA: Pacific Southwest Research Station, Forest Service, U.S. Department of Agriculture. pp. 311-315.

King, J.N., Hunt, R.S., 2004. Five needle pines in British Columbia, Canada: Past, Present and Future. In: Sniezko, R.A., Samman, S., Schlarbaum, S.E., Kriebel, H., (Eds.) Breeding and genetic resources of five-needle pines: growth, adaptability, and pest resistance, Proceedings: of the IUFRO Five-Needle Pines Working Party Conference. July 23-27, 2001, Medford, OR. USDA Forest Service, Rocky Mountain Research Station, Fort Collins, CO, RMRS-P-32. pp. 12-19.

King, J.N., David, A., Noshad, D., Smith, J., 2010. A review of genetic approaches to the management of blister rust in white pines. For. Pathol. 40, 292–313.

King, J.N., DeBell, J., Kegley, A., Sniezko, R.A., McDonald, G., Ukrainetz, N., 2018. Provenance variation in western white pine (*Pinus monticola*): The impact of white pine blister rust. In: Schoettle, A.W, Sniezko, R.A, Kliejunas, J.T. (Eds.) Proceedings of the IUFRO joint conference: Genetics of five-needle pines, rusts of forest trees, and Strobusphere; 2014 June 15-20; Fort Collins, CO. Proc. RMRS-P-76. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. pp. 63-80.

Kinloch Jr., B.B., 2003. White pine blister rust in North America: Past and prognosis. Phytopathology 93, 1044–1047.

Kinloch Jr., B.B., Sniezko, R.A., Barnes, G.D., Greathouse, T., 1999. A major gene for resistance to white pine blister rust in western white pine from the Western Cascade Range. Phytopathology 89 (10), 861867.

Kinloch Jr., B.B., Dupper, G.E., 2002. Genetic Specificity in the white pine-blister rust pathosystem. Phytopathology 92 (3), 278–280.

Kinloch Jr., B.B., Sniezko, R.A., Dupper, G.E., 2003. Origin and distribution of *Cr2*, a gene for resistance to white pine blister rust in natural populations of western white pine. Phytopathology 93 (6), 691–694.

Kinloch Jr., 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 (7), 751–758.

Kinloch Jr., 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 Genet. Genomes 4, 65–74.

Kinloch, B.B., Jr., Burton, D., Davis, D.A., Westfall, R.D., Dunlap, J., Vogler, D., 2012. Strong partial resistance to white pine blister rust in sugar pine. In: Sniezko, R.A., Yanchuck, A., Kliejunas, J., et al. tech. coords. (Eds.) Proceedings of the fourth international workshop on the genetics of host-parasite interactions in forestry: Disease and insect resistance in forest trees. Gen Tech Rep PSWGTR-240. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station: pp. 80–91.

Kinloch, B.B., Jr., Sniezko, R.A., Savin, D.P., Danchok, R., Kegley, A., Burton, D., Dunlap, J., 2018. Patterns of variation in blister rust resistance in sugar pine (*Pinus lambertiana*). In: Schoettle, A.W., Sniezko, R.A., Kliejunas, J.T. (Eds.) Proceedings of the IUFRO joint conference: Genetics of five-needle pines, rusts of forest trees, and Strobusphere; 2014 June 15-20; Fort Collins, CO. Proc. RMRS-P-76. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. pp. 124-128.

Komor, A.C., Badran, A.H., Liu, D.R., 2017. CRISPR-based technologies for the manipulation of eukaryotic genomes. Cell 169 (3), 559. https://doi.org/10.1016/j.cell.2017.04.005.

Krakowski, J., Gutsell, R., Jones, B., 2022. Reach for the top: implementing whitebark and limber pine recovery in Alberta. Proceedings of the IUFRO 2019 Joint Conference: Genetics of Five-needle Pines & Rusts of Forest Trees. Special Report ##. BC Ministry of Forests, Lands and Natural Resource Operations and Rural Development in press.

Kriebel, H.B., 2004. Genetics and breeding of five-needled pines in the eastern United States. In: Sniezko, R.A., Samman, S., Schlarbaum, S.E., and Kriebel, H.B., (eds.) Breeding and Genetic Resources of Five-Needled Pines: Growth, Adaptability and Pest Resistance. USDA For. Serv., Fort Collins, CO, RMRS-P-32. pp. 20-27.

Liu, J.-J., Ekramoddoullah, A., 2003. Isolation, genetic variation and expression of TIR-NBS-LRR resistance gene analogs from western white pine (*Pinus monticola* Dougl. ex. D. Don.). Mol Genet Genomics 270, 432–441.

Liu, J.-J., Ekramoddoullah, A., Zamani, A., 2005. A class IV chitinase is up-regulated upon fungal infection and abiotic stresses and associated with slow-canker-growth resistance to *Cronartium ribicola* in western white pine (*Pinus monticola* Dougl. Ex D. Don). Phytopathology 95 (3), 284–291.

Liu, J.-J., Ekramoddoullah, A., 2007. The CC-NBS-LRR subfamily in western white pine (*Pinus monticola* D. Don.): targeted identification, gene expression and genetic linkage with disease resistance against *Cronartium ribicola*. Phytopathology 97, 728–736.

Liu, J.-J., Sniezko, R.A., Ekramoddoullah, A.K., 2011. Association of a novel *Pinus monticola* chitinase gene (*PmCh4B*) with quantitative resistance to *Cronartium ribicola*. Phytopathology 101, 904–911.

Liu, J.-J., Hammett, C., Sniezko, R.A., 2013a. *Pinus monticola* pathogenesis-related gene *PmPR10-2* alleles as defence candidates for stem quantitative disease resistance against white pine blister rust (*Cronartium ribicola*). Tree Genet Genomes 9 (2), 397–408.

Liu, J.-J., Sturrock, R.N., Benton, R., 2013b. Transcriptome analysis of *Pinus monticola* primary needles by RNA-seq provides novel insight into host resistance to *Cronartium ribicola*. BMC Genomics 14, 884.

Liu, J.-J., Zamany, A., Sniezko, R.A., 2013c. Anti-microbial peptide (AMP): nucleotide variation, expression, and association with resistance in the white pine-blister rust pathosystem. Planta 237, 43–54.

Liu, J.-J., Sniezko, R.A., Sturrock, R.N., Chen, H., 2014. Western white pine SNP discovery and high-throughput genotyping for breeding and conservation applications. BMC Plant Biol. 14, 380.

Liu, J.-J., Sturrock, R.N., Sniezko, R.A., Williams, H., Benton, R., Zamany, A., 2015. De novo sequencing of *Cronartium ribicola* transcriptome for understanding molecular white pine blister rust interactions. BMC Genomics 16, 678.

Liu, J.-J., Schoettle, A.W., Sniezko, R.A., Sturrock, R.N., Zamany, A., Williams, H., Ha, A., Chan, D., Danchok, D., Savin, D.P., Kegley, A., 2016a. Genetic mapping of *Pinus flexilis* major gene (*Cr4*) for resistance to white pine blister rust using transcriptome-based SNP genotyping. BMC Genomics 17, 753.

Liu, J.-J., Sniezko, R.A., Murray, M., Wang, N., Chen, H., Zamany, A., Sturrock, R.N., Savin, D., Kegley, A., 2016b. Genetic diversity and population structure of whitebark pine (*Pinus albicaulis* Engelm.) in western North America. PLoS ONE 11 (12), e0167986.

Liu, J.-J., Sniezko, R.A., Zamany, A., Williams, H., Wang, N., Kegley, A., Savin, D.P., Chen, H., Sturrock, R.N., 2017a. Saturated genic SNP mapping identified functional candidates and selection tools for the *Pinus monticola Cr2* locus controlling resistance to white pine blister rust. Plant Biotechnol. J. 15, 1149–1162.

Liu, J.-J., Williams, H., Li, X.R., Schoettle, A.W., Sniezko, R.A., Murray, M., Zamany, A., Roke, G., Chen, H., 2017b. Profiling methyl jasmonate-responsive transcriptome for understanding induced systemic resistance in whitebark pine (*Pinus albicaulis*). Plant Mol. Biol. 95, 359–374.

Liu, J.-J., Schoettle, A.W., Sniezko, R.A., Yao, F., Zamany, A., Williams, H., Rancourt, B., 2019. Limber pine (*Pinus flexilis* James) genetic map constructed by exome-seq provides insight into evolution of disease resistance and a genomic resource for genomics-based breeding. Plant J. 98 (4), 745–758.

Liu, J.-J., Sniezko, R.A., Sissons, R., Krakowski, J., Alger, G., Schoettle, A.W., Williams, H., Zamany, A., Zitomer, R., Kegley, A., 2020a. Association mapping and development of marker-assisted selection tools for the resistance to white pine blister rust in the Alberta limber pine populations. Front. Plant Sci. 11, 557672.

Liu, J.-J., Williams, H., Zamany, A., Li, X.-R., Gellner, S., Sniezko, R.A., 2020b. Development and application of marker-assisted selection (MAS) tools for breeding of western white pine (*Pinus monticola* Douglas ex D. Don) resistance to blister rust (*Cronartium ribicola* J.C. Fisch.) in British Columbia. Can. J. Plant Pathology 42(2), 250-259.

Liu, J.-J., Fernandes, H., Zamany, A., Sikorski, M., Jaskolski, M., Sniezko, R.A., 2021a. In-vitro anti-fungal assay and association analysis reveal a role for the *Pinus monticola* PR10 gene (*PmPR10-3.1*) in quantitative disease resistance to white pine blister rust. Genome 64 (7), 693–704.

Liu, J.-J., Sniezko, R.A., Zamany, A., Williams, H., Omendja, K., Kegley, A., Savin, D.P., 2021b. Comparative transcriptomics and RNA-seq-based bulked segregant analysis (BSR-Seq) reveals genomic basis underlying *Cronartium ribicola vcr2* virulence. Frontier in Microbiology 12, 602812.

Liu, J.-J., Schoettle, A.W., Sniezko, R.A., Williams, H., Zamany, A., Rancourt, B., 2021c. Fine dissection of limber pine resistance to *Cronartium ribicola* using targeted sequencing of the NLR family. BMC Genomics 22 (1), 567.

Liu, J.-J., Schoettle, A.W., Sniezko, R.A., Waring, K.M., Williams, H., Zamany, A., Johnson, J., Kegley, A., 2021d. Comparative association mapping reveals conservation of major gene resistance to white pine blister rust in southwestern white pine (*Pinus strobiformis*) and limber pine (*P. flexilis*). Phytopathology. https://doi.org/10.1094/PHYTO-09-21-0382-R.

Lorenz, W.W., Ayyampalayam, S., Bordeaux, J.M., Howe, G.T., Jermstad, K.D., Neale, D. B., Rogers, D.L., Dean, J.F., 2012. Conifer DBMagic: a database housing multiple de novo transcriptome assemblies for 12 diverse conifer species. Tree Genet. Genomes 8 (6), 1477–1485.

Lorrain, C., Gonçalves Dos Santos, K.C., Germain, H., Hecke,r A., Duplessis, S., 2019. Advances in understanding obligate biotrophy in rust fungi. New Phytol. 222(3), 1190-1206. doi: 10.1111/nph.15641.

Lu, P., Derbowka, D., 2009. Breeding eastern white pine for blister rust resistance: A review of progress in Ontario. The Forestry Chronicle. 85 (5), 745–755.

Ma, Z., Liu, J.-J., Zamany, A., 2019. Identification and functional analysis of an effector secreted by *Cronartium ribicola.* Phytopathology 109 (6), 942–951.

Mahalovich, M,F., Burr, K,E,, Foushee, D,K. 2006. Whitebark pine germination, rust resistance, and cold hardiness among seed sources in the Inland Northwest: Planting strategies for restoration. In: Riley, L.E., Dumroese, R.K., Landis, T.D., tech. coords. 2006. National Proceedings: Forest and Conservation Nursery Associations - 2005. Proc. RMRS-P-43. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. p. 91-101.

McDonald, G.I., Zambino, P., Sniezko, R.A., 2004. Breeding rust-resistant five-needle pines in the western United States: lessons from the past and a look to the future. In: Sniezko, R.A., Samman, S., Schlarbaum, S.E., Kriebel, H.B. (Eds.), Breeding and genetic resources of five-needle pines: growth, adaptability, and pest resistance; 2001 July 23–27; Medford, OR, USA. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fort Collins, CO, pp. 28–50.

Maloy, O.C., 1997. White pine blister rust control in North America: A case history. Annu. Rev. Phytopath. 35, 87–109.

Menon, M., Bagley, J.C., Friedline, C.J., Whipple, A.V., Schoettle, A.W., Leal-Sàenz, A., Wehenkel, C., Molina-Freaner, F., Flores-Rentería, L., Gonzalez-Elizondo, M.S., Sniezko, R.A., Cushman, S.A., Waring, K.M., and Eckert, A.J., 2018. The role of hybridization during ecological divergence of southwestern white pine (*Pinus strobiformis*) and limber pine (*P. flexilis*). Mol. Ecol. 27, 1245-1260.

Murray, M.P., Strong, W., 2021. Disease screening for endangered whitebark pine ecosystem recovery in Canada. J. Ecosyst. Manage. 21 (1), 1–7. http://jem.forrex.org/index.php/jem/article/view/609/.

National Academies of Sciences, Engineering, and Medicine, 2019. Forest health and biotechnology: Possibilities and considerations. Washington, DC. The National Academies Press. https://doi.org/10.17226/25221.

Neale, D.B., 2022. The whitebark pine genome project. For. Ecol. Manage. this issue.

Ngou, B.P.M., Jones, J.D.G., Ding, P., 2022. Plant immune networks. Trends Plant Sci. 27 (3), 255–273. https://doi.org/10.1016/j.tplants.2021.08.012.

Omdal, D., Ramsey, A., Sniezko, R.A., 2018. Examining genetic variation and white pine blister rust resistance for whitebark pine (*Pinus albicaulis*): Field trials in Washington and Oregon. Washington Department of Natural Resources. Report WCFHM 18–01, 5 p.

Pike, C.C., Berrang, P., Rogers, S., David, A., Sweeney, C., Hendrickson, J., 2018. Improving the resistance of eastern white pine to white pine blister rust disease. For. Ecol. Manage. 423, 114–119.

Pruitt, R.N., Locci, F., Wanke, F., Zhang, L., Saile, S.C., Joe, A., Karelina, D., Hua, C., Fröhlich, K., Wan, W.L., Hu, M., Rao, S., Stolze, S.C., Harzen, A., Gust, A.A., Harter, K., Joosten, M.H.A.J., Thomma, B.P.H.J., Zhou, J.M., Dangl, J.L., Weigel, D., Nakagami, H., Oecking, C., Kasmi, F.E., Parker, J.E., Nürnberger, T., 2021. The EDS1-PAD4-ADR1 node mediates Arabidopsis pattern-triggered immunity. Nature 598 (7881), 495–499.

Reid, I.R., 2020. Whitebark pine (*Pinus albicaulis*) resistance to white pine blister bust: a cost-effective approach to progeny testing for restoration. University of British Columbia, Vancouver, British Columbia. Masters Thesis.

Samman, S., Schwandt, J.W., Wilson, J.L., 2003. Managing for healthy white pine ecosystems in the United States to reduce the impacts of white pine blister rust. Report No. R1-03-118. USDA Forest Service, Northern Region, Forest Health Protection, Missoula, MT. 10 p.

Schoettle, A.W., Sniezko, R.A., Kegley, A., Danchok, R., Burns, K.S., 2012. Patterns of Resistance to *Cronartium ribicola* in *Pinus aristata,* Rocky Mountain Bristlecone Pine. In: Proceedings of the fourth international workshop on the genetics of host-parasite interactions in forestry: Disease and insect resistance in forest trees. Sniezko, R.A., Yanchuk, A.D., Kliejunas, J.T., Palmieri, K.M., Alexander, J.M., Frankel, S.J., and tech. coords (2012). Gen Tech Rep PSW-GTR-240. Albany, CA. Pacific Southwest Research Station, Forest Service, U.S. Department of Agriculture. pp. 336.

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.

Schoettle, A.W., Sniezko, R.A., Kegley, A., Burns, K.S., 2018a. Genetic resistance to *Cronartium ribicola* in limber pine (*Pinus flexilis*). In: Schoettle, A.W., Sniezko, R.A., Kliejunas, J.T., eds. Proceedings of the IUFRO joint conference: Genetics of five-needle pines, rusts of forest trees, and Strobusphere, 2014 June 15-20, Fort Collins, CO. Proc. RMRS-P-76. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. pp. 165-166.

Schoettle, A.W., Sniezko, R.A., Kegley, A., Burns, K.S., 2018b. Patterns of resistance to *Cronartium ribicola* in Rocky Mountain bristlecone pine (*Pinus aristata*). In: Schoettle, A.W., Sniezko, R.A., Kliejunas, J.T. (Eds.) Proceedings of the IUFRO joint conference: Genetics of five-needle pines, rusts of forest trees, and Strobusphere, 2014 June 15-20, Fort Collins, CO. Proc. RMRS-P-76. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. pp. 163-164.

Schwandt, J.W., 2006. Whitebark pine in peril: a case for restoration. US Forest Service Forest Health and Protection Report R1-06-28, US Forest Service, Missoula, Montana.

Sniezko, R.A., Kegley, A., Danchok, R., Long, S., 2007. Variation in resistance to white pine blister rust among whitebark pine families from Oregon and Washington - early results and implications for conservation. In: Goheen E.M., Sniezko R.A., tech. coords. Proceedings of the conference whitebark pine: a Pacific Coast perspective. August 27-31, 2006. Ashland, OR. R6-NR-FHP-2007-01. Portland, OR: Pacific Northwest Region, Forest Service, U.S. Department of Agriculture; pp. 82-97.

Sniezko, R.A., Kegley, A.J., 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., 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: Keane, R.E., Tomback, D.F., Murray, M.P., and Smith, C.M., eds. The future of high-elevation, five-needle white pines in western North America: proceedings of the high five symposium. Proceedings RMRS-P-63. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. pp. 246–264.

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.

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. Can. J. Forest Research 46 (9), 1173–1178.

Sniezko, R.A, Kegley, A., Danchok, R., Long, S., 2018. Blister rust resistance in whitebark pine (*Pinus albicaulis*) - early results following artificial inoculation of seedlings from Oregon, Washington, Idaho, Montana, California, and British Columbia seed sources. In: Schoettle, A.W.; Sniezko, R.A., Kliejunas, J.T., eds. Proceedings of the IUFRO joint conference: Genetics of five-needle pines, rusts of forest trees, and Strobusphere; 2014 June 15-20; Fort Collins, CO. Proc. RMRS-P-76. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. pp. 129-135.

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, 57–68.

Sniezko, R.A., Liu, J.-J., 2021. Prospects for developing durable resistance in populations of forest trees. New Forest. Published on line on Nov. 30, 2021. https://doi.org/10.1007/s11056-021-09898-3.

Sniezko, R.A., Krakowski, J., Sissons, R., Alger, G., Liu, J-J., Lewien, M., Danchok, R., Zitomer, R., Kegley, A. 2022a (in press). Finding and using genetic resistance to white pine blister rust in Alberta populations of limber pine (*Pinus flexilis*). Proceedings of the IUFRO 2019 Joint Conference: Genetics of Five-needle Pines & Rusts of Forest Trees. Special Report ##. BC Ministry of Forests, Lands and Natural Resource Operations and Rural Development, Victoria, B.C.

Sniezko, R.A., Gleason, J., McTeague, B., Danchok, R., Lewien, M., Kegley, A., 2022b (in press). The search for white pine blister rust in foxtail pine (*Pinus balfouriana*). Proceedings of the IUFRO 2019 Joint Conference: Genetics of Five-needle Pines & Rusts of Forest Trees. Special Report ##. BC Ministry of Forests, Lands and Natural Resource Operations and Rural Development, Victoria, B.C.

Sniezko, R.A., Chadwick, K., Darbyshire, R., Hooke, J., Jensen, C., Omdal, D., et al. 2022c (in press). Partnerships in the Pacific Northwest to help save an endangered species, whitebark pine (*Pinus albicaulis*) - Research, rust resistance and restoration. Proceedings of the IUFRO 2019 Joint Conference: Genetics of Five-needle Pines & Rusts of Forest Trees. Special Report ##. BC Ministry of Forests, Lands and Natural Resource Operations and Rural Development, Victoria, B.C.

Stephan, B.R., 1986. The IUFRO experiment on resistance of white pines to blister rust (*Cronartium ribicola*) in northern Germany Vol. 1, 80–89.

Stephan, B.R., 2004. Studies of genetic variation with five-needle pines in Germany. In Breeding and genetic resources of five-needle pines: growth, adaptability, and pest resistance; 2001 July 23-27; Medford, OR, USA. Edited by Sniezko, R,A., Samman, S., Schlarbaum, S,E., and Kriebel, H.B., U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fort Collins, CO. pp. 98–102.

Stevens, K.A., Wegrzyn, J.L., Zimin, A., Puiu, D., Crepeau, M., Cardeno, C., Paul, R., Gonzalez-Ibeas, D., Koriabine, M., Holtz-Morris, A.E., Martínez-García, P.J., Sezen, U.U., Marçais, G., Jermstad, K., McGuire, P.E., Loopstra, C.A., Davis, J.M., Eckert, A., de Jong, P., Yorke, J.A., Salzberg, S.L., Neale, D.B., Langley, C.H., 2016. Sequence of the sugar pine megagenome. Genetics 204, 1613–1626.

Syring, J.V., Tennessen, J.A., Jennings, T.N., Wegrzyn, J., Scelfo-Dalbey, C., Cronn, R., 2016. Targeted capture sequencing in whitebark pine reveals range-wide demographic and adaptive patterns despite challenges of a large, repetitive genome. Front. Plant Sci. 7, 484.

Tomback, D.F., Achuff, P., 2010. Blister rust and western forest biodiversity: ecology, values and outlook for white pines. Forest Pathol. 40 (3–4), 186–225.

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.), an imperiled high-elevation western North American forest tree. Forest Ecology and Management. doi: 10.1016j.foreco.2021.119939.

Van Schie, C.C., Takken, F.L., 2014. Susceptibility genes 101: How to be a good host. Annu. Rev. Phytopathol. 52, 551–581.

Vázquez-Lobo, A., Amanda, R., Martínez-García, P.J., Vangestel, C., Wegzryn, J.L., Ćalić, I., Burton, D., Davis, D., Kinloch, B., Vogler, D., Neale, D.B., 2017. Finding loci associated to partial resistance to white pine blister rust in sugar pine (*Pinus lambertiana* Dougl.). Tree Genet. Genomes 13 (5), 1–7.

Velásquez, A.C., Castroverde, C., He, S.Y., 2018. Plant-pathogen warfare under changing climate conditions. Curr. Biol. 28 (10), R619–R634.

Verma, S.S., Yajima, W., Rahman, M.H., Shah, S., Liu, J.-J., Ekramoddoullah, A.K., Kav, N.N.V., 2012. A cysteine-rich anti-microbial peptide from *Pinus monticola* (PmAMP1) confers multiple disease resistance in canola (*Brassica napus*). Plant Mol. Biol. 79, 61–74.

Villari, C., Sniezko, R.A., Rodriguez-Saona, L.E., Bonello, P., 2017. Accelerating dynamic genetic conservation efforts: Use of FT-IR spectroscopy for the rapid identification of trees resistant to destructive pathogens. In: Sniezko R.A., Man G., Hipkins V., Woeste K., Gwaze D., Kliejunas J.T., McTeague B.A., tech. cords. 2017. Gene conservation of tree species—banking on the future. Proceedings of a workshop. Gen. Tech. Rep. PNW-GTR-963. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station. pp. 69.

Weiss, M., Sniezko, R.A., Puiu, D., Crepeau, M.W., Stevens, K., Salzberg, S.L., Langley, C. H., Neale, D.B., De La Torre, A.R., 2020. Genomic basis of white pine blister rust quantitative disease resistance and its relationship with qualitative resistance. Plant J. 104 (2), 365–376.

Whitebark Pine Ecosystem Foundation. 2018. National whitebark pine restoration plan. https://whitebarkfound.org/national-whitebark-pine-restoration-plan/.

Zamany, A., Liu, J.-J., Ekramoddoullah, A., Sniezko, R.A., 2011. Antifungal activity of a *Pinus monticola* – antimicrobial peptide 1 (Pm-AMP1) and its accumulation in *Cronartium ribicola*-infected western white pine. Can. J. Microbiol. 57, 667–679.

Zamany, A., Liu, J.-J., Ekramoddoullah, A., 2012. Comparative proteomic profiles of *Pinus monticola* needles during early compatible and incompatible interactions with *Cronartium ribicola*. Planta 236, 1725–1746.

Zhang, X.Y., Lu, Q., Sniezko, R.A., Song, R.Q., Man, G., 2010. Blister rusts in China: hosts, pathogens, and management. Forest Pathol. 40, 369–381.