Whitebark pine (*Pinus albicaulis*) resistance to white pine blister rust: a cost-effective approach to progeny testing for restoration

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Abstract

Endangered whitebark pine (*Pinus albicaulis* Engelm.), native to high elevation forests of western North America, is declining mainly due to the introduced pathogen Cronartium ribicola J.C. Fisch, causing the disease white pine blister rust. This decline is compounded by the impacts of climate change, mountain pine beetle, and fire suppression. Low levels of genetic resistance to blister rust are present in whitebark pine. Traditional methods of inoculating seedlings to determine family-level resistance to the rust are costly, labour and time intensive. Due to the need for resistant material for planting, this presents a bottleneck in the process of restoring whitebark pine stands that have been heavily infected by the rust. In this study I tested an alternative approach to controlled inoculations that would be an effective way to screen large numbers of families without as many costs and limitations. A large genetic sample comprising 214 open-pollinated families from 44 provenances were screened at Skimikin Nursery, British Columbia, to determine: (1) the effectiveness of natural rust inoculation from Ribes nigrum L. in a common garden; (2) family and provenance level resistance to blister rust; and (3) climate variables related to height and rust resistance. Eighty-one families previously screened in Dorena, Oregon using artificial inoculation methods were also planted at Skimikin to compare with the natural inoculation. The natural inoculation was effective, with 73% of seedlings displaying stem symptoms of the disease, and 95% showing rust infection. A clear relationship was found between distance from the Ribes and severity of blister rust. Linear mixed models with spatial correlations were fitted to height and rust data using ASReml-R to estimate breeding values, heritability, and among-population differentiation (O_{ST}). Resistance was highest in the Cascade Mountains of Washington, while the most susceptible families were located mainly in the BC Coast Mountains. Q_{ST} values revealed low genetic differentiation for height (0.07) and moderate differentiation for rust (0.28) while heritability was higher for height (0.42) than for rust resistance (0.23). This method of screening should be used more widely to determine families resistant to white pine blister rust and increase the availability of resistant seedlings for restoration.

Lay Summary

Endangered whitebark pine (*Pinus albicaulis*) is undergoing rapid decline mainly from an introduced disease known as white pine blister rust. Low levels of genetic resistance to the rust are present in whitebark pine, though determining which trees are resistant is limited by space in testing facilities, cost, and time. The objective of this thesis is to present an alternative method to finding rust resistant whitebark pine trees for restoration, by utilizing an outdoor nursery bed and natural infections for screening seedlings. Results suggest that this method of determining resistant whitebark pine parent trees is effective, and could be used at a larger scale to increase the production of rust resistant seedlings to plant in areas where whitebark pine has been decimated by rust.

Preface

This thesis is part of a larger research project established by Charlie Cartwright, Michael Murray, and Nick Ukrainetz of the British Columbia Ministry of Forests, Lands, Natural Resource Operations and Rural Development (FLNRORD). Study design of the common garden at Skimikin Nursery was established by FLNRORD in 2015. Data collection methods were based on those used by staff at Dorena Genetic Resource Centre, Oregon. Field data was collected with help from Leah Rettenbacher. Additional data were provided by Richard Sniezko of Dorena Genetic Resource Centre, including resistance grades for 81 whitebark pine families screened at Dorena using artificial inoculation methods. Charlie Cartwright of FLNRORD provided study design methods, preliminary data, and the locations of whitebark pine families and populations in the study. Sally Aitken, Richard Hamelin, and Tongli Wang provided input on data analyses. Climate data was obtained using *ClimateNA*, a program created in part by Tongli Wang. All data processing and analyses as well as writing were completed by myself. A paper with Sally Aitken, Richard Hamelin, Richard Sniezko, and Tongli Wang as co-authors will be submitted for publication based on Chapter 2 of this thesis.

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List of Symbols

AIC	Akaike information criterion
cm	centimetre
°C	degrees Celsius
$F_{\rm ST}$	proportion of genetic variation due to differences among populations
$G_{ m ST}$	$F_{\rm ST}$ extended for 3 or more alleles
h^2	heritability
ha	hectare
m	metre
mm	millimetre
$\sigma^2{}_{\rm f}$	family variance
$\sigma^2_{\ p}$	population variance
$\sigma^2{}_u$	units (residual) variance
$Q_{ m ST}$	among population differentiation
r^2	coefficient of determination
%SS	percent stem symptoms

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1 White pine blister rust and whitebark pine

1.1 Introduction

North American forests in the Anthropocene are undergoing rapid changes due to climate change, introduced species, forest fire, commercial logging, biotic disturbances, and development (Bengston and Dockry 2014). Rust fungi (Pucciniales) are a large and diverse group of forest pathogens causing widespread infection and mortality of conifers in North America (Tomback and Achuff 2010). Approximately 7800 species of rust fungi have been identified worldwide; however, the two most important rust fungi affecting conifers in North America are the fusiform rust caused by the native pathogen *Cronartium quercuum* (Berkeley) Miyabe ex Shirai infecting southern pines, and white pine blister rust caused by the invasive pathogen *Cronartium ribicola* J. C. Fisch infecting five-needle pines (Helfer 2014; Sniezko et al. 2014b). These pathogens have resulted in substantial economic losses in the forest industry as well as ecological changes. As a consequence, a great deal of effort has been expended trying to minimize the impacts of these rust fungi through a variety of means, including eradicating alternate hosts, fire, pruning, careful selection of planting sites, and genetic resistance (Zambino 2010; Sniezko et al. 2014b). Of these methods, the most likely to be effective in the long term is genetic resistance (Sniezko et al. 2014b).

Although some rust species are invasive, low levels of resistance are present in the host species affected (Helfer 2014; Sniezko et al. 2014b). A variety of resistance mechanisms to these rusts have been found so far, ranging from partial to complete (King et al. 2010). Many more are yet to be determined. Due to the co-evolved nature of fusiform rust, there appear to be higher levels of genetic resistance in its hosts compared to North American species infected by white pine blister rust (Sniezko et al. 2014b).

Rust fungi are obligate parasites requiring a living host to complete their life cycle. Rusts have been categorized into three life cycles, macrocyclic, demicyclic, and microcyclic (Petersen 1974). Additionally, rusts can be classified as heteroecious, requiring two unrelated hosts, or autoecious, where the entire life cycle can be completed on one species (Petersen 1974). For conifers, rust fungi can enter their hosts either through the leaf stomata or by directly penetrating

the stem (Sniezko et al. 2014b). From there, cankers form underneath the bark of the host, killing the cambium, and eventually cutting off the flow of water and nutrients, causing mortality of any living material above the canker if it encircles the branch or stem (McDonald and Hoff 2001).

One group of trees that has been directly affected by rust fungi is the five-needle pines (*Pinus* subgenus *Strobus*). Nine species of five-needle pines occur in North America, and of these all but one have been affected by white pine blister rust (Dunlap 2012). In addition to rust, many five-needle pine species are in decline due to the interacting and mainly anthropogenic processes of fire suppression, mountain pine beetle (*Dendroctonus ponderosae* Hopkins) and other insects, and climate change (Tomback and Achuff 2010; Shepherd et al. 2018). One such species native to mountainous areas of Western North America, whitebark pine (*Pinus albicaulis* Engelm.), is now listed as endangered in Canada under the Species at Risk Act and has been proposed for endangered listing in the United States as well (Environment and Climate Change Canada 2017). Numerous partnerships spanning different levels of government, universities, industry, and non-profit organizations have been created to help ensure the continued survival of this important species.

This thesis will focus on white pine blister rust, its effects on whitebark pine, and a method to screen families to determine resistance to the rust. With an increasing need for rust-resistant seedlings to plant for restoration purposes, efficient methods of screening seedlings for resistance is of utmost importance.

1.2 White Pine Blister Rust

1.2.1 Background

Non-native pests can cause significant problems in the ecosystems to which they are introduced, a good example of which is the disease white pine blister rust. White pine blister rust has caused widespread and significant ecological damage and economic losses in North American forests. It is caused by a macrocyclic, heteroecious rust fungus with teliospores and basidiospores produced on the alternate host (primarily *Ribes*) and aeciospores produced on the main host *Pinus* (Geils et al. 2010). During cool and wet conditions, teliospores germinate on the alternate host to produce basidia which in turn produce basidiospores. Basidiospores are generally fragile and require high humidity to ensure viability (McDonald and Hoff 2001). The

basidiospores disperse via wind and enter the main host (*Pinus*) through the needle stomata. After four to six months, yellow to red spots develop on the needles where infection took place (Hoff et al. 1980). The pathogen gradually makes its way to the branches and main stem as fungal mycelia grow in to the phloem causing swelling and forming diamond shaped cankers (Geils et al. 2010). Aecia containing aeciospores develop in the phloem, and after two years will burst through the bark (McDonald and Hoff 2001). These aeciospores can travel vast distances via wind and will infect susceptible alternate host species. Yearly eruptions of aeciospores forming in newly produced rings kill the cambium and eventually damage the xylem (Eckert 2007). The flow of water and nutrients is cut off once the canker encircles the branch or stem, thereby killing any living material above the canker and causing flagging or topkill (McDonald and Hoff 2001). If girdling of the bole takes place from rust, death of the entire tree will occur. Other symptoms of white pine blister rust include roughened bark, oozing sap, and rodent chewing (Shepherd et al. 2018).

White pine blister rust was introduced to North America in the early 1900s from Asia via Europe on a shipment of eastern white pine seedlings (Maloy 1997). In western North America, it is suspected that a single introduction occurred at Point Grey, in Vancouver, BC in 1910 (Mielke 1943).

The main hosts of white pine blister rust are the white pines (*Pinus* subgenus *Strobus*). Its primary alternate host, *Ribes*, is found mainly in moist sites, and as a result there is a higher prevalence of rust in moist, cool areas (Kinloch Jr. et al. 2003; Shepherd et al. 2018). Of the *Ribes* species in North America, *Ribes hudsonianum* Richardson *var. petiolare* (Douglas) Jancz. is most susceptible (Kimmey 1938; Zambino 2010). *Ribes nigrum* L. is another species that has been responsible for the spread of the rust and the focus of control efforts (Maloy 1997; Zambino 2010). More recently, other alternate hosts have been discovered, including native species of *Castilleja* (paintbrush) and *Pedicularis* (lousewort) (McDonald et al. 2006; Mulvey and Hansen 2011). Since suitable host species occur in North America and have little defense, this pathogen has devastated many species of five-needle pines and has infected all but Great Basin bristlecone pine (*Pinus longaeva* Bailey) in wild stands (Dunlap 2012). Although the rust does not directly kill the alternate host, for *Ribes* it has been noted to cause defoliation, decrease winter survival, and reduce fruit production (Pluta and Broniarek-Niemiec 2000).

Eradication and containment programs have not been successful and the rust continues to spread (Maloy 1997; Smith et al. 2013a). White pine blister rust has spread at a rate of 7.7-10.6 km/year in Colorado and Wyoming over the past four decades (Jacobi et al. 2018). Economically valuable trees such as sugar pine (*Pinus lambertiana* Dougl.), eastern white pine (*Pinus strobus* L.), and western white pine (*Pinus monticola* Dougl.) have been nearly wiped out in areas due to the pathogen, to the point where harvesting has been curtailed and companies are reluctant to plant them (Tomback and Achuff 2010; Sniezko et al. 2014b). Additionally, non-commercial species such as limber pine (*Pinus strobiformis* Engelm.) are undergoing the same declines, resulting in the loss of important ecological processes and services, as well as the former two being listed as endangered species (COSEWIC 2010, 2014).

Due to the long life cycle of these trees, natural selection and regeneration for rustresistant individuals is not keeping pace with mortality due to the rust (Bower and Aitken 2008). This is exaggerated for whitebark pine, where dispersal is limited to the corvid Clark's nutcracker (*Nucifraga columbiana* Wilson), and reaching reproductive maturity can take 30-50 years (COSEWIC 2010). Because of this, extensive resistance testing is going on to find genotypes resistant to the pathogen for use in restoration planting (Sniezko et al. 2014b). So far, either partial, complete, or both types of resistance have been found in all white pine species studied (King et al. 2010; Schoettle et al. 2014).

1.2.2 Historical Management

Historically, eradication of *Ribes* was thought to be the best option to reduce the spread of blister rust (Geils et al. 2010). The removal of *Ribes* plants of all species up to 275m from trees was recommended in areas where pines were to be protected (Zambino 2010). Removal was done by hand and was a labour intensive process. Herbicides were used as well to aid with eradication (Zambino 2010). After clearcutting, burning was also used as a method to reduce *Ribes* abundance (Moss and Wellner 1953). It was found that stands with eradication did have slightly lower rust incidence. After 70 years of controlling white pine blister rust through *Ribes* eradication in Maine, disease incidence was lower (3.8%) than in areas with no eradication (9.1%) (Ostrofsky et al. 1988). However, this method has been determined to be less viable in the long term, partly because *Ribes* is a fast growing, early successional plant that is nearly

impossible to completely remove, and also because rust spores can travel long distances via wind dispersal (Maloy 1997; Zambino 2010). Therefore, rust from *Ribes* not immediately in or around the stand being protected can easily travel into it. Additionally, other alternate hosts to blister rust were discovered such as *Pedicularis* and *Castilleja*; therefore, it would be very difficult to completely eliminate the risk of blister rust from eradication of *Ribes* alone (McDonald et al. 2006; Mulvey and Hansen 2011).

Although controversial, the use of antibiotic fungicides was also employed as a control for white pine blister rust in the late 1950's and early 1960's (Maloy 1997). A formula using the chemical cycloheximide dissolved in fuel oil would be sprayed on the lower 5-6 feet of the trunk (Maloy 1997). This was known as basal stem treatment and was widely used in some areas of the US for a few years (Maloy 1997). Aerial application of fungicides occurred as well (Viche et al. 1962). However, fungicides were determined to be ineffective and their use was ended (Maloy 1997).

In Western North America, breeding for genetic resistance to white pine blister rust began in Idaho in 1949 for *Pinus monticola* (Bingham et al. 1959). Breeding work for rustresistance has expanded to all North American five-needle pine species (Hoff et al. 1980; Sniezko et al. 2008). Proactive intervention methods now taking place in five-needle pine restoration seek to increase the frequency of rust resistance and tolerance (Schoettle and Sniezko 2007). These include methods to help increase rust-resistant individuals in five-needle pine populations and minimize the spread of rust.

1.2.3 Spread in North America

After its introduction to Vancouver in 1910, white pine blister rust spread during what are known as "wave" years. These wave years occur during cool and moist spring and summers where suitable conditions cause rapid germination, multiplication, and infection of rust spores. Wave years in North America during the early spread of blister rust were likely 1913, 1917, 1921, 1923, 1927, and 1937 (Mielke 1943). Although limited data exist, it appears rust first spread south into the Cascade Mountains of Washington and Oregon and east into the Rocky and Columbia Mountains of Washington, Idaho, southeast British Columbia, and Montana (Figures 1.1 & 1.2) (Mielke 1943; McDonald and Hoff 2001). The spread has gradually expanded south,

north, and east most recently infecting trees in Colorado and Wyoming (Jacobi et al. 2018). Highest infection levels are in areas of southeastern BC, southwest Alberta, northwest Montana, and the Cascade Mountains of Washington (Rochefort et al. 2018; Shepherd et al. 2018). In British Columbia, rust incidence increases from west to east (Zeglen 2002). Generally rust incidence is lower in warmer and drier areas coinciding with lower abundance of the primary alternate hosts, *Ribes* spp., and less suitable conditions for spore germination (Van Arsdel et al. 1956). In BC, no strong relationships were found between climate and blister rust incidence (Campbell and Antos 2000). In Wyoming and Colorado, *Ribes* densities and probabilities of occurrence could be predicted by type of overstory, elevation, and site classification such as riparian (Kearns et al. 2008). Many susceptible species of *Ribes* exist in Colorado further south so there is a high potential for white pine blister rust to spread in this region (Kearns et al. 2008). In California, latitudinal trends in white pine blister rust have been found with greater incidence further north, which may relate to the introduction location being further north (i.e. Vancouver, BC) (Maloney 2011).

1.2.4 Population Genetics

Strong genetic differentiation exists between eastern and western North American populations of white pine blister rust. Since only a single introduction has been documented in western North America, low genetic diversity exists in this region; however, due to multiple introductions in eastern North America, higher genetic diversity is present (Brar et al. 2015). The main barrier to gene flow between western and eastern populations is the lack of suitable host species in the Canadian Prairies and American Great Plains (Brar et al. 2015). Low genetic differentiation was found among populations within both eastern and western regions; however, western populations are somewhat differentiated due to topographic barriers such as mountain ranges (Brar et al. 2015).



Figure 1.1: Spread of white pine blister rust over time in North America (Sammam et al. 2003). Dark green represents the distribution of five-needle pines while coloured boundaries indicate the extent of blister rust in 1920 (light blue), 1925 (dark blue), 1953 (yellow), and currently (red).



Figure 1.2: Spread of white pine blister rust in western North America from 1925 to 1998. Striped area represents the range of whitebark pine (McDonald and Hoff 2001).

1.2.5 Genetic Resistance

Low levels of resistance have been found in all North American five-needle pine species affected by rust (Kinloch Jr. and Dupper 2002; Sniezko et al. 2014b). Breeding for genetic resistance has been going on for over six decades with early efforts focused on commercially valuable western white pine and sugar pine. Breeding programs were initiated in Northern Idaho for western white pine (1949), Washington and Oregon for western white pine and sugar pine (1956), and in California for sugar pine (1957) (Bingham et al. 1959; McDonald et al. 2001). Current resistance testing of white pines for genetic resistance to white pine blister rust typically consists of six steps as outlined by Sniezko et al. (2014b). The first step is selection of candidate trees, ideally in areas where rust infection and mortality are high so that more natural selection has taken place. This also helps ensure that those trees without rust are likely showing resistance and have not just escaped infection. Next is the collection of seed from these candidate trees to grow for use in controlled inoculations. Once seedlings are showing symptoms of blister rust, the next step is to determine the types of resistance present. The fourth step is to select the healthiest families and individuals or best parents for orchards for breeding. To confirm results from controlled screenings and assess durability of resistance and response to climate, field trials are very useful. Lastly, rust resistant seedlings are used in restoration plantings, and resistant parent trees are closely monitored and protected.

Extensive seed collections and screening of five-needle pine seedlots, including whitebark pine, for rust resistance is occurring at the USDA Forest Service's Dorena Genetic Resource Centre and Coeur D'Alene Nursery, as well as the Kalamalka Forestry Centre near Vernon, BC. The process of controlled inoculations involves taking seedlings into a controlled-environment chamber where *Ribes* leaves containing rust telia are spread over top. A relatively even spore load falls onto the seedlings thereby exposing them to the rust uniformly. At Kalamalka, BC and Dorena, Oregon target inoculum densities are approximately 3000 basidiospores/cm², temperature is maintained at 16-17^oC, and humidity levels are kept at 100% in the inoculation chamber (Danchok et al. 2004). Seedlings are then taken back outside where rust develops on them if susceptible. The first signs of rust appear as needle spots while cankers develop the following year. Detection of a hypersensitive response (complete resistance) is usually done 6-12 months after inoculation, whereas phenotyping for partial resistance is done after seedlings have grown for three to five years (Sniezko et al. 2014b).

1.2.6 Complete Resistance

Complete resistance has been found to white pine blister rust in four of the white pine species studied, and is usually the result of a single major gene causing a hypersensitive response (HR) in the needles (Schoettle et al. 2014). These major resistance (R) genes have been discovered in four different species through genetic mapping and Mendelian segregation (Kinloch Jr. and Dupper 2002; Liu et al. 2017b). They are Cr1 (sugar pine), Cr2 (western white pine), Cr3 (southwestern white pine), and Cr4 (limber pine) (Schoettle et al. 2014). The Cr1 gene was discovered in the 1970's in sugar pine as a phenotype with Mendelian inheritance, indicative of control by a single gene, which displayed distinct needle spots termed 'fleck' spots (Kinloch Jr. et al. 1970). These spots, unlike the susceptible reactions which were yellow and large, developed necrosis at the margins limiting fungal spread, and were darker in colour (Figure 1.3). This indicated the presence of a hypersensitive response to the rust (Kinloch Jr. and Littlefield 1977). Seedlings that displayed a hypersensitive reaction did not develop further stem symptoms, as the rust did not spread beyond the needles. A similar hypersensitive reaction has been found in the other pine species mentioned above (Kinloch Jr. and Dupper 2002; Schoettle et al. 2014).



Figure 1.3: Susceptible reaction (a) and hypersensitive reaction (b, c, d) to white pine blister rust in needles of western white pine (*Pinus monticola*). Necrosis is clearly visible in the bottom right photo (Sniezko et al. 2014b).

A major gene for rust-resistance in western white pine, named Cr2, was discovered in a stand in southwest Oregon called the Champion Mine site (Kinloch Jr. et al. 1999). This location contained very high tree mortality from rust due to the abundance of the alternate host Ribes and the site's location in a steep, narrow and moist canyon perfect for the spread of the pathogen (Kinloch Jr. et al. 2003). Therefore, strong natural selection for resistant individuals had taken place already. The Cr2 gene was discovered through Mendelian segregation of full-sib families (Kinloch Jr. et al. 1999). Once again, a similar hypersensitive response was observed where needle spots would not spread and stem symptoms did not develop. In a range-wide study of western white pine, Cr2 was highest in frequency in the Sierra Nevada and central Cascades of Oregon though overall was very rare (Kinloch Jr. et al. 2003). The Cr1 gene was also concentrated in the Sierra Nevada though at higher frequencies than Cr2 (Kinloch Jr. 1992). Cr2 was not detected in the Coast Mountains, Rocky Mountains, and North Cascades, though this could have been due to fewer samples from those regions (Kinloch Jr. et al. 2003). Zygotic frequencies of Cr2, defined here as the proportion of seed parents heterozygous for Cr2 relative to the total number of seed parents tested, at the Champion Mine site were nearly 20 times higher than in other areas of the central Cascades, highlighting the effect of strong natural selection for resistant individuals that took place at Champion Mine (Kinloch Jr. et al. 2003).

Not only have resistance genes been discovered for commercially important tree species, they have also been found in southwestern white pine and limber pine, both of which have little timber value. The Cr3 gene for resistance in southwestern white pine was found through Mendelian segregation, and more recently the Cr4 gene for resistance in limber pine was discovered from inoculation trials using tree seed collections (Kinloch Jr. and Dupper 2002; Schoettle et al. 2014). Unlike what was found for the other pine species, a hypersensitive response in the needles was not always obvious in limber pine and took longer to develop (Schoettle et al. 2014). Spot type was not always a good indicator of R-gene resistance in the early stages of infection post-inoculation. Additionally, major gene resistance in limber pine was found in Canadian populations as well as American populations (Sniezko et al. 2016a). This is the first instance of a major resistance gene in Canada and the furthest north R-gene resistance found to date in all five-needle pine species (Sniezko et al. 2016a).

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Trade-offs between growth and defenses have been found in several tree species and will be important going forward when selecting traits for tree breeding (Bigler and Veblen 2009). Limber pine seedlings exposed to blister rust and expressing the Cr4 resistance gene displayed significantly reduced growth compared to uninoculated seedlings, indicating a possible trade-off (Vogan and Schoettle 2016). However, no differences were found in carbon relations between limber pine containing the resistance allele and those without it (Vogan and Schoettle 2016). Understanding the costs of resistance alleles on physiology for each species is important for management planning, especially if selecting for a diversity of other resistance mechanisms helps offset these costs. Ongoing work may reveal more resistance genes in other pine species. Relying on complete resistance based on a single gene to protect trees from rust is risky, as it can be overcome more quickly by more virulent rust strains or a change in the pathogen than polygenic resistance mechanisms (Sniezko and Koch 2017).

Virulence to the major resistance genes Cr1 (sugar pine) and Cr2 (western white pine) is evident in white pine blister rust though has not yet been discovered for Cr3 and Cr4 genes (Kinloch Jr. et al. 2004). The vcr1 allele overcomes Cr1 in sugar pine and vcr2 overcomes Cr2 in western white pine. The distribution of these virulent alleles has been determined and it appears that the ranges of vcr1 and vcr2 barely overlap (Kinloch Jr. et al. 2004). The vcr2 allele is concentrated in the central Cascades of Oregon, whereas the vcr1 allele was found only in the Siskiyou Mountains (Kinloch Jr. et al. 2004). Additionally, the distribution of these virulence alleles revealed the limited amount of gene flow among *Cronartium ribicola* populations (Kinloch Jr. et al. 2004). Testing of the vcr2 allele against the Cr4 resistance gene in limber pine revealed that Cr4 could not be overcome by vcr2 (Schoettle et al. 2014). Additionally, neither of the two virulence alleles could overcome the Cr3 gene in southwestern white pine (Kinloch Jr. and Dupper 2002). This suggests that Cr3 and Cr4 are distinct from the Cr1 and Cr2 genes.

Although these single major gene resistances have been found, there appears to be little synteny between the loci after looking at their positions on the chromosomes of each species (Liu et al. 2016). Cr1, Cr2, and Cr4 have been anchored to different linkage groups (Liu et al. 2017a). This may indicate that these resistance mechanisms evolved independently, likely before white pine blister rust was introduced to North America (Liu et al. 2016). Advantages of these complete resistance mechanisms, however, is their simple inheritance and distinct phenotype,

making them easy to distinguish and breed rust resistant offspring (Sniezko et al. 2014b). Advances in genomic and molecular methods have provided greater possibilities in terms of pinpointing genes causing disease resistance and virulence. The entire white pine blister rust genome has been sequenced and the Cr1, Cr2, and Cr4 genes for resistance have been genetically mapped (Jermstad et al. 2011; Liu et al. 2016, 2017a). Identifying resistance related genes underlying each Cr locus will prove useful for marker-assisted selection and may reduce the length of resistance screening.

1.2.7 Partial Resistance

Partial resistance refers to all non-HR types of resistance and tolerance (no-spots, needle shed, short shoot reaction, latency of stem infection, bark reaction, survival with stem infection) which are the result of multiple genes and are quantitative rather than qualitative in nature (Hunt 1997; Sniezko et al. 2014b). Since trees are long lived, determining partial or quantitative resistance in all species affected by rust may be beneficial in the long term as it is a more durable form of resistance (Sniezko and Koch 2017). Complete resistance runs the risk of being overcome by more virulent rust strains, whereas having multiple types of resistance increases the likelihood that the tree will survive through different strains of rust. These different types of resistance and breeding approaches are also referred to as vertical resistance, for complete or Rgene resistance, and horizontal resistance, for polygenic or partial resistance (Van Der Plank 1966). It is still to be determined whether partial resistance is really an effective mechanism of resistance to rust, and if it is genetically grounded or a coincidence of environmental conditions. So far field trials of seedlings that have shown partial resistance have had good success in that they are less affected by rust than the susceptible controls (Sniezko et al. 2014b). Crossing the top performing parents is likely to help boost resistance. Recent genomic studies have found evidence to suggest that partial resistance phenotypes for blister rust are associated with several genes. A genome-wide association study in sugar pine found one gene related to normal active cankers and another two genes associated with blight case phenotypes (Vázquez-Lobo et al. 2017). Blight case phenotypes were those where self-pruning of infected branches stopped canker growth into the stem. However, survival could not be correlated with partial resistance, likely because of the complex inheritance of the rust and slow time to infection (Vázquez-Lobo

et al. 2017). Future studies ought to utilize genome-wide association methods to pinpoint genes associated with partial quantitative resistance in five-needle pines.

1.2.8 Mountain Pine Beetle Interactions

A topic of study showing variable results is the effect of mountain pine beetle on altering the ability of *Pinus* to defend itself against white pine blister rust and *vice versa*. Holtz and Schoettle (2018) hypothesized that limber pine, which contains an unusually high frequency of Cr4 resistance gene in the southern Rocky Mountains, had undergone natural selection for resistant individuals due to mountain pine beetle. However, they found that resistance to mountain pine beetle did not result in an evolutionary mechanism causing high frequencies of Cr4 allele, and that containing the Cr4 allele did not result in higher resistance to mountain pine beetle where white pine blister rust was absent (Holtz and Schoettle 2018). Attack density of mountain pine beetle was found to be lower on heavily infected blister rust trees; however, the beetles that emerged from those trees were larger in size (Dooley and Six 2015). This could indicate that fewer beetles are needed to kill trees already infected by white pine blister rust. In the Greater Yellowstone Ecosystem, mountain pine beetle preferentially selected whitebark pine trees displaying higher severity of blister rust infection (Bockino and Tinker 2012). Interactions between mountain pine beetle and white pine blister rust will be important going forward to understand patterns of rust infection and for successful management of five-needle pines.

1.2.9 Field Trials

Field trials are needed to assess the durability of resistance to rust. So far field trials have been established for most of the North American five-needle pine species; however, results are yet to come in due to the young age of the trials (McLane and Aitken 2012; Sniezko et al. 2014b). The longer these trials are in place, the more useful they are as a measure of resistance durability under field conditions, and the more exposure they have to extreme weather such as drought and heat which can effect tree performance and probability of infection. Field trials of western white pine and sugar pine, the two largest and most important white pine species from a commercial forestry perspective, have shown that the R-gene resistance from Cr1 and Cr2 can be overcome by a virulent race of rust containing vcr1 and vcr2 alleles (Kinloch Jr. et al. 2008; Kolpak et al. 2008). However, families with partial resistance, which is less common in sugar pine compared to western white pine, were not as vulnerable to these virulence alleles (Kinloch Jr. et al. 2008). Reduced mortality was observed in trees containing more genes for partial resistance (Kolpak et al. 2008). A more virulent race of blister rust in China that has not yet made it to North America represents a big threat to the five-needle pine work going on currently (Zhang et al. 2010). Field trials in China using rust resistant seedlings from North America would be beneficial to understand how well resistance holds up to this more virulent rust. Monitoring field trials regularly over time will provide important data for resistance testing.

1.2.10 Climate Change

Generally, temperature and humidity affect tree growth status and vigour, thereby altering their susceptibility to disease (Boland et al. 2004). Climate change induced warmer temperatures, more frequent periods of drought, and the rapid expansion of climate migrants such as mountain pine beetle occurring in western North America are likely altering host susceptibility to diseases such as white pine blister rust (Sturrock et al. 2011; Dooley and Six 2015). Additionally, higher levels of atmospheric carbon dioxide (CO₂) caused by climate change may modify host response to disease. Runion et al. (2010) found that higher CO₂ reduced host susceptibility to fusiform rust and pitch canker fungus.

Although the ranges of suitable host species to white pine blister rust are likely to shift in the long term, the pace of climate change is occurring faster than trees can migrate (Aitken et al. 2008). Assisted migration is being proposed for many tree species as a way to help them adapt to climate change and to ensure genetic diversity is maintained (Aitken et al. 2008; Williams and Dumroese 2013; Aitken and Bemmels 2016). In British Columbia, climate-based seed transfer is starting to be implemented which is allowing seed to be moved further north or higher in elevation based on future predicted climatically suitable habitat (O'Neill et al. 2017). A thorough look at biotic interactions, especially in places where tree species will be introduced to new areas and come into contact with pathogens they have not yet been exposed to, would be necessary before assisted migration takes place. Before this can be done, detailed mapping of current and predicted pathogen ranges which factor in climate change models would be a worthwhile endeavor. Alternate hosts for white pine blister rust already occur further north than five-needle pines, so if pines are moved north using assisted migration, rust is likely to be a threat. In an assisted migration trial of Fremont cottonwood (*Populus fremontii*) it was found that populations

from cooler sites moved further north had nearly four times faster growth, three times greater survival, and were eight times more resistant to shoot blight fungi (*Venturia sp.*) (Grady et al. 2015). Further studies ought to address the interactions of effects of pathogens at the transplant site.

There is little information on how the range of blister rust may shift with the impacts of climate change. This may be partly due to the many interaction effects present, including the susceptibility and changing ranges of host species. White pine blister rust is defined as a pathogen directly affected by climate, which means its life cycle is impacted by temperature and precipitation (Sturrock et al. 2011). *C. ribicola* thrives in cool, moisture-saturated environments (Zambino 2010). With a trend towards drier and hotter climates in its current range, the frequency of periods suitable for infection is likely to decrease, including a reduction in wet periods in spring and summer (Sturrock et al. 2011). White pine blister rust, however, is likely to adapt more quickly to climate change compared to host tree species due to its faster rate of reproduction and shorter life span (Sturrock et al. 2011).

1.3 Whitebark Pine

1.3.1 Taxonomy and Distribution

Whitebark pine (*Pinus albicaulis*) is a western North American high elevation fiveneedle pine of subsection *Cembrae*. Closely related species include other stone pines of the subgenus *Strobus*, section *Strobi*, and subsection *Cembrae*, including Swiss pine (*Pinus cembra* L.), Korean pine (*Pinus koraiensis* Sieb. et Zucc), Japanese stone pine (*Pinus pumila* Regel), and Siberian pine (*Pinus sibirica* Du Tour), all of which are wind pollinated and bird dispersed (McCaughey and Schmidt 2001). The geographic range of whitebark pine spans from the Rocky and Coast Mountains of central British Columbia in the north, to the Sierra Nevada of California in the south, and the Rocky Mountains of Colorado and Wyoming to the East, covering 18° latitude and 21° longitude (Gernandt et al. 2005). Whitebark pine has little commercial value due to its small size, often multistemmed and forked morphology, and often difficult to access habitat. Nonetheless, the species does get harvested as by-catch in some parts of its lower elevation range limit where it mixes with more commercially valuable species such as lodgepole pine and interior spruce. Additionally, it is sometimes harvested to make way for development projects such as mines and ski resorts (Environment and Climate Change Canada 2017).

1.3.2 Ecology

Dispersal of this species is entirely reliant on Clark's nutcracker (*Nucifraga columbiana*) which is regarded as a mutualism (Tomback 1982). Clark's nutcrackers open the cones of whitebark pine, then extract and cache the large, wingless seeds in their home ranges. Estimates of maximum distances between harvest trees and cache locations for Clark's nutcracker vary from 12.5km (Tomback 1978) up to 32.6 km away from the source tree as a winter food supply (Lorenz et al. 2011). In the Cascade Range, it has been determined that more seed caches are located above ground compared to below, and of those above ground caches, most are placed in living trees (Lorenz et al. 2011). Each cache contains 1-15 seeds and when located below ground is buried 1-3cm deep (Tomback and Linhart 1990). Many of these seed caches are left untouched and germinate producing solitary seedlings or clusters (Tomback 1982). Delayed seed germination has been observed in whitebark pine following fire (Tomback et al. 2001a). Whitebark pine may take 30-50 years to reach maturity and start producing cones, but large numbers of cones are not produced until trees reach 60-80 years of age (COSEWIC 2010). Many Clark's nutcracker caching sites are located in open or recently disturbed locations such as burns or clearcuts (Tomback 1986).

Whitebark pine is considered a keystone species in upper treeline environments where its seeds provide an important food source to grizzly bears, squirrels, and a variety of bird species including Clark's nutcracker (Mattson and Reinhart 1997; Tomback et al. 2001b). In the Greater Yellowstone Ecosystem, grizzly bear mortality nearly doubles in non-mast seed years due to an increase in human-bear conflicts as a consequence of seed shortage (Pease and Mattson 1999). Additionally, whitebark pine is a pioneer of harsh alpine sites, sheltering less cold and wind tolerant conifers (Tomback et al. 2001b). Whitebark pine seedlings are extremely hardy due to their long taproots, high tolerance of exposure and drought, and are often the first to establish following disturbance (Arno 1986; Tomback 1986). Near treeline, whitebark pine often grows in krummholz form and in tree-islands, patches of dwarfed and deformed trees occurring within high elevation tundra vegetation (Marr 1977; Resler and Tomback 2008). In northern Montana, Resler and Tomback (2008) found that whitebark pine was more often the sole initiator of tree-

island formation compared to other conifer species. Additionally, leeward microsites of whitebark pine experience lower wind speeds, and more moderate soil temperatures conducive to the establishment of other alpine species (Pyatt et al. 2016). Although whitebark pine encompasses a large latitudinal range, it is generally found at high elevations from the upper subalpine to treeline. This can be attributed to its ability to withstand cold and direct sunlight, as well as its poor competitive ability and slow growth (Tomback et al. 2016). Whitebark pine is generally thought to help increase snowpack retention and delay snowmelt. Its presence near treeline causes the redistribution and higher accumulation of snow throughout the winter which helps ensure more even runoff until late in the summer, though more empirical studies are needed (Tomback et al. 2016). Additionally, whitebark pine helps to reduce soil erosion due to its presence at high elevations and in poor soils rarely tolerated by other conifers (Tomback et al. 2016).

1.3.3 Quantitative Genetics

Due to the bird dispersed nature of whitebark pine and cache structure of seed dispersal, multiple trees germinating from a single cache are often fused at the base and are more closely related to each other than to other trees. Clark's nutcrackers fill their pouches with approximately 35-150 seeds from only one or a few parent trees (Tomback 1978, 1982). As, a result there is high potential for inbreeding and repeated founder effects. Additionally, whitebark pine inhabits a narrow range of climatic conditions in often small and isolated populations. F_{ST} or G_{ST} values are low and range from 0.004 to 0.088 (Table 1.1). In the Greater Yellowstone Ecosystem, whitebark pine contains low population and species level genetic differentiation with a mean F_{ST} of 0.025. F_{ST} reported in the Great Basin area was a bit higher at 0.088 (Yandell 1992) as well as in British Columbia (0.061) (Krakowski et al. 2003). Among 164 US sources, F_{ST} was low (0.026) (Mahalovich and Hipkins 2011). Low differentiation was found in the Lake Tahoe Basin (F_{ST} =0.0069) although strong evidence suggests local adaptation to the dry climate of this region (Lind et al. 2017). Among three watersheds in the Sierra Nevada of California, genetic differentiation was found to be very low ($F_{ST}=0.004$), while between high elevation krummholz form and lower elevation upright growth form F_{ST} was somewhat higher (0.051) (Rogers et al. 1999). Strong differentiation was found among individual clusters and krummholz within a sample site ($F_{ST}=0.334$).

Author(s)	$F_{\rm ST}$ or $G_{\rm ST}$	# Loci	Location	# Populations
Rogers et al. 1999	0.004	21	Sierra Nevada, California	3
Lind et al. 2017	0.0069	N/A	Lake Tahoe Basin	8
Bruederle et al. 1998	0.025	19	Greater Yellowstone Ecosystem	9
Mahalovich and Hipkins 2011	0.026	16	Inland Northwest	117
Jorgensen and Hamrick 1997	0.034	20	USA Range and Northern AB	30
Bower et al. 2011	0.038	N/A	Range Wide	85
Richardson et al. 2002	0.046	3*	Range Wide	18
Bower et al. 2011	0.059	N/A	Olympic Peninsula	9
Krakowski et al. 2003	0.061	10	British Columbia	17
Stuart-Smith 1998	0.062	N/A	Canadian Rockies	29
Yandell 1992	0.088	13	USA Great Basin	14

Table 1.1: Summary of F_{ST} (proportion of genetic variation due to differences among populations) or G_{ST} (F_{ST} extended for 3 or more alleles) values found for whitebark pine (*Pinus albicaulis*) populations. Data are from isozymes unless otherwise noted.

*Used chloroplast DNA microsatellites

Low among population differentiation (Q_{ST}) values have also been found in whitebark pine for growth-related traits and moderate values for cold adaptation-related traits (Table 1.2). Low to moderate heritability for cold injury traits have been found in whitebark pine and are most closely related to mean coldest month temperature (Bower and Aitken 2006). Whitebark pine has been found to be more cold tolerant than other conifer species. These low values, as well as low levels of local adaptation in whitebark pine, may allow seed to be transported further distances to planting sites as compared to other conifer species. Heritability of different traits range from 0.18 to 0.92 (Table 1.3). Rust-related traits show moderate to high heritability, while height shows more moderate heritability and cold hardiness lower heritability. Published studies on estimating breeding values for whitebark pine parent trees are lacking.

Height growth is often used as a phenotypic trait of interest in tree common garden studies as it provides a measure of juvenile fitness and the ability of individuals and populations to grow competitively, and is an important trait for commercial forestry (Aitken and Bemmels 2016). Additionally, due to the long-lived nature of trees, assessing lifetime fitness is unrealistic. However, for whitebark pine, height growth provides less of a competitive advantage especially in the low density open subalpine stands it often occupies, and is a less important trait for breeding since this species is not harvested commercially (Arno and Weaver 1990).

Table 1.2: Summary of Q_{ST} (among population differentiation for phenotypic traits) values found for whitebark pine (*Pinus albicaulis*) growth, cold hardiness, germination, survival, and blister rust-related traits.

Author(s)	$Q_{ m ST}$	Trait(s)
Bower and Aitken 2008	0.07-0.14	growth
	0.37-0.47	cold hardiness
McLane 2011	0.07	height
	0.15	germination
Mahalovich and Hipkins 2011	0.12-0.19	height
	0.10-0.11	cold hardiness
	0.14	% rust resistance
	0.07	survival

Table 1.3: Summary of heritability values found for whitebark pine (*Pinus albicaulis*) and western white pine (*Pinus monticola*) for growth, cold hardiness, and blister rust-related traits.

Species	Author(s)	Heritability (h ²)	Trait(s)
whitebark pine	Bower and Aitken 2006	0.18, 0.28	cold hardiness
	Hamlin et al. 2011	0.57-0.83	height
	Mahalovich et al. 2006	0.56	rust resistance
		0.64	survival
		0.85	height
		0.50	cold hardiness
	Sniezko et al. 2014a	0.69, 0.78	height
		0.92	# stem symptoms
		0.39	# spots
western white pine	McLane 2011	0.27	height
	Steinhoff and Hoff 1971	0.28, 0.43	annual height growth
	Meagher and Hunt 1996	0.77	spot frequency per seedling
	Mahalovich 2010	0.23	spots
		0.60	early stem symptoms
		0.64	bark reaction
		0.55	canker tolerance

1.3.4 Threats

White pine blister rust represents the biggest threat to whitebark pine across its range; however, other threats include mountain pine beetle, fire suppression and resulting encroachment of lower elevation species, and climate change. Blister rust alone is predicted to cause over 50% decline in whitebark pine populations over the next 100 years (COSEWIC 2010). The species is listed as endangered in Canada under the Species at Risk Act (2012) and was nominated for endangered listing in the United States as well (2011), but was not listed due to a lack of resources for species recovery. According to the proposed recovery strategy for whitebark pine put forward by the Government of Canada, the objective for rehabilitation of the species is to institute a "self-sustaining, rust resistant population of whitebark pine throughout the species'

range that demonstrates natural seed dispersal, connectivity, genetic diversity, and adaptability to changing climate" (Environment and Climate Change Canada 2017).

Monitoring over the past ten years has shown the cascading effects of white pine blister rust, including an increase in infection and mortality, as well as a reduction in cone production and subsequent visitation by Clark's nutcracker (Mckinney et al. 2009; Shepherd et al. 2018). A loss of cone-producing branches due to rust reduces seed production and dispersal capability for the tree (McKinney and Tomback 2007). This is because Clark's nutcracker occurrence declines substantially in areas with cone densities below 300 cones/ha (Mckinney et al. 2009). A threshold of 1000 cones/ha, or 5.0 m²/ha basal area, has been suggested as a requirement for high likelihood of nutcracker presence during seed dispersal time (Mckinney et al. 2009). A loss in dispersal in some stands where cone production is very low will likely have a significant negative impact on regeneration so that those stands may become functionally extinct (Mckinney et al. 2009). In some areas of the southern Rocky Mountains, the probability of infection and mortality due to white pine blister rust exceeds 90% (Shepherd et al. 2018). Over 13 years of monitoring, infection and mortality have increased at an average of 3% per year in the Canadian Rocky Mountains (Smith et al. 2013a). In the North Cascades of Washington, blister rust infection on whitebark pine is increasing at 1.5% per year in Mt. Rainier National Park and 2.3% per year in North Cascades National Park (Rochefort et al. 2018). Simulation modelling of whitebark pine forests into the future show that the species will continue to decline over the next several decades primarily from the impacts of white pine blister rust and a smaller climate change component (Keane et al. 2017). Because of the trees' slow regeneration time, the natural selection of rust resistant trees and their subsequent regeneration will not keep up with the pace of tree mortality due to the pathogen (Bower and Aitken 2008). Additionally, although many areas have avoided infection so far, it is unlikely that these areas will escape infection in the future (Kinloch Jr. et al. 2003).

Rust incidence on whitebark pine varies based on topographic and climatic features. In dry and cold climates of Montana, tree-island whitebark pine have higher canker intensity and likelihood of infection compared to solitary trees (Smith-Mckenna et al. 2013). Solar radiation, aspect, and moisture related variables all affected likelihood of infection by blister rust (Smith-Mckenna et al. 2013). In Wyoming and Colorado, a survey of nearly 19000 limber pine revealed that likelihood of infection was higher on larger diameter trees and in more northerly and easterly plots (Kearns and Jacobi 2007). Within the Greater Yellowstone Ecosystem, the best predictors of white pine blister rust infection on whitebark pine included August and September relative humidity and temperature (Thoma et al. 2019). Probability of infection increased after relative humidity surpassed 50% and temperature reached 11°C (Thoma et al. 2019).

Mountain pine beetle is also a substantial threat to whitebark pine ecosystems. Warmer winter temperatures have caused outbreaks in areas such as the Greater Yellowstone Ecosystem and Central British Columbia, and an increase in beetles at higher elevations (Macfarlane et al. 2013). For whitebark pine, minimum winter temperatures were likely the limiting factor for mountain pine beetles in colder areas before outbreaks occurred (Buotte et al. 2017). Increased winter temperatures and reduced summer precipitation will lead to an increase in suitable climates for outbreaks in whitebark pine (Buotte et al. 2017). Mountain pine beetle have caused the mortality of many individual whitebark pines including candidate trees marked for cone collections, and this mortality is increasing (Logan et al. 2010; Macfarlane et al. 2013). Beetles attack large, mature whitebark pine, often by spreading up in elevation from lodgepole pine stands below. In Montana, whitebark pine that survived mountain pine beetle attack had larger resin ducts and slower growth compared to those killed (Kichas et al. 2020). The antiaggregation pheromone verbenone, often utilized by stapling pouches to tree boles, has been known to decrease whitebark pine mortality from mountain pine beetle outbreaks (Perkins et al. 2015). Compared to untreated control plots, Fettig et al. (2012) saw an average reduction in whitebark pine mortality of 78% as a result of using Verbenone Plus.

Fire suppression and changes in fire regimes also represent threats to whitebark pine ecosystems. Throughout the twentieth century, fire suppression has reduced the frequency and severity of fires in the subalpine where historically fires occurred every thirteen to four-hundred years (Arno 1980; Larson et al. 2009). Fires reduced the abundance of subalpine fir and created open stands more conducive to the establishment of fire-tolerant whitebark pine (Arno 1986). As a result of fire exclusion, lower elevation species such as lodgepole pine and late-successional species such as subalpine fir have begun encroaching into whitebark pine habitat (Sala et al. 2001). Additionally, areas suitable for Clark's nutcracker caching have been reduced due to establishment of later successional species. Restoration actions such as prescribed burning and thinning are required to ensure the long-term viability of whitebark pine forests (Keane et al. 2017). Wildfires also have to potential to burn healthy stands of whitebark pine including candidate trees identified as having resistance to white pine blister rust. Due to fire suppression, a build-up of organic material has taken place and is now causing larger and higher severity fires that more often reach into subalpine forests than previously (Rollins et al. 2001). The frequency and severity of these fires are further exacerbated by a warming climate in western North America (Schoennagel et al. 2017).

On top of threats such as altered fire regimes and mountain pine beetle outbreaks which are indirect effects of climate change, whitebark pine is experiencing a major shift in its climatic niche. Species distribution models predict that only 11% of current climatically suitable areas will remain suitable by 2085 thereby resulting in a reduction in whitebark pine frequency (T. Wang, unpublished). Since the tree is found most often near treeline, and is a poor competitor, it has more limited available space to move upwards in elevation as current lower elevation habitat becomes unsuitable. With limited soil higher in elevation, and increased competition from lower elevation species below, whitebark pine is experiencing a narrowing ecological niche (McLane and Aitken 2012). Cold temperatures have been found to be a strong predictor of whitebark pine presence with higher temperatures corresponding to reduced probability of occurrence (Clason et al. 2020). Additionally, whitebark pine has a migration lag relative to its climate niche, as it does not currently occupy its entire climatic niche, with areas further north of its current range having suitable climates to support its presence (McLane and Aitken 2012; I. Reid, S. McLane and S. Aitken, unpublished). Clason et al. (2020) suggest that whitebark pine is not constrained by cold at its northern limit but rather by other factors such as dispersal limitation by the Clark's nutcracker or other biotic interactions. Bower and Aitken (2008) found a relatively low degree of local adaptation and geographic differentiation in whitebark pine, with low $Q_{\rm ST}$ values for growth traits and moderate values for cold adaptation traits (Bower and Aitken 2008). To avoid maladaptation, transfer distances of up to 1.9°C MAT in the northern range of the species and 1.0°C MAT in the U.S Rocky Mountains are recommended (Bower and Aitken 2008).

1.3.5 Restoration Strategies

Whitebark pine decline can be mitigated through implementing restoration strategies including the collection of seed from putatively rust-resistant trees, identification of rust resistant

genotypes, planting rust resistant seedlings, thinning competing species, prescribed fire, protection from mountain pine beetle using pheromones, and assisted migration informed by climate and species distribution modelling (McLane and Aitken 2012; Shepherd et al. 2018). Arguably, the identification and planting of rust resistant seedlings is the most important of these restoration strategies for the long-term survival of the tree. Seed collections and screening of whitebark pine for rust resistance is already occurring at the USDA Forest Service's Dorena Genetic Resource Centre and Coeur D'Alene Nursery, as well as the BC Ministry of Forests, Lands, Natural Resource Operations, and Rural Development's (FLNRORD) Kalamalka Forestry Centre near Vernon, BC where field-collected seed is being grown, seedlings are control inoculated with rust spores, and rust resistant parent trees are being identified. Long-term field trials are needed to assess the durability of resistance (Sniezko et al. 2011). Results from rust resistance trials can be used to identify parents for seed collections for restoration planting, or to graft into seed orchards.

Although complete resistance has not been found in this species, there may be sufficient levels of partial resistance to ensure higher survival of planted seedlings (Sniezko et al. 2014b). Mechanisms of resistance controlled by single-gene recessives documented in whitebark pine are rare and include the phenotypes no-spot and needle shed (<1% frequency), and short shoot (5.2% frequency) (Landguth et al. 2017). Short shoot fungicidal reaction is a resistance mechanism that has also been documented in western white pine and occurs when fungal hyphae reach the base of the needle fascicle triggering necrosis and stopping the fungus from reaching the stem (Hoff and McDonald 1971). Resistance screening trials are underway and as of 2010, 650 families had been inoculated with hundreds more collected since then (Sniezko et al. 2011). Early screening of 225 families from 21 geographic locations tested at Dorena Genetic Resource Centre found that seedlings from Oregon and Washington populations showed more resistance compared to those from Idaho, Montana, California, and British Columbia (Sniezko et al. 2018). In a trial at Coeur D'Alene, among 108 seed sources from the interior US mountains, the percentage of rust resistance was 48% after four rust screenings (Mahalovich et al. 2006). It has generally been found that seedlings from parent trees located in warmer, wetter areas show higher levels of resistance. This is presumably due to the higher amounts of blister rust present in those areas having resulted in more natural selection for resistant parent trees.
There is a great need for rust resistant seedlings for restoration planting along with other restoration tactics such as prescribed burning, thinning competing species, and protection from mountain pine beetle if whitebark pine is to persist in the future (Keane et al. 2017; Shepherd et al. 2018). In the Greater Yellowstone Ecosystem, where planting of whitebark pine has been occurring for three decades, growth rate of trees contained a positive correlation with actual evapotranspiration (Laufenberg et al. 2020). Direct seeding has been shown to be a relatively successful method of growing whitebark pine and may be an alternative to planting seedlings in remote locations or when seedlings are not available (McLane and Aitken 2012; Pansing and Tomback 2019). Additionally, whitebark pine may be one of the first tree species to undergo assisted range expansion in Canada to aid in its adaptation to climate change (McLane and Aitken 2012). A careful study of the biotic interactions the tree may face outside of its current range, including blister rust hazard and alternate host ranges, as well as the potential for a range shift in Clark's nutcrackers, essential for seed dispersal from and expansion of migrated populations, should be undertaken.

1.3.6 Thesis Objectives

The process of controlled inoculations for screening whitebark pine for rust resistance is costly, time and labour intensive. Additionally, the number of families that can be tested at a time is restricted by the size of facilities and staff required to complete the process. Due to the urgency of whitebark pine decline, time intensive screening is a limiting factor when it comes to the restoration of this species. Research into alternative methods of screening whitebark pine for resistance to blister rust is lacking and streamlining this process may help to increase availability of material for planting. To investigate this, the objective of this research is to determine the effectiveness of an alternative approach to inoculating and screening whitebark pine families for resistance to white pine blister rust. This objective was examined by answering the following research questions:

• How effective is natural inoculation from *Ribes nigrum* in a common garden experiment for identifying genetically resistant whitebark pine?

- Are there genetic differences among families or provenances for height or rust resistance?
- Are there environmental variables related to height and resistance to white pine blister rust?

2 A new approach to determining family-level resistance to white pine blister rust in whitebark pine (*Pinus albicaulis*)

2.1 Introduction

Cronartium ribicola, the introduced pathogen causing white pine blister rust, is primarily responsible for the dramatic increase in whitebark pine mortality in recent decades (COSEWIC 2010; Shepherd et al. 2018). Whitebark pine has little resistance to this disease, which is now present throughout its range (Schoettle and Sniezko 2007; Smith et al. 2008). Additional threats to whitebark pine include the combined impacts of mountain pine beetle, altered fire regimes, and climate change (COSEWIC 2010; Smith et al. 2013b). In some locations, white pine blister rust is present on over 90% of trees with a mortality exceeding 50% (Smith et al. 2008; Shepherd et al. 2018). Whitebark pine is an important foundation species in mountain environments of western North America, where it helps slow the speed of snowmelt, acts as a pioneer at, and above, treeline, and provides food to many wildlife species including bears, squirrels, and birds (Tomback et al. 2001b). Whitebark pine is reliant on the corvid, Clark's nutcracker, to disperse its seeds (Tomback 1982). In some areas, white pine blister rust has caused cascading effects such as a loss in cone production, resulting in reduced Clark's nutcracker visitation and seed dispersal (Mckinney et al. 2009). Although different approaches have been utilized to reduce the spread and impact of white pine blister rust (such as eradication of the primary alternate host, *Ribes*), genetic resistance represents the most promising conservation strategy to ensure the continued existence of whitebark pine (Ostrofsky et al. 1988; Sniezko et al. 2014b).

Although the frequency of resistance to blister rust is very low in all five-needle pines, it is present and heritable (Schoettle and Sniezko 2007). Previous studies have found moderate heritability for height and white pine blister rust infection in whitebark pine (Mahalovich et al. 2006; Mahalovich 2010; Hamlin et al. 2011; Sniezko et al. 2014a). For commercial white pines, breeding programs have successfully increased the availability of resistant material for planting. Although trait heritability is relatively high for whitebark pine, among-population differentiation for phenotypic traits is quite low (Bower and Aitken 2008).

The process of identifying rust-resistant genotypes of whitebark pine is traditionally done through controlled inoculations (Danchok et al. 2004; Sniezko et al. 2014b). This process

involves growing seed collected from phenotypic selections of trees displaying no rust, often in high infection stands, until seedlings are large enough to display signs of rust. Seedlings are placed in a controlled-environment chamber where *Ribes* leaves containing rust telia are suspended over the seedlings, leading to a controlled rust infection. Seedling assessments take place over a five-year period post-inoculation. Over 1000 whitebark pine families have been subjected to controlled inoculation testing so far (Sniezko et al. 2016b). However, space, personnel, and equipment constraints render this process costly, time and labour intensive. Since whitebark pine continues to decline, research into more rapid alternative screening methods for resistance to blister rust is necessary, and streamlining this process may increase the availability of material for planting.

The British Columbia Ministry of Forests, Lands, Natural Resource Operations, and Rural Development (FLNRORD) planted a common garden experiment of one-year-old whitebark pine seedlings at Skimikin nursery near Salmon Arm, British Columbia in 2015. It contains 217 open pollinated families and 44 provenances from across the species range. A western white pine progeny test interplanted with *Ribes nigrum* adjacent to the whitebark pine common garden has caused blister rust infection of many of the whitebark pine seedlings through wind dispersal of basidiospores. The six-year-old seedlings were phenotyped for signs of blister rust infection, including needle spots and stem infections. Seedling height, which is a trait of interest in many forest genetics studies, was measured to determine if there is a trade-off between disease resistance and growth. Growth-defense trade-offs have been observed in other plant and tree species, especially for biotrophic pathogens such as white pine blister rust (Loehle 1988; Bigler and Veblen 2009). Additionally, measuring seedling height allows for comparisons of population differentiation and genetic clines with other species and studies. For example, Aitken and Bemmels (2016) were able to compare 18 species from 23 data sets for genetic clines in height growth in relation to temperature gradients.

This common garden study tests a potential alternative to controlled inoculations which could save time and money for whitebark pine and other five-needle pine recovery. By exposing whitebark pine seedlings to blister rust in a field common garden rather than in an enclosed facility, family differences in rust resistance can still be determined and the potential to screen many more families at once is therefore possible. The objectives of this study are to: (1) determine the effectiveness of wind-dispersed natural inoculation of whitebark pine by the fungus causing white pine blister rust; (2) evaluate whether proximity to *Ribes nigrum* plants affects the likelihood of seedling infection in a nursery setting; (3) identify the most blister rust-resistant/tolerant whitebark pine progeny and geographic distribution of parent trees; and (4) determine what environmental factors of provenance location are related to height and rust resistance in whitebark pine seedlings. Results from this study will facilitate selection of rust resistant parent trees and inform development of a whitebark pine seed orchard. I predicted that seedlings in the common garden closer to the *Ribes* would be more severely infected by blister rust and have higher mortality. Additionally, I expected the provenance-mean rust resistance level to reflect time since arrival of rust after its introduction to Vancouver, BC in 1910. Climate variables affecting rust resistance were predicted to be those determining the presence of *Ribes*, such as higher moisture and warmer temperatures, and those closely affecting the blister rust life cycle.

2.2 Methods

2.2.1 Common Garden Experiment

Data were collected in a common garden experiment located at the FLNRORD Skimikin Nursery (50.79°N and -119.43°W), approximately 13 km northwest of Salmon Arm, British Columbia. The nursery site is located at approximately 550m above sea level, and is within the Interior Douglas-fir moist warm (IDFmw2) biogeoclimatic zone variant (Pojar et al. 1987). This site harbours warmer climates and longer growing seasons than are typical of natural whitebark pine habitat, with a mean annual temperature (MAT) of 7.0°C and frost-free period (FFP) of 145 days (estimated using the software *ClimateNA* - Wang et al. 2016). Mean annual precipitation (MAP) at the nursery is estimated to be 602 mm. In contrast, provenance locations in this study contained an average MAT of 1.6°C, FFP of 81 days, and MAP of 1247mm. The common garden is located on relatively flat ground with minimal topographic variation.

The Skimikin common garden contained 4282 seedlings comprised of 217 openpollinated whitebark pine families from 44 provenances throughout the species range. A list of provenances can be found in Appendix 1. Seeds were collected from healthy parent trees at least 50 metres apart and, in some locations, where rust infection was high. Most of the seed was obtained from the *ex situ* reserve at the FLNRORD Tree Seed Centre in Surrey, British Columbia. Additional seed was obtained from the US Forest Service's Dorena Genetic Resource Centre, Oregon and Coeur D'Alene Nursery, Idaho, as well as the British Columbia Ministry of Environment. Over half of parent trees (116) were from BC, while the remainder (101) were from the US including Washington, Oregon, Idaho, Montana, Wyoming, and Nevada. Approximate locations of parent trees are known and range from 39.32⁰N to 55.02⁰N in latitude and -110.45°W to -127.28°W in longitude. Seed was first stratified by FLNRORD in November 2013. Stratification methods included soaking of seeds in oxygenated water, five weeks of warm stratification (20°C), 12 weeks of cold stratification (2°C), and nicking the radicle end of the seed coat to facilitate germination. Seed was sown in March 2014 and seedlings were grown throughout the 2014 and 2015 growing seasons. One-year-old seedlings were planted at Skimikin in 2015 by FLNRORD. Families planted at Skimikin were also planted at field test sites throughout BC in 2015, though these were not surveyed in this study.

The experimental design established by FLNRORD included 8 columns and up to 595 rows per column, and had an alpha (resolvable block) design with 20 replications. Each family had up to 20 seedlings in the test, with one seedling per block per family. Thirty-two of the 44 provenances were represented by three or more families (Appendix 1, Table 1). The common garden was beside an experiment of western white pine interplanted with *Ribes nigrum*. Column one was located three metres from the *Ribes nigrum* and western white pine plantation and three metres from column two. All subsequent columns were two metres apart with the furthest being 18m from the *Ribes*. Within columns, seedlings were 11 to 14 cm apart.

Proximity to the western white pine and *Ribes* trial allowed for the natural inoculation of white pine blister rust into the whitebark pine seedlings via wind dispersal of basidiospores. Seedlings were watered at the nursery via drip irrigation, minimizing drought. Cold injury was unlikely due to the low elevation of the nursery site compared to the natural range of whitebark pine.

Eighty-one of the whitebark pine families included in the Skimikin common garden were previously tested for blister rust resistance at the Dorena Genetic Resource Centre (hereafter referred to as "Dorena"), Oregon using standard controlled inoculation procedures (Danchok et al. 2004). During controlled inoculations in a closed chamber, *Ribes* leaves containing blister rust basidia are spread evenly over top of seedlings, allowing basidiospores to fall down onto them. Target inoculum densities are approximately 3000 basidiospores/cm². Humidity levels are kept at 100% and temperature is maintained at 16-17°C in the inoculation chamber to ensure successful infection (Danchok et al. 2004). These 81 families were selected for inclusion in the Skimikin experiment based on available, viable seed and a wide geographic distribution. Although they were random selections of families that were previously tested, some of the seed parents were phenotypic selections from healthy trees in high infection stands. These families were used to compare the effectiveness of the natural inoculation at Skimikin to controlled inoculations.

2.2.2 Data Collection

Phenotyping of seedlings and blister rust identification in the spring of 2019 were based on procedures used at Kalamalka and Dorena. Every seedling at Skimikin was closely examined from the root collar to the top for evidence of white pine blister rust and other diseases. Insect damage and unusual growth forms were noted. The following were recorded for each seedling: height (cm), presence/absence of needle spots, cause of damage, severity of damage, vigour, presence/absence of bole infections, presence/absence of limb infections, presence/absence of needle flush, presence/absence of aecia, and notes (Appendix 2). Spots, bole infections, limb infections, needle flush, and aecia were recorded as binary variables while damage, severity, and vigour were recorded on a scale (Appendix 2). Height of living or recently dead seedlings was measured to the top of previous year's growth and rounded to the nearest 0.5 cm. Seedlings were classified as alive if any needles were still green (even if chlorotic), and dead if all needles were brown. Rust cankers were not counted since the merging of adjacent cankers create unreliable counts. Blister rust resistance ratings were also obtained from Dorena for the 81 families also present at Skimikin, with categorical grades from A (most resistant) to F (most susceptible) for each of the families. Grades were mainly based on the percentage of infected seedlings per family (%SS) at the second assessment one year after inoculation. Cut-offs for each grade were determined from plotting the family means for %SS and looking for any natural breaks. Data collection at Skimikin occurred in mid-May 2019 to ensure aecia was clearly visible. Estimates of provenance climates were obtained from ClimateNA version 5.60 using coordinates of parent trees and the 1981-2010 climate reference period (Wang et al. 2016).

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2.2.3 Data Analysis

The final dataset contained 4100 seedlings from 214 families and 44 provenances. Twenty-five seedlings had their roots removed by rodents and were removed from the blister rust analysis but were retained for height analysis. Missing seedlings, seedlings with missing tags, and those that could otherwise not be assessed were also removed (n=182). Raw height data were normally distributed and were used for analysis. Blister rust data, originally categorical with ten levels, were first re-classified into 4 levels (1=no rust (originally 0), 2=originally categories 1 through 4, 3=originally categories 5 through 8, 4=dead from rust (originally 9)). The ordered discrete categories for rust severity were then converted to normal scores before estimating breeding values for resistance to account for the subjective nature of assessor-specific phenotyping (Gianola and Norton 1981; Cappa and Varona 2013). This method of transforming values to normal scores has been used by tree breeding programs in Sweden (Ericsson et al. 1994).

Data were analyzed using the statistical analysis program R, version 3.5.1 (R Core Team 2018). Linear mixed models were fit using the R package ASReml-R (Butler 2019) to include the spatial correlation between column and row. Replication was removed from rust models since it had no significant effect; however, it was retained for the height models as a fixed effect. A pedigree was created to fit individual tree models (also known as "animal models", Wilson et al. 2010) for height and rust. The individual tree model uses pedigree information to create a matrix and estimate additive genetic effects. The following individual tree model was used for blister rust analysis:

$$y_{ijk} = \mu + u(i) + p(j) + t(k) + e$$

Where μ is the overall mean, u(i) is the effect of units (x, y position of seedling), p(j) is the effect of provenance, t(k) is the effect of the individual tree pedigree matrix including family, and *e* is error term containing spatial correlation. For height, the same model was used except that r(i)(replication) was added as a fixed effect:

$$y_{ijkl} = \mu + r(i) + u(j) + p(k) + t(l) + e$$

Both provenance and the seedling matrix were random effects enabling variance component estimation for heritability (h^2) and among-population differentiation for phenotypic traits (Q_{ST})

estimation. During model fitting, the 12 provenances represented by fewer than three families were also removed when estimating Q_{ST} , h^2 , and breeding values. The individual tree model assumes a coefficient of relatedness of 1/4 between half-sib seedlings. Since open pollination of whitebark pine results in some seeds from self-pollination and mating among relatives (Krakowski et al. 2003), offspring are likely more closely related to each other than half-sibs (Bower and Aitken 2008). To account for more relatedness, additive genetic variance (V_A) was multiplied by 0.75, assuming a coefficient of relatedness of 1/3 rather than 1/4. Narrow sense heritability was estimated using the individual tree model as:

$$h^2 = (3/4\sigma_f^2) / ((3/4\sigma_f^2) + \sigma_p^2 + \sigma_u^2)$$

Where σ_{f}^{2} is the family variance, σ_{p}^{2} is the provenance variance, and σ_{u}^{2} is the variance for units. Among-population differentiation was estimated as (Whitlock 2008):

$$Q_{\rm ST} = \sigma_{\rm p}^2 / (\sigma_{\rm p}^2 + 2(3/4 \sigma_{\rm f}^2))$$

To obtain breeding values for backward parental selection, provenance was left as a fixed effect in the individual tree models while the seedling matrix (including family) was kept random. Provenance BLUEs (best linear unbiased estimators of fixed effects) were added to family BLUPs (best linear unbiased predictors of random effects) to obtain estimated breeding values for each family. Breeding values for rust were multiplied by -1 so that higher breeding values corresponded to higher resistance. Wald tests were performed on fixed effects, including provenance in the rust model and replication in the height model, to determine their significance (Wald 1943). Families were then ranked for rust resistance based on breeding values. Heatmaps were created using the re-classified data for rust severity as well as residuals from the linear mixed models to demonstrate the effectiveness of removing spatial patterns.

Climate variables were tested for their importance in explaining provenance variation in phenotypic traits based on their coefficient of determination (r^2) and Akaike information criterion (AIC) values using an iterative function in R where all annual and seasonal variables were separately fitted to a quadratic model with average provenance blister rust breeding values or height as the response variable. After ranking, variables were selected based on biological significance and choosing among those that were highly correlated $(r^2 \ge 0.9)$. Selected variables were fit to both simple linear models and quadratic models to determine the best fit for the data. Forward stepwise selection was performed on selected variables by adding those that contributed the most to the model, starting with the top ranked single variable. To prevent overfitting, adjusted r^2 and predicted r^2 were calculated after adding each new variable. A Principal Component Analysis was also performed on the top ranking variables to compare the first two principal components (PC1 and PC2) with individual climate variables. Bonferroni adjusted alpha levels were calculated based on the number of comparisons tested.

Family mean blister rust grades (A to F) from controlled inoculations at Dorena were compared to breeding values at Skimikin. A one-way ANOVA was performed on the grades versus breeding values followed by post-hoc Tukey HSD pairwise tests to compare groups.

2.3 Results

2.3.1 Infection Levels

Overall, 73.4% of seedlings were cankered and 95.0% of seedlings showed signs of rust (needle spots or cankers). The majority of living seedlings showed needle spots (85.6%) while only some (21.5%) showed aecia. More seedlings had branch cankers (71.5%) than bole infections (66.8%). Some seedlings had needle spots but no cankers (20.8%). Mortality from rust was 27.5%.

Average rust severity as well as seedling mortality was highest in columns closer to the *Ribes* and gradually decreased as distance from them increased (Figure 2.1). The relationship between average rust severity and distance from *Ribes* was linear and strong ($r^2 = 0.97$) (Figure 2.1).



Figure 2.1: Linear relationships between: a) average rust severity of whitebark pine seedlings per column and distance from *Ribes nigrum* ($r^2 = 0.97$); b) average percent mortality of whitebark pine seedlings from rust per column and distance from *Ribes nigrum* ($r^2 = 0.96$) at Skimikin Nursery, BC.

2.3.2 Heritability and Family Rankings for Rust Resistance

Family breeding values for rust resistance were normally distributed and ranged from -0.92 to 1.31 (Appendix 3, Table 1). Eighty of the 194 tested families had positive breeding values for resistance. Moderate heritability was estimated from the individual tree model for rust resistance ($h^2 = 0.23$, S.E. = 0.044).

Family rankings for resistance based on raw data were very similar to those based on results from the *ASReml-R* model containing a spatial correction. In both cases, families 275 (Mt. Rainier, WA) and 255 (Freezeout Ridge, WA) were ranked highest for resistance (Appendix 3, Table 1). Wald test for provenance as a fixed effect showed that it was significant in the *ASReml-R* model (p<0.01).

Estimated breeding values had a strong relationship with percent stem symptoms per family of original data (prior to spatial adjustment), with higher breeding values corresponding to lower infection ($r^2 = 0.87$) (Figure 2.2). Heatmaps of the spatial distribution of blister rust at

Skimikin illustrate the effectiveness of first transforming data to normal scores, and then using the ASReml-R model to correct for spatial effects (Figure 2.3).



Figure 2.2: Linear relationship between spatially corrected family estimated breeding values and percent stem symptoms per family of original data for whitebark pine seedlings at Skimikin Nursery, BC ($r^2 = 0.87$). Each point represents one family. Families with breeding values higher than zero have positive breeding values for resistance.



Figure 2.3: Heatmaps of whitebark pine seedlings at Skimikin Nursery, BC. From top left to bottom right: a) rescaled distribution of rust severity with 4 levels, before normalization; b) normal score transformed rust distribution; c) residuals from model without spatial correction; d) residuals from spatially corrected model. Blue represents higher mortality and infection from blister rust while yellow represents healthier seedlings. Each row and column position represents one seedling.

Average family height of seedlings ranged from 18.4 cm (Moyie Mountain, BC), to 45.9 cm (Deschutes National Forest, OR). Average provenance height ranged from 23.0 cm (Hudson Bay Mountain, BC) to 39.6 cm (Gifford Pinchot/Mt. Adams, WA). Heritability for seedling height ($h^2 = 0.42$, S.E. = 0.075) was much higher than for rust resistance, while among-population differentiation was very low ($Q_{ST} = 0.07$, S.E. = 0.030). Breeding values for height were normally distributed and ranged from -16.4 to 16.5 (Appendix 3, Table 1). No relationship was found between breeding values for rust resistance and height.

2.3.3 Geographic Distribution of Rust Resistance

Breeding values for rust resistance were highest in families from the Cascade Mountains of Washington and Oregon, as well as the southern Columbia Mountains of BC, Kettle River Range of Washington, and Rocky Mountains of northern Idaho and northwest Montana (Figure 2.4). Higher susceptibility was found in provenances from further north in the North Cascades, Coast Ranges, and Rocky Mountains of British Columbia as well as in the far southeast of the range in Idaho and Wyoming. Moderate among-population differentiation was estimated for rust resistance ($Q_{ST} = 0.28$, S.E. = 0.069).



Figure 2.4: Map of estimated breeding values for rust resistance by provenance based on seedling performance at Skimikin Nursery, BC. Blue indicates higher resistance to blister rust. Circle size represents number of families per provenance. Provenances containing fewer than three families were removed from this analysis and are marked as black triangles on the map.

2.3.4 Climate Correlates of Rust Resistance

Provenance climate was a slightly better predictor of rust resistance than of height. The top ranked variables for rust resistance were related to temperature (Table 2.1 & 2.2). Provenance end of frost-free period (eFFP) was the most significant single predictor of breeding values for rust resistance ($r^2 = 0.29$, p = 0.0024), with provenances with a later end to the frost-free period having higher resistance (Figure 2.5). The number of frost-free days in spring

(NFFD_sp) as well as autumn degree days above 5°C (DD5_at) were also significant predictors containing positive relationships with rust resistance (Table 2.1). Two-variable models were marginally better with the top model containing eFFP and spring solar radiation (Rad_sp) ($r^2 =$ 0.33, p = 0.0046) (Table 2.2). Principal components 1 and 2 explained 82.5% of the variation in the data. Principal components had weaker relationships with rust resistance compared to individual variables (Table 2.1). Provenance geographical variables, including latitude, longitude, and elevation were not significant predictors of rust resistance. Additionally, distance from rust introduction (i.e. Point Grey, Vancouver, BC) was not a significant predictor of rust resistance. After performing forward stepwise selection and calculating predicted r^2 , it was determined that the best supported climate model for rust resistance is the single variable quadratic model containing eFFP.

Table 2.1: Relationship between select climate variables and the top principal components from Principal Component Analysis with breeding values for resistance to white pine blister rust of seedlings at Skimikin Nursery, BC. Models are single variable linear models unless otherwise noted ($\alpha = 0.05/6 = 0.0083$). Climate data was estimated using *ClimateNA* version 5.60 (Wang et al. 2016).

Variable	Description	Coefficients	Adjusted r ²	p-value	AIC
eFFP*	End of frost-free period	-0.272 (x) $0.00056 (x^2)$	0.29	0.0024	21.1
NFFD_sp	Number of frost-free days in spring	0.031	0.26	0.0017	21.7
PC1	Principal component 1	0.0520	0.26	0.0017	21.8
DD5_at	Autumn degree days above 5°C	0.0029	0.20	0.0057	24.1
Tmax_sm*	Maximum summer temperature	0.65 (x) -0.019 (x^2)	0.081	0.11	29.6
PC2	Principal component 2	0.015	-0.019	0.53	32.0

*Fit using a quadratic model ($y = x + x^2$)



Figure 2.5: Provenance end of frost-free period (eFFP) versus average breeding value per provenance for rust resistance of seedlings at Skimikin Nursery, BC (r^2 =0.29, p = 0.0024). Each point represents one provenance. Climate data was estimated using *ClimateNA* version 5.60 (Wang et al. 2016).

et al. 2016).
models ($\alpha = 0.05/4 = 0.0125$). Climate data was estimated using <i>ClimateNA</i> version 5.60 (Wang
white pine blister rust of seedlings at Skimikin Nursery, BC. Models are two-variable quadratic
Table 2.2: Relationship between select climate variables with breeding values for resistance to

Variable	Description	x Coefficier	ts x^2	Adjusted r ²	p-value	AIC
eFFP +	End of frost-free	-0.377	0.00077	0.33	0.0046	21.2
Rad_sp	period + spring solar	1.58	-0.044			
	radiation					
DD5_at +	Autumn degree days	0.00778	-0.000011	0.31	0.0064	22.1
SHM	above 5°C + summer	-0.00969	0.0000007	5		
	heat-moisture index					
eFFP +	End of frost-free	-0.342	0.00070	0.30	0.0081	22.7
Tmax_sm	period + maximum	0.550	-0.016			
	summer temperature					
eFFP +	End of frost-free	-0.43	0.00086	0.29	0.0086	22.8
DD5_at	period + Autumn	0.011	-0.000034			
	degree days above					
	5°C					

Provenance climate variables most strongly correlated with height were related to moisture (Table 2.3 & 2.4). Precipitation as snow (PAS) was the top ranking single climate predictor, and positively associated with height ($r^2 = 0.15$, p = 0.0062) (Figure 2.6). Some combinations of two variables were also significant correlates (Table 2.4). The combination of PAS and summer relative humidity (RH_sm) contained the strongest two-variable relationship with height ($r^2 = 0.27$, p = 0.0022). Elevation when combined with PAS also contained a significant relationship with height ($r^2 = 0.21$, p = 0.0089). The first two principal components from PCA explained 82.7% of the variance. Once again, principal components had weaker relationships with height compared to individual climate variables (Table 2.3 & 2.4). After forward stepwise selection, the best-supported multivariate climate model for height included RH_sm plus its square, frost free period (FFP), PAS squared, and the interaction between RH_sm and FFP ($r^2 = 0.32$, p = 0.0013).

Table 2.3: Relationship between select climate variables and the top principal components from Principal Component Analysis with height of seedlings at Skimikin Nursery, BC. Models are single variable linear models unless otherwise noted ($\alpha = 0.05/6 = 0.0083$). Climate data was estimated using *ClimateNA* version 5.60 (Wang et al. 2016).

Variable	Description	Coefficients	Adjusted r ²	p-value	AIC	
PAS	Precipitation as snow	0.0056	0.15	0.0062	235.7	
NFFD_wt	Number of frost-free days in winter	0.4624	0.13	0.010	236.7	
RH_sm*	Summer relative humidity	-4.74(x) 0.037(x ²)	0.10	0.046	239.0	
PC2	Principal component 2	0.452	0.090	0.027	238.4	
FFP*	Frost-free period	0.34(x) -0.00019(x^2)	0.045	0.15	241.5	
PC1	Principal component 1	0.26	0.056	0.066	240.1	
*Fitusing a quadratic model $(y - x + x^2)$						

*Fit using a quadratic model $(y = x + x^2)$



Figure 2.6: Provenance precipitation as snow (PAS) versus height (cm) of seedlings at Skimikin Nursery, BC (r^2 =0.15 p=0.0062). Each point represents one provenance. Climate data was estimated using *ClimateNA* version 5.60 (Wang et al. 2016).

Table 2.4: Relationship between select climate variables and top principal components from Principal Component Analysis with height of seedlings at Skimikin Nursery, BC. Models are two-variable quadratic models unless otherwise noted ($\alpha = 0.05/6 = 0.0083$). Climate data was estimated using *ClimateNA* version 5.60 (Wang et al. 2016).

Variables	Description	x	Coefficients	x^2	Adjusted r ²	p-value	AIC
PAS +	Precipitation as snow	-0.00830	0.000	0087	0.27	0.0022	231.3
RH_sm	+ summer relative	-4.54	0.035				
	humidity						
PAS +	Precipitation as snow	-0.00304	0.000	0058	0.21	0.0089	234.7
Elevation	+ elevation	0.0395	-0.00	00086			
PAS +	Precipitation as snow	-0.00164	0.000	0047	0.21	0.010	235.1
FFP	+ frost free period	0.360	-0.00	21			
PAS +	Precipitation as snow	-0.00082	9 0.000	0045	0.20	0.012	235.4
Rad_sp	+ spring solar	8.94	-0.23				
_	radiation						
PAS +	Precipitation as snow	-0.0050	0.000	0058	0.18	0.021	236.9
NFFD_wt	+ number of frost	0.14	0.015				
	free days in winter						
PC1 +	Principal component	0.263	1	N/A	0.15	0.013	236.4
PC2*	1 + principal	0.452	1	N/A			
	component 2						
*Fit using a si	mpla linear model						

*Fit using a simple linear model

2.3.5 Dorena Comparison with Controlled Inoculations

Breeding values were calculated for 69 of the 81 families contributed from Dorena since some provenances contained less than three families. A to F grades from Dorena for these previously screened families matched well with the estimated breeding values of the same families at Skimikin (Figure 2.7). All A and B grade families at Dorena had positive breeding values in the Skimikin trial, while C grade families contained a range of positive and negative breeding values. D, E, and F grade families contained mostly negative breeding values at Skimikin though some discrepancies did exist. Dorena grades were significant in the one-way ANOVA (p<0.01). Tukey HSD pairwise tests revealed significant differences between the most resistant classes (A and B) and the intermediate class (C); however, of the susceptible classes, only D was significantly different from the intermediate class (C) (Table 2.5). After combining D, E, and F into one "susceptible" class, significant differences were found between it and class C (p = 0.01). No differences were found between classes A and B or between classes D, E, and F.



Figure 2.7: Comparison between spatially corrected family breeding values for rust resistance at Skimikin Nursery, BC with A to F grades from controlled inoculations of the same families done at Dorena, OR. Families per grade: A: 10, B: 11, C: 10, D: 12, E: 11, F: 15.

Table 2.5: Results from Tukey HSD pairwise testing of Dorena A to F grades from controlled inoculations versus Skimikin breeding values for rust resistance estimated from natural inoculation of the same whitebark pine families. Results are reported as the difference between means with the p-value in parentheses. Families per grade: A: 10, B: 11, C: 10, D: 12, E: 11, F: 15.

	В	С	D	Ε	F
Α	-0.25 (0.58)	-0.74 (0.00016)	-1.21 (<0.0001)	-1.16 (<0.0001)	-1.00 (<0.0001)
В		-0.49 (0.021)	-0.96 (<0.0001)	-0.92 (<0.0001)	-0.76 (<0.0001)
С			-0.47 (0.025)	-0.43 (0.066)	-0.27 (0.41)
D				0.046 (1.00)	0.20 (0.65)
Е					0.16 (0.86)

2.4 Discussion

The Skimikin common garden trial successfully demonstrates a simple, inexpensive alternative to controlled artificial inoculations for the purposes of identifying blister rust resistant families of whitebark pine. As this endangered species continues to decline, efficient methods of screening large numbers of families for rust resistance are of the utmost importance to ensure adequate genetic diversity and blister rust resistance, and therefore long-term viability of seedlings planted for restoration.

2.4.1 Effectiveness of Natural Inoculation

Controlled inoculations of whitebark pine with *Cronartium ribicola* typically result in close to 100% infected seedlings (Sniezko et al. 2014b). With natural inoculation, Skimikin came close to this with 95% of seedlings showing needle spots. Additionally, with 73% of seedlings showing rust cankers, family level differences in resistance could be determined. This demonstrates that despite uneven wind dispersal of rust basidiospores, the vast majority of seedlings were exposed to the rust, with few escaping it. However, the number of rust spores that each seedling was exposed to varied substantially with distance from the *Ribes* plants, as evidenced by the spatial patterns of blister rust infection severity (Figure 2.3). Although some families are resistant to low levels of rust, they may have been susceptible beyond certain spore densities. It is possible that spots of *Dothistroma septosporum* Dorog., which was also present at Skimikin, could have been mistaken for rust spots though spots of *Dothistroma* typically are more red to brown in colour and develop bands (Bradshaw 2004). *Cronartium ribicola*, however, is the only likely pathogen that could have caused the branch and stem cankers.

I found a clear, strong negative relationship between distance from *Ribes* and rust severity in this trial. This spatial pattern is informative in showing the dispersal capability of rust basidiospores from *Ribes nigrum*. Also, this can be useful for determining hazard ratings in wild stands by measuring proximity of trees to *Ribes* plants; however, since only short distances were examined from a single common garden, further testing would be required. It has been recognized that when a discrete inoculum source exists, rust canker abundance decreases as distance from source increases (Van Arsdel et al. 2006).

The linear regression between rust severity and distance contained a limited number of data points since columns of seedlings were only located at eight different distances from the *Ribes*. Typically, dispersal of rusts is leptokurtic, following a steep negative slope followed by a long tail (Zambino 2010). Whitebark pine infection declined sharply in this trial with distance from *Ribes* which is typical; however, the trial was not large enough to detect the tail of the distribution. Estimates of basidiospore dispersal distances range widely and vary based on local topography, air flow patterns, and weather (Buchanan and Kimmey 1938). Dispersal distances of white pine blister rust basidiospores range from 15-18 m (Buchanan and Kimmey 1938) up to 16-27 km (Van Arsdel 1965). Infection has been shown to decrease with distance due to dilution, filtering by vegetation, and a loss of basidiospore viability (Mielke 1943; Gregory 1945; Van Arsdel et al. 2006). These factors could have affected infection at Skimikin where seedlings were planted in close proximity to one another causing filtering of spores, and were located mostly in the open with little protection from sun and wind. Additionally, the Skimikin common garden is on flat ground. Whitebark pine often inhabits steep slopes which would change basidiospore dispersal patterns and distances. Due to *Ribes* being native to the forest surrounding Skimikin, it is possible that small numbers of spores were carried from nearby forest to the trial, though this was not tested in this study.

Future natural inoculation trials would benefit from interplanting *Ribes* within the whitebark pine seedlings rather than on one side. *Ribes* should be interplanted at a spacing that allows for relatively even inoculation but without causing such high mortality that family differences cannot be determined. Based on the regression found here, I would recommend planting *Ribes* approximately five metres apart within the whitebark pine seedlings as a more effective method than used at Skimikin. It would be worthwhile to do a similar test to Skimikin

using other alternate hosts, such as blister rust susceptible species of *Castilleja spp*. and *Pedicularis spp*., to determine their effectiveness at inoculating whitebark pine seedlings (McDonald et al. 2006).

Due to the uneven dispersal of rust spores via wind and strong spatial patterns present in the data, spatial correlations between position within the common garden and rust infection were included in the model. When comparing original rust data (avg. family rust severity) with the breeding values estimated from the *ASReml-R* model, family rankings did not change substantially. This indicates that randomizing families within replications was effective at reducing the effect of wind dispersal and distance from *Ribes*, and that a more simple linear model would have still given a relatively good estimate of resistant versus susceptible families. The *ASReml-R* model not only corrected for the effect of distance from *Ribes*, but also for the patchiness of infection, such as areas at the east and west ends (top and bottom of heatmaps) of the trial that had healthier seedlings and likely were exposed to fewer rust basidiospores. The spatial modelling approach used in this study could be useful for future rust screening trials of whitebark and other five-needle pines for blister rust infection.

Based on pairwise testing (and as shown in Figure 2.6), the Skimikin trial did a good job of classifying resistant, moderately susceptible, and very susceptible families but was not sufficiently sensitive to distinguish some of the most resistant and susceptible classes. This comparison to the Dorena grades demonstrates that inoculating seedlings simply by positioning them close to *Ribes* plants in a nursery setting can be nearly as effective as control inoculations at determining resistant families. Varying levels of seedling exposure to rust spores at Skimikin compared to even inoculation at Dorena may have caused the discrepancies in a few families between Skimikin breeding values and Dorena grades. These discrepancies could have also been a result of the different climates present at Skimikin and Dorena, with Dorena having a warmer more moderate climate. Spatial correction of data helped to minimize the effect of wind dispersal. Natural inoculation can therefore be an effective approach for determining parental selections.

2.4.2 Heritability and Breeding Values

Heritability for rust resistance was lower (0.23) compared to height showing that variables other than genetics explain more variation of this trait among families. This heritability

is also lower than what has been found in previous studies for whitebark pine rust-related traits which range from 0.39 to 0.92 (Mahalovich et al. 2006; Sniezko et al. 2014a). The heritability estimated in this study for height (0.42) falls within the range of estimates found in other studies which range from 0.27 to 0.85 (Mahalovich et al. 2006; Hamlin et al. 2011; McLane 2011; Sniezko et al. 2014a). Mahalovich et al. (2006) similarly found higher heritability for height than for rust resistance in whitebark pine. It is possible that since blister rust was measured on a severity scale, it contains more subjectivity and random measurement error causing a lower heritability.

Breeding values estimate the genetic quality of parent trees and are the additive component of the genotypic value (Xie and Yanchuk 2003). For Skimikin seedlings, breeding values estimated from the linear mixed effects models for height and rust resistance showed normal distributions. For rust resistance, family breeding values were closely associated with the percent stem symptoms (%SS) of original rust data, showing the high effectiveness of the model. Most families at Skimikin were represented by 18-20 seedlings. More seedlings per family would have reduced error from genetic and environmental sampling, giving more accurate breeding values. However, obtaining whitebark pine seed and growing seedlings is expensive and labour intensive. These breeding values based on progeny performance should be used for selection of parent trees, and collecting more seed from those parents with higher breeding values for resistance. The results of this study, validated with resistance grades from Dorena from controlled inoculations for some families, add to the base of blister rust resistant parent trees that can be used for restoration purposes, including the potential development of a whitebark pine seed orchard for British Columbia.

2.4.3 Provenance Variation & Geographic Patterns of Resistance

In this study, the most resistant families were located in the Cascade Mountains of Oregon and Washington as well as further east in the Columbia and Rocky Mountains near the Canada/U.S. border in B.C., Idaho, and Washington. The highest susceptibility was found in the Chilcotin and Coast Ranges of British Columbia, as well as in the far southeast of the range. Outliers such as the McBride Peak provenance were present, which showed high resistance but is located in the Northern Rockies of British Columbia. This distribution of resistance does not seem to follow any particular latitudinal, longitudinal or elevational pattern; however, it may relate to the spread of blister rust over time. Since its introduction to Vancouver in 1910, the rust first spread south into Washington and Oregon as well as directly east towards the Columbia and Rocky Mountains from 1913 to 1942 (Figure 1.2) (Mielke 1943; McDonald and Hoff 2001). Since then, the rust has gradually spread south into California and Nevada as well as southeast in the Rockies down to Colorado, and north into the Coast Ranges (Smith and Hoffman 2000; Kearns and Jacobi 2007; Maloney 2011). Since areas such as the Cascade Mountains were infected first by white pine blister rust, more natural selection for resistant individuals has likely taken place in these areas. When seeds were collected for this study, healthy trees were selected in areas with a range of rust infection. In those areas with high infection or that were exposed to blister rust earlier, phenotypic selection may have resulted in the collection of more seed from resistant individuals while in areas with low or no infection, blister rust had not yet infected or killed many susceptible trees so phenotypic selection would not have played as much of a role. Additionally, breeding values could only be calculated for families of 32 provenances as the remainder were represented by too few families to generalize results. Having more parent trees tested in undersampled regions of the species range in this study may have revealed more patterns in the distribution of resistance.

At Skimikin, seedling height did not vary significantly by provenance and did not follow any geographic pattern as found in other conifer species. In general, previous research indicates that provenances from lower elevations and warmer climates grow better than those from higher elevation colder climates (Aitken and Bemmels 2016). The lack of association between provenance geography and height in this study may be due to the low levels of amongpopulation differentiation found in whitebark pine and the fact that seedlings were grown at a low elevation site (Bower and Aitken 2008).

Higher population differentiation was found for blister rust resistance (0.28) than for height in this study. Estimates of population differentiation for a single locus (F_{ST}) found in whitebark pine are low and average 0.041 indicating most neutral genetic variation occurs among individuals among populations (Bower and Aitken 2008; Bower et al. 2011). The Q_{ST} estimated here for rust resistance was substantially higher than F_{ST} estimates from other studies possibly indicating differential directional selection and more adaptation to local environments. The Q_{ST} estimated for height (0.07) was very similar to those previously found for whitebark pine which range from 0.07 to 0.19 (Bower and Aitken 2008; Mahalovich and Hipkins 2011; McLane 2011). Low Q_{ST} values for height indicate limited population differentiation for this trait. This could be a result of whitebark pine being wind pollinated and bird dispersed. Q_{ST} values for height found at Skimikin were slightly higher than the average but within the range of F_{ST} values previously found for whitebark pine indicating differentiation could be explained by genetic drift rather than selection for local adaptation. The Q_{ST} found here for height is also lower than has been found in a number of other tree species for growth related traits including limber pine at 0.17 (Gass 2016). Alberto et al. (2013) found a mean Q_{ST} of 0.32 for height increment based on 36 studies of northern tree species. Other traits similarly had a higher mean Q_{ST} , including fall frost hardiness (0.58), root allocation (0.34), bud flush (0.25), and bud set (0.39) (Alberto et al. 2013).

2.4.4 Influence of Provenance Climate

The climate variables that had the strongest correlations with rust resistance were related to temperature and growing season length including the end of the frost-free period, the number of frost-free days in spring, and autumn degree days above 5°C. When pairs of variables were tested in multivariate regressions, the strongest correlations once again came from those related to temperature and growing season. Additionally, some of the top two-variable models included spring and autumn variables. Other studies have found that August and September site climate conditions were good predictors of rust infection including the interaction between relative humidity and temperature (Thoma et al. 2019). However, for limber pine, early season site climate conditions were better predictors of white pine blister rust presence (Kearns et al. 2014). Moisture can influence presence and intensity of blister rust, and infection levels are generally higher in wetter areas due to the higher abundance of *Ribes* and more suitable conditions for blister rust spore production (Van Arsdel et al. 1956; Shepherd et al. 2018), but I did not detect evidence of more selection for rust resistance in wetter provenances. Climate variables more strongly predicting resistance in this study may also influence the blister rust life cycle. For example, teliospores and basidiospores are normally produced on Ribes in late summer and autumn which is also when infection of pines takes place (McDonald and Hoff 2001). This may explain the strong relationship I found between the end of the frost free period, which typically occurs in late summer or early autumn, and rust resistance.

For height, both single climate variables and pairs of variables were significant predictors, though were not as strongly associated as the top ranked climate variables for rust resistance. Height was most strongly related to precipitation and moisture related variables. I found a relatively strong relationship between height and precipitation as snow. Additionally, the two-variable quadratic model including precipitation as snow and summer relative humidity contained a strong relationship with height. Generally, tree growth in the subalpine and alpine is dictated by snowpack and snowmelt. Locations receiving greater precipitation as snow would be expected to have less growth due to adaptation to later snow melt and a shorter growing season. However, snow also acts as an insulator in the winter and protects seedlings from wind scouring and frost damage (Mellmann-Brown 2005; McLane and Aitken 2012). Additionally, snowmelt throughout the summer helps keep soil moist. Clason et al. (2020) similarly found that precipitation as snow strongly predicted whitebark pine occurrence and abundance though in differing directions. Higher precipitation as snow was positively correlated with juvenile abundance, yet negatively related to adult abundance (Clason et al. 2020). For both height and rust resistance, principal components had weaker relationships than individual climate variables. Generally, principal component analysis is effective for large datasets with many variables in reducing noise and redundancy; however, variables become more difficult to interpret after PCA since correlations between specific features in a dataset are lost.

Several explanations exist for the weaker trends between seedling height and provenance climate and geography in this study. First, the climate at Skimikin Nursery is atypical of what whitebark pine normally experiences in nature with a longer growing season and warmer winter temperatures. Therefore, seedlings may be more influenced by the environment at Skimikin rather than the genetic effect of the provenance environment. Additionally, whitebark pine occupies a relatively narrow range of climates even though it is widely distributed in western North America. The species is typically found in the subalpine and near treeline since it is more cold tolerant and a poor competitor at lower elevations (Tomback et al. 2001b). Although areas occupied by whitebark pine vary substantially in elevation as a function of latitude, climate associated with whitebark pine locations may not differ as much as for other conifer species because it is the same narrow band around treeline. At a given location, whitebark pine has one of the narrowest elevation distributions of any five-needle pine (Tomback et al. 2016). For example, mean annual temperature of provenances in this study ranged from -1.0°C to 4.3°C.

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Previous work by Bower and Aitken (2008) found that whitebark pine shows relatively weak local adaptation and could be moved up to 4.6° in latitude, or 505 km, in the northern part of its range and 320 metres in elevation in the Rocky Mountains. They recommended that in the southern region, seed can be moved freely with the exception of transfer between mountain ranges (Bower and Aitken 2008). Changing climates may alter estimates of seed transfer causing local seed sources to become unsuitable for local climates (Bower and Aitken 2008). Mahalovich and Hipkins (2011) similarly found relatively weak population differentiation for a number of traits including cold hardiness, survival, height, and certain rust symptoms. As a result, a reduction in the number of seed zones was recommended in the Inland West of the U.S. (Mahalovich and Hipkins 2011). If whitebark pine undergoes assisted range expansion, rust resistant seedlings will need to be planted since alternate hosts of white pine blister rust collectively occupy a much larger geographic range than the tree itself does. More provenance trials of whitebark pine, such as those planted by McLane and Aitken (2012) would be informative in determining suitable transfer distances for planting seedlings and seed sources to use.

Trade-offs between growth and defense are thought to occur in many tree species, especially for biotrophic pathogens, as resources must be allocated between them to maximize survival and reproduction (Loehle 1988; Albrecht and Argueso 2017). The fact that no relationships were found between resistance and height indicates that seedlings with rust exhibited comparable growth to those without rust and suggests no trade-offs were made between height growth and rust resistance. Mahalovich et al. (2006) similarly found no trade-off between height growth and rust resistance in young whitebark pine seedlings. However, it is notable that many of the seedlings at Skimikin with blister rust that were still alive were not flushing and had no new growth. This lack of a trade-off is consistent with the life-history strategy of whitebark pine in which it grows in relatively sparsely treed areas at higher elevation. Therefore, allocating resources to height growth, rather than protection against abiotic stressors such as cold and wind hardiness, may not provide much of a competitive advantage. In a study of 18 species in the Pinaceae, Moreira et al. (2016) determined that slow growing species that occupy harsh environments allocated more resources to constitutive rather than induced defenses. Additionally, since whitebark pine has not co-evolved with white pine blister rust, it may be less likely to have a growth-defense trade-off. Future studies could determine if tradeoffs are present between cold hardiness and rust resistance.

2.4.5 Implications for Restoration

Whitebark pine is an important species for restoration as it is federally endangered in Canada under the Species at Risk Act (SARA). It is also a challenge to restore as it is slow growing, often inhabits difficult to access areas, and it can be difficult to collect large amounts of seed. (Environment and Climate Change Canada 2017). This makes restoration efforts costly, time consuming, and labour intensive. Finding ways to increase efficiency in finding, growing, and planting rust-resistant trees is of the utmost importance for the long-term viability of the species. The method used in this study of screening seedlings in a nursery bed common garden trial with uncontrolled inoculation from *Ribes* provides a faster, cheaper option for identification of rust-resistant seedlings. However, this approach presents several drawbacks as well. First, screening families will be limited by space and willingness of nurseries or research facilities to use land for a non-commercial species. Most tree nurseries and seed orchards are occupied by economically important species which have a greater return on breeding programs. Additionally, the effectiveness of inoculation is in part a function of the conditions outside during growth. During artificial inoculations, temperature and humidity levels can be controlled to ensure successful production and dispersal of white pine blister rust basidiospores. It is possible that suitable conditions for spore germination and dispersal would not occur every year in an outdoor setting, and that rust infection would occur in waves. This indicates that the time needed to identify resistant families could be longer than in controlled inoculations. Since this study was not replicated with additional common gardens exposed to Ribes, it is difficult to know if the high efficacy is repeatable over years and sites. Putting all of the families screened at Skimikin through controlled inoculations would help to further confirm the effectiveness of this method of determining rust resistance. Field trials are already in place throughout British Columbia with the same families as those at Skimikin. Long term monitoring of these trials will help determine the durability of resistance as well as potential seed transfer guidelines with climate change. Finally, both seed collection and planting efforts for whitebark pine should target areas where blister rust and mountain pine beetle have caused the highest mortality of trees, as those areas are in greatest need of restoration, and the surviving trees have experienced the strongest natural selection for blister rust resistance.

3 Conclusion

Although whitebark pine is threatened by climate change, mountain pine beetle, and altered fire regimes, white pine blister rust still represents the most prominent threat to the species. Introduced to Vancouver, BC in 1910, white pine blister rust continues to expand its range and infect new whitebark pine trees every year. Some areas of the Rocky Mountains have experienced infection levels in excess of 90% (Shepherd et al. 2018). Although not commercially valuable, whitebark pine provides a range of important ecological functions to mountainous regions of western North America such as providing a food source for wildlife, regulating snowmelt, and initiating tree-islands (Tomback et al. 2001b). To ensure viable populations of whitebark pine into the future which produce cones in sufficient numbers to support Clark's nutcracker dispersal, screening for rust resistance and breeding programs must continue. Historical methods of blister rust control such as *Ribes* eradication have not been successful.

Currently, rust resistance screening is done in enclosed facilities in a controlled manner. The Skimikin study analyzed here used a field common garden to expose seedlings to uncontrolled white pine blister rust inoculation by planting whitebark pine beside *Ribes nigrum*. This was an effective method to inoculate seedlings and identify rust resistant families without as many costs and equipment constraints as controlled inoculations. Correcting for spatial effects due to uneven inoculation across the experiment, such as those at Skimikin, can be done by utilizing the R package *ASReml-R*. However, future screening trials using natural inoculation would benefit from interplanting *Ribes* evenly throughout the seedlings, rather than on one side, to ensure a more even rust infection. Planting *Ribes* approximately five metres apart would be sufficient to inoculate seedlings, while ensuring gaps between plants experience relatively even spore loads. Future natural inoculation seedling-screening trials such as Skimikin should make use of unused nursery space or locations where control-inoculation whitebark pine testing is already being done. To validate screening trials and evaluate resistance durability, field testing should take place.

Using alternative methods to screen families for blister rust resistance, such as those presented here, may increase the availability of seedlings for restoration planting. Restoration plantings should target areas that have experienced high mortality due to white pine blister rust and mountain pine beetle such as the Greater Yellowstone Ecosystem and the Crown of the Continent Ecosystem. Relatively low levels of local adaptation have been found in whitebark pine; therefore, resistant seedlings can be planted at substantial distances from provenance locations. Planting should take into account changing climates, including the shifting climatic niche of whitebark pine. Planting whitebark pine into areas it does not currently occupy, but that are climatically suitable, should be considered. A large region of northwestern British Columbia does support the establishment of the species (McLane and Aitken 2012). Restoration plantings should be monitored over time to evaluate their success. Additionally, planting should be combined with other restoration tactics such as protecting candidate trees from mountain pine beetle, prescribed burning, and thinning competing species.

Breeding values for rust resistance estimated from Skimikin were highly variable, indicating considerable opportunity for artificial selection to increase levels of resistance in restoration plantings. Mean provenance breeding values for resistance were highest in the Cascade Mountains of Washington and Oregon, as well as in southeast BC, northeast Washington, northern Idaho, and northwest Montana. Results from Skimikin may be used for the potential creation of a whitebark pine seed orchard by grafting resistant parent trees. No seed orchards exist currently for whitebark pine in British Columbia; therefore, there is potential to substantially increase the availability of rust-resistant material.

Restoring this important species will not be possible without a collaborative approach that incorporates the many different viewpoints of indigenous groups, scientists, federal and provincial government, conservation-oriented non-governmental organizations, consulting companies, and industry. Various working groups, committees, and a dedicated organization called the Whitebark Pine Ecosystem Foundation already exist, and have developed important management strategies as well as raised awareness of the issues facing whitebark pine. Once a final recovery strategy is published for whitebark pine in Canada, specific recovery actions will hopefully be implemented in a more coordinated and efficient manner.

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Appendix 1

Table 1: List of whitebark pine (*Pinus albicaulis*) provenances present at the Skimikin Nursery common garden ordered from north to south including number of families, geographic location, and select climate variables. Climate data estimated using *ClimateNA* version 5.60 (Wang et al. 2016).

Provenance			Elev.			MAT	FFP	MAP
ID	Location	Families	(m)	Lat.	Long.	(°C)	(days)	(mm)
1	Mt. Sidney Williams, BC	10	1580	55.02	-125.59	-0.6	65	1035
2	Hudson Bay Mtn, BC*	1	1500	54.77	-127.28	0.1	71	1094
3	McBride Peak, BC	6	2100	53.34	-120.12	-0.2	74	1557
4	Heckman Pass, BC	10	1909	52.53	-125.82	-0.5	48	1207
5	Perkins Mtn, BC	5	1940	51.83	-125.04	-1.0	44	1256
6	Sapeye Mtn, BC	3	2027	51.82	-124.81	-0.8	46	1143
7	Niut Mtn, BC*	2	2003	51.67	-124.52	-0.9	46	982
8	Nemaiah Mtn, BC	9	2010	51.49	-124.12	-0.5	57	867
9	Jesmond, BC	6	1850	51.31	-121.92	0.5	80	686
10	Kickinghorse, BC	5	2000	51.30	-117.06	-0.1	73	1480
11	Taseko Mtn, BC	6	1950	51.26	-123.54	0.0	68	1272
12	Lime Mtn, BC*	1	1935	51.09	-121.66	0.6	69	568
13	Mt. Carson, BC	3	2000	50.80	-121.72	0.1	72	548
14	Blackcomb, BC	6	1893	50.06	-122.90	0.8	86	1830
17	Mt. Stevens, BC	9	2200	49.84	-115.57	-0.3	63	969
16	Bear Lake, BC*	2	2200	49.77	-115.47	-0.4	62	1081
15	Puddingbum, BC	7	2340	49.59	-116.01	0.1	94	864
18	Apex Mtn, BC	5	2100	49.37	-119.92	0.9	92	667
19	Moyie Mtn, BC	10	2078	49.26	-115.77	1.0	93	923
20	Mt. Baldy, BC	4	2050	49.17	-119.25	0.8	74	732
21	Blackwall/Manning, BC	3	2005	49.10	-120.76	1.2	78	1298
22	Colville Forest, WA	10	2036	48.65	-118.21	2.9	111	906
25	Wenatchee, WA	10	2102	48.34	-120.20	1.5	83	1122
23	Baree Mtn, MT	3	1829	47.96	-115.55	3.3	120	1714
24	Olympic, WA	6	1766	47.81	-123.14	3.1	103	2364
26	Morrell Lookout, MT	3	2217	47.19	-113.35	2.2	77	1387
27	Freezeout Ridge, WA	3	1783	47.01	-116.04	4.1	122	1426
44	Baker-Snoqualmie, WA*	2	1908	46.94	-121.49	2.6	95	1888
29	Mt. Rainier, WA	6	1962	46.91	-121.58	2.6	94	2001
28	John King Creek, MT	3	2426	46.84	-110.67	1.0	62	844

	Gifford							
36	Pinchot/Mt.Adams, OR*	2	2069	46.15	-121.49	2.1	84	2153
30	Mt. Hood, OR	6	1834	45.35	-121.69	3.6	120	2949
	Wallowa-Whitman,							
33	OR*	2	2389	44.95	-118.24	3.3	114	953
32	Umatilla, OR	8	2315	44.72	-118.58	3.9	119	935
31	Warm Springs OR*	2	1957	44.66	-121.69	4.3	90	2189
35	Deschutes, OR	8	2056	44.01	-121.57	4.0	91	1106
34	Galena Summit, ID	3	2705	43.87	-114.72	1.5	64	820
41	Umpqua, OR	6	2256	43.21	-122.15	2.9	54	1542
37	Crater Lake, OR*	2	2197	42.89	-122.07	4.2	95	1275
40	Pine Grove, WY	3	2917	42.41	-110.45	1.1	62	917
39	Winema, OR*	2	2184	42.40	-122.29	4.3	87	1500
38	Fremont, OR	9	2268	42.39	-120.53	4.3	92	704
42	Jarbridge, NV*	1	2653	41.80	-115.49	2.9	82	916
43	Mt. Rose, NV*	1	2578	39.32	-119.90	4.1	80	1214

*Removed from final analysis due to low sample size

Appendix 2

Rust Inspection Code Sheet

Code sheet for Skimikin Nursery whitebark pine common garden blister rust inspections. Adapted from the Dorena Genetic Resource Centre code sheet.

Damage (dm)	<u>Severity (sv)</u>	<u>Vigor (vig)</u>			
0 = none	0 = no infection				
 1 = animal 2 = mechanical 3 = disease (non- blister rust) 4 = blister rust 5 = insect 9 = unknown 	 1-3 = rust present, but little impact 4-6 = intermediate (4 = not all the way around bole, 5 = all the way around but not spreading) 7-8 = fairly severe impact (large lesion growth vertical, and encircling stem) 9 = dead from rust (usually massive cankering, but sometimes not) 	 Tree alive and vigorous Tree alive and sickly Tree recently dead (bark intact, easy to count cankers) Tree dead more than a few years Tree dead or missing; unable to determine presences of rust Top dead from rust 			
<u>Flush</u>	Bole Infections	Some standard abbreviations:			
0 - no new shoots present	0 – no bole infections	ID = insect damage			
1 - new shoots present	1 – bole infections present	MD = mechanical damage			
Spots	Limb Infections	FK = forked			
0 = no spots	0 – no limb infections	MT = multi-top			
1 = has spots	1 – limb infections present	CHL = chlorotic			
Aecia		CG = compact growth			
0 = no aecia		PC = pollen cones			

1 = aecia present

Severity	Description
0	No infection
1	Small infection on one limb, infection not touching bole, limb is small relative to size of tree
2	Small-medium size infections on 2-3 small limbs, or one medium size infection on a medium to large limb (encircling limb), infections not touching bole
3	More than three small limbs with infections, or one large infection on a larger limb relative to tree size, infections touching bole but not in bole
4	Infection in bole but not encircling bole, or one large limb completely cankered. If tree forked or multi-topped, one of the tops has a small bole infection but other is healthy
5	Infection in bole and completely encircling but not spreading up or down. If forked or multi-topped, one of tops has bole infection that is spreading up or down with the other(s) healthy
6	Infection in bole and usually limbs and is spreading up or down (10-25% of bole height). If forked or multi-topped either all boles have small infections or one of boles has large infection (over 25% of bole height)
7	Infection in bole and usually limbs - bole infections 25-50% of bole height. For multi-top or forked trees, infections are in all boles and spreading up or down
8	Infections in boles and limbs. Most or all limbs/tops infected and bole infections are over 50% of bole height
9	Dead from rust

Table 1: Detailed description of severity scale (0 to 9) for blister rust inspections of whitebark pine seedlings at Skimikin Nursery, BC.



Figure 1: Examples of severity scale for Skimikin blister rust inspections from 1 (small limb infection) to 9 (dead from rust). A severity code of 0 (not displayed) indicates a healthy seedling with no rust symptoms. Detailed descriptions can be found in Appendix 2, Table 1.

Appendix 3

Table 1: List of whitebark pine families tested at Skimikin Nursery, BC including geographic location, estimated breeding values (EBV) for rust resistance and height, as well as average rust severity. Families are ordered from most resistant to blister rust to most susceptible. Families from provenances not used in breeding value estimation are located at the bottom of the table.

					EBV	EBV	Avg.
Fam.	Provenance	Lat.	Long.	Elev. (m)	(Rust)	(Height)	Severity
275	Mt. Rainier, WA	46.91	-121.51	2028	1.31	4.90	0.22
255	Freezeout Ridge, WA	47.01	-116.04	1783	1.30	-4.59	0.75
376	Umpqua, OR	43.37	-122.20	2195	1.29	-3.01	0.89
210	Colville Forest, WA	48.55	-117.16	1768	1.20	-3.22	0.95
277	Mt. Rainier, WA	46.92	-121.66	1944	1.20	0.43	0.76
188	Moyie Mtn, BC	49.26	-115.77	2078	1.18	-2.61	0.95
181	Moyie Mtn, BC	49.26	-115.77	2078	1.16	2.51	0.83
318	Deschutes, OR	43.47	-121.86	2121	1.11	2.20	2.26
22	McBride Peak, BC	53.34	-120.12	2100	1.07	5.62	1.25
204	Colville Forest, WA	48.66	-118.47	2067	1.05	2.85	1.25
203	Colville Forest, WA	48.66	-118.47	2067	1.03	-4.42	1.37
272	Mt. Rainier, WA	46.91	-121.50	2001	1.01	-0.68	1.40
183	Moyie Mtn, BC	49.26	-115.77	2078	1.00	3.11	1.13
217	Baree Mtn, MT	47.96	-115.55	1829	0.98	16.48	1.53
262	Mt. Rainier, WA	46.91	-121.50	1950	0.95	6.82	1.31
206	Colville Forest, WA	48.66	-118.47	2067	0.94	-2.64	2.33
189	Moyie Mtn, BC	49.26	-115.77	2078	0.92	-6.33	1.50
207	Colville Forest, WA	48.70	-118.47	2164	0.87	0.53	1.57
271	Mt. Rainier, WA	46.91	-121.65	1890	0.87	8.77	1.30
374	Umpqua, OR	43.15	-122.22	2195	0.85	-2.64	2.53
24	McBride Peak, BC	53.34	-120.12	2100	0.84	6.57	2.47
186	Moyie Mtn, BC	49.26	-115.77	2078	0.84	6.87	2.00
211	Colville Forest, WA	48.55	-117.16	1768	0.81	-3.00	1.89
286	Mt. Hood, OR	45.33	-121.71	1832	0.77	-1.17	2.80
23	McBride Peak, BC	53.34	-120.12	2100	0.77	6.34	2.47
283	Mt. Hood, OR	45.33	-121.68	1851	0.75	-0.25	2.53
187	Moyie Mtn, BC	49.26	-115.77	2078	0.75	-13.44	2.28
317	Deschutes, OR	44.26	-121.75	2149	0.70	3.49	2.65
208	Colville Forest, WA	48.70	-118.47	2164	0.70	3.70	2.40
279	Mt. Rainier, WA	46.92	-121.66	1960	0.70	-4.55	1.90
341	Fremont, OR	42.07	-120.25	2335	0.67	-3.42	3.00
282	Mt. Hood, OR	45.33	-121.68	1850	0.62	1.99	2.44
202	Colville Forest, WA	48.65	-118.48	2067	0.61	-9.22	2.39
284	Mt. Hood, OR	45.33	-121.68	1844	0.57	1.59	3.06

71	Nemaiah Mtn, BC	51.49	-124.12	2010	0.50	5.70	3.95
251	Freezeout Ridge, WA	47.01	-116.04	1783	0.47	-1.52	3.12
205	Colville Forest, WA	48.66	-118.47	2067	0.46	5.30	3.00
147	Puddingbum, BC	49.59	-116.01	2340	0.44	5.29	3.60
193	Mt. Baldy, BC	49.17	-119.25	2050	0.44	-0.80	4.22
185	Moyie Mtn, BC	49.26	-115.77	2078	0.43	0.26	2.94
216	Baree Mtn, MT	47.96	-115.55	1829	0.41	2.84	3.41
25	McBride Peak, BC	53.34	-120.12	2100	0.38	-9.25	4.15
83	Jesmond, BC	51.31	-121.92	1850	0.38	2.89	3.89
215	Baree Mtn, MT	47.96	-115.55	1829	0.37	-11.70	4.14
39	Heckman Pass, BC	52.53	-125.82	1909	0.32	-1.21	4.63
81	Jesmond, BC	51.31	-121.92	1850	0.32	-1.13	4.60
285	Mt. Hood, OR	45.33	-121.70	1812	0.32	4.10	3.53
173	Apex Mtn, BC	49.37	-119.92	2100	0.31	3.06	4.60
263	John King Creek, MT	46.84	-110.67	2426	0.31	-7.19	4.16
36	Heckman Pass, BC	52.53	-125.82	1909	0.30	-10.30	4.61
35	Heckman Pass, BC	52.53	-125.82	1909	0.28	5.42	4.60
174	Apex Mtn, BC	49.37	-119.92	2100	0.28	5.09	4.20
134	Blackcomb, BC	50.06	-122.90	1893	0.26	5.74	4.28
44	Perkins Mtn, BC	51.83	-125.04	1940	0.25	3.29	4.70
191	Mt. Baldy, BC	49.17	-119.25	2050	0.25	3.18	4.81
82	Jesmond, BC	51.31	-121.92	1850	0.25	1.78	4.84
349	Fremont, OR	42.39	-120.23	2298	0.25	-5.01	3.81
180	Moyie Mtn, BC	49.26	-115.77	2078	0.23	-4.50	4.05
38	Heckman Pass, BC	52.53	-125.82	1909	0.22	4.94	4.50
294	Umatilla, OR	44.72	-118.58	2313	0.21	0.06	4.75
344	Fremont, OR	42.61	-120.94	2118	0.21	2.98	4.42
316	Deschutes, OR	44.40	-121.63	1890	0.20	1.89	5.24
43	Perkins Mtn, BC	51.83	-125.04	1940	0.19	-8.66	4.56
184	Moyie Mtn, BC	49.26	-115.77	2078	0.19	-11.08	4.16
121	Mt. Carson, BC	50.80	-121.72	2000	0.17	-13.25	4.94
87	Jesmond, BC	51.31	-121.92	1850	0.16	-8.88	4.73
209	Colville Forest, WA	48.70	-118.47	2164	0.16	3.40	4.41
375	Umpqua, OR	43.15	-122.22	2195	0.11	8.01	4.29
373	Umpqua, OR	43.15	-122.22	2195	0.11	0.56	4.45
223	Olympic, WA	47.81	-123.14	1712	0.10	-2.13	4.84
145	Puddingbum, BC	49.59	-116.01	2340	0.09	-6.12	4.90
342	Fremont, OR	42.07	-120.25	2335	0.09	-2.56	5.58
281	Mt. Hood, OR	45.42	-121.68	1814	0.07	-0.35	4.63
298	Umatilla, OR	44.72	-118.57	2313	0.07	-5.69	5.26
230	Wenatchee, WA	47.99	-120.40	2012	0.06	9.25	4.68

363	Pine Grove, WY	42.41	-110.45	2917	0.04	-2.10	5.79
123	Mt. Carson, BC	50.80	-121.72	2000	0.03	3.86	5.25
222	Olympic, WA	47.81	-123.14	1717	0.02	-9.88	5.33
226	Olympic, WA	47.82	-123.14	1829	0.01	-2.55	6.44
362	Pine Grove, WY	42.41	-110.45	2917	0.00	-6.46	5.39
164	Mt. Stevens, BC	49.84	-115.57	2200	0.00	-0.74	5.70
78	Nemaiah Mtn, BC	51.49	-124.12	2010	-0.01	3.88	5.44
163	Mt. Stevens, BC	49.84	-115.57	2200	-0.02	-1.98	5.72
235	Wenatchee, WA	48.66	-119.95	2182	-0.02	3.55	5.65
182	Moyie Mtn, BC	49.26	-115.77	2078	-0.02	8.10	4.35
225	Olympic, WA	47.82	-123.14	1800	-0.03	2.77	5.50
297	Umatilla, OR	44.72	-118.57	2313	-0.04	0.81	5.95
93	Kickinghorse, BC	51.30	-117.06	2000	-0.05	2.57	6.00
241	Morrell Lookout, MT	47.19	-113.35	2217	-0.06	0.07	5.40
165	Mt. Stevens, BC	49.84	-115.57	2200	-0.09	3.56	5.79
144	Puddingbum, BC	49.59	-116.01	2340	-0.09	4.83	5.59
295	Umatilla, OR	44.72	-118.58	2313	-0.10	4.03	5.95
345	Fremont, OR	42.61	-120.94	2103	-0.10	7.09	5.53
195	Mt. Baldy, BC	49.17	-119.25	2050	-0.11	-5.98	4.89
1	Mt. Sidney Williams, BC	55.02	-125.59	1580	-0.11	-8.99	5.89
348	Fremont, OR	42.30	-120.16	2507	-0.11	-2.40	5.24
237	Wenatchee, WA	48.66	-119.95	2182	-0.11	0.06	5.79
92	Kickinghorse, BC	51.30	-117.06	2000	-0.13	-10.07	5.78
31	Heckman Pass, BC	52.53	-125.82	1909	-0.13	0.61	5.52
315	Deschutes, OR	44.40	-121.63	1951	-0.14	-0.82	5.21
306	Galena Summit, ID	43.87	-114.72	2705	-0.14	-9.52	5.94
238	Wenatchee, WA	48.75	-120.07	2214	-0.14	3.29	5.95
198	Blackwall/Manning, BC	49.10	-120.76	2005	-0.15	4.22	5.74
307	Galena Summit, ID	43.87	-114.72	2705	-0.16	2.31	5.84
371	Umpqua, OR	43.22	-122.04	2377	-0.16	-0.45	5.17
76	Nemaiah Mtn, BC	51.49	-124.12	2010	-0.16	3.11	5.67
77	Nemaiah Mtn, BC	51.49	-124.12	2010	-0.17	-0.30	6.11
101	Taseko Mtn, BC	51.26	-123.54	1950	-0.17	5.05	5.60
233	Wenatchee, WA	47.99	-120.40	2012	-0.17	-7.34	6.05
95	Kickinghorse, BC	51.30	-117.06	2000	-0.18	0.81	5.89
265	John King Creek, MT	46.84	-110.67	2426	-0.18	-0.91	6.29
314	Deschutes, OR	44.10	-121.62	1987	-0.18	-10.68	5.63
4	Mt. Sidney Williams, BC	55.02	-125.59	1580	-0.19	-16.37	6.14
30	Heckman Pass, BC	52.53	-125.82	1909	-0.19	-4.35	5.83
343	Fremont, OR	42.61	-120.94	2111	-0.20	-3.96	6.00
47	Perkins Mtn, BC	51.83	-125.04	1940	-0.21	1.38	5.79

52	Sapeye Mtn, BC	51.82	-124.81	2027	-0.22	-5.85	5.93
299	Umatilla, OR	44.72	-118.57	2313	-0.22	-4.95	5.53
261	John King Creek, MT	46.84	-110.67	2426	-0.24	3.44	6.11
133	Blackcomb, BC	50.06	-122.90	1893	-0.24	1.45	5.95
245	Morrell Lookout, MT	47.19	-113.35	2217	-0.24	3.36	5.95
51	Sapeye Mtn, BC	51.82	-124.81	2027	-0.24	-4.72	6.10
103	Taseko Mtn, BC	51.26	-123.54	1950	-0.25	0.75	6.65
2	Mt. Sidney Williams, BC	55.02	-125.59	1580	-0.25	6.51	5.68
312	Deschutes, OR	43.68	-121.26	2264	-0.25	-1.31	6.47
167	Mt. Stevens, BC	49.84	-115.57	2200	-0.26	1.70	6.26
137	Blackcomb, BC	50.06	-122.90	1893	-0.26	2.45	6.00
21	McBride Peak, BC	53.34	-120.12	2100	-0.26	4.18	5.33
148	Puddingbum, BC	49.59	-116.01	2340	-0.26	8.68	5.95
311	Deschutes, OR	43.69	-121.19	2099	-0.27	14.32	5.95
169	Mt. Stevens, BC	49.84	-115.57	2200	-0.27	-4.69	6.30
221	Olympic, WA	47.81	-123.14	1701	-0.27	9.52	5.94
42	Perkins Mtn, BC	51.83	-125.04	1940	-0.27	-2.89	6.24
131	Blackcomb, BC	50.06	-122.90	1893	-0.27	-4.17	5.90
143	Puddingbum, BC	49.59	-116.01	2340	-0.28	-2.18	5.95
33	Heckman Pass, BC	52.53	-125.82	1909	-0.28	5.48	6.05
175	Apex Mtn, BC	49.37	-119.92	2100	-0.28	1.39	5.90
293	Umatilla, OR	44.72	-118.58	2309	-0.28	0.19	6.16
34	Heckman Pass, BC	52.53	-125.82	1909	-0.29	2.62	6.00
37	Heckman Pass, BC	52.53	-125.82	1909	-0.30	2.21	6.00
135	Blackcomb, BC	50.06	-122.90	1893	-0.30	1.58	6.60
75	Nemaiah Mtn, BC	51.49	-124.12	2010	-0.31	-2.81	6.24
132	Blackcomb, BC	50.06	-122.90	1893	-0.31	-2.67	6.22
231	Wenatchee, WA	47.99	-120.40	2012	-0.32	-2.54	6.11
347	Fremont, OR	42.30	-120.15	2488	-0.32	6.08	6.53
142	Puddingbum, BC	49.59	-116.01	2340	-0.32	8.97	6.90
73	Nemaiah Mtn, BC	51.49	-124.12	2010	-0.33	1.50	6.15
243	Morrell Lookout, MT	47.19	-113.35	2217	-0.35	-3.61	6.21
53	Sapeye Mtn, BC	51.82	-124.81	2027	-0.36	-2.91	6.47
196	Blackwall/Manning, BC	49.10	-120.76	2005	-0.37	-4.86	6.89
41	Perkins Mtn, BC	51.83	-125.04	1940	-0.37	3.23	5.68
70	Nemaiah Mtn, BC	51.49	-124.12	2010	-0.37	-3.41	6.15
122	Mt. Carson, BC	50.80	-121.72	2000	-0.38	5.12	6.58
91	Kickinghorse, BC	51.30	-117.06	2000	-0.38	4.83	6.47
102	Taseko Mtn, BC	51.26	-123.54	1950	-0.39	-0.95	6.35
292	Umatilla, OR	44.72	-118.58	2316	-0.40	-3.37	6.11
372	Umpqua, OR	43.22	-122.04	2377	-0.41	3.67	5.93

224	Olympic, WA	47.82	-123.13	1839	-0.41	3.72	6.68
97	Kickinghorse, BC	51.30	-117.06	2000	-0.42	-2.43	7.00
162	Mt. Stevens, BC	49.84	-115.57	2200	-0.43	-0.90	6.50
72	Nemaiah Mtn, BC	51.49	-124.12	2010	-0.44	-2.15	6.55
11	Mt. Sidney Williams, BC	55.02	-125.59	1580	-0.44	-3.18	7.21
172	Apex Mtn, BC	49.37	-119.92	2100	-0.44	-10.86	6.55
160	Mt. Stevens, BC	49.84	-115.57	2200	-0.46	7.02	6.50
7	Mt. Sidney Williams, BC	55.02	-125.59	1580	-0.46	5.97	7.20
84	Jesmond, BC	51.31	-121.92	1850	-0.46	3.97	6.45
239	Wenatchee, WA	48.75	-120.06	2195	-0.46	-3.24	6.67
236	Wenatchee, WA	48.66	-119.95	2182	-0.47	1.30	6.35
161	Mt. Stevens, BC	49.84	-115.57	2200	-0.47	-0.53	7.00
234	Wenatchee, WA	47.99	-120.40	2012	-0.48	0.88	6.40
141	Puddingbum, BC	49.59	-116.01	2340	-0.48	-9.34	6.76
104	Taseko Mtn, BC	51.26	-123.54	1950	-0.49	3.67	6.70
296	Umatilla, OR	44.72	-118.57	2326	-0.50	-2.31	6.58
32	Heckman Pass, BC	52.53	-125.82	1909	-0.50	-2.00	6.50
88	Jesmond, BC	51.31	-121.92	1850	-0.52	4.64	6.83
346	Fremont, OR	42.60	-120.94	2117	-0.52	4.51	6.55
5	Mt. Sidney Williams, BC	55.02	-125.59	1580	-0.52	10.56	6.63
194	Mt. Baldy, BC	49.17	-119.25	2050	-0.56	-5.13	7.00
8	Mt. Sidney Williams, BC	55.02	-125.59	1580	-0.57	-2.84	6.78
171	Apex Mtn, BC	49.37	-119.92	2100	-0.58	2.93	7.37
26	McBride Peak, BC	53.34	-120.12	2100	-0.58	-2.31	6.45
105	Taseko Mtn, BC	51.26	-123.54	1950	-0.61	2.92	7.32
3	Mt. Sidney Williams, BC	55.02	-125.59	1580	-0.62	2.66	7.05
74	Nemaiah Mtn, BC	51.49	-124.12	2010	-0.65	-2.20	7.11
253	Freezeout Ridge, WA	47.01	-116.04	1783	-0.65	-6.64	6.60
197	Blackwall/Manning, BC	49.10	-120.76	2005	-0.66	-4.54	7.32
305	Galena Summit, ID	43.87	-114.72	2705	-0.66	2.28	7.10
107	Taseko Mtn, BC	51.26	-123.54	1950	-0.68	-3.42	7.15
361	Pine Grove, WY	42.41	-110.45	2917	-0.70	1.72	7.10
168	Mt. Stevens, BC	49.84	-115.57	2200	-0.70	0.98	7.26
6	Mt. Sidney Williams, BC	55.02	-125.59	1580	-0.76	0.43	7.58
10	Mt. Sidney Williams, BC	55.02	-125.59	1580	-0.83	4.11	7.55
313	Deschutes, OR	44.10	-121.62	1989	-0.88	1.05	7.00
232	Wenatchee, WA	47.99	-120.40	2012	-0.92	0.01	7.79
15	Hudson Bay Mtn, BC	54.77	-127.28	1500	NA	NA	7.25
62	Niut Mtn, BC	51.67	-124.52	2003	NA	NA	8.11
63	Niut Mtn, BC	51.67	-124.52	2003	NA	NA	7.50
111	Lime Mtn, BC	51.09	-121.66	1935	NA	NA	4.26

151	Bear Lake, BC	49.77	-115.47	2200	NA	NA	3.56
152	Bear Lake, BC	49.77	-115.47	2200	NA	NA	2.15
200	Baker-Snoqualmie, WA	46.93	-121.49	1908	NA	NA	0.82
201	Baker-Snoqualmie, WA	46.94	-121.50	1908	NA	NA	1.84
290	Warm Springs OR	44.66	-121.69	1947	NA	NA	1.65
291	Warm Springs OR	44.65	-121.69	1966	NA	NA	1.58
300	Wallowa-Whitman, OR	44.95	-118.24	2388	NA	NA	3.37
301	Wallowa-Whitman, OR	44.95	-118.24	2390	NA	NA	3.83
	Gifford Pinchot/Mt.Adams,						
321	OR	46.15	-121.49	2057	NA	NA	2.59
	Gifford Pinchot/Mt.Adams,						
322	OR	46.15	-121.49	2081	NA	NA	2.20
331	Crater Lake, OR	42.89	-122.07	2196	NA	NA	5.53
332	Crater Lake, OR	42.89	-122.07	2197	NA	NA	5.32
350	Winema, OR	42.44	-122.30	2325	NA	NA	6.53
351	Winema, OR	42.36	-122.27	2042	NA	NA	4.43
400	Jarbridge, NV	41.80	-115.49	2653	NA	NA	4.94
405	Mt. Rose, NV	39.32	-119.90	2578	NA	NA	4.73