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Fort Collins, Colorado June 15–20, 2014

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Abstract

Proceedings from the 2014 IUFRO Joint Conference: Genetics of five-needle pines, rusts of forest trees, and Strobusphere in Fort Collins, Colorado. The published proceedings include 91 papers pertaining to research conducted on the genetics and pathology of five-needle pines and rusts of forest trees. Topic areas are: ecology and climate change, common garden genetics, genomic resources, rust resistance, and rust biology, ecology, and management.

Keywords: genetic variation, genetic conservation, restoration, *Pinus*, *Populus*, rust fungi, disease resistance, climate change, *Cronartium ribicola*

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Cover photo captions: (clockwise from the upper left) Uredinia and telia of *Cronartium flaccidum* on *Paeonia tenuifolia* (J. Kaitera); Wild *Populus nigra* infected with *Melampsora larici-populina* in the field (P. Frey); *Cronartium ribicola* on *Pinus flexilis* in the field (A. Schoettle); *Pinus flexilis* in Rocky Mountain National Park (C. Holtz); male pollen cones (strobili) on *Pinus albicaulis* seedling at Dorena Genetic Resource Center (A. Schoettle); Mature seed cone of *Pinus strobiformis* (A. Schoettle); *Pinus flexilis* mortality from *Cronartium ribicola* on Vedauwoo rock formation during field trip #1 (A. Schoettle); field trip #3 in *Pinus aristata* forest in Mt Goliath Research Natural Area (R. Sniezko).

Pesticide Precautionary Statement

This publication reports research involving pesticides. It does not contain recommendations for their use, nor does it imply that the uses discussed here have been registered. All uses of pesticides must be registered by appropriate State and/or Federal agencies before they can be recommended.

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Conference participants at Vedauwoo Recreation Area, Medicine Bow National Forest. Photo by Justin Hof, USDA Forest Service.

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The Organizers would like to extend a heart-felt thanks to all the entities that contributed funds, support, and energy to help make this first joint conference and proceedings happen.

Many thanks to:

USDA Forest Service, Rocky Mountain Research Station

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USDA Forest Service, Western Wildland Environmental Threat Assessment Center

USDA Forest Service, Forest Health Protection, Rocky Mountain Region

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Preface

This conference was a joint international conference of three groups: IUFRO 2.02.15 (Breeding and Genetic Resources of Five-Needle Pines), IUFRO 7.02.05 (Rusts of Forest Trees), and Strobusphere. The International Union of Forestry Research Organizations (IUFRO) is a nonprofit, nongovernmental international network of forest scientists that promotes global cooperation in forest-related research and enhances the understanding of the ecological, economic, and social aspects of forests and trees. This was the first time these three groups have met together to share research in genetics-pathology of five-needle pines and rusts of forest trees.

IUFRO Working Group 2.02.15—Breeding and Genetic Resources of Five-Needle Pines

Our Working Party on Breeding and Genetic Resources of Five-Needle Pines is concerned with research cooperation and exchange of information on all aspects of genetic research on the five-needle pines. This includes provenance and progeny testing, gene conservation, landscape genomics, breeding, species hybridization, clonal propagation and testing, molecular genetics, and the genetics of host-pathogen interactions, as well as ecology, evolutionary dynamics, and management of these species. Increasingly, though, we are using this knowledge to address issues related to climate change, land use pressure, and conservation. We welcome new members interested in these topics. Since 2001, this group has met in the United States, Romania, the Republic of South Korea, and Russia and was also a sponsor and organizer of the 2012 International Workshop on Disease and Insect Resistance in Forest Trees (IUFRO Working Group 2.02.15 Website).

IUFRO Working Group 7.02.05—Rusts of Forest Trees

Our Working Party aims to bring together scientists and investigators working on tree rusts. Our goal is to foster scientific discussion and exchanges relating to tree rust epidemiology, biology, host-pathogen interactions, resistance, control and management, and genomics. Our working group meets approximately once every 4 years in locations in Europe, North America, or Asia. We usually meet in locations that allow us to discuss our scientific findings and have field trips in a friendly and relaxed environment that is conducive to exchanges and debates. We want to place a strong emphasis on participation of young investigators and students, as these meetings provide unique experiences to meet and exchange information with the related community (IUFRO Working Group 7.02.05 Website).

Strobusphere

The Strobusphere working group arose as a collaborative effort among researchers that began with a multinational White Pine Genomic Resource Workshop held in 2008. The objective of this workshop was to discover and identify research objectives, strengths, scope, and resources among the various working agencies. This collaborative effort is designed as a foundation to build wider scientific participation with a scope that spans molecular to landscape models, from host to pathogens and alternative hosts (Strobusphere Website).

Contents

Ecology and Genetics of *Pinus strobiformis*

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Abstract—Extending from the Canadian province of Alberta to the Transverse Volcanic Plateau of Mexico, *Pinus strobiformis* is part of a species complex with two geographically adjacent, but nonsympatric, species, namely *P. flexilis* and *P. ayacahuite*. *Pinus strobiformis* is classified as being of least concern and at lower risk according to the guidelines of the International Union for the Conservation of Nature and Natural Resources (IUCN) Red List (IUCN 2014). In Mexico, this frequent tree species is protected and included in Mexican Regulation NOM-059-2010. *Pinus strobiformis* appears to have broad environmental tolerances and fire resistance when mature. It is often found in mixed-conifer forests that are valuable summer habitat for big and small game animals, rodents, and game and nongame birds. At the large scale, analyses of spatial genetic structure of *P. strobiformis* indicated a very weak spatial distance–genetic distance relationship, probably resulting from a strong seed interchange, likely by birds, particularly the Mexican jay (*Aphelocoma wollweberi*), and from a continuous and broad geographic distribution of this tree species. The highest genetic diversity was found in the southern populations, indicating the recent colonization of the populations at the northern distributional limit. Recently, interest in *P. strobiformis* has increased due to the discovery of white pine blister rust (causal agent: *Cronartium ribicola*) in the Sacramento Mountains of New Mexico (USA).

DESCRIPTION

Pinus strobiformis, subgenus *Strobus*, section *Quinquefoliae* (Gernandt et al. 2005), is commonly called Mexican white pine, Arizona white pine, Chihuahuan white pine, southwestern white pine, pino blanco, pinabete, and pino enano. Extending from Alberta (Canada) to the Transverse Volcanic Plateau of Mexico, *P. strobiformis* is part of a species complex that includes two geographically adjacent, but nonsympatric, species, namely *P. flexilis* and *P. ayacahuite* (Farjon and Styles 1997; Moreno-Letelier and Piñero 2009), as well as other species such as *P. strobiformis* subsp. *veitchii*, and *P. stylesii* (Gymnosperm Database 2014) (fig. 1). The conservation status of *P. strobiformis* is classified as "least concern and lower risk" according to the guidelines of the International Union for the Conservation of Nature and Natural Resources (IUCN) Red List of Threatened Species™ (IUCN 2014). In Mexico, this frequent tree species is protected and included in Mexican Regulation NOM-059-2010.

Pinus strobiformis is a native, evergreen conifer with an open, irregular crown. It can grow to 27 m tall with a diameter up to 1 m; the bark is thin, rough, and furrowed. Branches are long and horizontal to pendant. Needles are 6 to 10 cm long in fascicles of five. The cones are 7 to 25 cm long with reflexed, thick scales;

In: Schoettle, Anna W.; Sniezko, Richard A.; Kliejunas, John T., eds. 2018. Proceedings of the IUFRO joint conference: Genetics of five-needle pines, rusts of forest trees, and Strobusphere; 2014 June 15–20; Fort Collins, CO. Proc. RMRS-P-76. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 245 p.

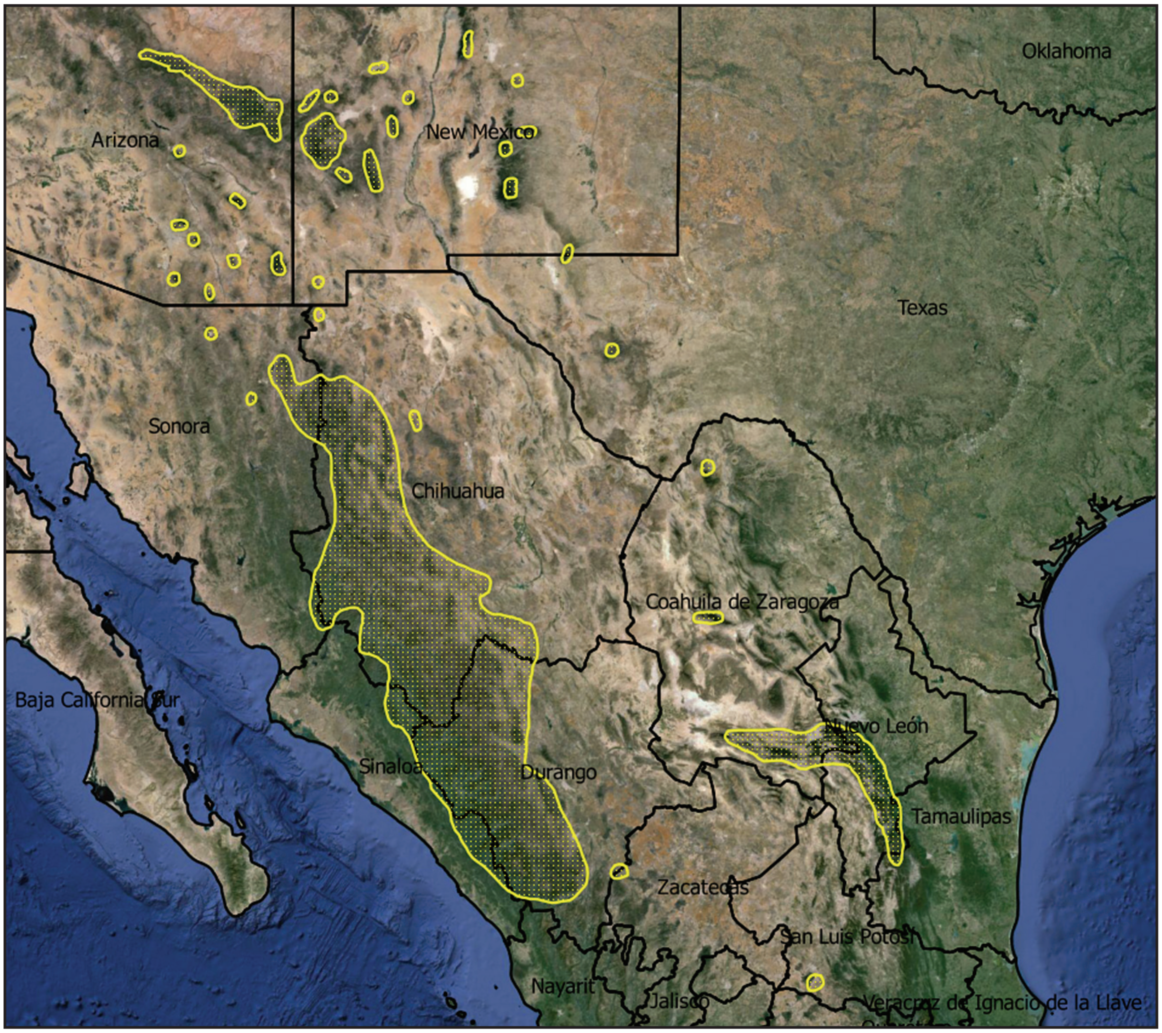

Figure 1—Natural range of *Pinus strobiformis* (from Little, E.L., Jr., 1971).

cones are dehiscent when mature (figs. 2 and 3). Seeds are essentially wingless and 10 to 12 mm long (Pavek 1993; Perry 1991).

DISTRIBUTION AND OCCURRENCE

Pinus strobiformis has a limited distribution. It occurs in the mountains of western Texas, New Mexico, Arizona, and southwestern Colorado (USA) (Jones 1974; Samano and Tomback 2003). It also extends along the mountains to central Mexico (Farjon and Styles 1997), ranging from northeastern Sonora to Western Chihuahua and Durango, northern Zacatecas, southern Coahuila, and central Nuevo Leon (U.S. Geological Survey 2006). The largest populations are found in Mexico (Pavek 1993). This species typically occurs in low densities in southwestern pine, mixed-conifer, and spruce-fir (*Picea* spp.-*Abies* spp.) forests (Stromberg and Patten 1991), which generally have been the subject of relatively few studies (Jones 1974).

This pine species is associated with several other pine species including *P. ponderosa*, *P. arizonica*, *P. leiophylla*, *P. engelmannii*, and *P. cembroides*. *Pinus strobiformis* also is associated with *Pseudotsuga menziesii*, *Populus tremuloides*, *Abies concolor*, *Abies bifolia*, *Picea pungens*, *Picea engelmannii*, *Juniperus deppeana*, *Cupressus arizonica*, *Arbutus arizonica*, and a variety of *Quercus* species. In Mexico, this tree species is associated with a wide variety of endemic Mexican species such as *Picea chihuahuana*, *Cupressus lusitanica*, *Abies durangensis*, *Abies vejari*, and *Abies religiosa* (Looney and Waring 2013; Simental-Rodríguez et al. 2014).

Figure 2—*Pinus strobiformis* trees (a and b) in Durango State, Mexico (photo: C. Wehenkel).

Figure 3—Female flowers (a) and cones (b) of *Pinus strobiformis* trees in Durango State, Mexico (photo: C. Wehenkel).

ECOLOGY

Pinus strobiformis appears to have broad environmental tolerances (Looney and Waring 2013) and fire resistance when mature (Dieterich 1983). It is widespread in mesic sites on ridges, slopes, and canyons of montane zones (Pavek 1993). These zones have a semiarid climate, with mild winters and warm summers. The average precipitation ranges from 487 to 1,143 mm per year. In its U.S. range, *P. strobiformis* typically grows at elevations ranging from 1,900 to 3,000 m above sea level (Pavek 1993). In Mexico, it is found at elevations from 2,200 to 3,600 m above sea level (Aguirre-Calderón et al. 2003; Fulé and Covington 1996).

The range of *P. strobiformis* is influenced by a variety of environmental factors, including elevation (Brady and Bonham 1976), moisture availability (Niering and Lowe 1984), and temperature (Laughlin et al. 2011). The species usually grows on moist, cool sites, where it is most likely to be dominant. It is preferentially located on dry rocky slopes in high mountains, or as a minor component in mixed-conifer forests. The species is found in sites with loamy soil textures ranging from shallow, gravelly loams to deep, sandy loams and stony silty clay loams (Pavek 1993). Depending on elevation, this species may occur on a variety of aspects and topographic settings (Looney and Waring 2013).

GENETICS

Moreno-Letelier and Piñero (2009) presented patterns of genetic variation between the taxa in the *P. strobiformis* complex by chloroplast haplotype (microsatellite) analysis, which supports the concept of significant distinctions between each of the taxa named here, and between the Sierra Madre Occidental, Sierra Madre Oriental, and Jalisco populations of *P. strobiformis* (Gymnosperm Database 2014). Two main genetic and geographic groups of *P. strobiformis* were separated by the Chihuahuan Desert. Little population differentiation and no isolation by distance within each of the two groups were found, suggesting genetic connectivity as a result of population expansions within these areas during glacial stages.

Quiñones-Pérez et al. (2014) observed a nonsignificant autocorrelation in fine-scale spatial genetic structure (SGS) using amplified fragment length polymorphism (AFLP), suggesting that the genetic variants of the tree species are randomly spatially distributed within sampled plots of 50 m × 50 m. On a larger scale, the SGS analyses of *P. strobiformis* also indicated a very weak spatial distance–genetic distance relationship, probably resulting from a strong seed interchange, likely by birds, particularly the Mexican jay (*Aphelocoma wollweberi*), and from a continuous and broad geographic distribution of this tree species (Looney and Waring 2013). The overall mean of genetic distances between *P. strobiformis* trees was higher than between *Picea chihuahuana* trees, *Populus tremuloides* trees, and *Pseudotsuga menziesii* trees in the rare Mexican *Picea chihuahuana* tree community.

Simental-Rodríguez et al. (2014) found no evidence that tree species diversity is related to adaptive AFLP structure and mean genetic diversity per AFLP locus of *P. strobiformis* at 12 locations in the *Picea chihuahuana* tree community on the Mexican Sierra Madre Occidental (in contrast to *Populus tremuloides*, *Picea chihuahuana*, and *Pseudotsuga menziesii*). As seen in Moreno-Letelier and Piñero (2009), the highest genetic diversity was found in the southern populations, indicating recent colonization of the populations at the northern distributional limit (Petit et al. 2003).

Villalobos-Arámbula et al. (2014) examined cross-species transferability and usefulness of six nuclear microsatellite markers developed in consubgeneric eastern white pine (*Pinus strobus*) with regard to ecologically and commercially important Mexican white pine species of conservation genetics concern: *Pinus chiapensis*, *P. flexilis*, *P. strobiformis*, *P. ayacahuite*, and *P. ayacahuite* var. *veitchii*. Four to six microsatellite loci were found to be polymorphic in different species.

MANAGEMENT CONSIDERATIONS

Pinus strobiformis is often found in mixed-conifer forests that are valuable summer habitat for big and small game animals, rodents, and game and nongame birds. As natural regeneration of clearcut mixed-conifer forests on south-facing slopes requires 50 to 100 years, these clearcut areas can be a valuable long-term forage resource for deer (*Odocoileus* spp.) and elk (*Cervus elaphus*) (Pavek 1993).

This pine species is the principal host of Apache dwarf mistletoe (*Arceuthobium apachecum*). Infected trees have reduced growth and increased susceptibility to other infections, insects, and mortality (Hawksworth 1978; Walters 1978). *Pinus strobiformis* is infected by red ring rot and by root and butt rots (Walters 1978). Recently, interest in *P. strobiformis* has increased due to the discovery of white pine blister rust (causal agent: *Cronartium ribicola*) in the Sacramento Mountains of New Mexico (Hawksworth 1990). *Cronartium ribicola* is a potentially lethal introduced pathogen that threatens white pine species (Conklin 2004; Schoettle 2004; Zeglen et al. 2010).

Despite the research to date on white pine blister rust, there is insufficient information to predict how this disease will affect *P. strobiformis* in the future (Looney and Waring 2013). Other disturbances of *P. strobiformis* include fire (Danzer et al. 1996; Villanueva-Diaz and McPherson 1995), and climate change (Seager et al. 2007). A few studies reported effects of insects or animals on this species (Hawksworth et al. 2002).

Wood of *P. strobiformis* is soft, not resinous, and white with a slightly darker heartwood. It can be used for cabinetry, doors, and window frames. Crooked stems and coarse branches make it undesirable for lumber (Pavek 1993).

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Climate Change Alters Distribution of Whitebark Pine (*Pinus albicaulis*) With Respect to Land Management Status: Are there Implications for Adaptation?

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INTRODUCTION AND METHODS

Climate-based predictions of species distributions provide information for understanding impacts of climate change. However, species distribution models do not include land management zones as input. Improving understanding of geographic shifts will require consideration of management zones where certain activities could facilitate or impede responses to climate change. For instance, assisted migration to overcome dispersal barriers could be challenged in areas designated for "untrammeled" character (e.g., wilderness). Alternatively, large tracts of land with uninterrupted disturbance regimes may offer opportunities for disturbance-facilitated establishment of species into new areas. We focused on current and future distributions of whitebark pine (*Pinus albicaulis*), a keystone species of treeline. Current and future "climate space" of whitebark pine were overlaid onto ecoregions and land management zones. We also used different thresholds of predicted presence to assess the sensitivity of patterns to varying levels of confidence.

RESULTS AND CONCLUSIONS

Geographic distribution of viable "climate space" for whitebark pine is predicted to contract 86 percent by 2060 across the western United States based on our most inclusive confidence estimate. As climate change reduces the total area of viable habitat, distribution of whitebark pine in designated wilderness is expected to nearly double. The U.S. Department of Agriculture, Forest Service climate model predicts that whitebark pine range occurring within wilderness boundaries will increase from 38 percent in 2010 to 62 percent by 2060. Climate adaptation and restoration strategies need to consider the influence of land management zones as species shift in response to climate change. From our analysis, we developed a simple conceptual model that managers could use when deciding what level of active restoration to perform in protected areas set aside to sustain "untrammeled" conditions. We discuss our results in the context of ongoing and predicted whitebark pine decline, climate change, restoration, and wilderness character.

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Genetic and Ecological Divergence in the *Pinus flexilis–Pinus strobiformis–Pinus ayacahuite* Species Complex: Clues to Understanding Speciation in Pines

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North America is a secondary center of diversification of the genus *Pinus*. Most of these speciation events are fairly recent, producing a pattern of widespread shared polymorphism and fuzzy species boundaries. Among five-needle pines, the relationships among *Pinus flexilis*, *Pinus strobiformis*, and *Pinus ayacahuite* have been particularly difficult to determine. To understand the speciation process and the phylogenetic relationships in this species complex, we evaluated genetic and ecological differentiation using multilocus sequence data and ecological niche model comparisons. Our results show Late Pliocene–Early Pleistocene divergence and a contrasting pattern of differentiation between genes and ecological niche. Cytoplasmic and housekeeping nuclear genes show a clear differentiation of *P. ayacahuite*. However, ecological niche differences and candidate genes for drought tolerance show a strong differentiation in *P. flexilis*. Significant correlation was found between genetic distance and environmental distance in some of these candidate genes, as well as evidence of natural selection in *P. flexilis*.

This discrepancy among genes could be evidence of a genomic mosaic product of ecological speciation in *P. flexilis*. In adaptive speciation, only those genes under natural selection would become differentiated, whereas in allopatric or parapatric speciation, differentiation would occur in the entire genome. Additionally, the ecological differentiation of *P. flexilis* could represent a barrier to gene flow with its sister species.

The differentiation of *P. flexilis* at the ecological and candidate gene level suggests that adaptive divergence has played a role in speciation. This means that despite their long generation times, pines can adapt to new environments relatively quickly, and that local adaptation could be an important factor in causing population structure and speciation in other five-needle pines. Considering the broad latitudinal range of this species complex, it is also possible that divergent selection could be acting on other genomic regions that were not sampled in this study, which in turn would explain the differences between the tropical *P. ayacahuite* and subtropical *P. strobiformis*. Therefore, more work is needed to characterize the genetic structure in these pines at the genomic level.

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Southwestern White Pine (*Pinus strobiformis*) Regeneration Ecology Along Disease and Management Gradients in the Southwestern United States

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Southwestern white pine (*Pinus strobiformis*, SWWP) is a component of dry and wet mixed-conifer forests in the southwestern United States and provides ecological function and diversity. The species is geographically limited to Arizona, New Mexico, parts of Texas, southern Colorado, and northern Mexico, but occurs on a wide range of habitats within its distribution. Historically, SWWP regeneration has not been well studied or monitored in mixed-conifer forests of the Southwest, although our understanding of the species' ecology is increasing. Introductions of *Cronartium ribicola*, the pathogen responsible for causing white pine blister rust (WPBR), are fairly recent compared to other areas in North America, and disease intensity is variable in the Southwest. Heavy disease occurs in south-central New Mexico, whereas many forests in Arizona are not affected yet. However, the disease continues to spread west through isolated mountain ranges in the Southwest.

Our research seeks to quantify SWWP regeneration responses to various silvicultural strategies across a range of WPBR intensities, and to determine how disease incidence has been affected by recent management in the Southwest. The opportunity to study regeneration in stands not yet affected by WPBR allows us to make baseline comparisons for management goals and to determine if stand trajectories in areas where WPBR has been present for several decades are occurring in areas with more recent infestations.

Fifty-five stands were surveyed across the region, including stands with no recent management and stands with recent uneven-aged (UEA) treatments (leaving two-aged and multi-aged structures). Seedling ages (by whorl count) demonstrate germination following treatments; survey results indicate that UEA treatments successfully regenerate SWWP in areas with and without WPBR. Results suggest that SWWP grows most successfully in two-aged structures, but models interpreting the effects of management, disease, and other species' presence on SWWP densities across the region are in progress. Regeneration stem mapping will also allow comparisons of species distributions and possible competitive interactions between similar and different size classes in the understory.

Regeneration success of species at risk of biotic invasions is critical to the sustainability of current and future distributions, and SWWP regeneration ecology must be better understood in order to make sound management decisions in the face of climate change and other disturbances. Previous work addressing proactive management in other North American five-needle pine systems advises that increases in regeneration diversity and densities are critical to maintaining populations on the landscape. These results will help guide management efforts in sustaining SWWP in the presence of an invasive pathogen.

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Limber Pine (*Pinus flexilis*) Stand Conditions After White Pine Blister Rust- and Mountain Pine Beetle (*Dendroctonus ponderosae*)-caused Mortality in the Central and Southern Rocky Mountains

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Mountain pine beetle (*Dendroctonus ponderosae*) and white pine blister rust (causal agent: *Cronartium ribicola*) are causing extensive crown dieback and mortality in limber pine (*Pinus flexilis*) in the central and southern Rocky Mountains. Ecologically valuable limber pines often grow in fragile ecosystems where few other trees can grow. The combined effects of mountain pine beetle, white pine blister rust, dwarf mistletoe (*Arceuthobium cyanocarpum*), and climate change could greatly reduce the biodiversity of these ecosystems. Current status and long-term monitoring of limber pine trees and seedlings are needed to advise land managers and to implement restoration. The study objectives were to: (1) assess site, stand, and health characteristics of seedlings and mature limber pines in Colorado, Wyoming, and Montana (USA); (2) determine factors that influence the occurrence and incidence of white pine blister rust, bark beetles, and dwarf mistletoe; and (3) determine factors that affect seedlings, including site, stand, and meteorological characteristics, and white pine blister rust. In 2011 and 2012, we assessed 22,700 limber pines on 508 plots in limber pine-dominated stands in 25 study areas in Colorado, Wyoming, and Montana. Mean density of live limber pine was 311 stems ha^{-1}. Fifty percent of standing limber pine trees were classified as healthy, 26 percent were declining or dying, and 24 percent were dead. White pine blister rust was the primary damage agent and was widespread, occurring in 23 of the 25 study areas, on 73 percent of plots with a mean incidence of 26 percent. Bark beetle-caused mortality occurred in all 25 study areas, and 18 percent of standing limber pines were killed by bark beetles. Limber pine dwarf mistletoe occurred within 20 study areas, on 29 percent of plots, with a mean incidence of 9 percent. Live limber pine seedling density averaged 141 stems ha^{-1}. Of all standing live and dead limber pine seedlings, 1.5 percent were dying, 4.4 percent were dead, and white pine blister rust occurred on 5.3 percent of live seedlings.

We used statistical modeling to determine the meteorological, macrosite, and microsite factors and stand factors that influenced the occurrence and incidence of white pine blister rust, bark beetles, and dwarf mistletoe on limber pines. We also used statistical modeling to determine factors that predict density (stems ha^{-1}) of limber pine seedlings and proportion of limber pine seedlings with white pine blister rust. The widespread occurrence of limber pine stands heavily affected by mountain pine beetle and white pine blister rust, combined with low regeneration density, suggests that these areas should be monitored so land managers can decide if restoration efforts are warranted.

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Present and Possible Past Distribution of Swiss Stone Pine (*Pinus cembra*) in the Austrian Alps—The Ups and Downs of Climate Change

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Abstract—Swiss stone pine (*Pinus cembra*) is currently restricted to the uppermost subalpine forest belt in the central and eastern Alps in Austria, where high mountain national parks are important for this species. Climate change threatens this habitat, as global and regional warming tends to enhance the competitiveness of other tree species—European larch (*Larix decidua*) and Norway spruce (*Picea abies*)—while an upslope migration "escape" is often not possible for orographic reasons. However, records suggest that this pattern has recurred throughout post-glacial history, and it is tempting to speculate whether it has resulted in the absence of the species from many mountain chains. Human overexploitation has certainly added to the species' current condition. This raises the question as to what approximate minimum number of trees is necessary to maintain these tree populations, especially when they are in isolation as a consequence of fragmentation of formerly continuous stone pine belts. Any such predictions will also have implications for genetic diversity.

We will present results from a survey of literature and data regarding past and present distribution of the species in Austria. A striking feature is that many places mentioned in older literature (about 1900) as rare outposts often do not seem to have any remaining Swiss stone pine trees, at least in a density relevant for forest inventory data. Orographic features (that is, whether the terrain allows the species to escape to higher elevations in warmer periods) may have an impact on its current distribution range on the eastern edge of the Alps, as there are dispersal limits that apparently affect the main distribution agent, the Eurasian nutcracker bird (*Nucifraga caryocatactes*). We are starting a genetic investigation in very small stands, trying to assess minimum viable population sizes and densities for this pine species.

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Forecasting Climate and Disease Impacts on Limber Pine (*Pinus flexilis*) in Rocky Mountain National Park

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Abstract—Five-needle pine management in the face of climate change is challenging because tree disease may exacerbate climate impacts in complex ways. Here, using climate-based ecological niche models that forecast species' potential distributions through 2100, plus near-term estimates of tree mortality from the U.S. Department of Agriculture, Forest Service 2013–2027 National Insect and Disease Risk Assessment, we quantify the potential impact of climate and disease on limber pine (*Pinus flexilis*) in Rocky Mountain National Park (Colorado, USA). Niche models trained and projected under a wide range of conditions and uncertainties all predict the distribution of limber pine in the park to move upslope in elevation, resulting in a patchwork of predicted range stability, loss, and colonization under future climate change. Disease risk projections further identify areas within the park where limber pine is expected to suffer declines in basal area, which, depending on the magnitude, may feed back into the niche models to either encourage persistence or accelerate loss. We rank the combined impact of climate and disease on limber pine throughout the park and explore how our results may be used to assess vulnerability and develop management scenarios.

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Projecting Future Whitebark Pine (*Pinus albicaulis*) Bioclimatic Envelope Distributions Within the Greater Yellowstone Ecosystem Under CMIP5 General Circulation Models for the Next Century

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Abstract—Projected climate change at a regional level is expected to shift vegetation habitat distributions over the next century. For the species whitebark pine (*Pinus albicaulis*), warming temperatures may result in loss of suitable bioclimatic habitat, reducing its distribution within its historical range. This research focuses on understanding the patterns of spatiotemporal variability for future projected *P. albicaulis* suitable habitat in the Greater Yellowstone Ecosystem (GYE) through a bioclimatic envelope approach. Because intermodal variability from general circulation models (GCMs) leads to differing predictions about the magnitude and direction of modeled suitable habitat area, the Coupled Model Intercomparison Project Phase 5 (CMIP5) used nine bias-corrected statistically downscaled GCMs to understand the uncertainty associated with modeled projections. Modeled *P. albicaulis* for the 1980–2010 climate showed strong presence/absence separations by summer maximum temperatures and springtime snowpack. Patterns of projected habitat change by the end of the century suggested a constant decrease in suitable climate area from the 2010 baseline for both representative concentration pathways (RCPs) 4.5 and 8.5 climate forcing scenarios. Percent suitable climate area estimates ranged from 29 to 2 percent and 10 to 0.04 percent by 2099 for RCP 4.5 and 8.5, respectively. Habitat projections between GCMs displayed a decrease of variability over the 2010–2099 time period related to consistent warming above the 1910–2010 temperature normal after 2070 for all GCMs. A decreasing pattern of projected suitable *P. albicaulis* habitat area was consistent across GCMs, despite strong differences in magnitude. Future ecological research in species distribution modeling should consider a full suite of GCM projections in the analysis to better explore range contractions/expansions generated by species niche modeling. The results suggest that restoration strategies such as planting of seedlings and controlling competing vegetation may be necessary to maintain *P. albicaulis* in the GYE under the more extreme future climate scenarios.

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Associations between Complete Resistance to White Pine Blister Rust and Abiotic Stress Tolerances in Limber Pine (*Pinus flexilis*)

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During the naturalization process of white pine blister rust (WPBR, caused by *Cronartium ribicola*), susceptible pine genotypes will be selected against, along with any traits that are associated with those genotypes, potentially causing a shift in the suite of traits in the post-selection population compared to the native population. Consequently, identifying associated traits with WPBR resistance is critical for effective restoration planning, especially in a changing climate. We sought to determine whether complete resistance to WPBR in limber pine (*Pinus flexilis*) is associated with abiotic stress tolerances, particularly those related to freezing and drought. Previous research has noted that proteins involved in partial resistance to *C. ribicola* also exhibit antifreeze capabilities in vitro and very likely enhance plant frost tolerance in vivo; these proteins are also expressed in response to cold and, occasionally, drought. It is not known if a similar association exists between the major gene in limber pine (*Cr4*) and tolerance of abiotic stress.

We raised 1,020 seedlings of limber pine from 10 rust-resistant (R) and 10 rust-susceptible (S) open-pollinated families (Schoettle et al. 2014), paired as 1 R and 1 S family from each site of origin to filter out potential geographic trait variation. In a series of studies, R families exhibited greater frost tolerance than S families, which may imply a genetic association between *Cr4* and cold tolerance gene(s) or a direct cryoprotective function of the *Cr4* protein product(s) itself. Wintertime freezing tolerance of R and S families was not affected by inoculation with *C. ribicola* the previous fall. This result suggests that differences in frost tolerance between R and S families were not induced by pathogen infection or resistance expression and instead frost tolerance is a constitutive trait of families (Vogan and Schoettle 2015).

We found a tendency for S family seedlings to retain somewhat greater stomatal conductance under modest drought than R families and no significant differences under severe (<–2.0 MPa) drought (Vogan and Schoettle 2015). Drought during the summer resulted in marginally, but not significantly, greater frost tolerance in seedlings the following winter, suggesting that mechanisms induced by drought may have enhanced cold hardiness in the ensuing months. The correlation between cold tolerance and rust resistance may provide an explanation of how *Cr4* resistance exists and persists in limber pine before the arrival of the corresponding pathogen as cold tolerance traits are usually under strong selection in forest trees.

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Quantifying Tree Species' Sustainability from Forest Inventory Data: Current Trends in the Pacific Northwestern United States

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Abstract—The task of guiding forests toward a sustainable future is perhaps one of the greatest challenges facing land managers today. Significant shifts in forest location and composition are predicted to increase, and trees are long-lived and sessile organisms so management decisions made now must confront uncertain environments decades from now. Economically important tree species are predicted to decline, disappear, or migrate. How do we make the most-strategic decisions now to ensure long-term sustainability of our forests? Characterization of current trends occurring in forest inventories is a key ingredient in forest management for an uncertain future. Nature is complex, and prediction from models of species distributions have uncertainty. What is occurring now may not necessarily be what models would otherwise predict. Here, we present an index that can quantify the sustainability of tree populations and meta-populations from forest inventory data to inform scientists, managers, and policy-makers and provide corroboration for predictions. We use the index to discover current trends for tree species in the Pacific Northwestern United States. We derive the index from data provided by the U.S. Department of Agriculture, Forest Service's Continuous Vegetation Survey and Forest Inventory and Analysis program. We examined only fairly commonly occurring species with a minimum of 50 occurrences in the plots. We find that current stands of *Juniperus occidentalis*, *Abies procera*, *Thuja plicata*, *Tsuga heterophylla*, *Pinus ponderosa*, and *Pseudotsuga menziesii* are on sustainable trajectories. Conversely, we find that *Pinus monticola*, *Arbutus menziesii*, *Pinus albicaulis*, *Abies lasiocarpa*, *Taxus brevifolia*, *Pinus contorta*, *Abies grandis*, *Picea englemanii*, and *Larix occidentalis* are not on sustainable trajectories. We quantify these findings by blending change in demography and basal area. We argue that our metric is simple, can help track the status of our forest resources in a changing climate, and provides information that agrees with what foresters and pathologists are noticing on the ground.

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Whitebark Pine (*Pinus albicaulis*) Growth Release Potential Following Overstory Disturbance

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Much of the information available on successional modeling for whitebark pine (*Pinus albicaulis*) communities is based on research conducted in an ecosystem influenced by maritime conditions (Pacific Northwest). The Greater Yellowstone ecosystem (GYE; Wyoming, Montana, and Idaho, USA) is subjected to more continental climatic conditions and as a result, the dynamics of whitebark pine populations in the GYE may in fact be very different from those of maritime populations. Recent survey work in the GYE shows establishment is common with variable recruitment of young trees in the understory of mature forests. Density and age distribution of these individuals vary greatly among stands across the ecosystem. By determining historical seedling and sapling growth rates and their relation to successional disturbances such as fire, bark beetles (*Dendroctonus* spp.), and disease, we will be better equipped to guide restoration efforts throughout the GYE and to ensure that management decisions are based on scientific information collected from the ecosystem in which restoration efforts are actually occurring.

Evidence for the occurrence of a growth release phenomenon in trees has been documented for several shade-tolerant and partially intolerant species following the creation of gaps in the forest canopy due to a variety of ecological impacts. By using ring width patterns taken from whitebark pine individuals, this project examines evidence for growth suppression and a hypothetical growth release event in whitebark pine understory individuals located in stands where canopy composition changed due to the mountain pine beetle (*D. ponderosae*) outbreak of the late 1970s to early 1980s in the Gallatin Range of the Gallatin National Forest (Montana). In some areas, natural regeneration of whitebark pine is clearly the optimal form of whitebark pine restoration following widespread mortality. To fully evaluate a stand for mechanical restoration, it will be important to gain an understanding of historical patterns in growth following major canopy disturbance as well as the current demographic structure of understory populations and the relationship between seedling/sapling age and recruitment potential into the canopy.

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White Pine Blister Rust Alters Facilitation Interactions at Treeline: Implications for Treeline Communities and Response to Climate Change

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In some Rocky Mountain treeline ecotones, whitebark pine (*Pinus albicaulis*) serves as a tree island initiator more frequently than Engelmann spruce (*Picea engelmannii*) or subalpine fir (*Abies lasiocarpa*). Tree island initiation begins with a solitary krummholz tree, which provides sufficient leeward protection so that another tree may become established, resulting in a tree island. More trees may establish on the lee side over time. Whitebark pine mortality from white pine blister rust (pathogen: *Cronartium ribicola*) reduces opportunities for such facilitation. Will declines in whitebark pine alter treeline response to climate warming? We have surveyed various treeline communities from the Greater Yellowstone area (Wyoming, Montana, and Idaho, USA) to whitebark pine's northern limit.

From 2010 to 2013 at Line Creek, Montana, and Divide Mountain, Montana, we examined facilitation mechanisms by simulating whitebark pine death from blister rust, measuring tree shoot lengths, and evaluating performance of common treeline leeward microsites with respect to seed germination, seedling survival, and 11 biophysical variables. In both study areas, plus Parker Ridge, Alberta (Canada), we sampled 80 randomized 15-m × 15-m vegetation plots to characterize community composition and structure with respect to local climate, hydrology, digital elevation models, and incidence of blister rust. Using predictive modeling, we examined possible climate and topographic correlates of whitebark as tree island initiator both within and across study areas, and used agent-based modeling (ABM) to examine response to climate change in the presence of blister rust.

At Line Creek and Divide Mountain, blister rust incidence was 19.2 and 23.6 percent, respectively. Among solitary conifers, whitebark pine was most abundant and had the greatest vigor and longest shoot lengths. Death of windward whitebark pine reduced shoot length and vigor in leeward trees, demonstrating facilitation.

In planting experiments, new seedlings had higher survival on whitebark microsites. Microsites leeward of whitebark pine and spruce, relative to rocks or exposed sites, had lower photosynthetically active radiation, sky exposure, and wind speeds, and more moderate soil and air temperatures. Thus, whitebark pine prevalence as a tree island initiator results from its hardiness, relative abundance, and the protective qualities of its leeward microsites. Modeling indicates that growing season temperature and slope curvature may predict the incidence of whitebark pine facilitation at local scales. In certain treeline ecotones, whitebark pine is abundant, but facilitation is less prevalent, very likely because of a more moderate climate. The use of ABM combining climate warming and blister rust indicated that the latter slowed the overall advance of trees while slightly favoring spruce and fir in relative abundance. Our combined studies suggest that whitebark pine tolerates the harsh treeline climate better than associated conifers, and losses to blister rust will affect treeline response to climate warming.

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Whitebark Pine (*Pinus albicaulis*) Direct Seeding Trials in the Northern Rocky Mountains: The Role of Planting Site and Cache Pilferage by Rodents

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Direct seeding is an emerging technique that could significantly reduce the labor and costs associated with whitebark pine (*Pinus albicaulis*) restoration, and may be compatible with wilderness values. Because other restoration methods—including outplanting of seedlings—are largely incompatible with maintaining wilderness as "untrammeled and natural," the adoption of direct seeding may open an additional 48 percent of whitebark pine habitat to restoration efforts in the future.

We conducted a large direct seeding experiment on White Calf Mountain, Glacier National Park, Montana (USA) and Tibbs Butte, Shoshone National Forest, Wyoming (USA) to determine: (1) germination and first-year survival rates of simulated caches and how rates vary between subalpine forest and the treeline ecotone, (2) which planting site characteristics are important for germination and survival, and (3) rates of cache pilferage by small rodents (Order Rodentiae). In 2012, we created simulated Clark's nutcracker (*Nucifraga columbiana*) caches near three different microsite types (trees, rocks, or no object) at randomly selected locations using randomly generated cache sizes (numbers of seeds per cache, range = 1 to 7). Caches were revisited in 2013 and 2014 to assess germination, seedling survival, and seed loss. In 2013, 31.9 percent of 717 relocated caches germinated (25.6 percent on White Calf Mountain, 38.0 percent on Tibbs Butte). Odds of germination were 1.78 (95-percent confidence interval [C.I.] = 1.29–2.44) times higher on Tibbs Butte than on White Calf Mountain. On Tibbs Butte, odds of germination were 1.71 (95-percent C.I. = 1.05–2.78) times higher at treeline than in the subalpine zone, and odds were increased for caches created near rocks or no object when compared to caches created near trees. About 54 percent of the caches created on both White Calf Mountain and Tibbs Butte were pilfered. At both study areas in the treeline zone, 62.1 percent of caches were pilfered; in the subalpine zone, 46.1 percent were pilfered. The estimated odds of pilferage at treeline were 3.94 (95 percent C.I. = 1.42–2.57) times the odds of pilferage at the subalpine sites. In 2014, 21.3 percent and 4.6 percent of the remaining caches germinated on Tibbs Butte and White Calf Mountain, respectively, for a total of 59.3 percent and 30.2 percent of caches created in 2012 germinating after 2 years.

Survival rates of seedlings germinated in 2013 were 66.9 and 35.6 percent on Tibbs Butte and White Calf Mountain, respectively. On Tibbs Butte, germination seems to be favored near rocks and in the open, and further investigation is needed to determine whether these findings are consistent throughout the range of whitebark pine. Because germination/survival rates exceeding 10 percent are considered successful for direct seeding efforts, observed germination rates suggest that large-scale direct seeding efforts should be considered for restoration. However, determining the regional drivers of successful germination and survival (climate, soils, seed source, and others) is necessary to ensure a full understanding of the processes required for germination and establishment to achieve the greatest success of direct seeding efforts.

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White Pine Blister Rust, Mountain Pine Beetles (*Dendroctonus ponderosae*), and Their Potential Impact on White Pines: A National Assessment From the National Insect and Disease Risk Map

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Abstract—The 2012 National Insect and Disease Risk Map (NIDRM) contains a nationwide strategic assessment of the hazard of tree mortality due to insects and diseases, displayed as a series of maps. The NIDRM is more than just maps: it is a nationwide, science-based, administrative planning tool resulting from the United States' forest health community working together to determine the severity and extent of potential tree mortality. The NIDRM's modeling process provides a consistent process through which interactive spatial and temporal hazard assessments can be conducted. The NIDRM serves as the U.S. Department of Agriculture, Forest Service (USDA FS), Forest Health Protection program's comprehensive analysis of pest hazards, and is referred to in Federal legislation regarding watershed restoration funding to the States. Five-needle pines, and their primary disturbance agents—white pine blister rust (causal agent: *Cronartium ribicola*) and mountain pine beetles (*Dendroctonus ponderosae*)—are among the NIDRM's interactions, which show a potential for catastrophic host impacts over the next 15 years.

This presentation will explain the design and construction of the NIDRM system, and the results for white pine ecosystems. These impacts are displayed in map and tabular formats, at various geographic scales and for different administrative units, for both the USDA FS and the U.S. Department of the Interior, National Park Service. The presentation will also explain the data layers used in the analysis, available for downloading, and other website resources where additional information and results can be found (https://www.fs.fed.us/foresthealth/applied-sciences/mapping-reporting/gis-spatial-analysis/national-risk-maps.shtml).

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Survey of *Pinus dalatensis* for Gene Conservation in Vietnam

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Abstract—*Pinus dalatensis* is a conifer species of conservation importance in Vietnam. Eighteen 2,500-m^2 plots were established in mixed-species forests at different elevations in Vietnam to evaluate the occurrence of *P. dalatensis*, site conditions, and forest structure; to identify primary associated species; to study the morphology of pine samples; and to collect *P. dalatensis* cones for ex situ gene conservation studies. Mature *P. dalatensis* trees were found at elevations between 1,070 and 1,900 m. The survey also showed that the number of *P. dalatensis* individuals was low, but it was the species in the upper canopy, with importance value ranging from 5.6 to 47.4 percent. The principal associated species were *Craibiodendron syellatum*, *Podocarpus imbricatus*, *Dacrydium elatum*, *Hellicia cochinchinensis*, *Symplocos poilanei*, and *Quercus langbianensis*. The N/D distribution of *P. dalatensis* forest on most plots was described by Mayer or Weibull distributions and suggested that the forests had been selectively thinned. There was no regeneration of *P. dalatensis* in the observed forests. Five-year survival and growth results in ex situ locations where seeds were planted from cones collected in the original survey suggest that *P. dalatensis* gene conservation areas should be established in forest gaps. Plantations of *P. dalatensis* could be successful, but the trees are expected to grow very slowly. Seedbanks could be an alternative method of gene conservation of *P. dalatensis*.

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Pinus strobiformis Gene Conservation and Genecology

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INTRODUCTION

Southwestern white pine (*Pinus strobiformis*, syn. *Pinus flexilis* var. *reflexa*) is a high elevation five-needle pine. *Pinus strobiformis* occurs in mixed species stands across a range of elevations and forest types in western Texas, southwestern Colorado, and throughout New Mexico and Arizona (USA), and continues along the mountains south to central Mexico (Alexander et al. 1984; Looney and Waring 2013; Moreno-Letelier and Piñero 2011; Steinhoff and Andresen 1971) (fig. 1). This species, like all species of North American five-needle pines, is susceptible to infection and mortality from the nonnative, invasive forest pathogen (*Cronartium ribicola*) that causes the disease white pine blister rust (WPBR) (Kinloch and Dupper 2002; Sniezko et al. 2011). White pine blister rust was first reported in natural stands of *P. strobiformis* in 1990 (Hawksworth 1990), and is spreading through the range in New Mexico and Arizona (Conklin 2004; Conklin et al. 2009; Fairweather and Geils 2011; Looney and Waring 2012). Across the area where WPBR was first discovered, about 40,500 ha have high WPBR incidence and trees with high severity infections including frequent “topkill” (a condition in which needles, branches, and stem tissue above the canker location are killed) (Conklin et al. 2009).

According to available species climate profile predictions, the suitable range of *P. strobiformis* is expected to decrease under most future climate change scenarios (Crookston and Rehfeldt 2008). Predictions of future WPBR distributions are not as well understood because of difficulty in modeling pathogen/host tree interactions (Sturrock et al. 2011), but high hazard areas exist in the Southwest (Conklin 2004; Geils et al. 1999).

The impacts of WPBR on five-needle pine species in northern Rocky Mountain and Pacific Northwest ecosystems illustrate the disastrous effects of the disease on susceptible hosts on high hazard sites (Fins et al. 2002; Tomback and Achuff 2010). Cascading negative effects of forest recovery and ecosystem services in these areas should be considered a preview of what could occur in some southwestern ecosystems with no intervention (Schoettle and Sniezko 2007; Tomback and Achuff 2010). Negative effects may be exacerbated both through reductions of *P. strobiformis* genetic diversity with declining suitable habitat and through local population extirpations from recent large, high-severity fires across the Southwest (fig. 1).

Intervention in this case refers to the use of proactive restoration, where silvicultural practices can be used to promote natural (stimulated) or artificial (planted) regeneration with some resistance to WPBR (Keane and Schoettle 2011; Schoettle and Sniezko 2007). Our program to sustain *P. strobiformis* in southwestern

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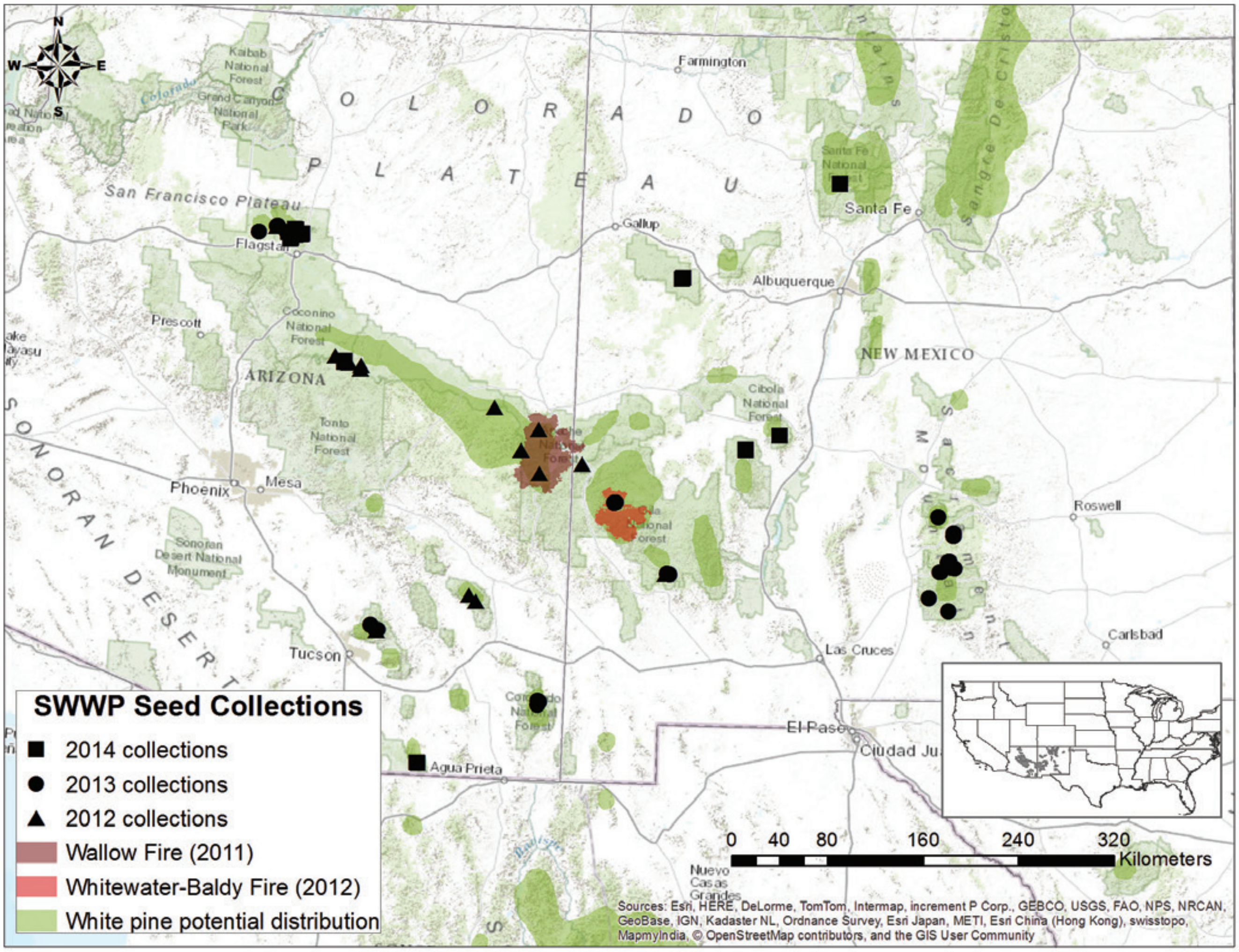


Figure 1—*Pinus strobiformis* (southwestern white pine, SWWP) gene conservation seed collection sites across USDA FS Region 3; collections from 2012–2014. Recent large fires in collection areas / *P. strobiformis* habitat included.

mixed-conifer forests includes gene conservation and studies of genecology and population genetics. Some of our other research directions not addressed here are regeneration ecology, silvics, sustainable management, and prediction of the future distribution of the species using landscape genetics and climate change modeling. Understanding ecophysiological and adaptive processes of this species will help inform genetic and silvicultural approaches to decrease forest tree vulnerability and increase adaptive capacities in the face of disturbances and a changing climate (Bower et al. 2011; Chmura et al. 2011).

GENE CONSERVATION

Our seed collections throughout the range of *P. strobiformis* in the Southwest began in 2012. Although our primary goal was to archive seed for the U.S. Department of Agriculture, Forest Service (USDA FS) Gene Conservation program (Mangold 2011), we have collected enough additional seed to support research and management projects. Sites were identified using the known range of *P. strobiformis* in Arizona and New Mexico in conjunction with information about local cone crop production in a given year. At each site, we collected cones from up to five trees separated by a minimum distance of 200 ft (61 m) and recorded major overstory and understory species, presence of *Ribes* shrubs (one of the key secondary hosts for WPBR), slope, and aspect (see Schoettle et al. 2011). Each collection tree was georeferenced and tagged at breast height (1.37 m) for relocation purposes; we also recorded total tree height, live crown proportion, diameter at breast height, presence and severity of WPBR

Table 1—*Pinus strobiformis* seed collection sites and year of collection(s) across National Forests in the Southwest. Collections from Indian Reservations not shown.

Collection year(s)	Location	District	State	Gene conservation sites
2012-2014	Coconino National Forest	Peaks	AZ	7
2012	Coconino National Forest	Mogollon Rim	AZ	2
2012, 2014	Apache-Sitgreaves National Forests	Black Mesa	AZ	3
2012	Apache-Sitgreaves National Forests	Springerville	AZ	1
2012	Apache-Sitgreaves National Forests	Alpine	AZ	1
2012	Apache-Sitgreaves National Forests	Lakeside	AZ	1
2012	Coronado National Forest	Safford	AZ	2
2013	Coronado National Forest	Santa Catalina	AZ	4
2014	Coronado National Forest	Sierra Vista	AZ	1
2013	Kaibab National Forest	Williams	AZ	1
2013	Gila National Forest	Reserve	NM	1
2014	Santa Fe National Forest	Jemez	NM	1
2013	Lincoln National Forest	Sacramento	NM	3
2014	Cibola National Forest	Magdalena	NM	2
2012, 2014	Cibola National Forest	Mt. Taylor	NM	2

infection, and insect infestation levels in cones and seeds for each collection tree. In areas where WPBR was present, we attempted to collect seeds from infection-free trees (putatively resistant), but seeds were also collected from trees with visible WPBR infections. To date, we have collected cones and seeds across much of the range of *P. strobiformis* in Arizona and New Mexico and along local elevational gradients where possible (fig. 1). Our seed collections for gene conservation represent eight national forests and two Indian Reservations (table 1). These collections consist of 111 trees (26 populations archived in the Gene Conservation program) from 2012 and 2013 and 59 trees (12 archived populations) from 2014. These are in addition to previous collections of 88 families and 3 bulk populations of *P. strobiformis* in USDA FS Region 3 (southwestern United States; Sniezko et al. 2011).

GENECOLOGY RESEARCH

To properly manage *P. strobiformis* in the face of climate change and WPBR, adaptive traits and responses to stressors must be quantified from populations across the range; these are considered key objectives in successful restoration efforts in other species of threatened five-needle pines (Bower et al. 2011; Hamlin et al. 2011). There is relatively little information about population differentiation of quantitative traits in *P. strobiformis* (but see Steinhoff and Andresen 1971). With known family and population variation in quantitative traits such as phenology, morphology, and drought tolerance, we can infer adaptation to geographic or climatic gradients, quantify heritability, and reduce risk of seedling maladaptation in seed transfer and restoration efforts (Bower and Aitken 2008; Bower et al. 2011). Because outplanting artificial regeneration with natural genetic resistance is a realistic option for managers, the risk of seedling maladaptation to new sites must be quantified with common garden studies (Bower and Aitken 2008; Keane and Schoettle 2011; Schoettle and Sniezko 2007).

We began a greenhouse common garden study in Flagstaff, Arizona, in spring 2012 with the specific objectives to determine family, population, and geographic variation in quantitative traits (growth, phenology, and the tolerance to low water treatments) of *P. strobiformis*. We used seed from our collections, as well as donated seed from previous collections (Region 3 Forest Health Protection 1997–2008; Conklin et al. 2009; Sniezko et al. 2011), in a design

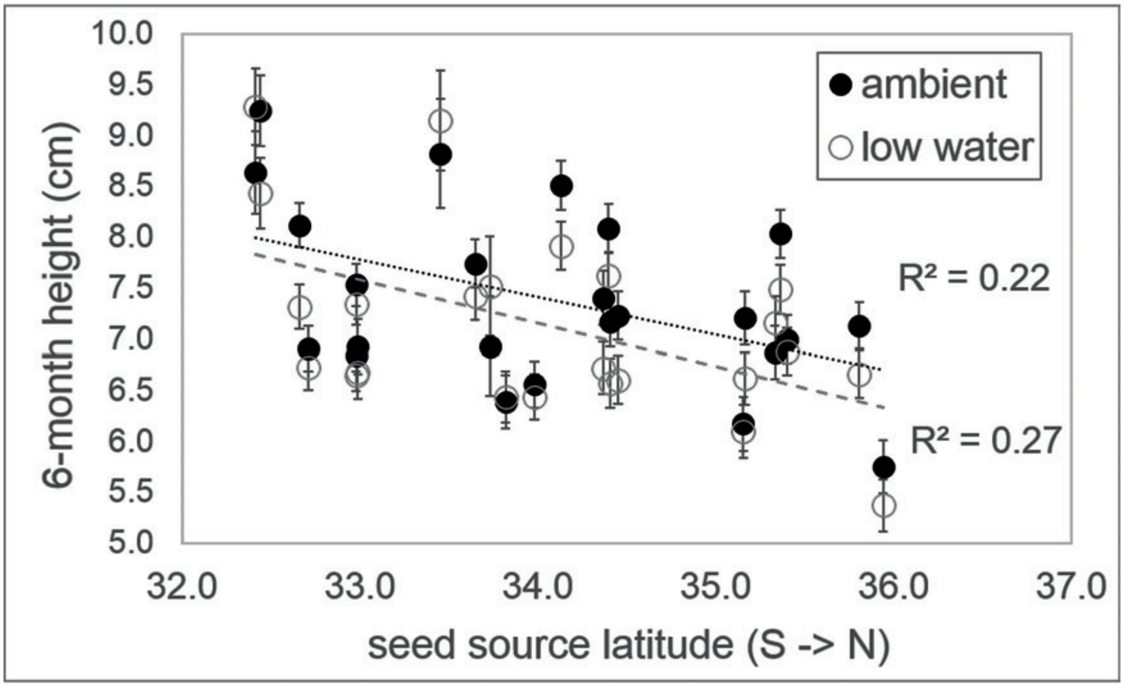


Figure 2—Relationship between 6-month-old seedling height means (± 1 standard error) and seed source latitude across two watering treatments in a greenhouse common garden study in Flagstaff, Arizona (ambient and low water). N = 23 populations, 2–15 families per populations, 18–36 seedlings per family.

that included 2 to 15 families (half or full-sibling offspring from a single mother tree) per population across 24 populations of *P. strobiformis*. Families were represented by 18 to 36 seedlings per family dependent upon germination rates, and half the seedlings from each family were subjected to low water treatments over 2 years. Growth, phenology, and physiological responses of all families, populations, and watering treatments were measured over two growing seasons. We hypothesized that (1) growth, phenology, and drought tolerance variation would be small within populations but large between populations because of the isolated, disjunct nature of *P. strobiformis* populations; (2) population seedling growth and phenology would parallel seed source climate (e.g., seedlings from source climates with fewer frost-free days would end growth earlier; seedlings from warmer source climates would grow larger); and (3) growth and survival under drought conditions would also parallel seed source climate.

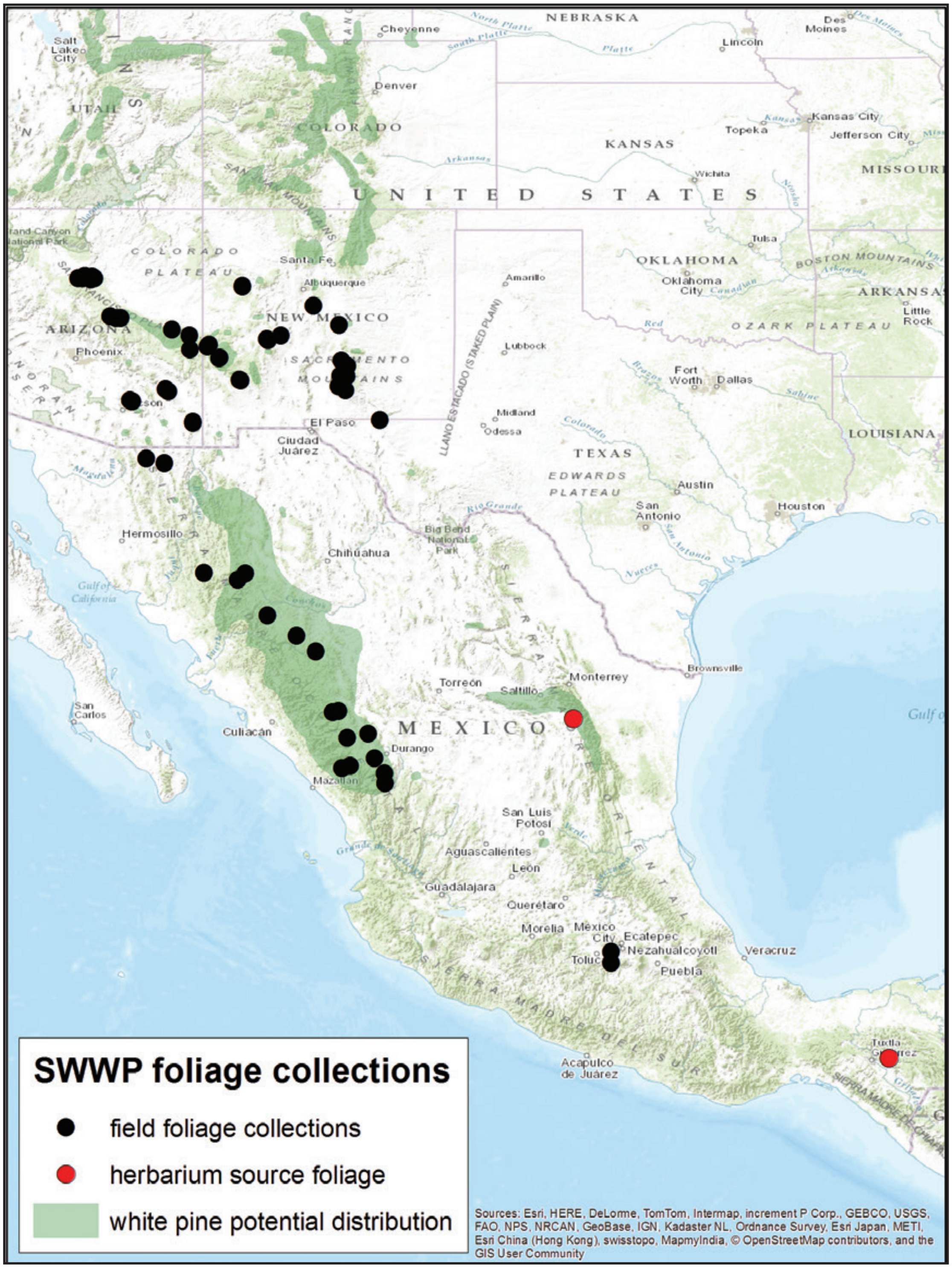
Preliminary results indicated that growth trait population means such as 6-month height, number of branches (potential allocation to growth processes other than height), and growth initiation (phases of bud and leaf development) were well differentiated and appeared to correlate weakly with seed source geographic and climatic clines (fig. 2, R^2 = 0.22 and 0.27 for ambient and low water seedlings, respectively), indicating local adaptation of populations for these traits, which will be evaluated with reciprocal transplant gardens in the future. Overall, populations from southern sources grew taller than populations from northern sources (fig. 2). These patterns continued in the second growing season; population means of 14-month-old seedling heights were also weakly correlated with seed source latitude and southern populations grew larger than northern populations (data not shown; R^2 = 0.23 and 0.21 for ambient and low water treatments, respectively). Latitude was a stronger correlate than elevation for all growth traits measured in the first season. No genotype-by-environment effects were observed in the first season for any measured trait and analyses are ongoing. Relationships between quantitative traits and climate variables are also being investigated.

ONGOING AND FUTURE RESEARCH DIRECTIONS

We are expanding the initial common garden from the greenhouse to the Southwest Experimental Garden Array (SEGA), a series of common gardens across an elevation gradient in northern Arizona. The first trial garden was planted in fall 2014 with 170 families from 44 populations (fig. 3). Many of these families are also included in disease resistance screening trials at the USDA FS Dorena Genetic Resource Center (DGRC, Cottage Grove, Oregon, USA) in a project funded by the USDA FS Forest Health Protection Special Technology Development Program (R3-2014-02). There are 39 populations of *P. strobiformis* represented by 154 families growing at DGRC slated to be inoculated in fall 2015 for long-term testing of partial resistance mechanisms to *C. ribicola*. A small proportion of these populations were inoculated in fall 2014 for shorter term major gene resistance tests. We are developing two outplanting sites on national forest and tribal land in Arizona and New Mexico to test the long-term durability of resistant seedlings in the field, and families to be included in the outplanting will be chosen following early results from resistance trials.

We are also investigating population genetics across the entire range of *P. strobiformis*, beginning with 70 populations across the southwestern United States and Mexico. In Mexico, foliage was collected from 19 populations and 169 individuals in the Sierra Madre Occidental and Trans Mexican Volcanic Belt in May 2014, with additional foliage obtained from the botanical collection at the Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional (CIIDIR, Interdisciplinary Research Center for Integrated Regional Development), Durango (fig. 4).

Figure 3—Initial common garden planting at The Arboretum, Flagstaff, Arizona. Site is part of the Southwest Experimental Garden Array. Six-month-old seedlings were planted in early October 2014.



Sources: Esri, HERE, DeLorme, TomTom, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community

Figure 4—*Pinus strobiformis* foliar collection sites across the Southwest United States (Region 3) and northern Mexico for use in restriction-site associated DNA sequencing (RADSeq) to identify single nucleotide polymorphisms across the genome.

Collections included one population of *P. strobiformis* var. *stylesii* from Sierra Madre Oriental and one population of *P. ayacahuite* from Sierra Madre del Sur. We also collected foliage from 49 populations across Arizona and New Mexico and from one *P. flexilis* (limber pine) population in southern Wyoming (fig. 4; Wyoming population not included in map). *Pinus flexilis*, *P. strobiformis* var. *stylesii*, and *P. ayachuite* populations were included to distinguish between closely related species and any hybridization that may occur.

Restriction-site associated DNA sequencing will be used to identify single nucleotide polymorphisms across the genome. We are conducting studies to understand the dynamics of gene flow, population structure, and hybridization to better understand the temporal and geographic severity of WPBR and climate change on *P. strobiformis*. Pines are well known for their long-distance gene flow and lack of incompatibility systems resulting in hybridization. It is therefore necessary to quantify the flow of genes related to disease resistance, drought tolerance, and other potentially adaptive traits across the entire range of *P. strobiformis* and hybrid zones with closely related species.

Future research plans will expand our seed collections across the range of the species (including more sites throughout Mexico), add three SEGA common gardens to the current experimental design, and add families and populations to disease resistance screening trials. Other ongoing and future objectives include determining linkages between adaptive traits and resistance frequencies, identifying the genetic bases for adaptive traits, and providing tools to better predict the potential distribution of *P. strobiformis* under future climate change. Our *P. strobiformis* research program combines basic and applied research using cross-collaborative efforts to sustain and conserve an important tree species in the southwestern United States and Mexico. Conserving the genetic diversity and defining management strategies to sustain *P. strobiformis* are critical in a changing climate and with the threat of WPBR. We envision a sustainable future for *P. strobiformis* with continued proactive and adaptive research and management.

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

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

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INTRODUCTION

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

Stomata mediate the exchange of oxygen, water vapor, and carbon dioxide (CO_2) with the atmosphere. Control of transpiration allows plants to photosynthesize in a wide range of changing environments, and was most likely a driving force behind the ecological diversification of plants (Hetherington and Woodward 2003). Variations in stomate size and density are generally correlated with environmental variables such as atmospheric CO_2 concentration, as well as geographic variables such as elevation (Holland and Richardson 2009; Schoettle and Rochelle 2000). The size and density of stomata influence the rate of conductance of carbon to sites of assimilation within the leaf and regulate many other ecophysiological functions of leaf surfaces (Franks and Beerling 2009). Stomate morphology may also play a significant role in interactions with pathogens and pests. Natural openings in leaf surfaces such as stomata are important entry points for many fungal pathogens, including *C. ribicola* (Patton and Johnson 1970). Consequently, plants have evolved to regulate the stomate opening as an important part of a general response to microbial invasion (Gudesblat et al. 2009; Zeng et al. 2010).

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

METHODS

Sample Selection and Inoculations

A subset of 30 families was selected from a common garden inoculation study examining resistance to white pine blister rust in 225 seedling families from much of the range of WBP. Parent trees of the seedlings examined here represent eight seed zones from throughout Oregon and Washington as well as individual populations from Idaho, Montana, California, Nevada, and British Columbia (fig. 1). The seeds were sown in 2007 (see Hamlin et al. 2011 for background on seedling culture) in 10-in^3 (164-cm^3) containers, which were then placed into a randomized complete block (RCB) design before inoculation with *C. ribicola*. The seedlings were inoculated in 2008 after their second growing season. Three blocks were inoculated in early September, and the other three blocks in early October; different geographic sources of rust were used for each inoculation. Infected *Ribes* leaves were placed on wire screens atop the WBP seedlings to allow basidiospore release. Basidiospore discharge was monitored on trap slides to ensure that all trees received similar inoculum exposures. Average spore densities were 3,359 and 6,013 cm^{-2} for the first and second inoculations, respectively. Seedlings from all 225 families were transplanted in October 2008 to wooden boxes (0.9 m × 1.2 m × 0.3 m) in the same RCB design with 12 families per box, and 20 boxes per block. Families were in row plots (up to 10 seedlings per family row plot).

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

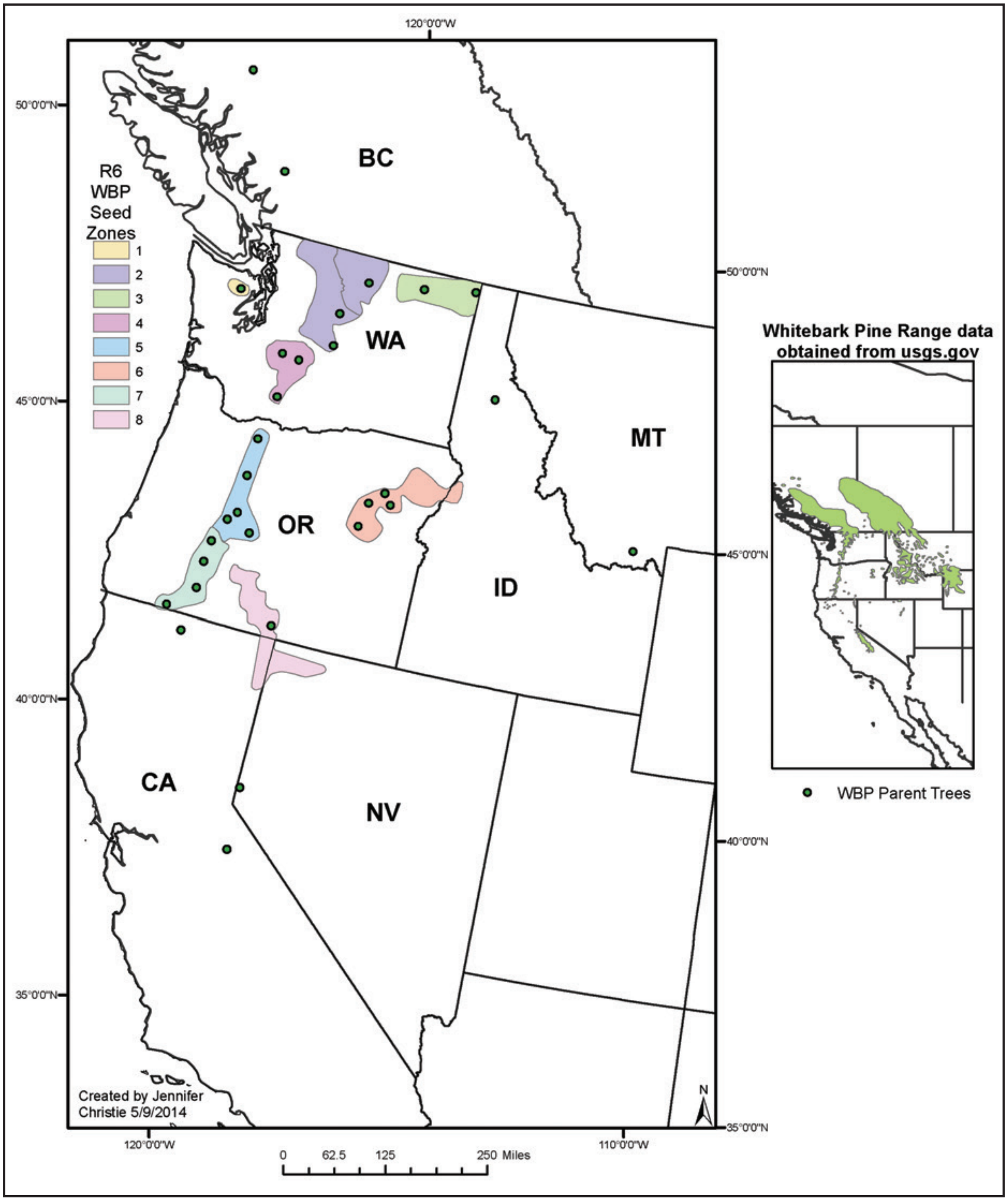

Figure 1—Map of parent tree locations and seed zone designations used in this study.

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

Needle Measurements and Statistical Analyses

Needles were attached to 3-inch × 5-inch (7.6-cm × 12.7-cm) index cards with double-sided tape and photographed with a Dino-Lite digital USB camera (AnMo Electronics Corp., Taipei, Taiwan). Needle dimensions and stomate densities were measured for 300 sampled seedlings (30 families × 10 seedlings per family) for both the 2008 and 2009 needle cohorts. For each seedling, four photographs were taken from both the abaxial (AB) and adaxial (AD) needle surfaces (eight photos total) at a magnification of 200× (fig. 2a). The abaxial side is the rounded outer surface of a needle, and it typically has two resin channels running alongside the stomate rows (fig. 2b). An individual needle has two adaxial surfaces; these are the inner sides of a needle (fig. 2b). Different needles were photographed for the AB and AD sides, and two to four needles were used for each seedling to obtain the photographs. An approximately 2-mm length of needle was used for measurements; the 2-mm segments were randomly chosen from each needle surface for quantification of stomata and rows of stomata (fig. 2). Needle width was measured in magnified images using the line measure tool in the Dino-Lite software (fig. 2a). Individual seedling means were calculated by averaging these measures for each seedling, and analyses of variance (ANOVAs) and linear regressions of needle attributes were performed on these means (accounting for block differences) using R (R Core Team 2016). Family means were calculated from the mean of block means, and pairwise correlations were performed by using these means and SAS® software (version 9.4, SAS Institute Inc., Cary, North Carolina) with the CORR procedure.

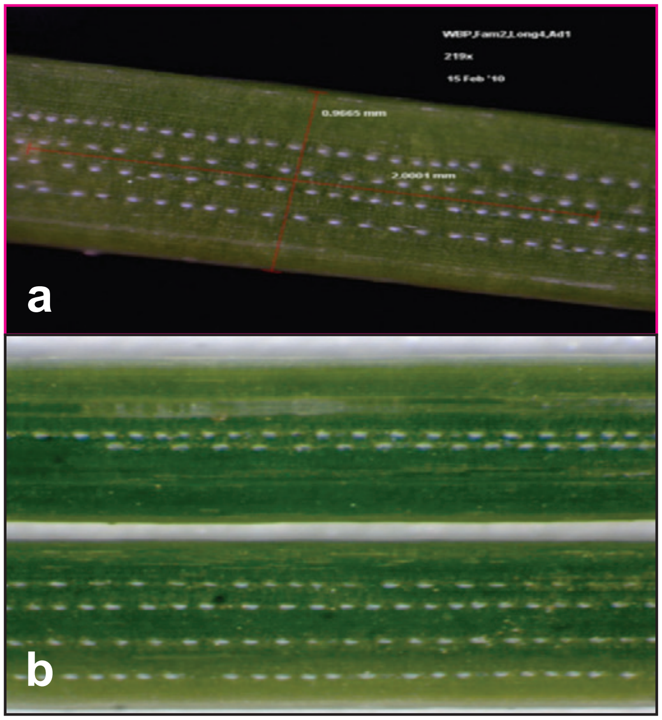

Figure 2—Magnified images of leaf surfaces: (a) width and length measurements shown on photograph of adaxial leaf surface; (b) abaxial (top) and adaxial (bottom) leaf surfaces.

A sequence of analyses was conducted to explore if stomate features mediate the influence of source climate of the seed (maternal) parent on early post-infection rust symptom development. Potential significant predictors and groups of intercorrelated predictors were explored by using 10-fold cross validation techniques with lasso and elasticnet (Friedman et al. 2008) in the glmnet package (Friedman et al. 2010) in R (R Core Team 2016). By using these penalized regression techniques, three sets of family mean stomate data (variables averaged across 2008 and 2009 needles, and those data for each year singly) were independently analyzed. Each dataset was analyzed in a two-part sequence to (1) identify stomate variables that significantly predicted disease development, and (2) identify seed source climate variables that explained variation in the stomate variables identified in step 1. Each step generated linear models to predict disease response from stomate traits and predict stomate traits from source climatic variables. Normalized (1961–1990) means of derived climate variables (table S1) were estimated for each source location by thin plate spline surfaces (Rehfeldt 2006).

RESULTS

Family (Geographic) Variation in Needle Morphologies

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

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

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

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

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

Family mean number of rows of stomata (nROWS) varied from 2.31 to 3.48, and from 1.31 to 2.14 on the AD and AB surfaces, respectively, in the 2008 cohort; family mean nROWS for the 2009 cohort ranged from 2.98 to 3.80 and 1.73 to 2.78 for the AD and AB surfaces, respectively (table 1); there were 1.60 to 1.69 times as many nROWS on the AD side relative to the AB side for the 2009 and 2008 cohorts, respectively. The number of rows on the AD surface for the 2008 and 2009 cohorts was significantly correlated with NW of both the AD and AB surfaces for the respective cohorts (table 2). There was also a moderate correlation between the nROWS on the AB surface in the 2008 cohort and RDEN on the AD surface of the 2008 cohort ($r = 0.374$, $P = 0.042$). nSTOM was generally highly correlated with nROWS on the corresponding needle surface within the same needle cohort; for example, nSTOM for AD surfaces in the 2009 cohort was highly correlated with nROWS for AD surfaces in the 2009 cohort ($r = 0.879$, $P < 0.0001$).

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

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

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

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

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

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

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

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

Needle Width

The width of AD and AB surfaces of needles (NW) from both cohorts varied significantly between families across the range of whitebark pines sampled for this analysis (ANOVA): $P = 0.0011$ (AD 2009), 0.0129 (AB 2009), <0.0001 (both AD and AB 2008) (table 1). The family means for NW on the AD surfaces averaged over the 2008 and 2009 cohorts was 0.897 mm; the NW for the AB surfaces averaged over the 2008 and 2009 cohorts was 1.046 mm. The coefficient of variation for NW was similar in magnitude within a cohort: 6.4 and 4.6 percent on the AD surfaces and 6.7 and 4.2 percent on the AB surfaces for 2008 and 2009, respectively.

Although the mean NW of the AB side was greater than that of the AD sides by a factor of 1.166 (due to its curvature), there were fewer stomata, as well as fewer rows of stomata on the AB side (1.92 rows AB vs. 3.15 rows AD). nSTOM and nROWS were correlated with NW on the AD and AB surfaces in the corresponding needle cohorts, except for nSTOM and nROWS for the AB side in the 2009 needle cohort (table 2).

Disease and Climate Correlations

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

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

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

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

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

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

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

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

group. The first group that correlated with the yearly averaged RDEN (AD) and RDEN (AB) included mean temperature in the warmest month (mtwm), degree-days >5 °C (dd5), and degree-days >5 °C accumulating within the frost-free period (gsdd5), each of which was a significant predictor of RDEN (AD) in univariate analyses, with mtwm providing the best fit. The second group included summer precipitation balance (smrpb) and summer/spring precipitation balance (smrsprpb), which were not significant predictors, but did significantly improve the model fit when combined with mtwm (Akaike's information criterion was used as the criterion). The third group of correlated predictors, Julian date of the last freezing date of spring (sday) and length of the frost-free period (ffp), were not significant singly and did not improve the overall fit when modeled with the previous variables. RDEN (AB) for 2009 was best predicted by mtwm alone with no improvement in fit with additional predictors. Elasticnet identified one group of climate variables, growing season precipitation (gsp) and spring precipitation (sprp), which correlated with the yearly averaged nROWS (AD), providing the best fit with sprp. nROWS (AD) for 2008 and 2009 singly showed mixed results; gsp and sprp were only weakly correlated with 2009 nROWS (AD), and sprp provided the best univariate model for 2008 nROWS (AD).

DISCUSSION

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

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

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

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

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

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

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

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

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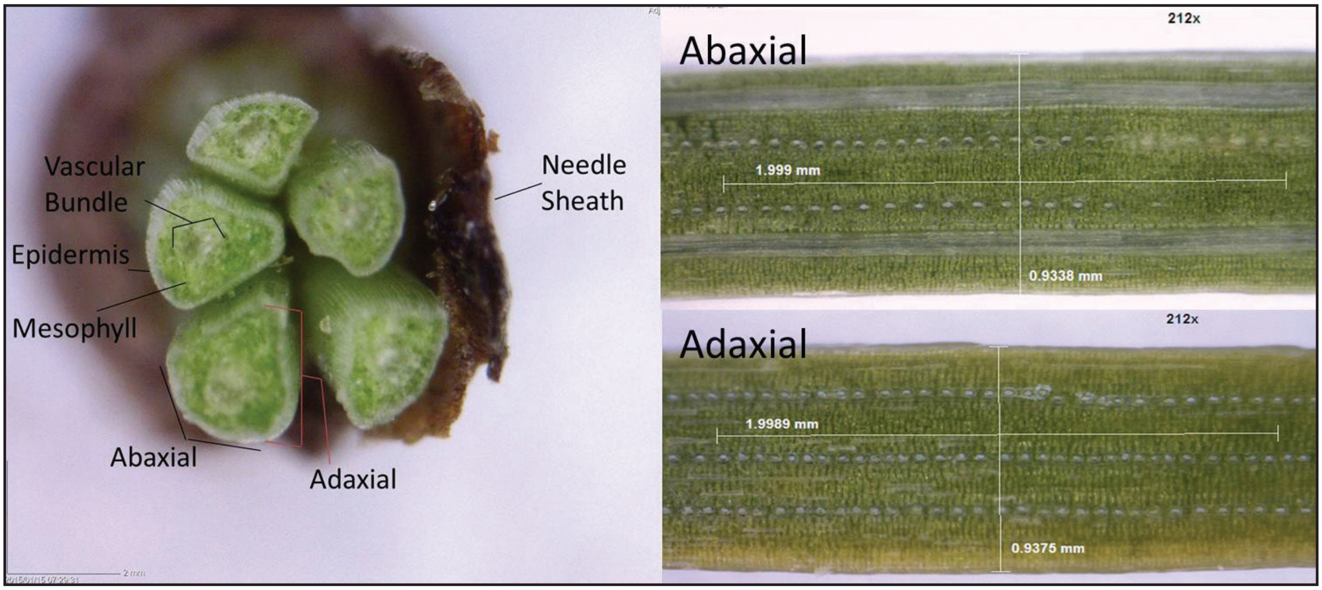

Figure 1—Cross section of a whitebark pine fascicle, illustrating how the one abaxial and two adaxial sides of each needle fit together to form the five-needle bundles, at approximately 205x magnification (left). The abaxial side at 212x magnification, exhibiting the typical two resin channels on either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

INTRODUCTION

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

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

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

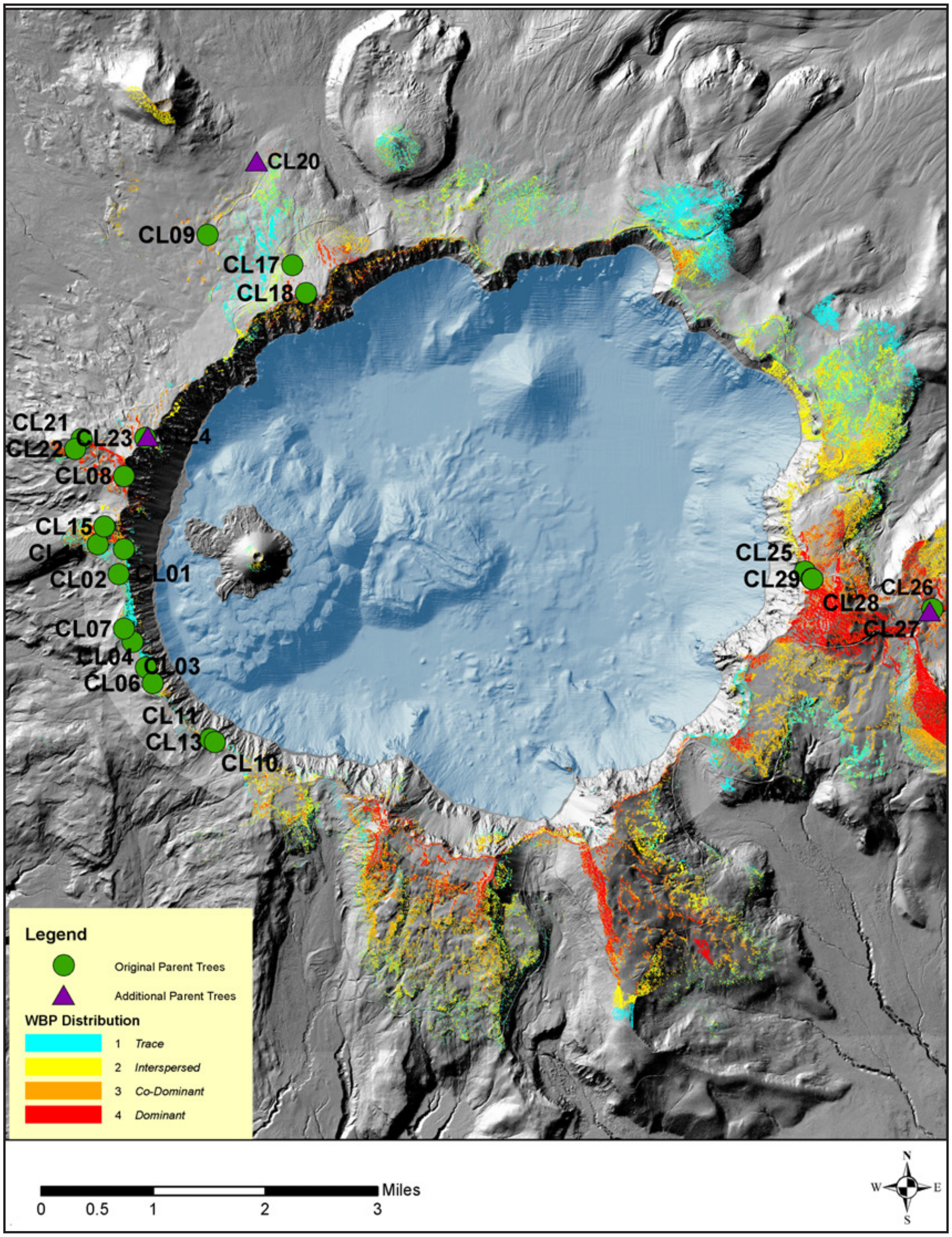

Figure 2—Map of Crater Lake National Park, showing the locations of the 25 whitebark pine parent trees whose progeny are in the 2007 common garden study. Also shown is the natural density of whitebark pine throughout the Park. Green circles signify the location of the subset of 22 families used for the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

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

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

METHODS

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

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

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

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

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

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

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

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

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

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

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

RESULTS

Family Variation

Needle Variation: Adaxial Versus Abaxial Side

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

Growth and Rust Infection Traits

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

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

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

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

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

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

Parent Variation (Geographic Variation)

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

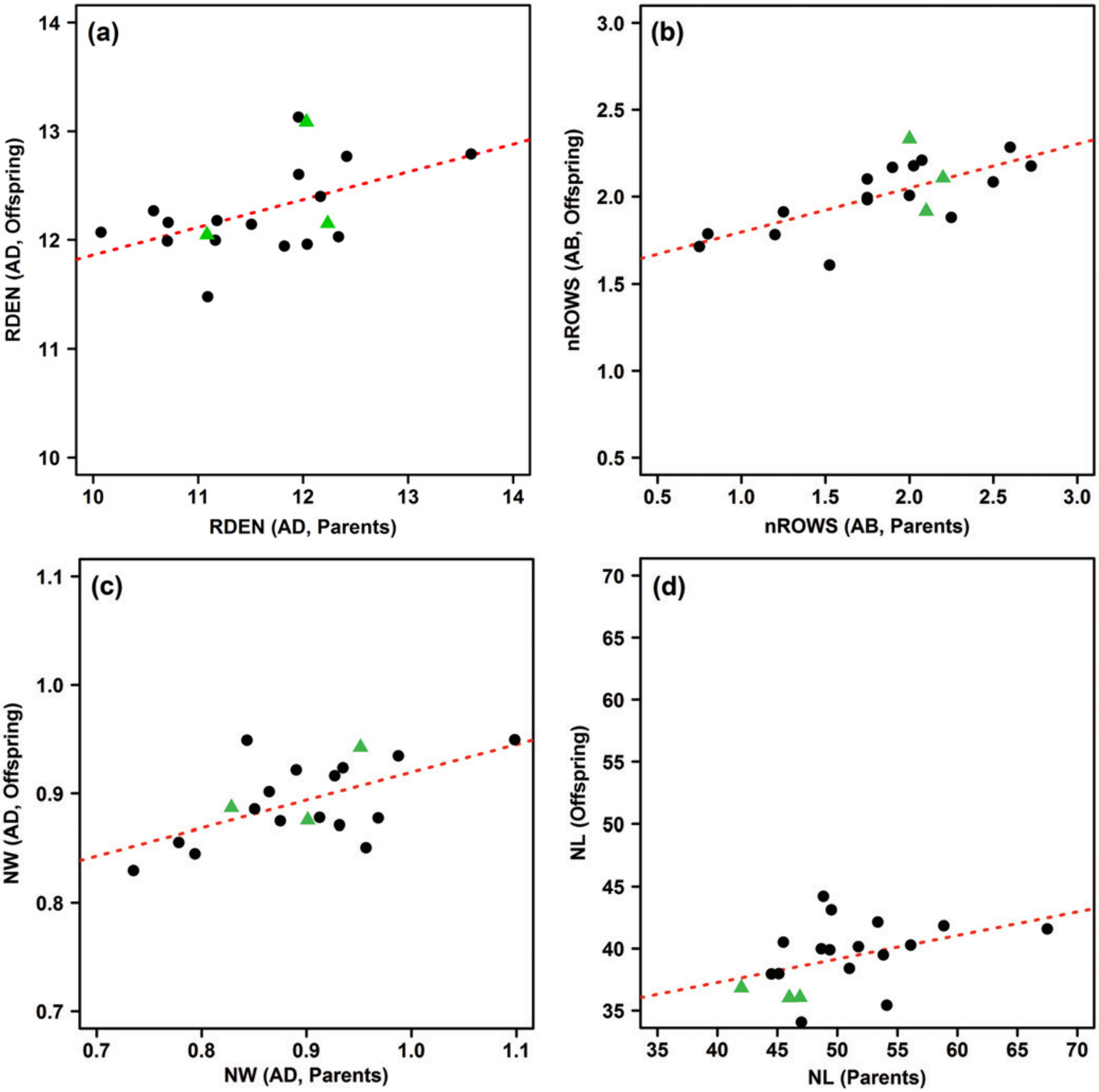

Figure 3—Relationship between offspring (y-axis) and parents (x-axis) for a variety of needle traits for 19 seedling families in a common garden test and the corresponding parent trees at Crater Lake National Park (CLNP). (a) stomatal density in rows (RDEN) on the adaxial (AD) side (r = 0.50); (b) number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

Correlation Between Traits: Progeny

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

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

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

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

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

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

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

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

Correlation Between Traits: Parents

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

Correlation Between Traits: Parents versus Progeny

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

East Side Versus West Side

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

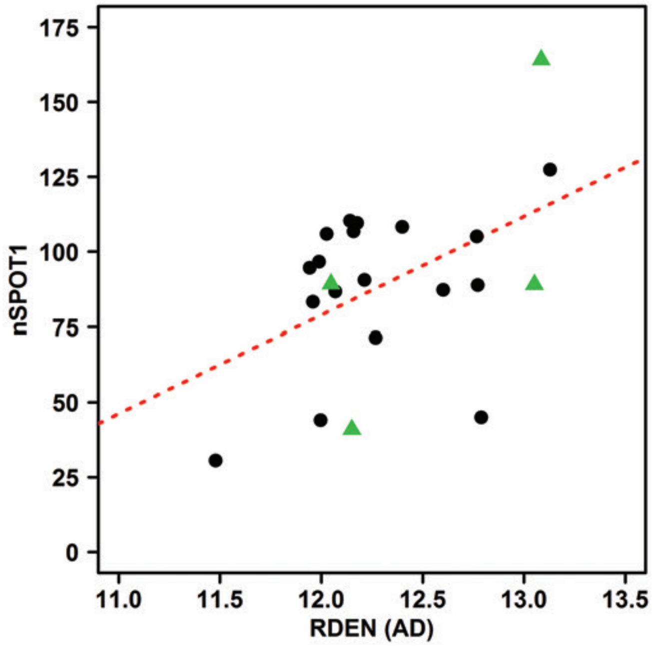

Figure 4—Relationship between 22 family means for stomatal density in rows (RDEN) on the adaxial (AD) side of the needle (x-axis) and the number of needle spots (nSPOT1, y-axis) after inoculation with blister rust spores ($r = 0.47$, $P = 0.026$). Green triangles represent parents on the east side of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

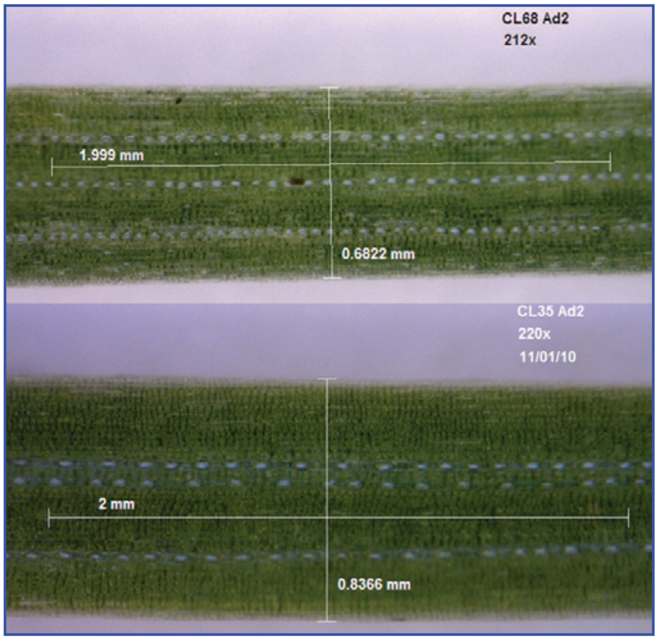

Figure 5—Micrographs of adaxial needles from the families with the highest (top) and lowest (bottom) within row density of stomates (RDEN). Family CL25 (top, 212x) had the highest stomatal row density as well as high spotting after inoculation with blister rust. Family CL02 (bottom, 220x) had the lowest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

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

Narrow-Sense Individual Tree Heritability

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

DISCUSSION

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

For any additional future efforts to examine needle traits, methods for micrographing and analyzing the needles may need to be reexamined and standardized. Although storage in the –20 °C freezer did not seem to affect the clarity of stomata, needle conformation could be affected (Wykoff 2002). The twistiness of the needles posed some problems. Several micrographs appeared to be needles with resin channels on the adaxial side; further investigation suggested that most of these needles had a reflective ridge on the needle that looked very similar to a resin channel. Others showed an unusually high number of stomate rows on the AD side. Although this may be an entirely natural event, it is also possible that because the angle between the two AD sides varies, both of the adaxial sides were captured and analyzed in a single micrograph. It is also possible that needle curvature caused part of the abaxial side to be captured as part of an adaxial photograph. Each photograph was examined by at least two people at different times and obvious problems were removed from the analysis. However, it is possible that some of these unusual observations were naturally occurring. In future investigations of needle properties, greater care should be taken to ensure only one side of the needle is being micrographed.

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

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

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

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Adaptive Variation Among Whitebark Pine (*Pinus albicaulis*) Populations from the Interior Northwestern United States in Relation to Climate

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Abstract—Provenance tests of 49 populations of whitebark pine (*Pinus albicaulis*) revealed moderate genetic differentiation for growth potential (growth under conditions approaching physiological optimum). Moderate genetic differentiation was also detected for survival after more than 10 years under warm-dry climate in the field but not for unconditional expected height, which was considered the best available predictor of fitness. Height was considered unconditional because survival over multiple years was explicitly included in the analysis. Genetic divergence for growth potential and survival was found to correspond with climatic clines. Population differentiation for growth potential was estimated to require a separation of about ±7.4 Julian days for timing of spring warming (D100) between climate of seed sources (≈ ±210 m elevation). Differentiation for survival was estimated to require about ±2.66 cm annual spring precipitation between climate of population origin. These results inform seed transfer and may provide insight into the genetic response of populations under a warming climate, particularly during early growth.

INTRODUCTION

Many forest tree species are composed of populations that are genetically adapted to only a portion of environmental variation that the species occupies as a whole. Knowledge from genecology studies on intraspecific patterns of genetic variation in relation to the environment is fundamental to gene conservation and reforestation (Morgenstern 1996). In particular, knowledge of how genetic variation is related to climate may be critical to identify threatened populations and match genotypes with suitable climate under rapid ongoing climate change (Joyce and Rehfeldt 2013; Rehfeldt and Jaquish 2010; Rehfeldt et al. 2014).

Whitebark pine (*Pinus albicaulis*) is a keystone and foundational species in western U.S. subalpine ecosystems that is threatened by population decline due to anthropogenic disturbance (Tomback et al. 2001). In recent years, several regional (Hamlin et al. 2008, 2011; Mahalovich et al. 2006) and rangewide (Bower and Aitken 2006, 2008) studies have advanced knowledge of the genecology of whitebark pine genecology. Nonetheless, information on genetic variation among populations for growth and survival in the field and in relation to climate of population origin remains limited. Thus, the objectives of the present study were to (1) assess genetic variation in growth and survival among populations from the interior northwestern United States in the field and (2) model this genetic variation in relation to climate at population origin. This paper reports only preliminary results.

METHODS

This study used seeds from wind-pollinated cones, collected during 1991–1997 from 49 wild whitebark populations in the interior northwestern United States (fig. 1). Collections were made from 5 to 20

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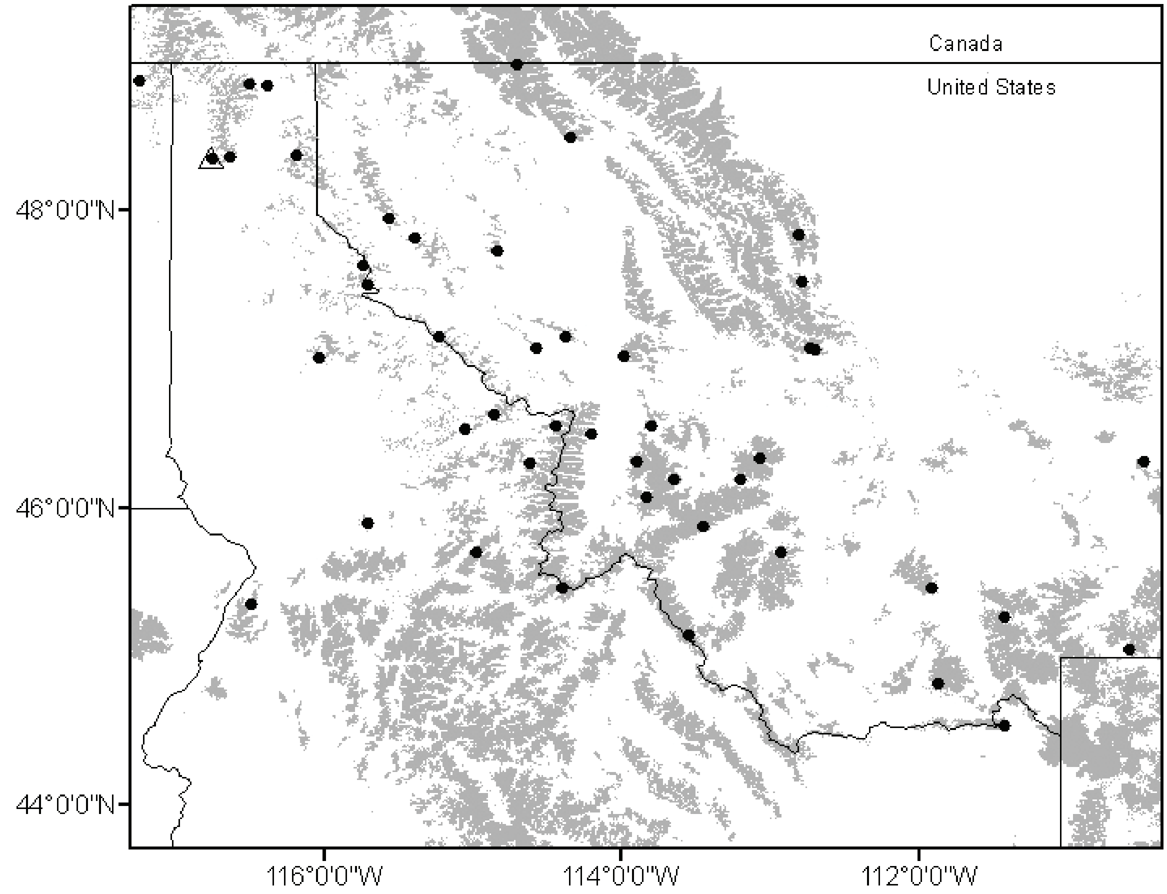

Figure 1—Location of whitebark pine (*Pinus albicaulis*) provenances (●) and study site at Priest River Experimental Forest (∆). Shaded area indicates predicted contemporary realized climate niche for whitebark pine (Warwell et al. 2007).

trees per population. Seeds were sown in 1998, and outplanted into two provenance tests in forest openings at Priest River Experimental Forest (lat. 48.3°N, long. –116.8°W) in northern Idaho (USA) (fig. 1). Test 1 was outplanted in April 24–26, 2000 with 2-year-old trees representing 46 populations. Test 2 was outplanted 2.2 km south of test 1 in October 2–5, 2001 with 4-year-old trees representing 42 populations (fig. 2). Thirty-nine populations were common to both tests. Soil at the test 2 site was rocky and shallow to a hard pan (Page-Dumroese 1993) and appeared to be harsher than the test 1 site, where soil was tilled at planting, unobstructed, and well drained. Both tests were located at a relatively low elevation (671 m) where temperatures were warmer (+4.4 to 9.1 °C mean annual temperature) and growing seasons were longer (+5 to 99 frost-free days) than found among native elevations (1,646 m to 2,774 m) of the study populations. A randomized complete block design was used in both tests. Each seed source was represented by 30 seedlings. Test 1 used five-tree row-plots per population within each of six blocks. Test 2 used 10-tree row-plots within each of 3 blocks. Individual trees were planted at a spacing of 1 m × 1 m at test 1 and 35.56 cm × 33.02 cm at test 2. Thinning to prevent competitive interaction among trees was not necessary despite close planting in test 2 due to high mortality and the species' characteristic slow growth. Total aboveground height measurements on 14-year-old trees were tallied in fall 2012 for both tests. Survival and shoot elongation were tallied in fall 2001–2005, 2011, and 2012 in test 1, and 2003–2006 and 2010–2012 in test 2.

The degree of differentiation among populations for survival and unconditional expected height was assessed with aster analysis (Geyer et al. 2007; see Shaw

Figure 2—Test 2 site in 2005, 3 years after outplanting. Trees are ~7 years old. Ten-tree row-plots are arranged from the foreground to the background edge.

et al. 2008) using the reaster function (Geyer 2013) in R (R Core Team 2012). Aster analysis permits unified analysis of multiple life history stages with appropriate statistical distributions. Thus, to assess unconditional height, where survival over multiple years was explicitly included in the analysis of height, survival was modeled using Bernoulli and height was modeled using normal distributions. For this procedure, unconditional expected height was assessed using seed source row-tree plot sums for height and mean survival to 2012. Differences in growth potential were assessed using general linear mixed model analysis using the lmer function (Bates et al. 2013) in R (R Core Team 2012). For this procedure, growth potential was assessed using seed source row-tree plot means for total shoot elongation in 2005.

To model genetic variation with climate, predictions for 26 climate variables (Rehfeldt 2006) representing the timing of moisture or temperature, or a combination, and their interaction at seed origins were tested by comparing the likelihoods of nested models following the statistical framework presented by Leites et al. (2012) and using a forward stepwise selection approach. Finally, regression analysis was used to describe climate based seed transfer guidelines following methods described by Rehfeldt (1983).

RESULTS AND DISCUSSION

After 12 and 11 years of growth in the field, mean height for the 14-year-old trees was 155 and 110 cm with 39- and 41-percent survival for tests 1 and 2, respectively. Differences between the two test sites were statistically significant ($P < 0.05$) for mean height but not survival. In test 1, survival among populations varied from 8 to 75 percent; mean apical shoot elongation in 2005 varied from 7.3 to 16.5 cm and mean height in 2012 varied from 90.4 to 214.4 cm. In contrast, populations in test 2 ranged from 19- to 77-percent survival and 4.6 to 13.7 cm for mean apical shoot elongation in 2005, and 18.8 to 124.3 cm for mean height in 2012.

In both tests, no statistically significant ($P > 0.05$) differences among seed sources were detected for unconditional expected height, the best available approximation of fitness. In test 2, however, a negative trend was apparent for fitness in relation to annual spring precipitation (SPRP) at seed source. Survival was statistically significantly ($P < 0.05$) in test 2 but not test 1. In test 2, survival was negatively related to SPRP at seed source. SPRP accounted for 13.2 percent of variation in survival among populations. Variation in growth potential was statistically significant ($P < 0.05$) and negatively related to annual dryness and winter coldness at the seed source (ADIMMINDD0) in test 1 and positively related to timing of spring warming (D100) at the seed source in test 2. In test 1, ADIMMINDD0 accounted for 9.8 percent of population variation in growth potential. In test 2, D100 accounted for 21.3 percent of population variation in growth potential. Clinal variation for growth potential and height generally decreased in a northwest to southeast direction and with decreasing elevation in the study region. Conversely, survival and fitness generally increased in a northwest to southeast direction.

Results from the present study indicate that reasonable confidence in population differentiation for growth potential requires a separation of about ±7.4 Julian days for D100 between climates of seed sources. This climate range corresponds to an elevation range of about ±210 m. These results are in general agreement with findings reported by Bower and Aitken (2008). In comparison with other western forest species, these results suggest that whitebark pine is an intermediate between specialist and generalist modes for growth potential (Rehfeldt 1994).

Reasonable confidence in population differentiation for survival under warm-dry climate requires a separation of about ±2.66 cm SPRP between seed sources. Differentiation of survival was assessed using young trees grown in a warm-dry climate for more than 10 years. The warm-dry climate conditions were similar to predicted future climate for the contemporary distribution of whitebark pine. Thus, results may provide insight into the genetic response of populations under a warming climate, particularly during early growth. To be sure, populations were tested outside of whitebark pine's native distribution; thus, results may not necessarily indicate performance in whitebark pine's native habitat.

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Phenotypic Selection on Growth Rhythm in Whitebark Pine (*Pinus albicaulis*) in Low Elevation Common Gardens

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Abstract—Growth rhythm represents the timing of annual plant growth and development in relation to the environment. Growth rhythm that is well synchronized with the local climate is understood to confer adaptation in plant species. Rapid ongoing climate change threatens to desynchronize growth rhythm for many plant populations, yet knowledge of how plant populations undergo selection on growth rhythm and how they will respond is limited. Therefore, to evaluate phenotypic selection on growth rhythm, seedlings from 49 populations of whitebark pine (*Pinus albicaulis*), representative of the interior northwestern United States, were grown in two common-garden field tests over a 12-year period under climate (+4.4 to 9.1 °C mean annual temperature) approximating projected climate change in the present century for the sampled species range. In addition, variation in growth rhythm among populations and its relation to the climate of the seed source were evaluated to clarify how growth rhythm varies over the study region. Height at the end of the study was used as the best available measure of fitness. Survival and the unconditional expected value for height were dependent on timing of apical shoot elongation rates within and among growing seasons. Comparison of models using survival versus unconditional expected height as the proxy for fitness showed that survival strongly influenced selection on elongation rates. The form and magnitude of selection on timing and rate of shoot elongation varied between test sites and over time. Analysis of the timing of selection detected directional and stabilizing selection on shoot elongation rates in the earliest years whereas only directional selection was detected for selection on elongation rate in later years. Differences among populations for growth rhythm were mild and were explained to a moderate extent ($r^2 = 0.08–0.28$) by climatic clines. Taken together, these findings suggest that growth rhythm in whitebark pine has been mildly selected for in relation to past climate and will undergo phenotypic selection in response to ongoing climate change. .

INTRODUCTION

Growth rhythm represents the timing of annual plant growth and development in relation to the environment. Growth rhythm that is well synchronized with the local climate is understood to confer adaptation in woody plant species (Dietrichson 1964; Howe et al. 2003; Rehfeldt 1992). Rapid ongoing climate change threatens to desynchronize growth rhythm for many tree populations in the western United States (see, for example, Rehfeldt 2004), resulting in maladaptation. Over the long term, the persistence of natural populations is determined by the process of evolutionary adaptation. Indeed, evolutionary adaptation is expected when the traits under selection vary within a given population and are heritable (Darwin 1859; Endler 1973; Falconer and Mackay 1996). In keeping with this understanding, evolutionary trajectory may be estimated when information about selection on the phenotype and trait inheritance is available. However, selection on phenotype is not well understood for many tree species under predicted rapid climate change.

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Whitebark pine *(Pinus albicaulis*) is of particular interest given its status as a keystone (Tomback and Kendall 2001) and foundational (Ellison et al. 2005) species in subalpine forest ecosystems of western North America. In addition, due to rapid ongoing climate change, whitebark pine's contemporary (1961–1990) realized climate niche is projected to be eliminated by mid-century for almost all of its distribution in the United States (Warwell et al. 2007). Therefore, the primary objectives of the present study were to (1) assess variation in growth rhythm among populations under warmer and drier climate than the climate of seed origin and (2) estimate the direction and magnitude of selection on variation in growth rhythm. This paper reports only preliminary results.

METHODS

The present study used whitebark pine provenance tests of 46 (test 1) and 42 (test 2) populations, with 39 populations common to both tests. The provenance tests were outplanted in forest openings at Priest River Experimental Forest (Idaho, USA) in April 2000 using 2-year-old trees in test 1 and October 2001 using 4-year-old trees in test 2. These trees were grown over a 12-year period under nonnative climate (+4.4 to 9.1 °C mean annual temperature) that approximated projected climate change in the present century for whitebark pine's contemporary distribution. Growing conditions were mild at the test 1 site, which was characterized by deep, well-drained soil that was tilled prior to planting. In contrast, growing conditions were harsh at the test 2 site. At that site, air temperature was 0.25 °C colder over a 17-month period while soil was shallow, poorly drained, and rocky. Both provenance tests used a randomized complete block design with populations planted in 5-tree row-plots in each of 6 blocks in test 1 and 10-tree row-plots in each of 3 blocks in test 2. Each provenance was represented by 30 seedlings. Measurements of annual incremental apical shoot elongation growth were recorded approximately weekly to biweekly from late March through July in 2001–2005 in test 1 and in 2003–2006 in test 2. Because sequential events of annual development tend to be intercorrelated (Dietrichson 1964), the timing and magnitude of apical shoot elongation were used as proxies for growth rhythm.

To evaluate selection on growth rhythm, the relationship between fitness and timing of intra-annual rate, cessation, initiation, and duration of apical shoot elongation was assessed with aster models (Geyer and Shaw 2008; Geyer et al. 2007) following a general multivariate selection approach (Lande and Arnold 1983). Aster, using the "reaster" function (Geyer 2013) in R (R Core Team 2012) was used to model unconditional expected height, which is an inference of population height that explicitly considers population survival. Normal distributions were used to model final height in 2012 conditional on survival to that year, and Bernoulli was used to model survival through each of multiple intervals concluding in 2012. Unconditional estimates of final height were used as the best available proxy for fitness because they provide a more complete measure of population fitness than either survival or height measures separately. Survival alone was also used as a proxy for fitness. Results from selection analyses using unconditional height and survival alone were compared to distinguish the influence of height from survival on inferred selection.

RESULTS AND DISCUSSION

Elongation rates at the test 1 site were greater than at the test 2 site throughout the study period. Total aboveground mean height of survivors in 2012 differed significantly ($P < 0.05$) between test sites, with 61.4 cm at the test 1 site and 45.3 cm at the test 2 site. Survival to 2012 was 39 and 41 percent in tests 1 and 2, respectively. Survival declined substantially in single episodes in the fall following relatively dry growing seasons in 2002 at the test 1 site and 2006 at the test 2 site.

Timing of initiation, timing of cessation, and duration of elongation were highly correlated. Higher elongation rates were correlated with earlier initiation, later cessation, and longer growth duration overall. Variation in the timing of initiation of shoot elongation spanned 2 weeks; timing of cessation of shoot elongation varied by as much as 3 weeks. Finally, total duration of shoot elongation for most individuals ranged from about 7 to 10 weeks.

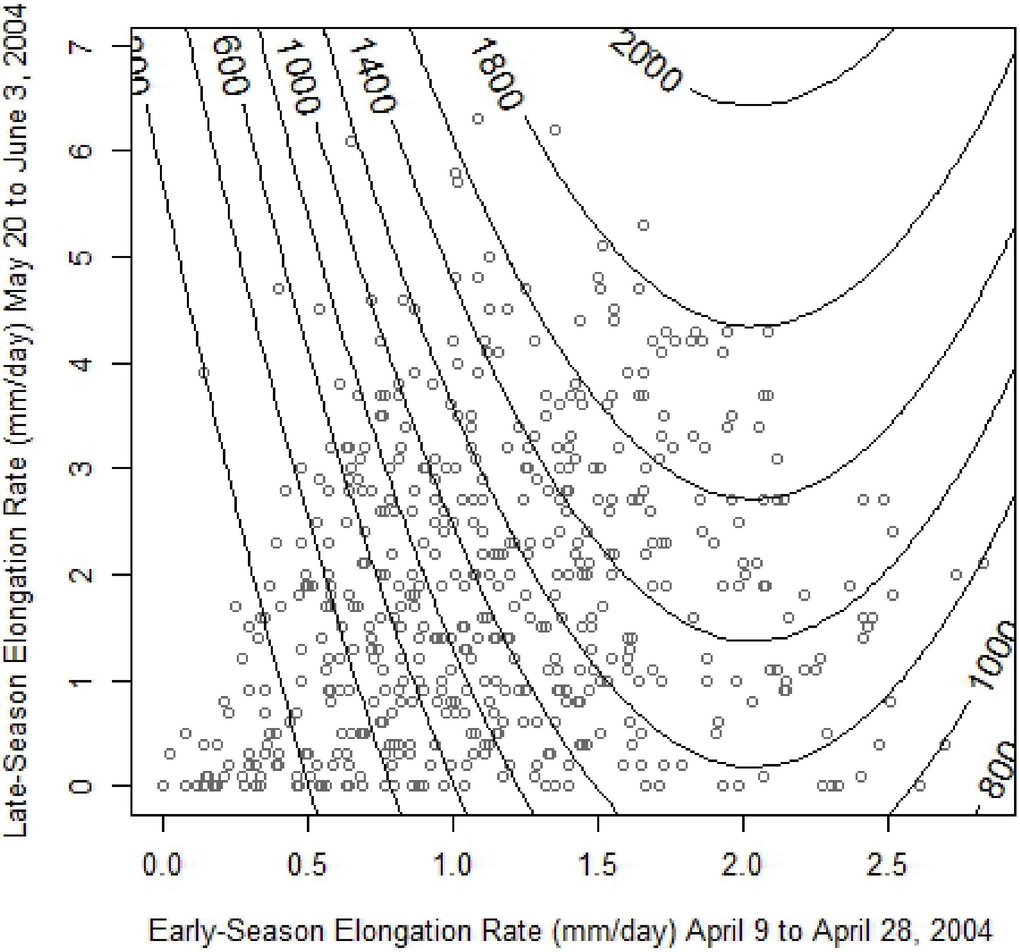

Figure 1—Fitness surfaces showing observed (open circles) apical shoot elongation rates (mm/day) early (Julian dates 108-127) and late (Julian dates 141-155) in the 2004 growing season in relation to modeled unconditional expected height (mm) in 2012 at test 1 site.

Survival and the unconditional expected value for height were dependent on timing of apical shoot elongation rates within and among growing seasons. A comparison of models using survival versus unconditional expected height as the proxy for fitness showed that survival strongly influenced selection on shoot elongation rates. The form and magnitude of selection on timing and rate of shoot elongation varied between test sites and over time. More complex selection was observed in test 1, which had more favorable growing conditions than test 2. Analysis of the timing of the form of selection detected directional and stabilizing selection in the earliest years, whereas only directional selection was detected for selection on elongation rate in later years (fig. 1).

A substantial range of variation in timing and rate of apical shoot elongation was observed within all populations. Differences among populations for growth rhythm were mild and were explained to a moderate extent ($r^2 = 0.08–0.28$) by climatic clines. Faster and longer growing populations originate from locations where winters were milder and precipitation was more balanced over the year. This climate tends to occur in an increasing southeast to northwest direction in the interior northwestern United States.

Taken together, study findings suggest that growth rhythm in whitebark pine has been mildly selected for in relation to past climate and is likely to undergo further selection in response to ongoing climate change. In addition, this study is an initial step toward assessing how growth rhythm in pine populations may evolve in response to ongoing climate change. Continuing research is needed to assess phenotypic selection of growth rhythm and other key adaptive traits of forest tree species during seed emergence, early establishment, and sexual maturity and under novel and contemporary climate.

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Provenance Variation in Western White Pine (*Pinus monticola*): The Impact of White Pine Blister Rust

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Abstract—The five-needle pines have been an important part of the landscape of North America, but they have been severely affected by the introduction of white pine blister rust (causal agent: *Cronartium ribicola*). However, most of these species have one or more types of some inherent resistance to this exotic disease, such as major gene resistance (complete resistance), and some forms of partial resistance. Long-established provenance trials in western white pine (WWP, *Pinus monticola*), established with unselected populations, allow us to investigate and compare genetic variation in our native populations for this resistance. These trials in British Columbia (Canada) and Washington and Idaho (USA) include seed sources that cover all the native range of WWP; the Canadian trials also include some of the selected populations from the ongoing genetic improvement effort. Trends shown here for growth (and survival) are similar to other reports on WWP—a large rather genetically undifferentiated northern population above latitude 45° N and a highly diverse but much less vigorous southern population. But also reported here, after 20 years of cumulative rust infection in the trials, is a trend in this northern population, sometimes significant, showing much higher levels of resistance in the Northern Cascades and lower elevation North Coastal sources compared to interior sources from Idaho and elsewhere. We describe several factors that could have caused these differences, such as natural selection after the early introduction; selection for traits involved in general constitutive defenses, which also gives resistance to this nonnative disease; or refugium differences in the primarily undifferentiated northern population. We also note the high effectiveness of the selection programs and discuss the inherent resistance defenses to this exotic pathogen that exist in native American species of white pine.

INTRODUCTION

Provenance testing is an important feature in understanding the background genetic variability of forest tree species (Morgenstern 1996). In the five-needle pines (also known as “white pines” and “soft pines”) of North America, this has been especially important since the accidental introduction of the exotic white pine blister rust (WPBR). The fungal pathogen *Cronartium ribicola*, the causal agent of WPBR, has been in North America for about 100 years. Although it appears very fragile, with a complex life cycle involving five different spore stages, alternating hosts, and exacting environmental conditions, it has proved remarkably successful in its colonization throughout most of the native ranges of the very susceptible North American white pines (Geils et al. 2010; Kinloch 2003). However, unlike other exotic pathogens that were also introduced in the early 20th century, in

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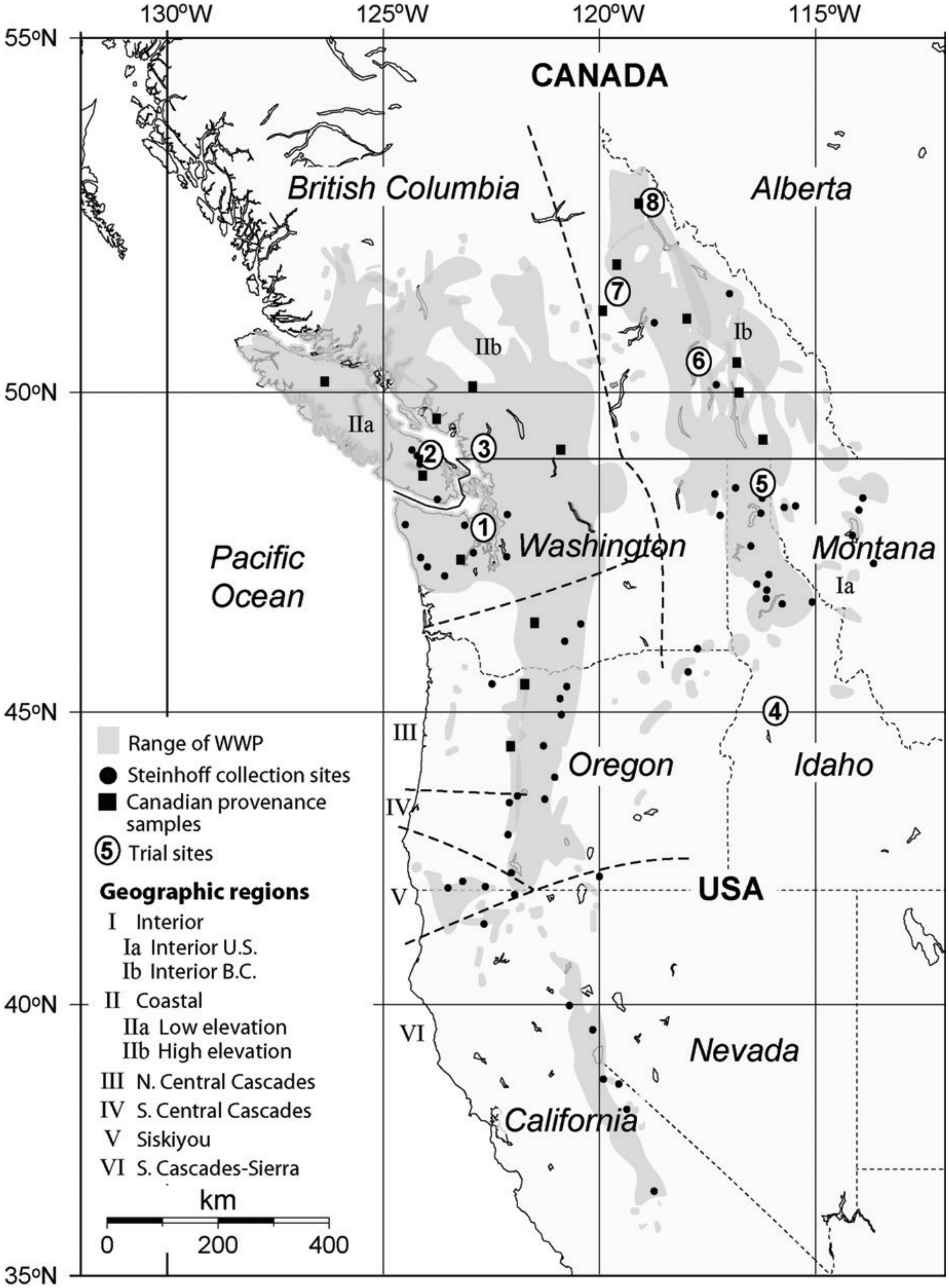

Figure 1—Western white pine distribution, sample locations, and trial sites.

particular chestnut blight caused by *Cryphonectria parasitica* on American chestnut (*Castanea dentata*), and Dutch elm disease caused by *Ophiostoma ulmi* and *O. novo-ulmi* on elm species (*Ulmus* spp.), there appears to be considerable native resistance in North American white pines to this exotic disease, albeit at low frequencies in natural populations (Kegley and Sniezko 2004; Kinloch et al. 2003; Sniezko et al. 2014).

Western white pine (WWP, *Pinus monticola*), has both a wide geographic distribution and one of the most comprehensive series of long-term provenance tests for the native North American five-needle pines. This paper summarizes results from these provenance trials, distributed both in the United States and Canada now that WPBR has had sufficient time to affect them. It draws some conclusions about geographic variability in genetic resistance in native WWP to the nonnative WPBR, and also about how this important tree species is perhaps evolving with this exotic pathogen.

Background to Provenance Research in Western White Pine

Two features were earlier noted in studies on the geographic variation and genecology of WWP:

1. There is a marked difference between the widely distributed northern populations (Regions I–III) and more narrowly distributed southern populations (fig. 1).

Regions for the southern populations, particularly in the Sierra Nevada and Warner Mountains (fig. 1: Region VI), are reported as quite distinct both with isozyme data (Steinhoff et al. 1983) and with growth and frost hardiness data (where it is noted these populations show lower growth potential but higher hardiness) compared to the northern populations (Rehfeldt et al. 1984). In both these studies the populations from Region V (Siskiyou) and Region IV, the southern Oregon (USA) Cascades, appear transitional between the northern population and the Sierra Nevada population.

The observations made from Steinhoff and colleagues' wide-ranging collections are also noted in other collections and studies and many observers have commented on the distinct difference in growth and needle characteristics of the Sierra Nevada white pine. In Canadian studies, Hunt (1994) and Meagher and Hunt (1998, 1999) noted southern sources of white pine (Southern Cascades, Siskiyou, and Sierra Nevada) are distinct from northern sources and are far more susceptible to WPBR. An evaluation of early growth and survival in a large progeny test of WWP in southwestern Oregon by Bower and Sniezko (2004) showed higher growth potential from northern sources of WWP. Bower and Sniezko (2004) also reported a distinct Californian population and a transition zone in the southern Oregon Cascades and confirmed Steinhoff's designation of a broad northern population from northern Oregon and eastward into Montana (USA).

2. There is exceptionally wide phenotypic plasticity for the northern populations (fig. 1: Regions I–III).

A commonly observed phenomenon is the wide transferability between the interior (Montana, Idaho, interior British Columbia [BC]) and coast for growth potential (Bower 1987; Steinhoff 1981), but a slightly less although still very wide amplitude for cold hardiness (Rehfeldt et al. 1984; Thomas and Lester 1992). This is highly unusual as strong genetic differences are typically noted between coastal and interior populations of most conifers; and strong latitudinal, elevational, and other environmental clines are the observed norm (Rehfeldt et al. 1984). Steinhoff et al. (1983) noted in an isozyme analysis that among-source population variability within this northern group was very low and even within-source variability was generally lower than in other regions. Even though populations from the Inland Empire (Idaho, eastern Washington, and Montana) and interior BC are now separated from the Northern Cascades and Coastal populations (fig. 1), the isozyme and the growth and adaptive trait data show a lack of strong differentiation within this large region, indicating they probably disseminated from a common gene pool after the last ice age (Critchfield 1984; Steinhoff et al. 1983).

Less has been noted on infection and survival differences among WWP provenances to exposure to WPBR, but it is interesting to note that the major R-gene (major gene resistance; MGR) that confers a hypersensitive response resistance (Kinloch et al. 1970) identified as Cr2 (Kinloch et al. 1999) appears absent throughout this northern population and shows higher frequencies in the Sierra Nevada (0.005–0.008) compared to only 0.001 in the southern Oregon Cascades (Kinloch et al. 2003). This observation fits well with Critchfield's hypothesis of a common gene pool and bottleneck that did not allow Cr2 to become established in the northern population. Another observation on resistance is based on assessment of various plantations and trials in BC (Hunt 1994, 2004). Hunt (2004) suggests that some of the resistance responses selected for in Idaho (e.g., needle shed) can do well in the shorter growing season of interior BC, but may not perform as well in the longer growing season found in coastal environments.

In a study of the genetic architecture of the Steinhoff WWP rangewide seed collections using amplified fragmented length polymorphism markers, Kim and others (2010) showed with nonmetric multidimensional scaling of genetic distance (Kim et al. 2010: fig. 4) a potential for a more diverse population structure than that described by Steinhoff and others (1983). They confirmed the divergence between a northern population of relatively low genetic diversity and the more diverse southern population at about 45° N latitude. Kim et al. (2010) also found that the transition areas between these populations (which they identified as clades) east and west of the ridge of the Southern Cascades could be quite marked (also Richardson et al. 2009).

METHODS

Provenance Samples and Field Trial Descriptions

Figure 1 shows the distribution of WWP, the provenance source population, and trial locations. Details of the U.S. provenance collection information are provided by Steinhoff et al. (1983), and the Canadian populations and trial sites are described in detail in Meagher and Hunt (1998). The Steinhoff collection (U.S. trials) had 60 populations in total with 3 to 25 trees per population. These were considered random samples, which means they did not come under the intense selection identified for the WPBR screening (King et al. 2010; McDonald et al. 2004). The Canadian collection of more than 100 samples (trees or mixed seedlots) included random samples encompassing more of the Canadian range of WWP (both Coastal and Canadian Rockies). The Canadian collection also used the Steinhoff collections for the U.S. Northern Rockies Region (Region I-a; fig. 1), and also some selected populations. This selected material came from various screening programs and included: (1) *The Porter families*—early intensive selections with some screening (as described by King and Hunt [2004]); (2) *The Westar families*—selections made in southern interior BC using the Idaho protocols (McDonald et al. 2004) without screening; (3) *Dorena Genetic Resource Center MGR seedlot*s from Oregon; and (4) some exotics, of which *P. koraiensis* is reported here.

Table 1 details the trials we report here with ages, final height measurements, and the overall percentage of trees with cankers from blister rust as well as blister rust frequency (incidence) for the Idaho collection (U.S. Northern Rockies Region I-a) subset of seedlots (as this was common to both the U.S. and Canadian trials). Rust incidence (presence or absence) was recorded as canker frequency in the *Results and Discussion* section (tables 2–7), and canker severity (counted number of cankers, up to 30) was also recorded in the U.S. trials. The U.S. trial sites have not previously been described, but are randomized complete block (RCB) designs of three 10-tree row blocks per seedlot (collection sample) and were established in 1983. The Whidbey Island site (Lone Lake) established by Washington State Department of Natural Resources (WA DNR) in cooperation with the U.S. Department of Agriculture, Forest Service (USDA FS) Moscow Forestry Sciences Laboratory (1 in fig. 1) and the Lost Valley site (located near McCall, Idaho outside the range of WPP on USDA FS land; 4 in fig. 1) both provided high survival. Another Idaho site located at Priest River Experimental Forest (USDA FS) lost two full blocks due to early plantation failure (5 in fig. 1). One block provided nearly full survival and was assessed. The Canadian sites described by Meagher and Hunt (1998) are also RCB with 25 replicated blocks of 4-tree row plots and were planted in 1988; they are described in greater detail later.

DATA ANALYSIS

Analysis of the trial information, which included both height and blister rust canker incidence (Canker) and severity (Canker Severity), was conducted by using a linear mixed-effects model that included the effects of

Table 1—Site descriptions of provenance trials reported.

	Site	Agency	Location	Latitude ^{o}N	Longitude ^{o}W	Elevation m	Mean height and (age)	% Blister rust incidence for overall plantation and (Idaho collection)	References to sites or materials
1	Whidbey Is.	WA DNR	Washington, Coastal	48^o 03'	122^{o}46'	50	123 –(20)	50% - (54%)	a,b
2	Ladysmith	Canadian For. Serv.	BC Coastal	49^o 00'	123^o 52'	75	62 – (12)	68% - (79%)	c,d,e,f
3	Sechelt	Canadian For. Serv.	BC Coastal	49^o 30'	123^o 44'	250	45 – (12)	91% - (96%)	c,d,e,f
4	Lost Valley	USDA For. Serv. Region 1	Idaho South	44^o 59'	116^o 30'	1800	35 – (21)	85% - (91%)	a,b
5	Priest River	USDA For. Serv. Region 1	Idaho North	48^o 20'	116^o 42'	730	95 – (20)	22% - (24%)	a,b
6	Nakusp	Canadian For. Serv.	BC Interior (South)	50^o 15'	117^o 46'	680	46 – (12)	35% - (45%)	c,d,e
7	Barrière	Canadian For. Serv.	BC Interior (Central)	51^o 19'	119^o 54'	725	52 – (12)	16% - (19%)	c,d,e
8	Valemount	Canadian For. Serv.	BC Interior (North)	52^o 41'	119^o 03'	760	49 – (12)	76% - (81%)	c,d,e

[a] Steinhoff et al. (1983).
[b] Rehfeldt et al. (1984).
[c] Hunt (1994).
[d] Meagher and Hunt (1998).
[e] Meagher and Hunt (1999).
[f] King and Hunt (2004).

Table 2—Results for canker frequencies (incidence and severity) at the Whidbey Island, Washington DNR Trial Site.

Trait	Canker 13 yrs		Canker 21 yrs		Canker_Severity 21	
Random effect	**variance**	**S.E.**	**variance**	**S.E.**	**variance**	**S.E.**
Block	0	n/a	0.0933	0.1039	0.6646 ns	0.6816
Population (Region)	0.1081	0.1908	0.2358.	0.0866	0.521 ***	0.1251
Fixed effect	**DF**	***P*** [a]	**DF**	***P*** [a]	**DF**	***P*** [a]
Region	5,66	<0.0001	5,57	<0.0001	5,57	0.0002
Region level	**Estimated canker incidence**	**Confidence limits**	**Estimated canker incidence**	**Confidence limits**	**Estimated canker severity**	**Confidence limits**
I N - Interior	0.028	0.017, 0.046	0.537	0.384, 0.682	**1.513**	-0.268, 3.294
II N - Coastal	0.050	0.028, 0.087	**0.302**	0.190, 0.444	**0.675**	-0.955, 2.305
III N - N. Cascades	0.019	0.006, 0.060	**0.200**	0.111, 0.334	0.574	-0.979, 2.126
IV T - S. Cascades	0.025	0.009, 0.067	0.488	0.327, 0.652	1.884	0.334, 3.433
V T - Siskiyou	0.046	0.011, 0.182	0.680	0.430, 0.857	3.097	1.362, 4.833
VI S – Sierra	**0.204**	0.136, 0.293	**0.712**	0.564, 0.826	2.328	0.757, 3.899
Comparison of regions	**Difference in canker incidence**	***P*** [b]	**Difference in canker incidence**	***P*** [b]	**Difference in severity score**	***P*** [b]
Interior vs. Coastal	-0.05	0.1445	**0.235**	**0.0001**	0.838	0.0141
Interior vs. N. Cascades	0.009	0.1450	**0.337**	**<0.0001**	0.940	0.0242
Coastal vs. S. Cascades	0.025	0.2374	-0.186	0.0187	-1.208	0.0107
Coastal vs. Siskiyou	0.004	0.9359	**-0.388**	**0.0115**	-2.422	0.0013
Coastal vs. Sierra	**-0.154**	**0.0408**	**-0.441**	**0.0007**	-1.653	0.0002

**** significant <0.0001,*** significant <0.001, ** significant <0.01, n.s. non significant >0.05, n.a. not available

[a] *P*-value for testing whether all Fixed Region effects are zero is derived from an *F* distribution.

[b] *P*-value for testing whether each difference is zero is derived from a *t* distribution.

interest: *Region (R)*, provenance *Population (P)*, *Trial* site *(T)*, and *Block (B)*. Region was based on the original seven regions as designated in Steinhoff et al.'s (1983) survey (shown in figure 1), but we also discuss the division in terms of northern populations (fig. 1: N, Regions I–III), southern Sierra populations (S, Region VI), and transitional populations (T, Regions IV and V). In the Canadian trials, which had larger sampling within the defined northern regions, we also looked at regions defined as: Coastal Low elevation (Region II-a), Coastal High elevation (or Northern Cascades, Region II-b), Idaho (Region I-a), and Interior BC (Region I-b) (fig. 1).

In the mixed model the random *Population* (trees sampled) is nested in *Region*, which is the principal fixed effect. The MIXED procedure of SAS® (SAS Institute Inc., Cary, North Carolina, USA) was used for the normally distributed height data and for canker severity; and the GLIMMIX procedure was used for rust frequency (incidence), a 1,0 binomial dataset (Littell et al. 1996; SAS Institute Inc. 2004). Contrast statements allowed testing hypotheses about the fixed regional effects or groupings of these fixed effects using t-tests. In the analysis of estimates of variance of the components associated with random effects of *Trial*, *Block*, and *Population*, the significance of each of these effects was tested by individually removing it from the model and assessing the change in the model fit via the observed log likelihood. The reduced model lies on the boundary of the parameters' space for the variance component; therefore, the *P*-value associated with these likelihood ratio tests was determined by using the technique described in Verbeke and Molenberghs (2000: section 6.3.4). All tests have a level of significance $\alpha = 0.05$. In the analysis of the Canadian sites, two models were run: one excluded the selected populations, and another included these populations.

Table 3—Results for heights in decimeters at the Whidbey Island Trial (Washington DNR).

Trait	Height 4 yrs		Height 13 yrs		Height 21 yrs	
Random effect	**variance**	***P* [a]**	**variance**	***P* [a]**	**variance**	***P* [a]**
Block	0.1600	0.2132	11.81	0.1986	22.12	0.2118
Population (Region)	0.4801	**0.0018**	24.06	**< 0.0004**	69.69	**< 0.0001**
Tree (Population Region)	0.4159	0.0014	146.74	< 0.0001	429.24	< 0.0001
Fixed effect	**F value**	***P* [b]**	**F value**	***P* [b]**	**F value**	***P* [b]**
Region	39.40	<0.0001	37.81	<0.0001	58.41	**< 0.0001**
Region level	**Least squares mean**	**Confidence limits**	**Least squares mean**	**Confidence limits**	**Least squares mean**	**Confidence limits**
I N - Interior	11.036	10.2, 11.9	70.17	63.2, 77.2	**123.64**	113.7, 133.6
II N - Coastal	10.545	9.67, 11.4	64.37	57.3, 71.5	114.19	103.9, 124.5
III N - N. Cascades	10.312	9.35, 11.3	64.56	57.1, 72.0	114.36	103.2, 125.5
IV T - S. Cascades	9.388	8.41, 10.4	54.94	47.4, 62.5	100.12	88.78, 111.5
V T - Siskiyou	7.775	6.34, 9.17	43.30	33.3, 53.3	70.38	54.39, 86.37
VI S – Sierra	5.493	4.60, 6,39	29.95	22.8, 37.1	41.96	31.25, 52.66
Comparison of regions	**Difference in least squares**	***P* [c]**	**Difference in least squares**	***P* [c]**	**Difference in least squares**	***P* [c]**
Interior vs. Coastal	0.492	0.2468	5.804	0.0823	9.45	0.0801
Coastal vs. S. Cascades	1.156	0.0321	9.430	0.0183	14.07	0.0282
Coastal vs. Siskiyou	2.790	0.0004	21.07	0.0002	43.81	<0.0001
Siskiyou vs. Sierra	2.262	0.0038	13.34	0.0131	28.42	0.0017

[a] *P*-value for testing whether each variance component is zero is derived from a mixture of χ^2 distributions.

[b] *P*-value for testing whether fixed Regional effects are zero is derived from an *F* distribution.

[c] *P*-value for testing whether each difference is zero is derived from a *t* distribution.

RESULTS AND DISCUSSION

The results are discussed in four sections: (1) the Washington State DNR trial at Lone Lake on Whidbey Island, which was one of the most informative trials for survival and completeness of seed sources; (2) the coastal sites including analysis of Canadian sites, plus in combination with the Whidbey Island (Lone Lake) site;(3) the Canadian interior sites analyzed separately; and (4) Idaho sites analyzed separately and combined.

Blister Rust and Height, Lone Lake on Whidbey Island, Washington

The Lone Lake trial site on Whidbey Island was the most comprehensive of the provenance trials analyzed, having the complete Steinhoff collection. White pine on this site had good vigorous growth and high overall survival but moderate (50 percent) rust incidence measured over several years.

The frequency (incidence) of cankers from blister rust was low at age 13, with no region having more than 21 percent, and most having less than 5 percent (Cankers 13 years, table 2). This allowed the trees to display vigorous growth on this productive site and get ahead of later rust development. A significant incidence of both branch and stem cankers was recorded only for Region VI (the Sierras), where a 20 percent incidence of blister rust cankers was significantly different from 0 percent. The 21-year assessment was quite different and by this time the overall incidence (proportions of stems infected) of cankering in the trial was 50 percent (table 2). Although the Sierra population samples again showed the highest frequencies (>70 percent), other populations were also higher than 50 percent. But the two local populations—the Coastal (Region II) and North Cascades (Region III)—had significantly lower rust incidence (20–30 percent) than most other regions (table 2). Noticeable within the broad region

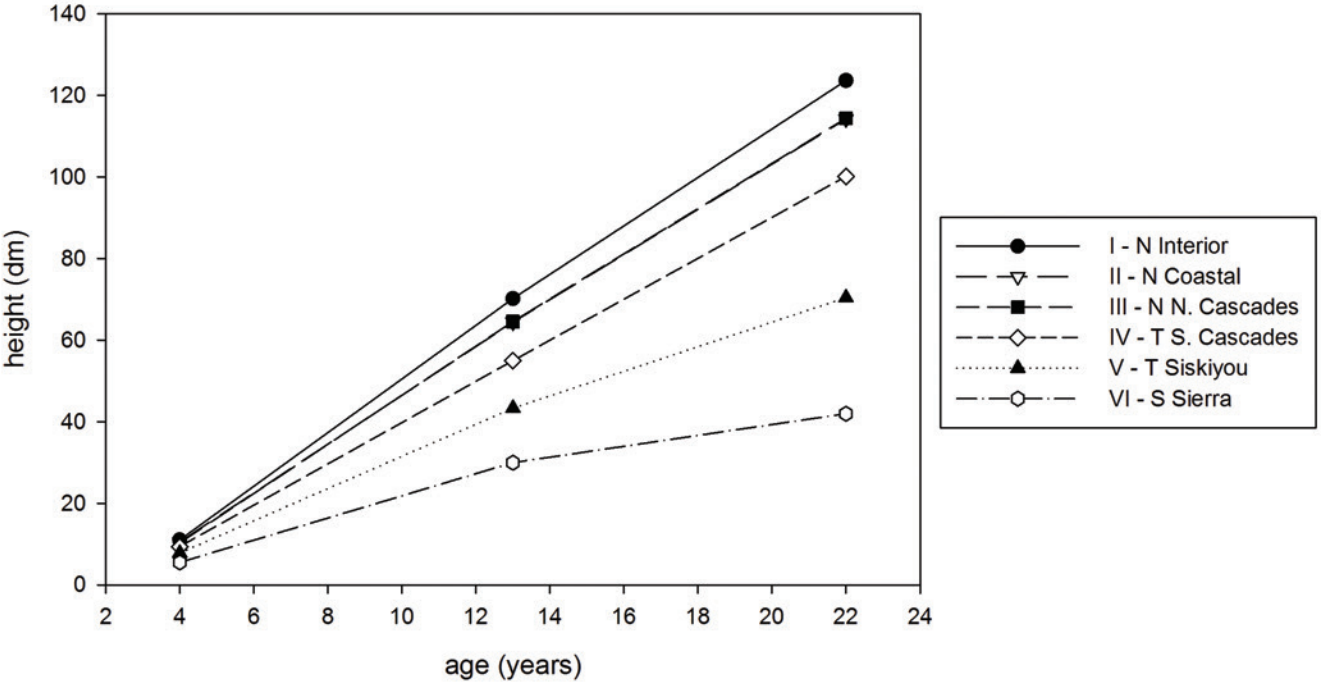

Figure 2—Height measurements (decimeters) over time at the Whidbey Island site.

for the northern populations is that interior populations have significantly higher rust incidence than the local Coastal populations (Pr > |t| 0.0001) and the North Cascades populations (Pr > |t| <0.0001, table 2). Canker incidence and canker severity (number of cankers) tracked each other closely on the U.S. sites where this was measured. The Idaho Region populations had twice the severity of the local Coastal or Northern Cascades populations (table 2).

Table 3 shows the three height measurements at the Whidbey Island site (diameter at age 21 was also recorded; it is not reported, but tracked height closely). The vigor especially of the Interior populations (>12 m on average at age 21) was very marked; they were on average 1 m taller than the local populations. The southern regions all showed poorer growth compared to local sources. The Sierra sources at one-third the height of the northern regions suffered poor survival caused not only by higher rust incidence but also by overgrowth of bigger trees. Very few of the Region VI trees were still alive at the 21-year assessment. Figure 2 shows the increasing height trends of the different regions over time.

Blister Rust and Height on Canadian and All Coastal Sites

The Ladysmith site, about 200 km north of Whidbey Island, is a similar low-elevation productive site with average height of more than 6 m at 12 years. Mean canker incidence was 68 percent, and showed significant discrimination between the regional groups, as did the Whidbey Island site (table 4). Five of the eight groups showed greater than 75-percent rust incidence; only the North Cascades among the unselected populations had a lower incidence (67 percent). This difference was significant compared to Northern Interior BC (Region I-b; Pr > |t| 0.0339). All the selected populations had a significantly lower incidence than the unselected, most notably the *Dorena MGR population* with only 27 percent versus nearly 70 percent overall. Also of interest were the *Porter families*, which were selected quite early in the outbreak and screened on rather simple resistance attributes; they showed statistically significant higher levels of resistance than the local unselected populations (83 percent vs. 46 percent, Pr > |t| 0.0006, table 4; King and Hunt 2004).

Table 4—Results for canker frequencies (incidence) at the Canadian sites in Coastal BC.

Trait	Sechelt 1995		Sechelt 2001		Ladysmith	
Random effect	**variance**	**S.E.**	**variance**	**S.E.**	**variance**	**S.E.**
Block	0.4848	0.1825	1.158	0.4874	0.7671	0.2766
Population (Region)	0.1084	0.7112	0.2173	0.2231	0.4005	0.1771
Fixed effect	**DF**	***P* [a]**	**DF**	***P* [a]**	**DF**	***P* [a]**
Region (only unselected)	4, 26	0.4343	4, 23	**0.3759**	4,24	0.0405
Region (with selected)	7, 66	0.0184	7, 52	0.2582	9,88	<.0001
Region level	**Estimated canker incidence**	**Confidence limits**	**Estimated canker incidence**	**Confidence limits**	**Estimated canker incidence**	**Confidence limits**
I-a Inland Empire	0.54	0.42, 0.67	0.96	0.91, 0.98	0.80	0.65, 0.89
I-b BC Interior unselected	0.49	0.39, 0.60	0.93	0.95, 0.97	0.86	0.78, 0.92
Westar families	0.39	0.30, 0.50	0.94	0.89, 0.97	0.76	0.68, 0.83
II-a - Coastal (unselected)	0.48	0.36, 0.60	0.95	0.89, 0.98	**0.83**	0.71, 0.90
Porter families	0.24	0.12, 0.41	0.91	0.74, 0.97	**0.46**	0.30, 0.62
II-b… high elevation	**0.58**	0.45, 0.70	0.97	0.91, 0.99	0.92	0.83, 0.96
III N - N. Cascades	0.44	0.30, 0.58	0.92	0.82, 0.97	**0.67**	0.45, 0.83
IV *T - S. Cascades (Dorena)*	**0.20**	**0.07, 0.45**	**0.72**	**0.33, 0.93**	**0.27**	0.08, 0.62
Comparison of regions	**Difference in canker incidence**	***P* [b]**	**Difference in canker incidence**	***P* [b]**	**Difference in canker incidence**	***P* [b]**
Coastal (II-a) vs Interior BC	0.01	0.7318	0.02	0.4187	0.03	0.4579
Coastal (II-a) unselect vs *Porter*	**0.24**	**0.0328**	0.03	0.6647	**0.37**	**0.0006**
Interior (BC) vs. *Westar families*	0.10	0.1379	0.01	0.6911	**0.10**	**0.0160**
N. Cascades (III) vs. Interior BC	0.05	0.2286	0.01	0.8123	**0.20**	**0.0339**
N. Cascades (III) vs. Idaho (I-a)	0.10	0.1951	0.01	0.1575	0.13	0.2019
Idaho – I-a vs. *Dorena*	**0.34**	**0.0200**	**0.24**	**0.0144**	**0.53**	**0.0052**

[a] *P*-value for testing whether all fixed Regional effects are significantly different is derived from an *F* distribution.

[b] *P*-value for testing whether each difference is zero is derived from a *t* distribution.

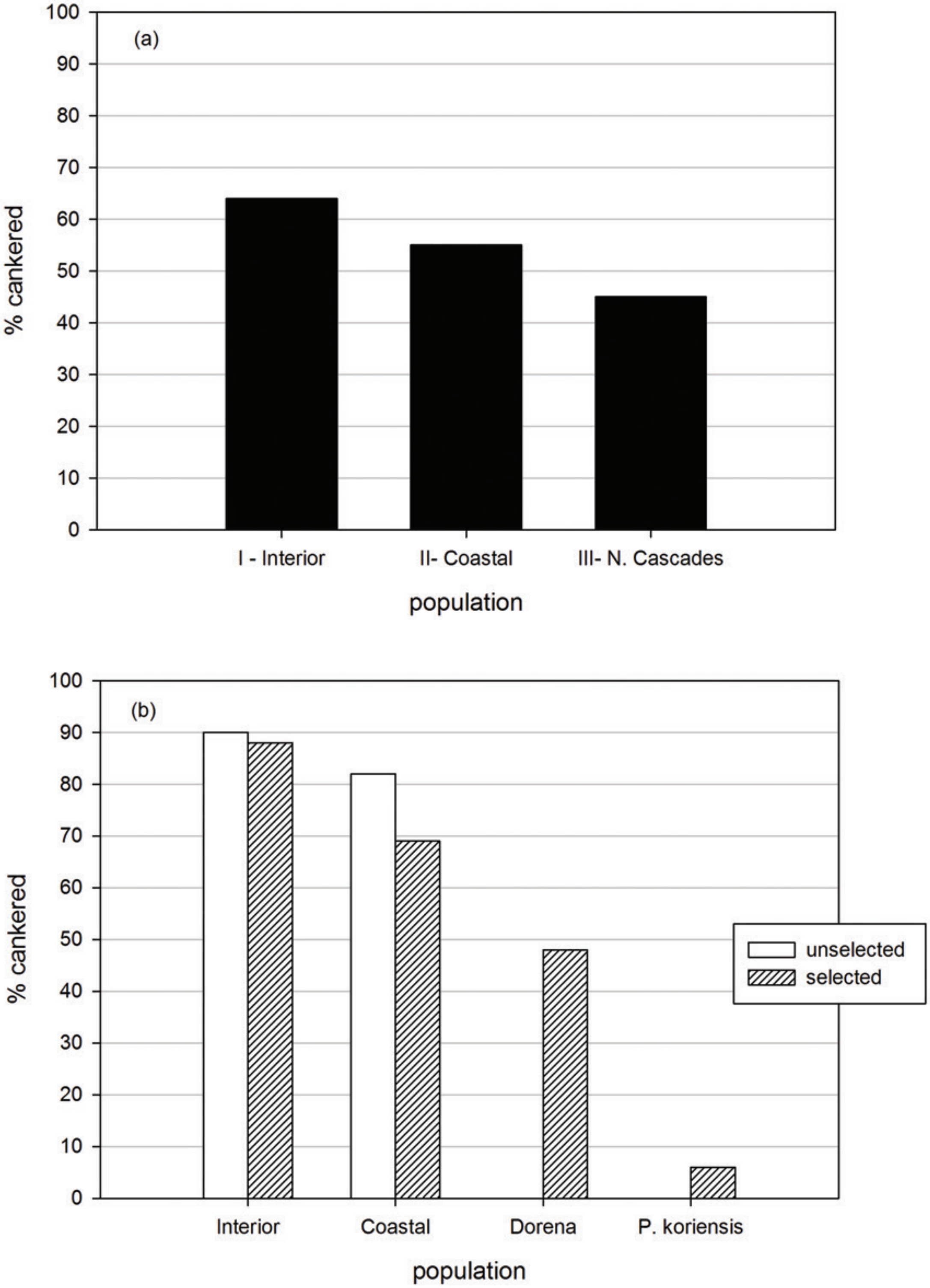

Figure 3—Canker frequencies in the coastal trials.

The Sechelt site is another low-elevation coastal site on the mainland side of the Salish Sea. This site had the highest incidence of rust of all the trials surveyed with greater than 90-percent infection at 12 years old, a dramatic increase from the 1995 assessment, when no group showed more than 58 percent infection. The regional differences are nonsignificant (Pr > F 0.3795) unlike at Ladysmith or Whidbey Island. Only the *Dorena MGR* seedlot at 72-percent infection (increasing from 20 percent at the 1995 assessment) had a significantly lower incidence than the others. Although a frequency of 72 percent is higher than what might be expected from the segregating Cr2 expression, this figure is not in fact significantly different from the expected 50 percent (confidence intervals of 33–93 percent, table 4). Thus, it does not conclusively indicate a breakdown of resistance due to vcr2; further monitoring would be needed to verify if in fact vcr2 was present. This site has not had any other detailed assessment, but a walk-through in 2008 indicated a still marked proportion of white pines in the stand structure (30 percent or greater), many living with pronounced cankers.

Results from a combined mixed model run of all the coastal sites—Whidbey, Ladysmith, and Sechelt—based on the last assessment for rust incidence demonstrate the difference between the unselected North Cascades (Region III—45 percent cankered) and the Interior (Region —64 percent cankered) (fig. 3a; Pr > |t| 0.0443). The local Coastal Region II was 54 percent cankered and was not significantly different from either of these. Figure 3b shows the combined summary of coastal Canadian sites and includes selected populations. Differences are noted between the Interior (Idaho and North BC at 90 percent) and the North Cascades at 82 percent. Selected populations also showed up well. The *Dorena MGR* at 48 percent had a significantly lower incidence of cankers than all other populations except the *Porter families* at 69 percent. The exotic *P. koriensis* had only a 6-percent infection rate. The *Westar families*, representing the selected interior families, were not much better than the Interior unselected and certainly not as good as the North Cascades (Region III). The *Westar families* representing selections from wild stands in the southern interior of BC were not screened.

Heights in the coastal Canadian trials showed the same trends as at Whidbey Island with the Idaho Region having some of the greatest growth, but unlike Whidbey these regional differences were not significant (table 5). However, if the variation in provenance samples within regions is investigated more closely, some significant trends did emerge. If we split off the Northern Rocky Mountain BC samples from the Southern Interior (I-b), it shows significantly poorer growth than Idaho, Southern Interior BC, and the local Coastal population (II-a). These are 18 parent trees sampled from areas such as Mount Revelstoke and Valemount at the very northern edge of WWP's inland distribution (e.g., trial site 8, fig. 1). It would indicate that although this broadly defined northern population (Regions I–III) is relatively homogeneous, some differentiation is occurring at the species' edge. Another feature on the more detailed redefinition of regions is the significant and superior growth of the selected *Porter families*. If this group is taken out of the Coastal high elevation group (II-b) and treated separately, it shows a marked effect. Individuals in the *Dorena MGR* seedlot (Region IV) as at Whidbey Island were significantly smaller at the Ladysmith site; but on the heavily rusted Sechelt site they performed well, as one of the few healthy populations on this site.

Blister Rust and Height on the Canadian Interior Sites

Among the three Canadian trial sites in Interior BC, Nakusp, a moderate productivity site in the Southern Interior, had just 35-percent overall infection. Barrière in the Thompson River Valley was a more northern and severe site but with only 16-percent overall infection. The Valemount site was more than 75 percent overall infected and located at the northern edge of WWP distribution. The regions (without any of the selected populations) were nonsignificant at all sites, but most discriminating at the Valemount site. The same trend as the coast was shown: interior populations (Region I) had a higher incidence of cankers than the low elevation Coastal (Region II-a) or North Cascades (Region III) populations (table 6).

This trend of regional differences is shown graphically for the Valemount site in figure 4, with the lower canker incidence of North Cascades and low elevation Coastal

Table 5—Results of the mixed-effects model for heights in decimeters for the Coastal Canadian sites.

Trait	**Ladysmith**		**Sechelt**	
Random effect	**variance**	***P*** [b]	**variance**	***P*** [b]
Block	7607	0.0005	1760	0.0128
Population(Region)	NE[a]		NE	
Tree (Seedlot Region)	36299	<.0001	32446	<.0001
Fixed effect	**DF**	***P*** [c]	**DF**	***P*** [c]
Region	5, 2900	0.2084	5, 1048	0.1583
Region (with selected)	7, 2900	<.0001	6, 1048	0.0077
Region level	**Least squares mean**	**Confidence limits**	**Least squares mean**	**Confidence limits**
I-a Inland Empire	63.09	58.82, 67.35	45.42	42.25, 48.60
I-b BC Interior - Westar	62.84	59.05, 66.63	45.74	43.29, 48.18
I-b BC Interior - Northern	58.22	53.54, 62.90	38.03	34.11, 41.95
II-a - Coastal	62.71	NE, NE	44.65	40.54, 48.75
II-b - Coastal high elevation	58.51	NE, NE	45.64	41.29, 49.84
II-a&b Coastal Porter	67.14	64.25, 70.03	47.93	42.28, 53.58
III N - N. Cascades	59.58	55.00, 64.15	41.51	37.52, 45.51
IV Dorena MGR	56.80	51.04, 62.55	44.67	36.28, 53.06
Comparison of regions	**Difference in least squares**	***P*** [d]	**Difference in least squares**	***P*** [d]
Local (II-a) vs Idaho	0	0.8341	0.7	0.7323
Local (II-a) vs N Interior	**4.5**	**0.0287**	**6.6**	**0.0097**
Local vs coastal high (II-b)	2.4	0.0776	0	0.7340
Local vs N. Cascades (III).	2.5	0.1160	3.1	0.2255
Local vs S. Cascades (IV).	**3.6**	**0.0276**	0	0.9957

[a] NE – Not estimable

[b] *P*-value for testing whether each variance component is zero is derived from a mixture of χ^2 distributions.

[c] *P*-value for testing whether all Fixed Region effects are zero is derived from an *F* distribution.

[d] *P*-value for testing whether each difference is zero is derived from a *t* distribution.

Table 6—Results of the analysis for canker frequencies (incidence) at the Canadian sites in Interior BC.

Trait	Nakusp		Barrière		Valemount	
Random effect	**variance**	**S.E.**	**variance**	**S.E.**	**variance**	**S.E.**
Block	0.0349	0.0366	0.0793	0.05692	0.2553	0.0916
Population (Region)	0.1190	0.07049	0.1600	0.0646	0.6147	0.1511
Fixed effect	**DF**	**P^a**	**DF**	**P^a**	**DF**	**P^a**
Region (only unselected)	5,25	0.0866	4,31	0.4395	4, 38	0.0852
Region (with selected)	9,92	<.0001	7,63	0.0738	8, 56	**0.0007**
Region level	**Estimated canker incidence**	**Confidence limits**	**Estimated canker incidence**	**Confidence limits**	**Estimated canker incidence**	**Confidence limits**
I-a Inland Empire	0.456	0.355, 0.561	0.186	0.134, 0.253	0.809	0.661, 0.902
I-b BC Interior	0.444	0.372, 0.520	0.198	0.152, 0.255	0.858	0.782, 0.911
Westar families	0.39	0.30, 0.50	0.127	0.094, 0.170	**0.713**	0.593, 0.809
II-a - Coastal	0.425	0.333, 0.522	0.203	0.147, 0.274	**0.662**	0.549, 0.759
Porter families	**0.181**	0.123, 0.258	**0.076**	0.033, 0.165	**0.451**	0.252, 0.667
II-b… high elevation	0.333	0.240, 0.440	0.151	0.099, 0.224	0.827	0.715, 0.902
III N. Cascades	0.338	0.229, 0.467	0.137	0.087, 0.210	**0.736**	0.510, 0.882
IV S. Cascades (Dorena)	0.190	0.080, 0.386	0.049	0.015, 0.152	**0.297**	0.074, 0.692
Comparison of Regions	**Difference in canker incidence**	**P^b**	**Difference in canker incidence**	**P^b**	**Difference in canker Incidence**	**P^b**
Interior vs. *Westar. Families*	0.06	0.0015	0.06	0.0309	0.145	**0.0121**
Interior vs. Coastal unselect	0.12	0.1271	0.04	0.2215	0.196	**0.0043**
Interior vs. Porter families	0.27	**<0.0001**	0.10	0.0891	0.407	**0.0001**
Interior vs. Dorena	0.25	**0.0275**	0.12	0.0115	0.561	**0.0019**

[a] *P*-value for testing whether all Provenance effects are zero is derived from an *F* distribution.
[b] *P*-value for testing whether each difference is zero is derived from a *t* distribution.

populations (70 percent) and the marked and significant effect in the selected populations—particularly the greater survival shown by the Region IV *Dorena MGR* population (30 percent), the *Porter families* selections (II-Coast select; 45 percent), and even the Westar compared to unselected Interior populations (70 vs. 85 percent).

Regional differences in height were nonsignificant at the moderate Nakusp site as they were on the coastal sites (table 7). But in the more northern sites the *Dorena MGR* seedlot begins to suffer in performance and at the Valemount site even the coastal low elevation sources (IIa) begin to suffer (5.1 m vs. 4.3 m (Pr > |t| <0.0001). A later walk-through of the Valemount site at 20 years showed the *Dorena MGR* seedlots still alive, but starting to be overgrown by the remaining white pine survivors.

Blister Rust and Height on the Lost Valley and Priest River, Idaho Sites

The Lost Valley site near McCall, Idaho was similar in scope to the Lone Lake site on Whidbey Island; it had the complete Steinhoff collection, good growth, and high overall survival but a high (85 percent) rust incidence measured at 21 years. Although outside the normal range of WWP, this area had one of the highest incidences of rust among the sites we investigated. The Priest River Experimental Forest trial site lost two full blocks due to early plantation failure; thus, only the one surviving block was assessed. The Priest River site was a low rust environment with just over 20 percent rust incidence overall.

Overall regional differences in canker frequency were nonsignificant in the highly rust-infected Lost Valley site (table 8). But contrasts in the Priest River site, and the overall analysis, picked up the same results as in the Whidbey Island and the Canadian trials. Unselected white pines in Idaho (Region I) had overall higher susceptibility than the unselected Northern Cascades (Region III) (79 percent vs. 49 percent cankered) (Pr > |t| 0.0092 in the combined analysis, table 8).

The Lost Valley and Priest River Planting 21-year data were also included in a separate analysis (McDonald, unpublished data, on file at USDA FS, Moscow

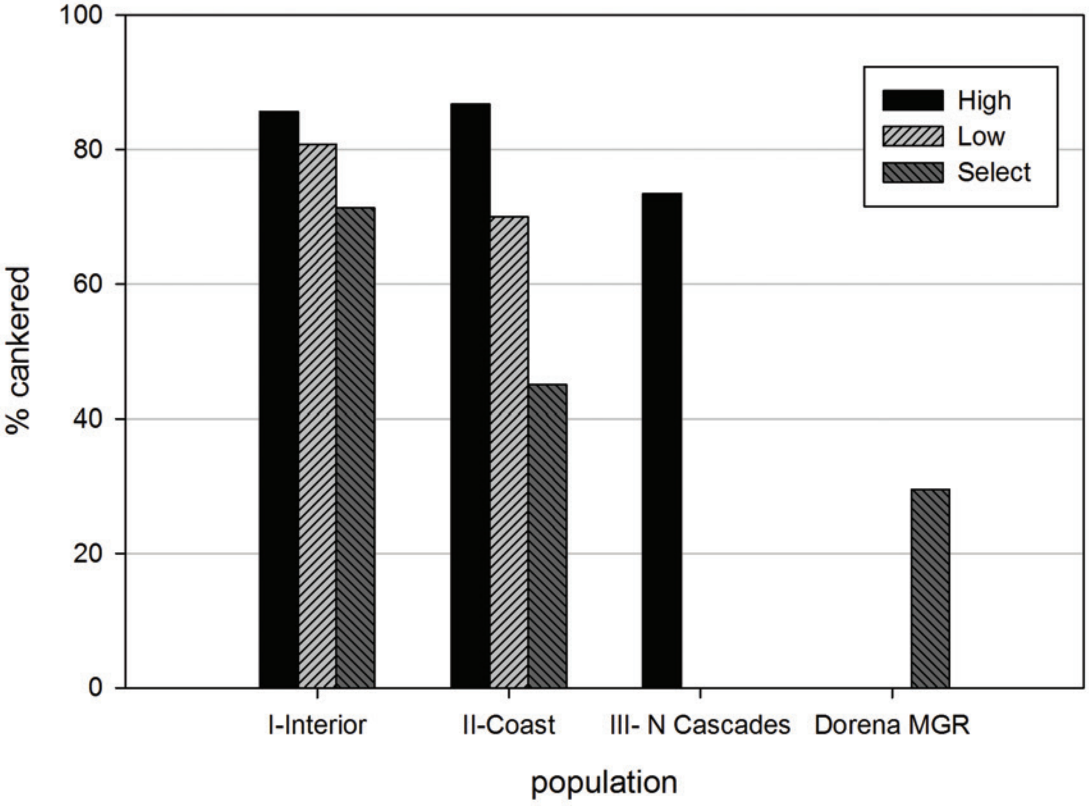

Figure 4—Canker frequencies at the Valemount site.

Forestry Sciences Laboratory, Moscow, Idaho). Seedlots in this analysis were portioned in a slightly different manner based on the separations shown in Kim et al. (2010: fig. 4) and data from an unpublished nursery and greenhouse experiment conducted at the Moscow Forestry Sciences Laboratory. Results suggest some small adjustments to the boundaries shown in figure 1 of this paper. These data suggest that the Region II and Region III boundary could be shifted to the Columbia River along the Oregon-Washington boundary. Other subdivisions concurrent with the present-day distribution of WWP are also noted: a North/South subdivision that follows the Bitteroot Divide in the United States and the Columbia River (Arrow Lakes) in Canada. Although this still may be considered a rather homogenous population, differences between the northern populations of Regions I–III are starting to emerge as was shown in the Canadian Northern Interior sites (Valemount). Molecular marker data suggest that a single refugia event may be an oversimplification for the northern Rocky Mountains.

DISCUSSION OF RESULTS

A result that is consistent but varying in magnitude indicates that in the random population selections, as in the Steinhoff collection, Region II (North Central Cascades) and III (low elevation Coastal) can have as much as one-half the level of infections compared to the Interior Region I samples or the higher elevation Region II (II-b in figure 1). Possible explanations include:

1. The effects of natural selection since the WPBR introduction to the Port of Vancouver in the early 20th century. The spread has been primarily south and west, so it is not surprising to see the Northern Cascades and low elevation North Coastal areas displaying higher native levels of resistance.

2. Details of mortality from WPBR are not shown here, but as noted in the Introduction, Hunt (2004) has suggested that some of the resistance responses valuable in the Interior (e.g., needle

Table 7—Results for height measurements for the Interior Canadian Sites.

Trait	**Nakusp**		**Barrière**		**Valemount**	
Random effect	**variance**	***P*** [a]	**variance**	***P*** [a]	**variance**	***P*** [a]
Block	21.0	0.0006	57.1	0.0005	30.8	0.0008
Seedlot(Region)	20.3	< 0.0001	0	n/a	9.09	0.0033
Tree * Seedlot (Region)	91.05	< 0.0001	156.68	< 0.0001	208.86	< 0.0001
Fixed effect	**DF**	***P*** [b]	**DF**	***P*** [b]	**DF**	***P*** [b]
Region	7,91	<0.0001	6,160	<0.0001	6, 47	<0.0001
Region level	**Least squares mean**	**Confidence limits**	**Least squares mean**	**Confidence limits**	**Least squares mean**	**Confidence limits**
I-a Inland Empire	45.7	41.1, 50.3	52.4	48.9, 55.8	48.6	44.7, 52.5
I-b BC Interior - Westar	**48.0**	45.5, 50.5	52.9	49.6, 56.2	51.5	48.5, 54.6
I-b BC Interior - Northern	44.9	41.7, 48.0	**49.5**	45.9, 53.0	**50.5**	47.2, 53.8
II-a - Coastal	47.5	44.5, 50.4	49.4	46.0, 52.8	**42.9**	39.8, 46.1
II-b… high elevation	45.6	42.0, 49.3	48.2	44.4, 52.0	47.1	43.1, 51.0
III N - N. Cascades	45.5	39.8, 51.3	49.4	45.7, 53.0	46.6	41.8, 51.3
IV S. Cascades (Dorena)	44.4	34.7, 54.0	46.1	41.7, 50.6	**35.9**	28.4, 43.5
Comparison of regions	**Difference in least squares**	**P** [c]	**Difference in least squares**	**P** [c]	**Difference in least squares**	**P** [c]
local vs. other interior	3.1	0.0475	3.4	0.0018	1.9	0.1316
local vs. coastal (II-a)	0.5	0.7095	0.1	0.9563	**7.5**	<0.0001
local vs. coastal high (II-b)	2.4	0.1904	1.3	0.3780	3.4	0.0998
local vs. N. Cascades (III).	2.5	0.3949	0.1	0.9454	3.9	0.1104
local vs. S. Cascades (IV).	3.6	0.4548	3.3	0.0777	14.6	0.0005

[a] *P*-value for testing whether each variance component is zero is derived from a mixture of χ^2 distributions

[b] *P*-value for testing whether all Fixed Region effects are zero is derived from an *F* distribution.

[c] *P*-value for testing whether each difference is zero is derived from a *t* distribution.

shed) that do well in the shorter growing season of the Interior may do less well in the longer growing season of the coast. We found the greatest discrepancy in mortality in the coastal sites.

3. Various constitutive defenses valuable against WPBR, but evolved against other unknown pathogens or pests, might have arisen faster in these more resistant populations after the postglacial northward migration.

4. The evolutionary history of the northern populations appears to be more complex than a single introduction, and a great deal more variability might exist (see following).

Besides the regional difference within the northern population for rust resistance, we also note that the northern population did not perform uniformly well for growth and survival across all sites. Samples from Southern Interior BC and Northern U.S. Rockies (Idaho I-a in tables) performed particularly well for growth across all sites—at the very favorable sites of Whidbey Island and Ladysmith (VI)—nearly 10 percent higher than the local populations. However, the Northern Canadian Rockies samples (I-b) (from the far north of its range) do poorly for growth on coastal sites, and Coastal populations have poor growth in the Northern Canadian Rockies. Kim et al. (2010) suggest that the glacial refugia in the Rockies and subsequent recolonizations might be more complex than a simple founder effect and that genotypic diversity is more likely to arise at the fringes of the range for a species (Kim et al. 2010; Petit et al. 2003) and McDonald (unpublished data, on file at USDA FS, Moscow Forestry Sciences Laboratory, Moscow, Idaho).

Table 8—Results of the GLIMMIX analyses for canker frequencies (incidence) at the Idaho sites.

Trait	Lost Valley		Priest River		Combined Idaho	
Random effect	**variance**	**S.E.**	**variance**	**S.E.**	**variance**	**S.E.**
Population (Region)	1.3839	0.4914	0.4442	0.3457	0.7646	0.2715
Block * Region					0	.
Fixed effect	**DF**	***P*** [a]	**DF**	***P*** [a]	**DF**	***P*** [a]
Region	5,52	0.6577	5,56	0.3457	5,53	0.1226
Region level	**Estimated canker incidence**	**Confidence limits**	**Estimated canker incidence**	**Confidence limits**	**Estimated canker incidence**	**Confidence limits**
I N - Interior	0.91	0.78, 0.97	0.24	0.16, 0.35	**0.79**	0.21, 0.98
II N - Coastal	0.90	0.73, 0.97	0.21	0.085, 0.43	0.78	0.21, 0.98
III N - N. Cascades	0.76	0.44, 0.93	0.03	0.004, 0.21	**0.49**	0.069, 0.92
IV T - S. Cascades	0.84	0.56, 0.96	0.22	0.094, 0.43	0.71	0.16, 0.97
V T - Siskiyou	0.92	0.39, 0.99	0.31	0.053, 0.79	0.83	0.24, 0.99
VI S – Sierra	0.89	0.69, 0.97	0.40	0.18, 0.68	0.83	0.28, 0.98
Comparison of regions	**Difference in canker incidence**	***P*** [b]	**Difference in canker incidence**	***P*** [b]	**Difference in canker incidence**	***P*** [b]
Interior vs. Coastal	0.01	0.8902	0.03	0.7795	0.10	0.8247
Interior vs. N. Cascades	0.14	0.1079	0.20	**0.0378**	0.30	**0.0092**

[a] *P*-value for testing whether all Provenance effects are zero is derived from an *F* distribution.
[b] *P*-value for testing whether each difference is zero is derived from a *t* distribution.

The results also show that early first generation phenotypic selection programs have been very effective against WPBR. The *Porter families* selected and screened in the 1950s (King and Hunt 2004) proved particularly effective as a group. This adds to the evidence from the unselected populations that low frequency, probably constitutive defenses, exist for resistance to this disease beyond just the MGR expression of Cr2. The value of ongoing selective breeding to take advantage of the inherent resistance found in the North American white pine and accelerate the natural selection process is well documented (King et al. 2010; Sniezko et al. 2014). In the most heavily infected sites (Sechelt at 90 percent and Valemount at 80 percent) the MGR seedlot from the Dorena program appeared the only effective resistant lots although many non-MGR trees still survived in later surveys of these severe sites with both active and non-active cankers. More recent trials that include seedlots with known partial resistance have shown good field resistance in very severely infected sites and in artificial inoculation trials (Sniezko et al. 2014; Ukrainetz and King, poster presented at IUFRO meeting, March 2016, Rotorua, New Zealand). In the long term this approach is likely to be more successful for stable resistance.

The existence of this genetic variation to WPBR is the foundation for the breeding work with western white pine. This paper shows that at least low-to-moderate differences exist in the level of resistance between geographic populations of WWP, with perhaps the highest incidence of resistance (but still relatively low) in the North Cascades populations. Previous work has shown geographic variation in one type of resistance, MGR (Kinloch et al. 2003). It also suggests that the efficacy of resistance might vary by site conditions (coastal or interior, high or low elevation) (Hunt 2004), and effectiveness under a changing climate will need to be monitored. Resistance programs seek to capture this resistance, and they are well underway in much of the range of WWP (King and Hunt 2004; McDonald et al. 2004; Sniezko et al. 2014). A new series of trials involving many of the best resistant seedlots known (from BC, Idaho, Oregon, and Washington) is being established in Oregon, Washington, and BC in 2014 and 2015 to further examine the efficacy of resistance from different geographic sources.

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Whitebark Pine (*Pinus albicaulis*) Field Screening for Blister Rust Resistance in British Columbia: Germination Results

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Abstract—Five hundred whitebark pine (*Pinus albicaulis*) parent trees from 80 populations were collected from throughout the range of whitebark pine, and are to be deployed in long-term field tests to screen for resistance to blister rust. The objective is to identify individuals to provide resistant seed for restoration of the species now undergoing catastrophic population declines throughout most of British Columbia (BC), Canada. Two series of four test sites, each with half of the open-pollinated families, are to be deployed across the range in BC with the first series to be established in 2015 and the second 2 years later. Survival and effects of rust will be assessed periodically for seedlings in the field, and total height will be measured. Along with resistance, it is anticipated that facets of genetic architecture, safe seed transfer distances, and likely impacts of climate change will be determined. To date, the first series has been sown and germination data collected. Stratification and nursery methods are discussed in light of the figures, in particular variation in germination based on seed source, consequences of storage and scarification, and efficacy of x-raying seed to determine viability.

INTRODUCTION

Although work with whitebark pine (*Pinus albicaulis*) has been underway for decades in British Columbia (BC), activities have increased in light of its classification as endangered in 2012 under the Government of Canada Species at Risk Act (SARA). This is in response to the report by the Committee on the Status of Endangered Wildlife in Canada (2010), in which it was concluded that the species would be reduced to a quarter of its range if remedial action were not taken. Prior to that there had been reports by forest pathologists (Campbell and Antos 2000; Zeglen 2002) documenting the results of province-wide surveys attributing catastrophic population declines largely to white pine blister rust (caused by the fungal pathogen *Cronartium ribicola*) and to a lesser extent mountain pine beetle (*Dendroctonus ponderosae*). From forest geneticists there had been studies suggesting how to ameliorate these challenges for whitebark (Bower and Aitken 2008; Krakowski and Kolotelo 2009) given success with related species (King 2010). Seed collections had been initiated building an ex situ reserve at the BC Ministry of Forests and Range Tree Seed Centre, and a limited number of individual tree seed lots had been sent for rust resistance screening at the U.S. Department of Agriculture, Forest Service (USDA FS), Dorena Genetic Resource Center in Cottage Grove, Oregon (USA).

As a consequence of the listing of whitebark pine in Canada, a recovery plan must be filed for Federal lands. Though much of the habitat in Canada is under provincial jurisdiction, language within SARA specifies that the Federal action must be supported. As a result, activities have become more organized and energized, and goals have been set. One of the goals is

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to identify blister rust-resistant trees and seed sources for use in restoration. To this end a proposal for establishment of two series of provenance trials with family structure by the provincial forest service was approved for funding. The first set of field tests, which will also elucidate genetic architecture, inform seed transfer guidelines, and ascertain long-term durability of the genotypes, was sown in early 2014. A second rust resistance screening proposal with controlled inoculation of 120 different families to be held in nursery beds was subsequently also approved (Murray and Berger, *Blister Rust Inoculation Trials for Whitebark Pine (Pinus albicaulis) in the Canadian Kootenay Region*, this proceedings). A description of the field testing project follows, along with some details of methods used and germination results.

Producing propagules effectively is of considerable consequence to the testing process. As noted by Leslie and Wilson (2011), sound whitebark pine seed is difficult to obtain because of erratic cone production, predation, and unpredictable seed development. It has been suggested that delayed seed germination as a result of underdeveloped embryos at the time of dispersal may be an ecological strategy that is a product of selection (Tomback et al. 2001). Complex dormancy is also an issue, and low germination rates are noted often (Arno and Hoff 1990; McCaughey and Schmidt 1990). These obstacles have led to varied and involved seed stratification methods, though seed are generally brought out of cold storage, x-rayed to determine their stage of development, dipped in fungicidal baths, soaked for several days in running water (Mahalovich et al. 2006), stratified for several months often at different temperatures (Burr et al. 2001), and then scarified (Leslie and Wilson 2011; Pitel and Wang 1990) before sowing into planting containers or germination trays.

Stratification of the whitebark seed for the field trials at the BC Ministry of Forests, Lands and Natural Resources Operations Kalamalka Forestry Centre nursery in Vernon follows a method for Siberian pine (*P. sibirica*) that had been in circulation at the facility for more than a decade, but is of unknown origin. Our investigations into refining protocols for growing whitebark pine started in 2012 with comparison of germination of scarified and unscarified seed of five trees (table 1). The practice of seed nicking prescribed by our approach was evaluated by staff (V. Berger, 2013, unpublished data, on file at Kalamalka Forestry Centre, Vernon, BC) and it was found that nicking improved germination by about 20 percent versus un-nicked seed. In another facet of the nursery trials, it was found that if care was taken to remove empty or rotten seeds when nicking, the germinants relative to the number of sown seed had a germination percent as much as 13 percent higher than for germinants relative to all seed put into stratification (V. Berger, 2013, unpublished data on file at Kalamalka Forestry Centre, Vernon, BC). This cull of bad seeds reduced nursery costs. The present study provides germination results for the stratification process used at Vernon.

METHODS

Seed Sources

Five hundred families will be screened across two series of field trials, the first of which is described here. A total of 257 wind-pollinated families from 46 populations went into stratification in mid-November 2013, of which 156 trees were from BC; 12 from across USDA FS Region 1; 8 from USDA FS Region 4; and 81 from USDA FS Region 6. Seed x-ray and germination data for the Region 6 families from earlier work were made available (R. Sniezko, 2012, unpublished data on file at the Dorena Genetic Resource Center, Cottage Grove, Oregon). Of the BC trees, 50 were from the Chilcotin, 44 from the Kootenays, 28 from the Coast-Interior Transition, 22 from the Interior North, and 12 from the Rocky Mountains. Survival, height, and health of the trees will be recorded in the nursery and then in the field, but only germination data are described here.

Stratification

Seed was stored at below-freezing temperatures for as long as 10 years, then x-rayed before use to estimate the numbers needed to produce the required seedlings. The seed was soaked in a 3 percent solution of hydrogen peroxide for no longer than an hour, though as little as 15 minutes. Seed were rinsed in running water to remove residue of the previous treatment and put into mesh bags. These were then placed in a bucket with running water allowed to gently flow over them. Aeration was enhanced with an aquarium pump (fish

Table 1—Comparison of percent germination of scarified and un-scarified seed.

Tree	Nicked seed	Discarded nicked seed	Seed not nicked	Germinated nicked seed	Germinated not nicked seed	Germinated all nicked seed (%)	Germinated of sound nicked seed (%)	Germinated not nicked seed (%)
1401 Moyie	165	13	185	103	88	62	68	48
1413 Baldy	169	5	179	129	94	76	79	53
1420 Baldy	179	10	171	99	47	55	59	27
1304 Panorama	191	6	164	140	112	73	76	68
1404 Puddingburn	172	15	167	128	96	74	82	57
					Average	68	73	51

Figure 1—Whitebark seeds soaking in running water with extra aeration.

tank oxygenator) and the bath continued for about 48 hours (fig. 1). Mesh bags were then placed as flat as possible on sterilized sand in plastic totes in a manner intended to maximize contact with the sand.

Sand is sterilized before use by adding enough water to make it moist, but not runny, covered with aluminum foil, then baked in an oven preheated to 94 °C until the center of the sand reaches 82 °C. Baking was continued for 30 minutes, with care not to overheat the sand. The first layer of bags was covered with more sand and a second layer was then laid out. Up to four layers can be placed in a 20-cm-deep tote (fig. 2). A lid was put on the tote at an angle such that there is air exchange, but moisture will not escape quickly. The sand was periodically checked so that it remained moist, but water did not sit in the bottom. The totes were kept at room temperature for a month at 15 to 20 °C, and then at 2 to 4 °C for 3 months.

Seed that germinated early are removed and placed in the growing containers; otherwise the mesh bags are removed and the seed scarified after cool stratification. Scarification entails cutting 1 to 3 mm from the radicle end of the seed with a razor or scalpel such that a bit of white is showing. The opening allows the opportunity to detect empty or rotten seed and discard them.

The seed for each open-pollinated family was sown into two 412A (short 77) Styroblocks™ (Beaver Plastics, Acheson, Alberta, Canada), which have a volume of 125 ml per cavity, with the intent of raising them in the nursery for two growing seasons. The blocks were single or double sown based on the proportion of good seed as determined by X rays. The growing medium was exclusively long-strand peat (premium grade) to assure ample air space to encourage root development. The seed was placed in the cavity and covered with forestry grit. The Styroblocks were misted and watered so that seeds were in constant contact with moisture. Upon germination, if there were two plants per cavity, one was transplanted to an empty cell. This was done before secondary roots appeared, at which point the operation is easy and successful. It would be possible to upgrade seed to remove more empty ones before sowing, or germinate them in a tray and then transplant to each cell of a Styroblock, but the approach described had financial advantages.

Data Collection and Analysis

Seeds were counted by tree (open-pollinated family) before stratification, and numbers discarded during scarification were recorded. The good seeds were sown and germinants counted on the 16th, 30th, and 49th day following. Seed was considered germinated when the radicle had achieved a length equivalent to the length of the seed. Seeds that did not germinate were sampled to determine if they were defective in order

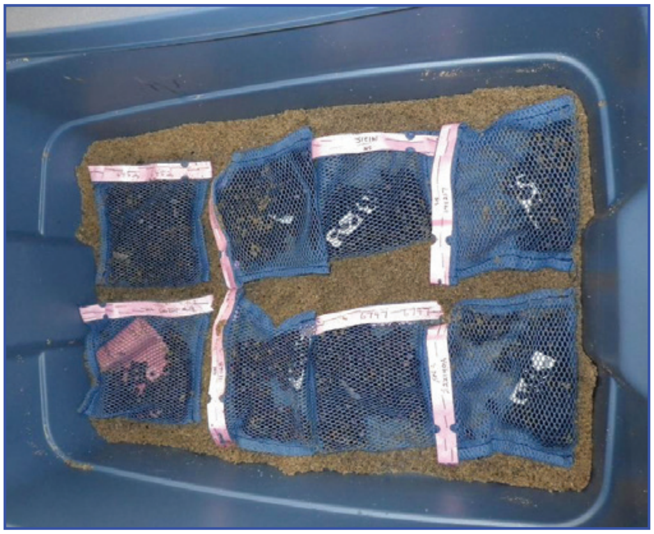

Figure 2—Mesh bags of seed to be buried in sterilized sand.

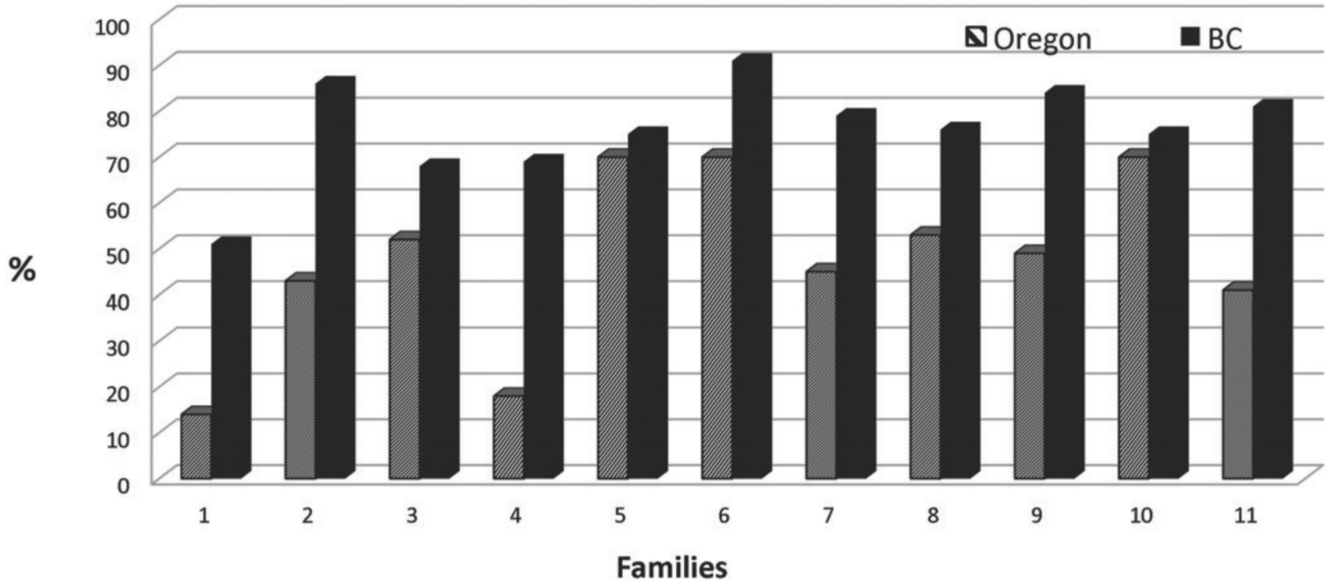

Figure 3—Percent germination of representative families (from Region 6) tested at nurseries in both Oregon and British Columbia.

to help account for germination rates. Descriptive statistics and Pearson correlation coefficients were determined by using SAS® software (Ver. 9.3, 2010; SAS Institute Inc., Cary, North Carolina, USA).

RESULTS AND DISCUSSION

Germination is described here as the number of germinants relative to the number of seed sown rather than compared to the number of seed before nicking. The average germination across all families was 49 percent, but 47 percent if it is calculated relative to the number of seed put into stratification. In previous sowings, the difference between these rates has been as much as 13 percent (V. Berger, 2013, unpublished data on file at Kalamalka Forestry Centre, Vernon, BC), but was accomplished with experienced technical staff. Due to the large quantity of seed (>60,000) to process, they were nicked by a rotating crew of six to eight nursery workers over 5 days. Nicking requires precision and practice to avoid damage and should be done in a warm and unhurried environment by competent staff.

Germination percent can be expected to vary by family, seed source, program, geographic descriptors, climate, and date of collection among other factors. Regionally there were very large differences, with the greatest being between Canadian (35 percent germination) and American (78 percent germination) sources. Differences among the United States regions were less consequential, ranging from 73 percent to 81 percent. Provenance-level variation was particularly pronounced for the Canadian populations and ranged from several sources with few germinants to some with more than 90 percent germination. Regionally the Kootenays had the best average germination at 43 percent, with the South Coast–Interior Transition sites the worst at 23 percent.

Family-by-family comparison of the same lots grown in this study and earlier in Oregon revealed a considerable differential. The current sowing averaged 79 percent germinations across the 81 Region 6 families used in common, but the mean for those grown previously in Oregon was 51 percent (fig. 3). It is likely that some features of the current study stratification were more effective. Both approaches used x-rays of the seed, sterilized them, and had stratification length that was similar and at similar temperatures. Features that are not common to both protocols included extra aeration in seed soaking, use of sand versus a naked stratification, and the nicking process.

Variable germination rates for BC regions are difficult to explain due to effects confounded by collection in different years, by different agencies with different storage. The large regional differences suggest that climate was important, but mean annual temperature and mean annual precipitation explained little of the variation in germination (R < 0.1) as was the case for Pearson correlations with latitude, longitude, and elevation. The strongest influence (R > 0.7) was the length of storage, which agrees with the findings of McCaughey and Schmidt (1990), though not with Bower et al. (2011) or Sniezko and Kegley (this proceedings). Seed most likely deteriorated in storage, though lower germination rates may be due also to excessive seed moisture in some lots, which was not consistently monitored in the BC collections in the past.

Random sampling of 300 ungerminated seed after the last assessment of germination revealed 23 percent were morphologically normal and sound but failed to germinate. A further 18 percent of good seed were rotten. Fifteen percent were empty, but were not detected during the nicking process; the same number had a full-length corrosion cavity but no embryo. Fourteen percent had no cavity, and another 3 percent had a short cavity. The remaining seeds were only partially filled. Though the sound seed might eventually have germinated, the rotten well-developed seed is consistent with deterioration in storage.

X-ray images of seed from families that germinated poorly often appeared more promising than the results, which would be consistent with undetected damage from storage or delayed germination. Data for x rays done at three different facilities were available. Those for BC seed originating from the ex situ reserve at the BC Tree Seed Centre showed a good correlation between germination percentage and sound seed as detected by x-ray (R = 0.8). For the seed from Oregon the information was clearly useful, but the images obtained at Kalamalka Forestry Centre were misleading, perhaps due to negative effects of storage.

Results suggest that fresher seed be used when possible and that monitoring seed moisture content should be undertaken before long-term storage. X-raying seed is beneficial, as may be the case for extra aeration during the soaking of seed and stratification in sand. Nicking appears to be effective in increasing germination rates and eliminates some unviable seeds, thereby reducing sowing costs. The process was expensive, accounting for over half of the first-year nursery expense. A more recent study (Robb 2014) suggests that with longer stratification, particularly at room temperature, scarification is not necessary for good germination.

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Monitoring of Blister Rust Resistance, Pathogen Virulence, and Genetic Adaptability of Western White Pine (*Pinus monticola*) at Six Sites in Western Washington, USA

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INTRODUCTION

Western white pine (WWP, *Pinus monticola*) is a wide-ranging, long-lived native conifer species in western North America. It is highly susceptible to the introduced fungal pathogen *Cronartium ribicola*, causative agent of white pine blister rust (WPBR). The impacts of the blister rust have reduced the incidence of WWP in natural ecosystems and have led to reluctance in using the species in both restoration and reforestation. Research and operational programs to develop genetic resistance to WPBR have been ongoing for more than five decades, with regional programs based in Oregon (USA), Idaho (USA), and British Columbia (Canada). Progeny of thousands of parent trees have been screened for rust resistance in short-term artificial inoculation trials, and several types of resistance have been uncovered (Sniezko et al. 2014). Seed orchards have been established using the products of the earlier resistance work, and breeding to increase the level of genetic resistance continues. However, relatively few field trials, and notably multisite field trials with a diverse array of geographic sources and types of resistance, have been established to examine field resistance and durability of resistance (Sniezko et al. 2012).

This series of field trials examines WWP families and orchard seedlots from a wide array of geographic origins (British Columbia, Idaho, Oregon, Washington) at six diverse sites in western Washington (USA). The group of families and orchard seedlots includes materials that should encompass most (if not all) of the known types of resistance to white pine blister rust in WWP, and represents among the best resistant seedlots currently available from the three operational screening programs working to develop resistance to WPBR. In addition to testing the efficacy and durability of the different types of resistance in the field, these sites can help increase our understanding of the dynamics of rust infection over time, refine rust hazard guidelines, and determine whether seedlots from other breeding zones are adapted to sites in western Washington.

OBJECTIVES

The current and future objectives for this study include:

1. Assessing the current rust incidence and impacts (mortality). Establishing baseline for long-term monitoring.

2. Examining growth, vigor, and reproductive status of western white pine. Establishing baseline for long-term monitoring.

3. Assessing impacts of abiotic and biotic agents on WWP seed sources from diverse geographic origins.

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4. Assessing the level and durability of partial resistance over an array of environments.

5. Monitoring durability of major gene resistance (MGR).

6. Providing updates to landowners and aerial surveyors in western Washington on the levels of rust incidence and field resistance currently available.

METHODS

Six 3-ac (1.21-ha) trials were established in 2006 across western Washington. Thirty-six seedlots were planted across the sites (two sites had 30), with 1,269 to 1,365 individual seedlings planted at each site. A randomized complete block design was used, with seven blocks per site and three to seven seedlings per seedlot per block. The seedlots originated from many areas across the region, including the Gifford Pinchot National Forest (NF), Mount Baker-Snoqualmie NF, Olympic NF, Wenatchee NF, and Colville NF in Washington; Mount Hood NF, Umpqua NF, and Confederated Tribes of Warm Springs in Oregon; British Columbia; and the Bingham F2 seed orchard in Idaho.

Incidence and severity assessments of WPBR were made in 2010, 2011, and 2013. During the assessments tree damage, tree vigor, and rust severity, including the number of WPBR cankers, were measured. During the 2013 assessments, tree height and reproductive status were also measured. HOBO® (Onset®, Bourne, Massachusetts, USA) weather stations were installed at each site in 2013 to measure temperature and relative humidity.

RESULTS AND DISCUSSION

In 2013, overall survival—taking into account all mortality agents, including root disease and animal damage—was 90 percent or greater at all sites, even in the known WPBR-susceptible seedlots (fig. 1a). WPBR mortality was generally less than 1 percent across all sites, with the greatest amount of mortality from rust at the Cayuse and Rocky Raccoon sites (fig. 1b). As expected, WPBR infection levels were higher than the mortality, with most seedlots having less than 25 percent of the trees infected, except at Shuwah Jigsaw (fig. 1c). At the Shuwah Jigsaw site, 47 percent of the trees were infected and there was an average of 4.3 infections per tree (fig. 1d). The susceptible control had the highest overall infection (45 percent) across the six sites and was the family with the highest percentage stem infection on four of the six sites, including 98 percent at the Shuwah Jigsaw site (where it averaged more than nine cankers per tree). The low-to-moderate level of rust infection is encouraging, but it is likely due to a combination of genetic resistance and initial low rust hazard. A few trees putatively originating from a parent homozygous for major gene (Cr2) resistance now show cankers, potentially indicating the presence of a low frequency of virulent genotypes (vcr2), or environmental conditions such that the resistance is not 100 percent effective. Further investigations are pending. As with other sites, the level of rust infection and mortality are expected to increase over time (Sniezko et al. 2012), hence the importance of a network of “permanent” field trials such as this to monitor rust infection over time in a changing environment.

Less than 10 percent of the trees on most sites had pollen or cones, but the trees on Shuwah Jigsaw stand out again, as 50 percent of some seedlots had reproductive features (fig. 1e). The height data show that generally the fast growing seedlots at one site are also fast growing at other sites; trees were notably shorter at the Caveman site (fig. 1f). Six similar sites will be established in eastern Washington in 2015 (and one site in western Oregon in December 2014). Future analyses will look at more details of the geographic origin of the parent trees and their performance in western Washington.

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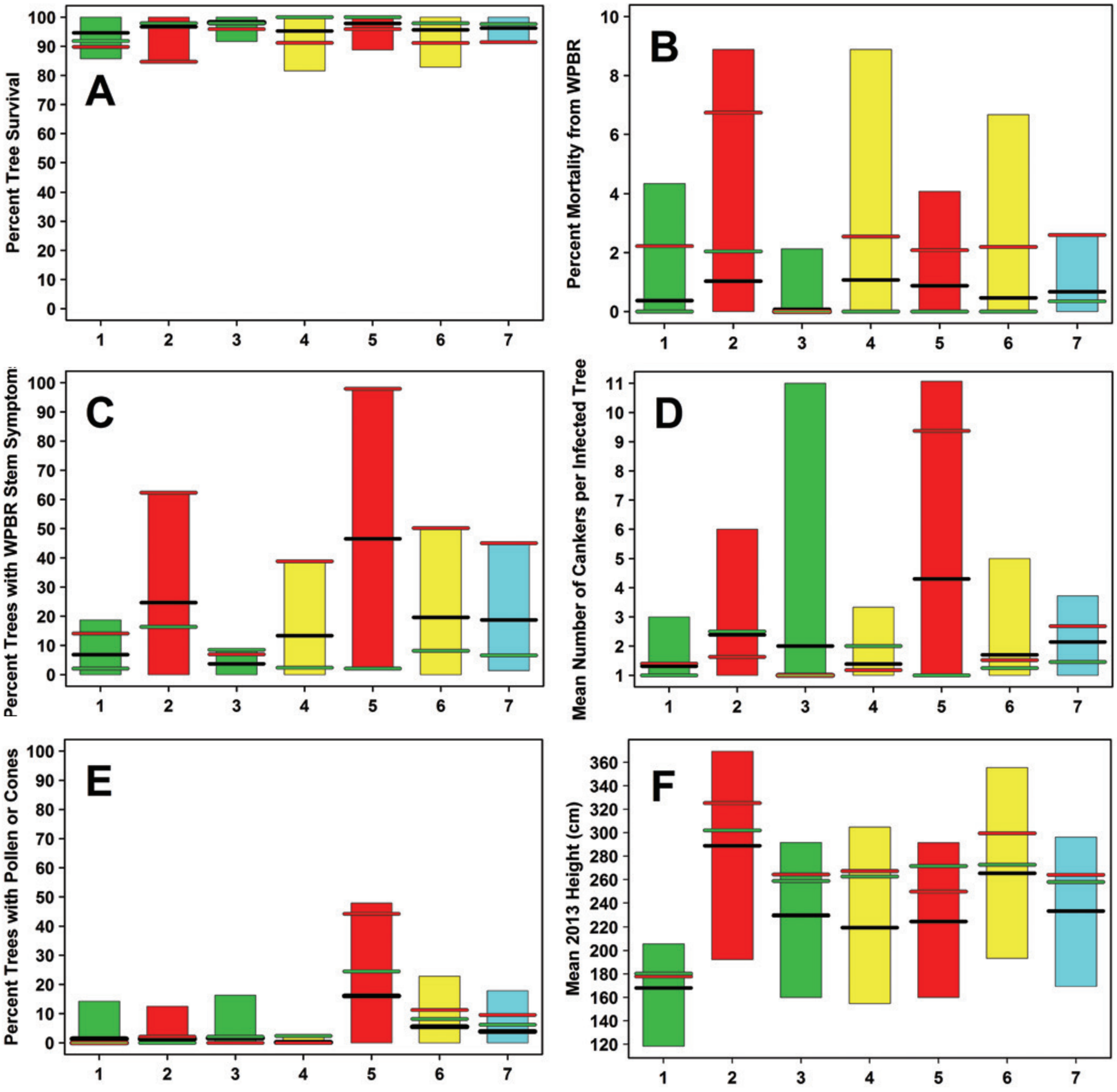

Figure 1—Growth, survival, white pine blister rust (WPBR) impacts and cone and/or pollen reproduction in six western white pine (*P. monticola*) genetic trials through 2013 (sites planted in 2006): (a) percent tree survival by site, (b) percent mortality from WPBR, (c) percent trees with WPBR stem symptoms, (d) mean number of cankers per infected tree, (e) percent trees with pollen or cones, and (f) mean 2013 height (cm). (Note: X-axis is site location/name: 1 = Caveman, 2 = Cayuse, 3 = High Chaparral, 4 = Rocky Raccoon, 5 = Shuwah Jigsaw, 6 = Stevens Left, 7 = Mean performance over all six sites. Each bar spans the range of the seedlot means at each site (30 or 36 seedlots, depending on the site) so that the minimum and maximum for each trait can be compared. The black line is the overall site average. The green line is the seedlot mean for a seedlot known to have Major Gene Resistance. The red line is the seedlot mean for a known susceptible seedlot. Green bars represent the 2 sites with lowest percentage of trees with stem symptoms, red bars for the highest 2 sites and yellow bars for the 2 intermediate sites.)

The Genetic Architecture of Local Adaptation and the Genomic Exploration of Rugged Evolutionary Landscapes Within Species of *Pinus* Subgenus Strobus

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Naturally occurring genetic diversity is the material upon which much of the biodiversity we attribute to forested ecosystems is based. The structuring of genetic diversity within natural populations of forest trees results from the interplay of numerous evolutionary forces, such as genetic drift, migration, and natural selection. Landscape genomics is concerned with testing hypotheses about how these interacting evolutionary forces change over geographical space and time to shape naturally occurring patterns of this diversity. Here, I will use a landscape genomic approach to illustrate how genetic drift, migration, and natural selection interact to determine patterns of genetic diversity, including the genetic diversity underlying fitness-related phenotypes, at multiple spatial scales within natural populations of forest trees.

I will accomplish this task by arguing that populations of sugar pine (*Pinus lambertiana*), western white pine (*P. monticola*), and whitebark pine (*P. albicaulis*) are adapted to local site conditions within the Lake Tahoe Basin of California and Nevada, USA. More specifically, I will test the hypothesis that local adaptation is apparent at local spatial scales (~600 km^2), despite large amounts of pollen-mediated gene flow among local populations. I will also highlight, however, several instances where clearly adaptive patterns of genetic diversity have been influenced by local demographic events. Building upon these results, I will then illustrate how the observed patterns of molecular genetic diversity for a varied set of functional genes are organized relative to the phenotypic variation used to highlight local adaptation. The drivers of these patterns are very likely the multilocus aspects of molecular genetic diversity as it contributes to local adaptation at fine spatial scales. Last, I will draw upon emerging examples from eastern white pine (*P. strobus*) and foxtail pine (*P. balfouriana*) to illustrate the promising role that species of *Pinus* subgenus *Strobus* will play in our understanding of the genetic architecture of local adaptation and the ability of this architecture to rapidly respond to environmental change within natural populations of forest trees.

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The Efficacy of Ex Situ Conservation of the Mexican and Central American White Pines (*Pinus ayacahuite* and *P. chiapensis*) in Developing Countries

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Mexican white pine (*Pinus ayacahuite*) and Central American white pine (*P. chiapensis*) are two of the largest tree species in the highlands of Mexico and Central America. *Pinus ayacahuite* occurs from northern Mexico to central Honduras as three varieties: brachyptera, veitchii, and ayacahuite. Its southernmost variety, *P. ayacahuite* var. *ayacahuite*, is the most threatened of the three in its native environment. *Pinus chiapensis* has a smaller geographic range than *P. ayacahuite*, from central Mexico to western Guatemala. Many of the remnant populations of *P. chiapensis* are endangered throughout its geographic range. Locally, these white pines are used for rustic furniture and woodcarvings.

Over the last three decades, the International Tree Breeding and Conservation Program (Camcore) at North Carolina State University has made small research seed collections of 9,250 mother trees in 380 populations of 25 different pine species in Mexico and Central America for the establishment of field tests and ex situ conservation plantings in Latin America and southern Africa. In its genetic testing and ex situ conservation work of the white pines, Camcore sampled 16 populations and 428 mother trees of *P. ayacahuite* and 16 provenance and 380 trees of *P. chiapensis* in Mexico and Central America. Genetic material was subsequently established in Brazil, Colombia, and South Africa.

The two white pines are some of the most difficult for which to successfully develop ex situ conservation programs because of their strict site requirements and slow initial growth that requires long-term weed control and trial maintenance. However, most detrimental to conservation success is that no markets for white pine wood have been developed in the southern hemisphere to interest the private sector or the small grower to establish commercial plantings of the species, even though Camcore members have seed stands of *P. chiapensis* and second-generation progeny trials available to continue low-level breeding and improvement work. The places where a species like *P. chiapensis* thrives as an exotic are also the same locations best suited for short-rotation species, such as *Eucalyptus grandis* and its hybrids.

This presentation broadly addresses ex situ conservation efforts of the Central American and Mexican pines and highlights the challenges of protecting these species. A case study is provided on how the accidental introduction of a disease (pitch canker, caused by *Fusarium circinatum*) into South Africa has changed the private sector's view of the importance of pine ex situ conservation, the need for having access to alternate species, and the benefits of developing pine hybrids as a defense against future potential threats to exotic plantation forests.

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Forest Health Protection Gene Resource Conservation: A Lasting Legacy

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Abstract—The U.S. Department of Agriculture, Forest Service (USDA FS) has a strong tradition of improving the resilience of forested landscapes. In 2007, a national workshop on genetic conservation classified nine native white pine species as important points for genetic conservation. Added support in 2008 and 2009 from Forest Health Protection and in-kind contributions from other branches of the USDA FS and its partners have facilitated and accelerated several gene conservation actions, including individual tree seed collections of *Pinus albicaulis*, *P. aristata*, *P. balfouriana*, *P. flexilis*, *P. longaeva*, and *P. strobiformis*. Forest Health Protection began a formal strategic approach to gene conservation in 2010. The USDA FS established a Gene Conservation Steering Committee and a charter, which was endorsed in December 2011. The program seeks to conserve tree species at risk from the negative impacts of mountain pine beetle (*Dendroctonus ponderosae*), white pine blister rust (caused by the pathogen *Cronartium ribicola*), and climate change, as well as other disturbance factors. Through this effort, more than 2,200 collections have been stored for conservation, many of which focus on pine. To help assess and prioritize other at-risk forest tree species, the USDA FS has just launched Project CAPTURE (Conservation Assessment and Prioritization of Forest Trees Under Risk of Extirpation). This 3-year effort will assess the vulnerabilities of major forest trees in the United States to help guide not just gene conservation, but also restoration and climate change mitigation actions.

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A Gene Conservation Program to Capture the Genetic Diversity of Rocky Mountain Bristlecone Pine (*Pinus aristata*)

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Abstract—Rocky Mountain bristlecone pine (*Pinus aristata*) has a narrow core geographic and elevational distribution and is threatened by rapid climate change, white pine blister rust (caused by *Cronartium ribicola*), and bark beetles (*Dendroctonus* spp.). The core distribution of *P. aristata* is in central and southern Colorado and extends into northern New Mexico with a disjunct population on the San Francisco Peaks in northern Arizona (USA). The first genetic diversity study of *P. aristata* revealed low species- and population-level genetic diversity (H_e = 0.070 and 0.062, respectively) and moderate among-population differentiation (F_{ST} = 0.131) (Schoettle et al. 2012). Genetic diversity correlated with longitude, latitude, and elevation, and a strong mountain island effect may contribute to substructuring and isolation. The combination of low genetic diversity, moderate population isolation, and a protracted regeneration dynamic puts populations at risk for extirpation by novel stresses (Schoettle et al. 2012), highlighting the need for ex situ gene conservation.

Using the knowledge of the variation and distribution of genetic diversity, we developed and executed an efficient gene conservation sampling design and collection. Ten populations within each of 6 collection areas corresponding to the observed genetic substructuring were identified; 10 individual tree seed collections and a bulk collection (20 seed trees equally represented) were targeted from each of the 60 populations. Seed trees are georeferenced and sampled for seeds and needle tissue; each collection is stored for gene conservation at –18 °C at the U.S. Department of Agriculture (USDA) Agricultural Research Service, National Center for Genetic Resources Preservation lab (Fort Collins, Colorado) with a working collection maintained by the USDA Forest Service, Rocky Mountain Research Station (Fort Collins, Colorado). Ecological data on each population were also recorded (site data including physical characteristics, stand density, species composition, and disturbance history) to complement the genetic collection.

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Germination of Whitebark Pine (*Pinus albicaulis*) and Other High Elevation White Pine Species Seed After Long-Term Storage: Ex Situ Gene Conservation Potential of High Elevation White Pine Species

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Abstract—Genetic variation in a plant species is a key to its ability to survive and evolve in the face of changing environmental pressures. Due to insect and disease impacts, changes in fire regimes, and a changing climate, many populations of high elevation white pine species continue to experience high mortality levels and potentially worrisome decreases in genetic variation. In recent years, some trees rated highly for resistance to the nonnative white pine blister rust (causal agent: *Cronartium ribicola*) have been killed by fire or mountain pine beetle (*Dendroctonus ponderosae*). Ex situ gene conservation offers the possibility to conserve the genetic variation within a species before much of it is lost. For many conifer species, freezer storage of seed offers a relatively inexpensive, long-term method of storing germplasm for potential future use. However, seed of some conifers does not store well for long time periods under currently used freezer technology, and few data are available for many species such as the high elevation white pine species of western North America.

The U.S. Department of Agriculture, Forest Service (USDA FS) and cooperators have embarked on a program to collect and conserve seed of these high elevation white pine species. Most of these seedlots have been collected since 2008. Knowledge of seed viability after one or more decades of storage would be useful for assessing the utility of the freezer storage for these species. A test was begun in 2010 for whitebark pine (*Pinus albicaulis*), limber pine (*P. flexilis*), southwestern white pine (*P. strobiformis*), Rocky Mountain bristlecone pine (*P. aristata*), and foxtail pine (*P. balfouriana*). Some organizations in the United States and Canada had seedlots available that had been in cold storage or freezer storage for 7 to 32 years and made seed available for a germination test at the USDA FS Dorena Genetic Resource Center (Cottage Grove, Oregon, USA). We report here on the results of germination testing of these oldest known seedlots of these five species and the implications for ex situ gene conservation efforts and future testing. Results indicate that at least some seedlots of species such as whitebark pine and foxtail pine can be stored for several decades and show very high germination percentage in subsequent testing.

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Genetic Relationship between Reproductive Morphology and Growth Characteristics of Korean Pine (*Pinus koraiensis*)

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Korean pine (*Pinus koraiensis*) is an important species for timber as well as pine nut production in Korea. The selection of "plus" trees began in 1959 and seed orchards have been established with grafts and seedlings propagated from these selected individuals. There are 300 "plus" trees and 100 ha seed orchards of *P. koraiensis* in Korea. Further, 244 open-pollinated families are being tested at four 18.5-ha sites. The main purposes of this study were: (1) to investigate the correlation among cone, seed, and growth traits of *P. koraiensis*; (2) to examine the possibility that such traits could be useful indicators for progeny testing and further selection; and (3) to provide information on genetic parameters for the establishment of advanced-generation seed orchards.

Open-pollinated cones of *P. koraiensis* plus trees were collected in 1981, and the morphological characteristics of cones and seeds were examined. The seeds were sown in a nursery in 1982 and grown for 4 years. In 1986, the seedlings were planted in three sites containing 20 trees in each of 6 replications with 1.8-m × 1.8-m spacing as a progeny test plantation. The correlations between morphological traits of cones and seeds from each plus tree and growth characteristics (height, diameter at breast height [d.b.h., diameter at 1.37 m] and volume) in plantations were investigated. Variance components and heritabilities for growth characteristics were estimated in the plantation at ages 10, 20, and 30 years.

Seedling height at the nursery bed showed a strong correlation with most cone and seed characteristics. Numbers of cones per tree and seeds per cone were negatively correlated with seedling height and statistically significant at the nursery bed. Cone diameter and cone weight showed a positive correlation with seedling height. Seed length, width, thickness, and weight were all positively correlated with seedling height at the nursery and in the plantation at age 5. From age 10, tree height, d.b.h., and volume were compared with cone and seed traits. None of the cone traits was correlated with growth characteristics from age 10. Some seed traits such as seed length, thickness, and weight were correlated with growth until age 10. After that age, the correlations between seed traits and growth characteristics were also nonsignificant, except between seed length and d.b.h. at age 20. Analyses suggest that the correlation between reproductive morphology and growth characteristics becomes weaker as trees grow older. It seems likely that cone and seed traits of *P. koraiensis*, which bears middle-sized seeds, are related until trees enter the mid-term growth phase.

Heritability estimation showed that growth characteristics were under genetic control. Family heritability was much higher than individual heritability, implying that family selection would be a better option for the establishment of advanced-generation seed orchards of *P. koraiensis*.

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Analysis of Genetic Diversity and Spatial Genetic Structure of the Endangered Dwarf Stone Pine at Mount Seorak by using Nuclear Microsatellite Markers

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Abstract—Dwarf stone pine (*Pinus pumila*), which occurs in northeastern Asia, is found only in a limited area at Daecheongbong (1,550–1,700 m above sea level) on Mount Seorak in South Korea. Dwarf stone pines are distributed over about 10 ha in this location. To establish strategies for adequate conservation and sustainable management of this endangered species, 57 individuals were selected at 12-m intervals in the study site (3.75 ha). Thirty-three nuclear microsatellite (nSSR) primers, developed from *P. koraiensis* and *P. strobus*, were tested and 10 of them revealed successful amplification products. However, only 3 of the 10 primer pairs turned out to be polymorphic. Fourteen alleles were observed in three polymorphic loci. The observed number of alleles per locus ranged from four to five. The observed heterozygosity (0.294) was very low compared to the expected heterozygosity (0.489), which resulted in a high value for the fixation index (0.4). This may have resulted from reproduction of progenies by crossing among genetically closely related individuals. Dwarf stone pines have been maintained as a single isolated relic population for such a long time (at least since the last ice age) that inbreeding among kinship individuals could not be avoided in the Daecheongbong area. This postulation could be supported by the high proportion of monomorphic loci (i.e., 7 of 10 nSSR loci). However, any apparent genetic patches were not observed in a previous study using I–SSR markers for spatial autocorrelation analysis of individuals 12 m apart. Failure to observe any spatial genetic structures at the study site may have resulted from either an inadequate sampling strategy developed for the earlier study or the limited number of nSSR loci analyzed in this study. Further work will be performed at a finer sampling scale (e.g., sampling individuals at 4-m intervals) and with additional polymorphic loci.

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Population and Evolutionary Genetic Studies of Five-Needle Pines in Russia

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Three species of stone pines are native to Russia: Siberian stone pine (*Pinus sibirica*), dwarf Siberian pine (*P. pumila*), and Korean pine (*Pinus koraiensis*). Genetic differentiation by allozyme loci placed *P. sibirica* in one clade with European stone pine (*P. cembra*), whereas the Far Eastern species *P. pumila* and *P. koraiensis* represent another lineage more closely associated with the Asiatic white pines that have winged seeds: *P. armandii*, *P. parviflora*, and *P. wallichiana*. Molecular data on the large divergence of *sibirica–cembra* and *pumila–koraiensis* clades are supported by analysis of needle anatomy. The taxonomic position of the North American stone pine (*P. albicaulis*) is intermediate between the Asiatic stone pines and the North American white pines *P. flexilis*, *P. monticola*, and others.

In the central parts of their ranges, stone pine gene pools are subjected to integrating factors: extensive gene flow and balancing selection in similar environmental conditions. Populations at the margins of their range experience differentiating forces: genetic drift, disruptive selection, and gene exchange with related species. When analyzing natural populations and clonal archives of *P. sibirica* ecotypes, we observed significant correlations between frequencies of particular alleles at allozyme loci and geographic coordinates as well as climatic conditions. Analyses of multilocus genotypes showed good correspondence with recognized forest seed zoning, in many cases refining borders of seed zones, regions, and subregions. The sympatry zone between *P. sibirica* and *P. pumila* is characterized by interspecific gene exchange through hybridization with bidirectional gene introgression of nuclear and mitochondrial markers. More genetically similar *P. pumila* and *P. koraiensis* do not form a hybrid zone. Formation of stone pine lineages and species putatively included multiple events of reticulate evolution. Analysis of allozyme and simple sequence repeat loci has proven that multitrunk forms in *P. sibirica* and clumps in *P. pumila* are different genotypes (but often related as half-sibs) and therefore represent the result of germination of several seeds from the caches of nutcrackers (*Nucifraga* spp.). A substantial level of heterozygote deficiency was recorded in several *P. pumila* stands that were characterized by a low ratio of plant height to the square of a clump. This indicates inbreeding, reflecting a high proportion of selfing and consanguineous mating due to the high probability of sedimentation of the pollen cloud within a clump of genetically related individuals.

In response to global climate change, it is evident that treelines for major stone pine species are shifting to the north on the plain and upslope in subalpine mountain belts. Descriptions of local populations and inventories of genetic resources of stone pines are urgently needed to ensure conservation of among- and within-species genetic diversity. Formation of genetic collections and seed banks should be based on knowledge of the genetic structure of populations at different types of markers (nuclear, cpDNA, mtDNA) and candidate genes responsible for adaptation and economically important traits.

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Using Historical Provenance Test Data to Understand Tree Responses to a Changing Climate in Sugar Pine (*Pinus lambertiana*)

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Climate change is a major concern for forest managers involved in ecological restoration, in part because they are responsible for planting trees that will be components of a healthy and resilient ecosystem for the next 100 to 200 years. Anticipated changes in climate include changes in temperature, precipitation, and length of the growing season. Trees locally adapted to their native climate may not be able to survive or grow in novel climates. Current afforestation strategies may thus require the use of seeds from populations already adapted to future climates.

Researchers can help to identify these populations thanks to provenance tests. Provenance tests are studies where genotypes from different sources (or provenances), coming from the entire range of the species or only a subset, are transplanted onto a common site. In fact, individuals are moved from one climate, are planted in a new one, and can be monitored for survival and growth. Therefore, this experimental design is ideal for understanding how trees respond to novel climates.

In this study we will focus on two sets of sugar pine (*Pinus lambertiana*) provenance tests. The first involves four test sites, two in California (USA) and two in Oregon (USA). Sixty-nine sources, spanning the entire range of this species from Mexico to Oregon, were planted in each of the four sites starting in 1984. The second test, Harrel, has only one planting site; 124 sources of sugar pine were planted on that site in 1992. Using information on where the seeds were collected, we can generate data for a set of climate variables (monthly average, minimum, and maximum temperatures; and monthly total precipitation) using available models.

The aim of our study will be to assess whether the growth and survival of the trees in the transplanted site is influenced by the climate at the home site. Our hypothesis is that trees are better adapted to the climate at the home site and thus, the growth and survival of transplanted trees will be negatively affected by a strong difference in climate between the home and the transplanted sites.

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Genetic Variation in Height and White Pine Blister Rust Resistance in Sugar Pine (*Pinus lambertiana*)—15-Year Field Trial Results of Three Six-Parent Half Diallels From Three Breeding Zones

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Sugar pine (*Pinus lambertiana*) is a long-lived conifer species found in forest ecosystems from southern California to northern Oregon (USA). It is highly susceptible to white pine blister rust (WPBR), caused by the fungal pathogen *Cronartium ribicola*. Two long-term operational programs to develop populations of sugar pine with genetic resistance to WPBR, one in Oregon and one in California, have been ongoing for more than four decades. Most of the resistance work involves artificial inoculation of young seedlings. A small percentage of sugar pine parents have complete resistance (a hypersensitive-like response in the needles in the absence of a virulent strain of the rust) under the control of a single major dominant gene. In addition, there is a low frequency of partial resistance in some sugar pine parents. Partial resistance is thought to be under the control of multiple genes and more durable than the hypersensitive-like needle resistance. Both types of resistance could potentially be combined to increase survival at most field sites. However, detailed field trials to examine partial resistance in sugar pine are relatively rare.

In 1998, a large field trial was established in Happy Camp, California, to examine partial resistance, its inheritance, and its durability. The trial was established with 39 full-sib families, produced from crosses among three six-parent half diallel mating designs (18 parents). The parents of the three diallels, all field selections from the early years of the Oregon program, represented three different breeding zones for sugar pine in southern Oregon. Prior rust resistance screening of seedling progeny showed only low levels of partial resistance (at least for 2-year-old seedlings) in these full-sib families. Post-planting survival was excellent, and height averaged 88, 312, and 537 cm in 2001, 2007, and 2012, respectively. Rust infection (stem cankers) was extremely low until 2007. In 2007, the site averaged 73-percent infection, with full-sib family means ranging from 50 to 96 percent; in 2012, the site averaged 89-percent infection, and family means ranged from 66 to 100 percent. The number of stem infections per tree averaged 4.7, and full-sib family means ranged from 1.9 to 14.3 in 2007; number of stem infections increased to 8.0 in 2012, with family means ranging from 3.2 to 19.1. Mortality from rust was relatively low through 2012, with full-sib family means ranging from 3 to 47 percent. The level of partial resistance exhibited by some sugar pine families is encouraging. The results will be used to help examine genetic differences in growth and rust resistance among the breeding zones, examine inheritance of some components of partial resistance, and provide possible selections for sugar pine seed orchards and for future breeding to increase rust resistance.

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Patterns of Population Structure and Environmental Associations Across the Range of Two Ecologically and Economically Important Five-Needle Pine Species

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Abstract—Under rapid global warming, it is critical for us to better understand the capacity of forest trees to adapt to a changing climate, especially for species that are more at risk such as five-needle pines. Western white pine (*Pinus monticola*) and eastern white pine (*Pinus strobus*) have fragmented ranges and have suffered declines due to harvesting, fire suppression, and the white pine blister rust (causal agent: *Cronartium ribicola*). We identified and compared patterns of genetic diversity and adaptation to climate in these two species by using a set of 267 orthologous genes, including candidate genes for growth, bud phenology, and resistance to biotic and abiotic stresses. Genotyping resulted in 158 and 153 successful single nucleotide polymorphisms (SNPs) for *P. monticola* and *P. strobus*, respectively. Each set of SNPs was genotyped on rangewide samples of 362 *P. monticola* individuals (61 populations) and 840 *P. strobus* individuals (133 populations). Analyses were conducted separately in each of the two species. STRUCTURE analyses identified two genetics clusters in each species, corresponding to north-south genetic discontinuities, as well as significant hierarchical substructure within each of those clusters. The use of F_{ST} outlier analyses revealed that about 6 percent and about 9 percent of SNPs were under selection in *P. monticola* and *P. strobus*, respectively. The use of different methods of analysis to detect environmental associations revealed that about 16 to 22 percent of *P. monticola* SNPs and about 15 to 30 percent of *P. strobus* SNPs were significantly associated with one or more climate variables. We looked for strong candidates by combining the results from all F_{ST} outlier and environmental association analyses. About 4 percent and about 5 percent of the SNPs in *P. monticola* and *P. strobus*, respectively, were detected by at least two different methods, highlighting candidate SNPs for future adaptation studies in western and eastern white pine.

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Population Projections and Resistance Gene Frequencies in the Presence of White Pine Blister Rust: A Population Genetic Model for High Elevation Five-Needle Pines

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The slow growth and long generation time of the five-needle pines have historically enabled these trees to persist on the landscape for centuries, but without sufficient regeneration opportunities these same traits hinder the species' ability to adapt to novel stresses such as the exotic disease white pine blister rust (WPBR). Increasing the frequency of resistance to WPBR is the foundation for options to sustain five-needle pine species in the presence of the pathogen *Cronartium ribicola*. The objective of management intervention in the high elevation ecosystems is to promote pine population resilience for many generations (Schoettle and Sniezko 2007; Schoettle et al. 2012). However, greater understanding of the regeneration cycle and the potential for increasing the frequency of resistance are needed. This is especially critical for the WPBR pathosystem as WPBR kills trees of all ages and therefore affects multiple stages of the regeneration cycle.

We developed a population genetic model, parameterized for high elevation five-needle pines, to improve our understanding of pine population dynamics in the presence of WPBR and identify key processes that affect resistance gene frequencies over time (Field et al. 2012). This model allows us to address questions such as: (1) What frequency of resistance to WPBR is enough to sustain a population under different initial conditions? (2) What regeneration densities are sufficient to promote increased frequency of resistance over time? (3) What is the effect of competition on population sustainability and frequency of resistance over time? (4) During what state of infestation and impact by WPBR is intervention most effective? The matrix model includes six age classes (cohorts): seeds, primary seedlings (1–4 yrs old), secondary seedlings (5–20 yrs), saplings (21–40 yrs), young adults (41–90 yrs), and mature adults (>90 yrs). The model includes nonlinear functions for the effects of competition (leaf area index) on germination and cone production. Population size, incidence of infection by WPBR, and frequency of a simply inherited dominant WPBR resistance gene by age class over time are all included in the model output. This presentation will demonstrate that regeneration is a stabilizing factor in populations and that how and when it is managed, in coordination with gene frequencies for resistance, will play an important role in the establishment of a new equilibrium for a host population and its ecosystems in the presence of a nonnative pest.

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Genome Sequencing in Conifers: Implications for Breeding and Gene Resource Management

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Abstract—The genomes of conifers were only recently sequenced due to their extremely large size and complexity. With the advent of next-generation sequencing technologies and improved sequence assembly methods, the genomes of Norway spruce (*Picea abies*), white spruce (*Picea glauca*), and loblolly pine (*Pinus taeda*) were reported in the last year. These genome sequences begin to reveal the size and complexity of conifer genomes and the path of genome evolution these ancient species have followed. Of more practical value, the genome sequences now provide the necessary "roadmap" to apply modern genomic technologies for future breeding and gene resource applications. In this presentation, I will begin with a description of how the PineRefSeq team generated the genome sequence for loblolly pine, what we have learned about conifer genomes, and how this knowledge is being applied to sequencing of the sugar pine (*Pinus lambertiana*) genome, the first white pine genome to be sequenced.

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Genomic Warfare in the Woods: Scaling Fusiform Rust Resistance From Molecules to Landscapes

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Abstract—Heteroecious rust fungi coevolve with two or more plant host species. The fusiform rust pathogen *Cronartium quercuum* f. sp. f*usiforme* (*Cqf*) incites rust symptoms on leaves of oak species (*Quercus* spp., the telial host) and gall symptoms on stems of pine species (*Pinus* spp., the aecial host). Genome sequencing of *Cqf* and loblolly pine (*P. taeda*) enabled us to identify candidate genes for corresponding avirulence and resistance loci, respectively. Because allelic variation at these interacting loci enables prediction of disease incidence in the field, markers should be useful for understanding co-evolution of pathogen and host species. We also envision the future development of a molecular toolkit for directly assessing pathogen allelic diversity at avirulence loci in *Cqf* spore collections. This information could be used to guide breeding and deployment of corresponding resistance loci in pine plantings.

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Identifying the Genetic Basis of Partial Resistance on Sugar Pine (*Pinus lambertiana*) Under White Pine Blister Rust Infection

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Abstract—Since its arrival in North America in the early 1900s, white pine blister rust (WPBR; causal agent: *Cromartium ribicola*) has infected and threatened natural populations of at least five white pine species. An important step toward learning how to control the rise of diseases is the identification of natural defense mechanisms of hosts. In genetic terms, plant resistance is classified as one of two types: the complete resistance or major gene resistance (monogenic) and the incomplete or partial resistance (polygenic). Both mechanisms have been documented in sugar pine (*Pinus lambertiana*) under WPBR infection, although their genetic basis remains unknown. To identify genes involved in pine partial resistance, a progeny of 1,000 individuals from a controlled pollination cross was exposed to inoculation of WPBR at Happy Camp Outplant Site (Klamath National Forest, California, USA). Phenotypes of young trees under infection were quantified by considering five different symptoms: normal active cankers (N), normal active blights (NBL), normal bark reaction (NBR), blights (BL), and bark reactions (BR). More than 50 percent of trees showed no sign of infection, and the most frequent symptom (in about 30 percent of trees) was the presence of normal active cankers overlapping with other symptoms. There is no statistically significant correlation between occurrences of symptoms, indicating that each symptom could be related with different genetic responses. The 2 parents and 93 individuals from progeny, including healthy and highly infected trees, were selected for genotyping using a 384-single nucleotide polymorphism (SNP) chip (Golden Gate Assay, Illumina®, San Diego, California), covering more than 260 genes of general functions, stress response, and candidates for disease resistance. Deviation from the expected segregation ratio revealed five genes associated with survivorship in early developmental stages. Associations between genetic variation and the five symptoms were detected through distribution of the minor allele frequency in affected cases and the use of full model association tests, to recognize association of genotypes with symptoms. Twenty-seven SNPs in 23 genes were associated with different symptoms. Annotations of these genes indicate similitude to genes previously associated with disease response in angiosperms. Most associations were related with allele frequency, although evidence for heterozygous advantage was found for some SNPs. Associated genes indicate that different responses to infection are related with proteins involved in general defense mechanisms such as signal transduction (protein kinases), inhibition of pathogen growth (proteases), and synthesis of plant antibiotics such as phytoalexins (chalcone synthases). These results provide evidence of the cellular responses of pines to infections and may be evaluated for use in breeding programs based on gene-assisted selection.

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Sequencing the Sugar Pine (*Pinus lambertiana*) Transcriptome

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Abstract—The PineRefSeq project is responsible for genome and transcriptome sequencing of three conifer species. Sugar pine (*Pinus lambertiana*) will be the second pine and largest genome sequenced, with an estimated size of 34 Gb. Few genomic resources currently exist to assist in the assembly and annotation of this large, complex genome. To generate these resources and define the gene space, samples were obtained for 14 different tissue types including those categorized as vegetative, reproductive, and early development. Vegetative organs (needle, stem, and roots) from two individuals were subjected to a variety of abiotic stressors, including temperature stress (both extreme heat and cold), water stress (drought and flooding), and salinity. Additionally, a biotic stress in the form of white pine blister rust (WPBR; causal agent: *Cronartium ribicola*) inoculations was used to generate sequencing libraries from vegetative organs.

Illumina® (San Diego, California) deep sequencing was conducted on all libraries in the form of HiSeq (150 bp PE reads) and MiSeq (300 bp PE reads). To combat existing challenges in transcriptome assembly from short reads, a new strategy known as "Iso-Seq" was applied to six libraries. This methodology uses single molecule technology from PacBio® (Pacific Biosciences of California, Inc., San Mateo, CA) to generate full-length transcripts that can reach 6 Kb in length. De novo assembly of all libraries (Illumina and PacBio) will generate the full reference transcriptome. Error correction provided by the short reads will improve the accuracy of the long transcripts obtained with the Iso-Seq approach. This will be the first attempt to generate full transcripts with next-generation sequencing techniques in conifers. The improved reference will facilitate expression analysis as well as the ability to compare against needle transcriptomes generated in several other white pine species, including whitebark pine (*P. albicaulis*), limber pine (*P. flexilis*), and western white pine (*P. monticola*).

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Using Genomics Tools to Assess the Phylogeography of Whitebark Pine (*Pinus albicaulis*)

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Abstract—The particularly heterogeneous climate, geology, and ecology of northwestern North America have created complex evolutionary histories for species, generally explained by patterns of ancient vicariance or dispersal in combination with more recent colonization from glacial refugia. Studies aiming to resolve the phylogeography of species in this region have been hampered by the limitations of species sampling as well as crude or biased analyses based on few molecular markers. Here, we are resolving these issues by using genomics tools that capture thousands of informative genetic markers, to analyze the geographic and evolutionary history of whitebark pine (*Pinus albicaulis*). Whitebark pine has a relatively unique geographic range that spans the western parts of the United States and Canada, providing the potential to test, within a single and consistent analysis, several alternative phylogeographic hypotheses posed for the region. Using the high-throughput restriction digest-based sequencing method genotype-by-sequencing (GBS), we are assessing the genetic structure across ≥35 whitebark pine populations representing the range of the species. This analysis will aid in the identification of those populations, across the species' geographic distribution, that are genetically unique and yet currently of conservation concern. Implementing species distribution modeling (ecological niche modeling) as an independent phylogeographic assessment, we are also constructing the historical geographic distribution of the species, during several points in time, to infer the influence of previous events on the species' present-day genetic structure. Last, we are combining these phylogeographic data with predictions about the future range of whitebark pine, as it is affected by climate change and white pine blister rust (causal agent: *Cronartium ribicola*), to further identify the most threatened and yet genetically distinct populations across the species' range.

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Targeted Capture Sequencing in Whitebark Pine (*Pinus albicaulis*) Reveals Rangewide Demographic and Adaptive Patterns Despite Challenges of a Large, Repetitive Genome

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Abstract—Whitebark pine (*Pinus albicaulis*) covers an expansive range in western North America, and is considered a keystone species of subalpine environments. Whitebark is susceptible to multiple threats—climate change, white pine blister rust (causal agent: *Cronartium ribicola*), mountain pine beetle (*Dendroctonus ponderosae*), and fire exclusion—and is suffering significant mortality rangewide, prompting the tree to be listed as “endangered” both by the International Union for Conservation of Nature (IUCN) and by the Canadian Government. Conservation collections (in situ and ex situ) are being initiated to preserve the genetic legacy of the species. Reliable, transferrable, and highly variable genetic markers are essential for quantifying the genetic profiles of seed collections relative to natural stands, and ensuring the completeness of conservation collections.

We evaluated the use of hybridization-based capture to enrich targeted genomic regions from the 30+ GB genome of whitebark, and to evaluate genetic variation across loci, trees, and geography. Probes were designed to capture 7,849 distinct genes, and screening was performed on 48 trees. Despite the inclusion of repetitive elements in the probe pool, the resulting dataset provided information on 4,452 genes and 32 percent of targeted positions (528,873 sites), and we were able to identify 12,390 segregating sites from 47 trees. Variation at single nucleotide polymorphism sites reveals strong geographic trends in heterozygosity and allelic richness, with trees from the southern Cascade and Sierra Nevada Ranges showing the greatest distinctiveness and differentiation. Our results show that targeted capture produces high-quality, codominant genotypes from the large genome of whitebark, and the resulting data can be readily integrated into management and gene conservation activities.

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Genetic Resources for Limber Pine (*Pinus flexilis*) and a Survey Focusing on Potentially Adaptive Single Nucleotide Polymorphisms

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Abstract—RNA-sequencing studies of limber pine (*Pinus flexilis*) and Engelmann spruce (*Picea engelmannii*) were conducted to identify single nucleotide polymorphisms (SNPs) in the Front Range of Colorado (USA). Each study was based on nine trees, five above treeline (3,353 m above sea level) on Niwot Ridge, and four trees near the lower limit of the local distribution (2,347 m above sea level), in the hopes of finding genetic markers with elevational clines in frequencies. Each study yielded more than 10,000 highly reliable SNPs. Annotations produced identifications for about 70 percent of the contigs. Comparisons with other conifer species revealed that unidentified sequences had been reported for other species, so although the genes have not been identified, they appear to be functional.

One of our objectives is to identify genes that play a role in site-specific adaptation, especially with respect to elevation. To increase the probability of choosing potentially adaptive genes, we have been screening for SNPs that change both the amino acid and its functional group (polar, nonpolar, acidic, basic); these sorts of changes are most likely to change the kinetic and physiological performance of the protein. Preliminary data will be presented on SNPs that change frequency along an elevational gradient in the Front Range of Colorado.

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Cross-Species Transferability of Microsatellites, and Genetic Diversity in Himalayan Five-Needle Pine (*Pinus wallichaia*) and Chir Pine (*P. roxburghii*) Forests

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Abstract—Pine forests in India are found in inland forests at elevations ranging between 400 and 2,500 m above sea level. Five species indigenous to India are chir pine (*Pinus roxburghii)*, blue pine (*P. wallichiana*), Khasi pine (*P. kesiya*), Chilgoza pine (*P. gerardiana*), and Teriasserian pine (*P. merkussi*). Of these, *P. roxburghii*, *P. wallichiana*, and *P. gerardiana* are found in the Himalayan Mountains, whereas *P. kesiya* and *P. merkussi* are indigenous to Assam and Myanmar. As part of our studies on population genetic analysis of *P. wallichiana* and *P. roxburghii*, we report here the results of detecting and testing microsatellites and genetic diversity in these species. Eighty simple sequence repeat (SSR) primers taken up from *P. thunbergii*, *P. sylvestris*, *P. resinosa*, *P. taeda*, *P. merkussi*, and *P. densiflora* were used for trans-specific amplification in *P. wallichiana* and *P. roxburghii*, which included 33 chloroplast and 47 nuclear microsatellite loci. In *P. wallichiana*, 26 primer loci showed positive amplification, revealing a transfer rate of 32.5 percent. Primers from *P. thunbergii* and *P. sylvestris* showed almost identical rates of transfer of 75.0 percent and 76.9 percent, respectively. A transfer rate of just 3.1 percent was observed from *P. taeda* whereas no transfer was seen from *P. resinosa*, *P. merkussi*, and *P. densiflora*. In *P. roxburghii*, very high transfer rates of primer pairs were observed from *P. thunbergii* (95 percent), *P. sylvestris* (100 percent), and *P. merkussi* (80 percent).

By using 12 chloroplast SSR (cpSSR) markers, 18 populations of *P. wallichiana* from the states of Uttarakhand, Himachal Pradesh, and Jamu and Kashmir, which together represent the natural range of distribution, were evaluated for genetic diversity. A maximum of 6 cpSSR alleles and 61 cpSSR size variants were found among 18 populations. The total gene diversity ranged from 0.45 to 0.68, which is moderate in comparison to other gymnosperms. The highest level of gene diversity was concentrated in populations from Himachal Pradesh. Similarly, 55 populations of *P. roxburghii* along the distribution range in the Himalayas were studied with 14 cpSSR markers. A maximum of 9 cpSSR alleles and 81 cpSSR size variants were found among 55 populations. The highest level of haplotypic diversity was concentrated in a population from Kiarala, Himachal Pradesh. The cpSSRs suggest that *P. roxburghii* maintains a high level of diversity (mean h = 0.65). Estimated gene flow between the populations was moderate in *P. wallichiana* (Nm = 0.43) and P. roxburghii (Nm = 0.58). Most of the variation resided within populations in both the species. Results are interpreted in the context of conservation of genetic resources of *P. wallichiana* and *P. roxburghii* in the Himalayas.

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Using Transcriptomics to Identify Candidate Genes Associated with Blister Rust Resistance in Whitebark Pine (*Pinus albicaulis*)

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Abstract—Whitebark pine (*Pinus albicaulis*) is an iconic high elevation tree species currently threatened by climate change, mountain pine beetle (*Dendroctonus ponderosae*), and white pine blister rust, a lethal disease caused by the invasive fungal pathogen *Cronartium ribicola* that afflicts all North American white pines. A major goal in managing pine populations vulnerable to blister rust is identifying sources of heritable resistance that can be used in operational breeding programs. U.S. Department of Agriculture, Forest Service (USDA FS) breeding programs have identified major resistance genes in four other *Pinus* species, but no such R gene is evident in whitebark pine. However, genetic variation in susceptibility to blister rust has been observed in naturally occurring populations. Although resistance phenotypes of whitebark pine differ from those in other pine species, certain seed parents are known to produce offspring that consistently develop fewer rust cankers compared to other seed parents under the same disease pressure, suggesting a genetic basis for partial resistance.

In collaboration with the USDA FS Dorena Genetic Resource Center (Cottage Grove, Oregon, USA), germplasm was collected from more than 50 mother trees, representing whitebark pine populations throughout the Pacific Northwest. In particular, seedling families yielded from both Mount Rainier (Washington, USA) and Mount Hood (Oregon) appear especially promising and will be the primary focus of bioinformatic investigation. Three days after inoculation with blister rust, needle tissue was sampled and flash-frozen to preserve each individual's RNA expression profile. Half of the seedlings from each family served as a control group and were inoculated only after sampling. This sampling strategy permits comparisons between both experimental conditions, while still revealing the resistance phenotype of each individual tree seedling. By using transcriptomics to analyze differential expression between these resistant and susceptible individuals, candidate genes associated with blister rust resistance can be detected. Additionally, comparisons between control and inoculated groups can reveal whether the resistance is constitutive or induced. Furthermore, co-expression analyses can be used to characterize gene networks and infer potential mechanisms of resistance. Once candidate resistance genes are identified, markers can be developed to assist breeders in screening whitebark pine individuals for resistance.

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Genomics Study of Western White Pine (*Pinus monticola*) Genetic Resistance Against White Pine Blister Rust

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Western white pine (WWP, *Pinus monticola*) is a long-lived conifer with an extensive geographic range in western North America. It is of high interest in ecological studies and forest breeding because of its high susceptibility to the invasive disease white pine blister rust (WPBR, caused by the fungus *Cronartium ribicola*). However, *P. monticola* lacks genomic resources and is evolutionarily far away from plants with available draft genome sequences. Use of high-throughput RNA-sequencing (RNA-seq) technology is a cost-effective strategy to generate substantial transcriptome data for global transcript profiling and DNA marker discovery. We report here the RNA-seq analysis results using Illumina® (San Diego, California, USA) deep sequencing of *P. monticola* infected with *C. ribicola*. De novo gene assembly was used to generate the first *P. monticola* consensus transcriptome of primary needles, which contained 39,439 unique transcripts with an average length of 1,303 bp and a total length of 51.4 Mb. About 23,000 *Pinus orthologous* genes (POGs) and 200 disease resistance gene analogs (RGAs) encoding NBS-LRR proteins were identified by BLAST search against the *Pinus* gene index database and plant R gene families, respectively. Comparison of transcriptomes from WPBR-susceptible and -resistant white pine genotypes revealed about 1,000 differentially expressed genes (DEGs) with statistical significance during early stages of the compatible and incompatible *P. monticola–C. ribicola* interactions.

In silico single nucleotide polymorphism (SNP) detection identified more than 100,000 high quality SNPs in the above three groups of candidate genes (POGs, RGAs, and DEGs) by a bulked segregation-based RNA-seq analysis. To estimate efficiency of in silico SNP discovery, 432 SNPs were selected to develop genotyping assays using Sequenom® iPlex (Sequenom, San Diego, California) technology. About 70 percent of in silico SNPs were successfully genotyped and 53 percent of the total SNPs were detected with predicted nucleotide variations in a collection of resistance germplasm. SNP clustering analyses consistently identified distinct populations, each composed of multiple full-sib seed families by parentage assignment. Linkage disequilibrium analysis identified 21 genes in significant association with major gene (Cr2) resistance. Genotyping verification by TaqMan™ (Thermo Fisher Scientific, Waltham, Massachusetts, USA) qPCR indicated that at least one SNP locus provided an excellent marker for Cr2 selection among populations across western North America (Oregon and Washington [USA] and British Columbia [Canada]).

The validated SNP markers can be used as novel genomic resources for genetic, evolutionary, and ecological studies in *P. monticola* and related five-needle pines. Our results demonstrate that integration of RNA-seq-based transcriptome analysis and high-throughput genotyping is an effective approach for discovery of a large number of nucleotide variations and for identification of functional gene variants associated with adaptive traits in a non-model species.

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Genetic Markers for Western White Pine (*Pinus monticola*): Enabling Molecular Breeding for Resistance to White Pine Blister Rust

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Abstract—Western white pine (*Pinus monticola*) is an economically and ecologically important conifer that has been severely affected by blister rust, a disease caused by the introduced fungal pathogen *Cronartium ribicola*. Because of this disease, the use of western white pine for reforestation is limited. The U.S. Department of Agriculture, Forest Service and others have resistance breeding programs to (1) identify the amount and type of genetic resistance in natural populations of western white pine and (2) develop durable disease resistance in breeding populations. These breeding programs can be enhanced by adopting advanced genomic technologies and new marker-assisted breeding approaches such as "breeding-without-breeding" (BWB) and "genomic selection." The BWB approach allows breeders to pursue advanced-generation breeding without crossing—that is, using wind-pollinated mating designs that avoid time-consuming activities and associated costs of controlled crosses. Furthermore, it may be possible to identify genetically resistant trees by determining the parentage of trees growing in field plantations that have been established with seed orchard seed. By using single nucleotide polymorphic (SNP) genetic markers to infer parentage, breeders may be able to select resistant genotypes from extensive operational plantations, field experiments, and nursery trials established using wind-pollinated seedlots from rust-resistant seed orchards. The success of BWB will depend on the characteristics of the (1) SNP markers (e.g., allele frequencies and genotyping success rate), (2) seed orchards (e.g., number and relatedness of the genotypes in the orchard), and (3) field and nursery tests (e.g., heritability of rust resistance traits). Our objectives are to (1) develop SNP genetic markers for western white pine, (2) use the SNP markers to conduct a proof-of-concept trial of BWB, and (3) transfer marker-assisted breeding technologies to tree breeders to accelerate resistance breeding and lay the foundation for genomic selection.

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Using Direct Amplification and Next-Generation Sequencing Technology to Explore Foliar Endophyte Communities in Experimentally Inoculated Western White Pines (*Pinus monticola*)

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Abstract—Fungal endophytes can influence disease severity and survival of infected trees. Here we characterized the endophyte community in western white pine (*Pinus monticola*), an important species in the northwestern United States that has largely been decimated by pathogenic fungi. We also assessed the ability to successfully inoculate seedlings with desirable endophytes, with the long-term goal of providing a protective microbiome and added defense against pathogens. *Pinus monticola* seedlings were inoculated in the field with potential pathogen antagonists and fungi isolated from healthy mature trees. After inoculations, direct amplification and next-generation sequencing were used to characterize fungal endophyte communities, and explore interspecific competition, diversity, and co-occurrence patterns in needle tissues. Negative co-occurrence patterns between inoculated fungi and potential pathogens, as well as many other species, suggest early competitive interactions. Our study explores early endophyte community assemblage and shows that fungal inoculations may influence tree growth.

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On the Road to Mapping Quantitative Trait Loci of Morphological and Aggressiveness Traits in the Poplar Rust Fungus

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Abstract—Poplar (*Populus deltoides*) is an important tree species in Europe, both for the wood industry and for its increasing contribution to energy production systems. The main disease limiting the growth of poplar is poplar rust, caused by the basidiomycete *Melampsora larici-populina* (*Mlp*). The selection of poplar cultivars with qualitative resistance has led to repeated failures since 1980, due to the breakdown of each resistance type by the rise of new virulence factors. The search for more-durable resistance has led breeders to target quantitative resistance. Meanwhile, pathologists have focused on the potential for rapid evolution of several fungal quantitative life history traits (LHTs) related to aggressiveness or dispersal potential, or a combination thereof (Lannou 2012). We used a quantitative genetics approach to determine whether these traits are heritable and directly affect the fitness of the pathogen as these characteristics can lead to rapid changes in pathogen populations in response to selection pressures exerted by the host. Mapping the traits of interest requires the construction of a high-resolution genetic map of the fungus and the measurement of segregating traits in progeny. Using techniques developed by our group to allow completion of the sexual cycle of *Mlp* in the laboratory, we adopted a strategy of genetic mapping by the resequencing (Huang et al. 2009), based on about 145,000 single nucleotide polymorphisms, of 96 offspring derived from selfing of the 98AG31 strain (reference genome, 462 scaffolds; Duplessis et al. 2011). Our preliminary analyses have already brought together 81 scaffolds in 23 linkage groups. A single virulence trait against *P. deltoides*, 'L270 -3,' segregates in the progeny, making it a potentially mappable qualitative LHT. Concerning the segregation of quantitative LHTs, we observed a clear segregation of both morphological traits of urediniospores (length, width, volume and length/width ratio) and aggressiveness traits (latent period, infection efficiency, sporulation capacity, lesion size), which is encouraging for future mapping of quantitative trait loci.

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Genomics, Transcriptomics, and Effectoromics of the Poplar Leaf Rust Fungus *Melampsora larici-populina*

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Melampsora larici-populina is causing leaf rust disease in poplar (*Populus* spp.) worldwide and poses serious problems to poplar breeders in France and in northern Europe. As with other rust fungi, its obligate biotroph status hinders laboratory investigations. Understanding the biology of rust fungi and deciphering the molecular mechanisms underlying biotrophy have long been a problem. Most of the knowledge gained was largely descriptive and limited to a few rust species; only recently has substantial progress been made in the characterization of avirulence determinants of the flax rust *Melampsora lini*.

The pioneer sequencing of the genomes of the poplar rust fungus *M. larici-populina* and of the wheat stem rust fungus *Puccinia graminis* f. sp. *tritici* in 2011 has opened great perspectives for the molecular study of this group of fungi. Comparative analyses with recent reports have confirmed the typical profile of rust fungi genomes. These genomes are rather large (>89 Mb) and contain many repetitive and transposable elements (~50 percent of the genome) and a very large number of predicted genes (>16,000), including many large gene families of unknown function with no homology outside the Pucciniales. Within the genomes are several hundreds of genes encoding candidate effectors that are likely to be involved in the fungal virulence. These effectors are small secreted proteins specifically expressed *in planta*.

In the present talk, we will briefly introduce some basics about the poplar rust genome. We will provide an overview of our current knowledge gained by genome-wide oligoarrays and RNA-Seq on *M. larici-populina* gene expression at different stages of the complex lifecycle of this heteroic and macrocyclic rust, particularly during successful infection of the telial host (poplar) and of the aecial host (larch, *Larix* spp.). We will present our current strategy to characterize virulence effectors and illustrate a few candidates under investigation. Finally, we will provide an overview of ongoing genomics projects including the refinement of the genome assembly through the construction of a genetic map and a population genomics approach to identify the gene(s) responsible for a breakdown in the major gene resistance in poplar plantations.

ACKNOWLEDGMENTS

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Ten-Year Results of a Comandra Blister Rust Screening Trial in the Central Interior of British Columbia

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Abstract—Improved resistance to comandra blister rust (causal agent: *Cronartium comandrae*) can improve lodgepole pine (*Pinus contorta* var. *latifolia*) survival and growth, but we lack information about resistance structure in this pathosystem. Incidence of comandra and other diseases was monitored annually in a 130-seedlot trial designed to both characterize the resistance structure in lodgepole pine and identify resistant families for inclusion in the tree improvement program in British Columbia, Canada. By 10 years of age, incidence of new comandra infection had largely stabilized on all three trial sites, having reached 79, 70, and 22 percent, respectively. However, close proximity to the alternate host plant, bastard toadflax (*Geocaulon lividum*), resulted in an increased risk of infection by comandra, and relatively high family-level variation between sites. Although most families appeared to be highly susceptible, a few families exhibited a moderate level of resistance. Based on these results, it is recommended that future additional screening of a much larger number of families be conducted to identify a sufficient number of families for either augmenting existing orchards or establishing a comandra-resistant orchard.

INTRODUCTION

Comandra blister rust (caused by *Cronartium comandrae*) is one of the most lethal diseases of young lodgepole pine (*Pinus contorta* var. *latifolia*) (van der Kamp and Spence 1987; Woods et al. 2000). It occurs throughout the Canadian province of British Columbia (BC), but is most common in the Sub Boreal Spruce (SBS) biogeoclimatic zone, and alternates between bastard toadflax (*Geocaulon lividum*) and lodgepole pine (Ziller 1974). Management options include increased composition of nonhost species in reforestation, but some of the colder, drier, or more nutrient-poor ecosystems may not be well suited for these other species (Reich and van der Kamp 1993). Increased planting density of lodgepole pine (proportional to risk) is practiced in some areas, but this can be costly where risk to comandra is very high (British Columbia Ministry of Forests 1996). Hazard rating for comandra (Jacobi et al. 1993) identified several key variables including proximity to alternate host. Numerous other authors previously reported that infection is typically found in close proximity to alternate hosts (Andrews and Harrison 1959; Applegate and Seay 1971; Cordell et al. 1969). Although resistance to comandra has not been thoroughly investigated in the past, a formal disease assessment of a BC Ministry of Forests progeny field test in 2000 showed significant and large differences in family infection rates. Based on this result, a field trial was designed specifically to test comandra resistance of genotypes established in the Bulkley Valley low-elevation seed orchard (Orchard 219).

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METHODS

Three sites within established pine plantations in the central interior of BC were selected based on high incidence of comandra, and abundant cover of *Geocaulon lividum*, an alternate host of comandra. Each 2-ha site (about 100 m × 200 m) was prepared in fall 2003 by mechanically mulching the existing trees. In June 2004, seedlings from 130 seedlots were planted on a 1.5-m-square grid, in single tree plots, with 50 blocks per site (Alpha design). Most seedlots (hereafter referred to as "families") consisted of 50 seedlings, typically from open-pollinated families, though several controlled-cross families were included to evaluate specific putative resistant families. A total of 7,440 seedlings, including internal buffer seedlings, were monitored.

Two sites, Endako and Thompson, were assessed starting in 2006, 24 months after planting, and the third site, Holy Cross, was assessed starting in 2007, 36 months after planting. Detailed annual assessments were continued through 2014, representing 10 full growing seasons in the field. Disease assessments in 2006 through 2008 consisted of recording tree condition, and counting the number of individual infections (severity) by comandra, as well as by western gall rust (causal agent: *Endocronartium harknessii*), and stalactiform blister rust (causal agent: *Cronartium coleosporioides*). From 2009 to 2014, the tree condition and only the occurrence (not the count of infections) of a new rust species on a tree were recorded. Starting in 2012, an additional form or severity was also recorded to facilitate rust impact modeling. For all rusts, either the estimated percent stem encirclement (for stem-infected trees), or the distance of branch infections from the stem (for branch-only infected trees), was recorded for the most lethal-appearing stem or branch infection per rust per tree. In 2007, a count of the number of *G. lividum* stems was recorded within each 1.5-m × 1.5-m tree cell to evaluate the source of spread for comandra. A geographic information system (GIS) exercise was later used to calculate the distance, in 1-m intervals, from each tree to the closest *G. lividum* plant for determining the risk of infection based on proximity to the alternate host plant. For evaluating the source of spread of stalactiform, the presence of common red paintbrush (*Castilleja miniata*) was also assessed within the 1.5-m grid on all three sites in 2007. For stalactiform, the presence and general abundance of both yellow rattle (*Rhinanthus minor*) and cow wheat (*Melampyrum lineare*) were also noted at the site level.

RESULTS

Comandra Occurrence by Site

Although the three sites were selected to maximize risk to comandra infection, only two of the three sites were infested with a sufficiently high incidence to allow reliable screening for comandra resistance (fig. 1). The two sites with the highest infection, Endako (79-percent incidence of infection) and Thompson (70-percent incidence), were located about 12 km apart. They apparently had very similar weather conditions, based on the similarity of the timing and incidence of comandra on an annual basis. The third site, Holy Cross, which was more than 100 km southeast of the other two sites, recorded significantly less comandra (22-percent incidence), and its timing was very different from that of the other two sites. By 2006, both of the western sites had about 35-percent comandra, with close to 0 percent noted at Holy Cross. By 2007, both of the western sites had about 50-percent cumulative comandra incidence, compared to only 2 percent at the Holy Cross site. Given that it takes 2 to 3 years for infections to become noticeable, this difference probably indicates very different weather, both in 2004 and 2005, between these two geographic regions. The incidence of comandra at all three sites had apparently stabilized by age 10, with very little new infection occurring. The vast majority of infections by comandra on lodgepole pine in BC are typically restricted to a zone less than 0.5 m above ground, so it is reasonable to assume that the lack of new infections is related to the development of age-based resistance.

Ranking Resistance to Comandra

The primary interest in this trial is the structure of resistance in the lodgepole pine population, and whether there are any resistant families that can be used to increase the resistance of the improved tree orchards for BC. Sorting families by increasing comandra incidence at the Thompson site allows a systematic evaluation of the family variation due to site (fig. 2). After 10 growing seasons, the vast majority of the

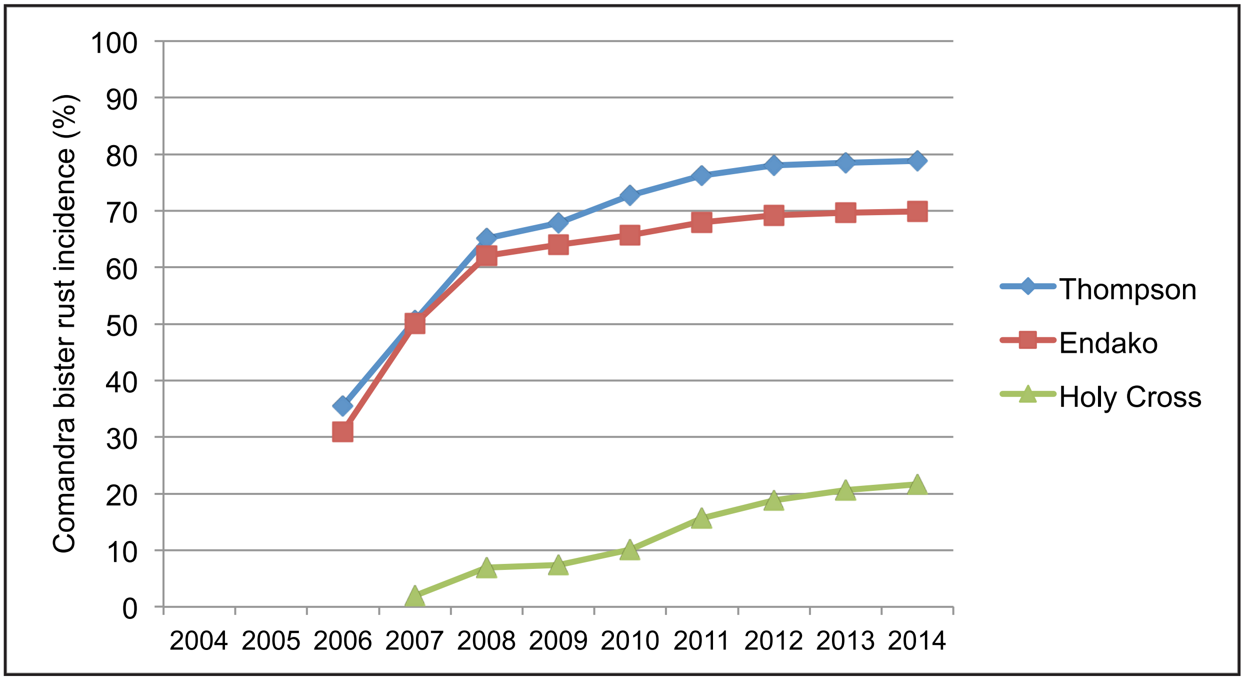

Figure 1—Cumulative comandra incidence at all three sites monitored annually.

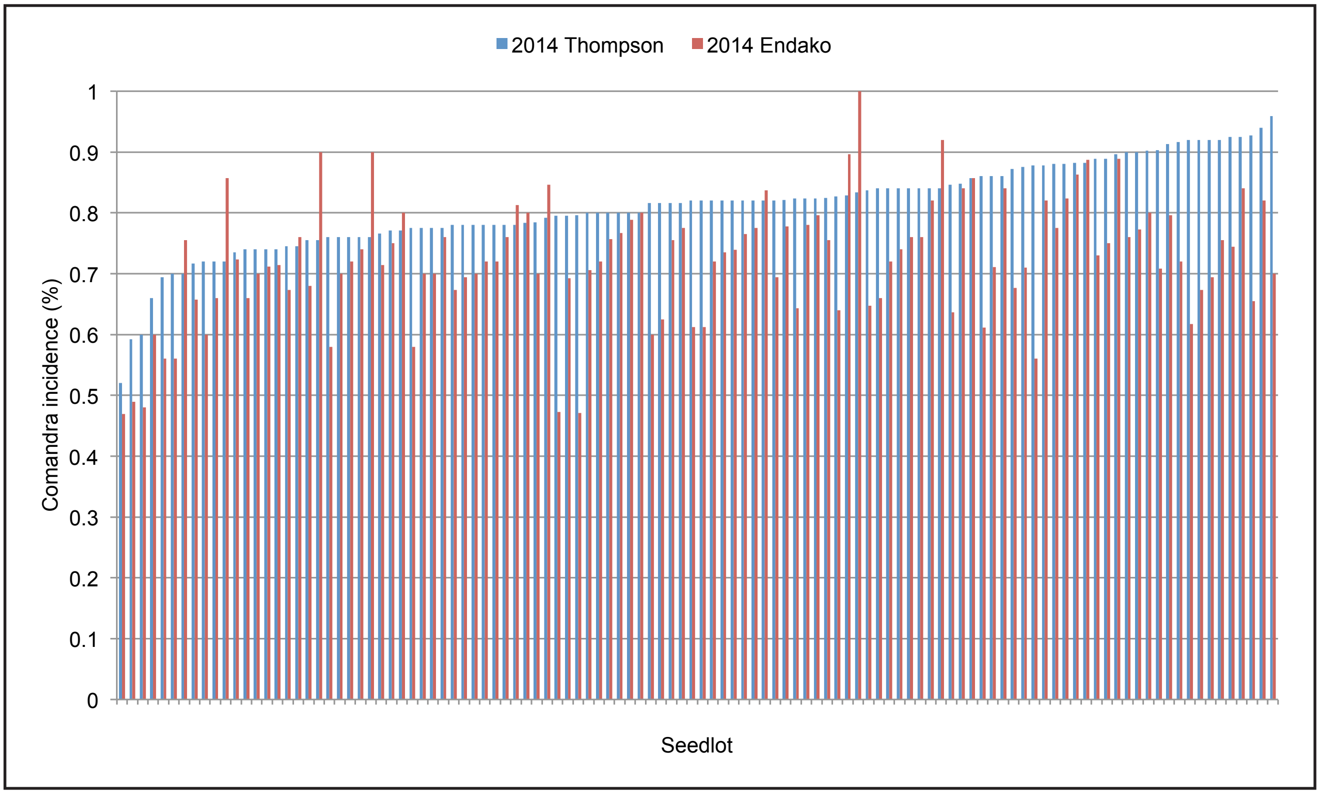

Figure 2—Cumulative mean family comandra incidence at Thompson and Endako, sorted by Thompson comandra incidence.

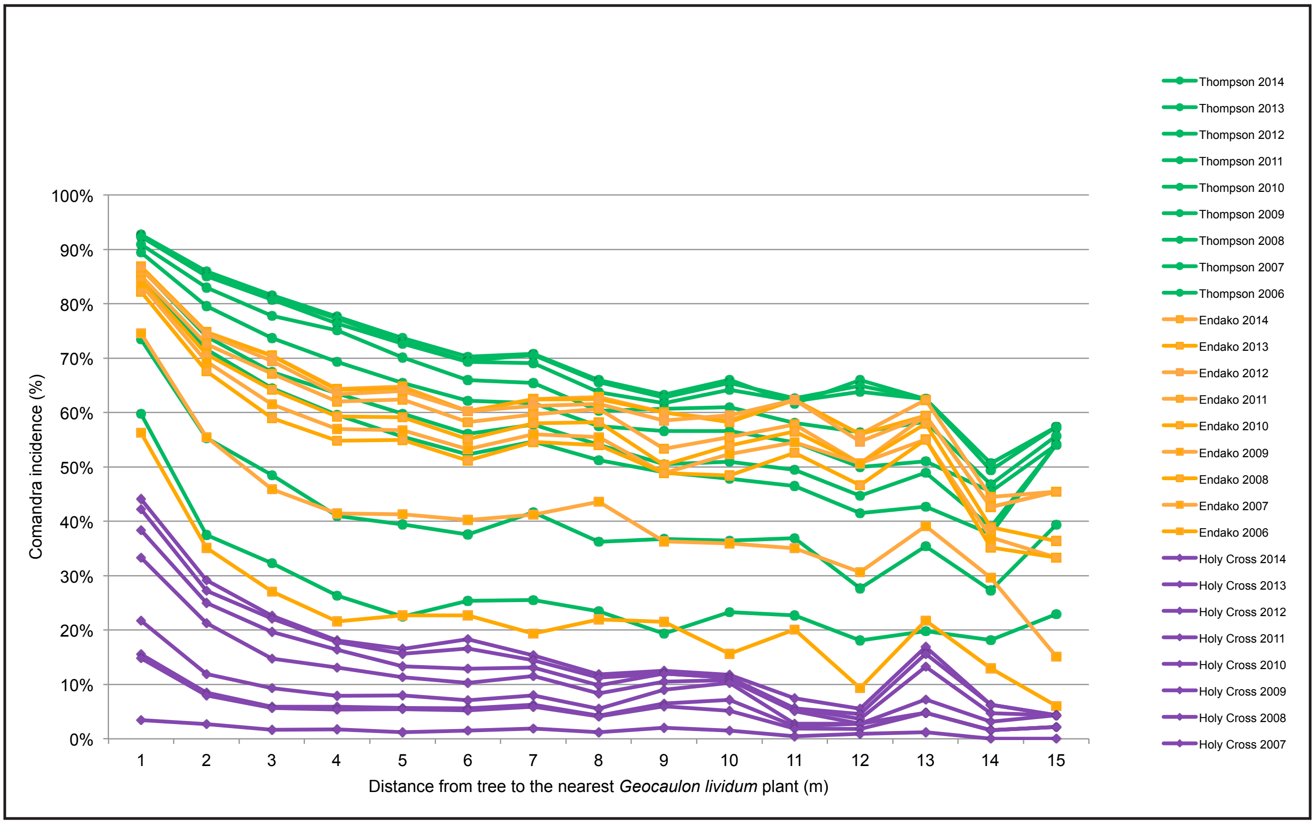

Figure 3—Cumulative comandra incidence by proximity to alternate host for all three sites, monitored annually.

families appear to be highly susceptible, with only a few showing somewhat lower comandra incidence. Especially given the large number of families in this trial, this result may suggest that there exists very low natural resistance in the lodgepole pine population as a whole. This would be reasonable to expect, especially if most of the pine population is not normally exposed to challenge by comandra on a regular basis.

The difference between the moderately resistant families and the highly susceptible ones is more distinct when comparing the severity of infection (count of infections per tree) rather than the mean incidence at the family level (Feng et al. 2013). The most susceptible families may be almost twice as susceptible as the least susceptible when basing the comparison on incidence, whereas the most susceptible families are close to six times more susceptible when using severity as the basis for comparison.

Although variability in family-level comandra incidence between sites should have been minimized through the robust trial design (50 open-pollinated seedlings planted in single-tree plots within 50 blocks per site), the family rankings varied considerably between sites. A correlation R^2 calculated annually for the two most highly infested sites never rose much higher than about 0.50. This would generally indicate that only about 50 percent of the variability was accounted for by genotype, which is considerably lower than expected for this type of study.

Spatial Correspondence of Comandra and its Alternate Host

At an early stage in the monitoring of this trial it became apparent that the highly clustered distribution of comandra incidence may be related to the highly clustered spatial distribution of the alternate host, and that this factor may affect the outcome of ranking families for resistance. In particular, ranking families using the average family incidence of all sites may introduce error, as trees within the same site may be exposed to dramatically different levels of challenge. Combining trees that are challenged at a low level with trees challenged at a high level is unlikely to yield reliable rankings, unless their proportions are similar.

A visual inspection of the mapped distribution and intensity of comandra incidence, in relation to the distribution and clustering of the alternate host, exhibited a distinct spatial correspondence on all three sites. Results of using the GIS technique to display the cumulative risk of infection by comandra in relation to the proximity to the nearest alternate host plant at each annual measurement show a sharp decrease in risk over a very short distance from the alternate host (fig. 3). The shape of this function appears to be relatively independent of the incidence or the site. This proximity relationship is explored in greater detail through the development of a flexible semi-parametric generalized additive model to reduce ranking error (Feng et al. 2013). This approach was used in an expanded, but unpublished, study by the same authors to more reliably rank families by modeling the effect of proximity to alternate host, abundance of alternate host, and other factors such as site topographic effects (e.g., slope, elevation, and aspect).

Other Rusts and Forest Health Factors

Several observations were made regarding the other two rusts. Western gall rust occurred at a lower than expected frequency and incidence on all three sites. Stalactiform blister rust occurred at a much higher than expected incidence at only one of the three sites. Stalactiform incidence at the Endako site appeared to follow a rapid and widespread invasion in 2004 or 2005 by *R. minor*, a known transient annual (Westbury 2004). *Rhinanthus minor* was not observed until 2010 at the Holy Cross site and not until 2012 at the Thompson site. Although *C. miniata* was highly clustered and somewhat abundant on all three sites, it did not appear to result in distinctly clustered stalactiform infection. It may not be as effective an alternate host for stalactiform as *R. minor*, though the trial was not designed to evaluate this factor. *Melampyrum lineare* was present only on the Holy Cross site, and was very abundant and widespread. It did not appear to cause widespread stalactiform on pine, though the weather may not have been suitable for infection by stalactiform.

Trial Design, Assessment Schedule, and Tree Condition

The results of this trial demonstrated the importance of careful site selection, the need for replication of test sites, and the dependence on suitable weather for successful screening using field trials. This trial also demonstrated the need for detailed annual monitoring given both the rapidly changing annual rate of new infection, and the rapid rate of mortality of trees infected at a very young age. Further, it demonstrated the value of mapping the alternate host distribution in climates where spatial infection gradients may develop over very short distances from the alternate host.

CONCLUSIONS

Detailed annual monitoring and subsequent analysis have shown that after 10 growing seasons, the comandra incidence has both stabilized and reached an adequate threshold for reliable screening on two of three sites. Although very few families demonstrated resistance in relation to the large number of families tested, this result may possibly indicate that the lodgepole pine population as a whole may contain a relatively low level of resistance to an endemic pathogen. Screening of additional lodgepole pine families to further bolster the number of comandra-resistant families is highly recommended.

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Patterns of Variation in Blister Rust Resistance in Sugar Pine (*Pinus lambertiana*)

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White pines exhibit both qualitative and quantitative resistance to white pine blister rust, which is caused by the fungal pathogen *Cronartium ribicola* (Sniezko et al. 2014). Species from several western U.S. States have a major gene at low frequencies that confers virtual immunity to the disease; and most also show heritable quantitative variation in infection frequency, as well as the ability to abort or heal infections that do occur (Kinloch et al. 2012). Here, we summarize extensive data from different tests, some previously published, showing the magnitude and pattern of variation in both mechanisms throughout the geographic range of sugar pine (*Pinus lambertiana*). We then discuss how this variation could be developed and deployed to effect durable resistance in synthetic populations of this ecologically and commercially important species.

The frequency of Cr1, the major gene for resistance in sugar pine, was assayed over many years following established protocols (Kinloch 1992). Open-pollinated seedlings from more than 15,000 seed parents in 50 seed zones were analyzed, covering most of the range of the species across more than 14 degrees of latitude from northern Oregon to Baja California (USA). Seedlings identified with Cr1 in these assays were outplanted at a test site near Happy Camp in northern California for a secondary screen for partial resistance (also called slow rusting resistance) (Kinloch et al. 2012). Development of different kinds of resistance was needed as defense against the possibility of virulence to Cr1 becoming widespread. At this site, a mutant virulent allele of the rust (vcr1, cognate to Cr1) had already arisen and become predominant as a result of selection by Cr1 host genotypes concentrated on the site. With Cr1 neutralized, different forms of partial resistance could become expressed and assessed, thus identifying individual trees with both Cr1 and partial resistance.

Field resistance was examined separately on an extensive collection of 64 seed sources, covering approximately the same range as the Cr1 assay, planted at mid-elevation in the central Sierra Nevada (Kitzmiller 2004; fig. 1). Major subregions represented included western Oregon/Cascades, Klamath Mountains, eastern Cascades, North Coast, northern and southern Sierra Nevada, South Coast, Transverse and Peninsula Ranges, and the Sierra San Pedro Martir. Three other sites planted with most of the same sources were also examined. Traits recorded were number of infections per tree, and whether the infections were normal

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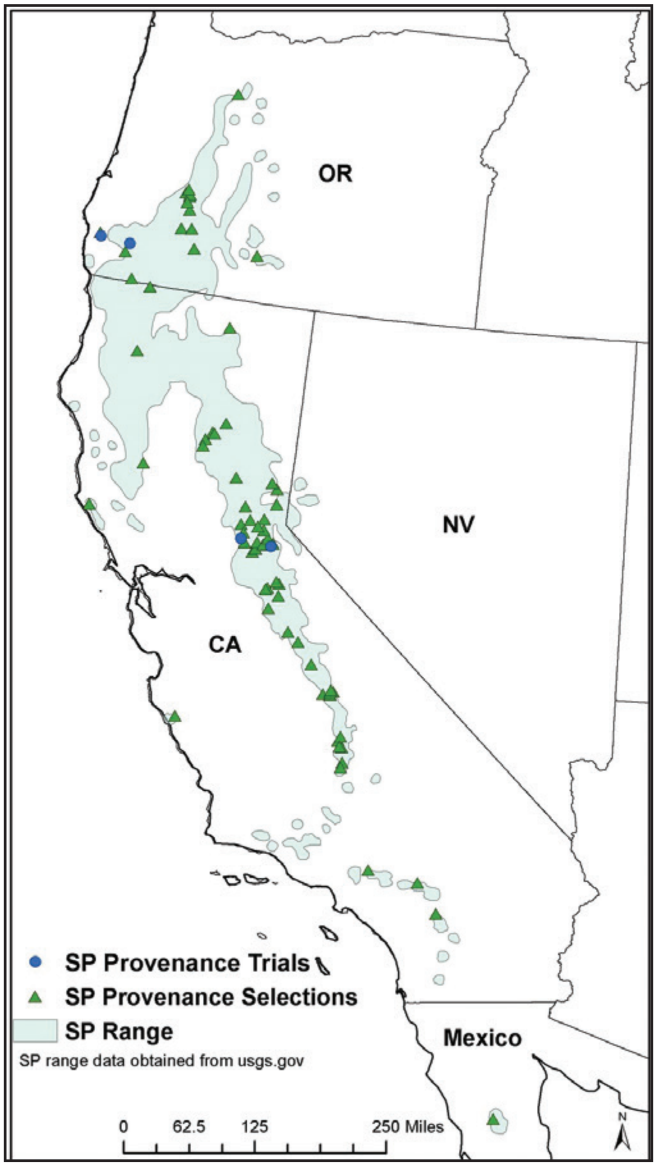

Figure 1—Locations of seed sources and plantations for sugar pine provenance trials.

or reactive; reactive responses show bark or blight responses to infection, leading to partial or complete remission of disease symptoms (Kinloch et al. 2012).

Mean frequency of Cr1 was 0.0208 (range: 0.0000–0.0886) (Kinloch 1992; J. Dunlap, unpublished data on file at USDA Forest Service, Pacific Southwest Region, Placerville Genetic Resources Unit, Placerville, California). Very low frequencies in northern latitudes increased almost clinally to the south, peaking around 0.06 in the southern Sierra, before dropping again in the Transverse and Peninsula Ranges and becoming undetectable at the southern extremity of sugar pine in the Sierra San Pedro Martir in Baja, Mexico (fig. 2). Cr1 frequency was negatively correlated (–0.62) with latitude. Several exceptions to this pattern were evident, including an anomalous high value (0.0886) in an outlier population on the south coast of California.

Challenge with blister rust was severe on three of the four field test sites, ranging from 84 to 92 percent of trees infected. Correlations of seed sources with infection among the sites were positive and generally high, but due to low survival and maintenance problems on other sites, only the site with the most seed sources, good survival, and highest overall infection (Fitch Rantz) (Kitzmiller 2004) is considered further. Percentage of trees infected among seed sources on this site ranged from 72 to 98, and was the reverse pattern of Cr1, with a highly significant (0.74) correlation with latitude. A much more sensitive index of susceptibility was infection frequency (counts of separate infections per tree), which ranged from 0 to over 100 for individual trees, and averaged from 3 to 29 among seed sources (fig. 3). No clear pattern emerged, but when seed sources were grouped over subregions, extreme northern and southern sources tended to have fewer, and Sierra Nevada sources more, infections (fig. 4).

A derivative parameter of individual infection counts is the proportion of trees with no active infections (NAI), that is, trees with complete remission of symptoms (resulting from bark, blight, or other reactions), or those that were never infected. This parameter is perhaps the clearest representation of partial resistance, as it integrates the several different components into one index (Kinloch et al. 2012). Mean NAI was almost 5 percent (range: 1.5–13 percent; fig. 5). Again, there was no obvious geographic pattern over seed zones or broader subregions, but all seed sources have abundant trees available for selection for this trait.

The generally wide distribution and strong inheritance of both major gene and quantitative resistances provide a clear and compelling strategy for developing durable resistance. Cr1 is nearly totipotent, and protects against all ambient variability in the rust population except genotypes carrying the vcr1 allele. Although Cr1 is specifically (and completely) vulnerable to this allele, partial resistance is not; it is no more vulnerable to vcr1 than wild type inoculum. Consequently,

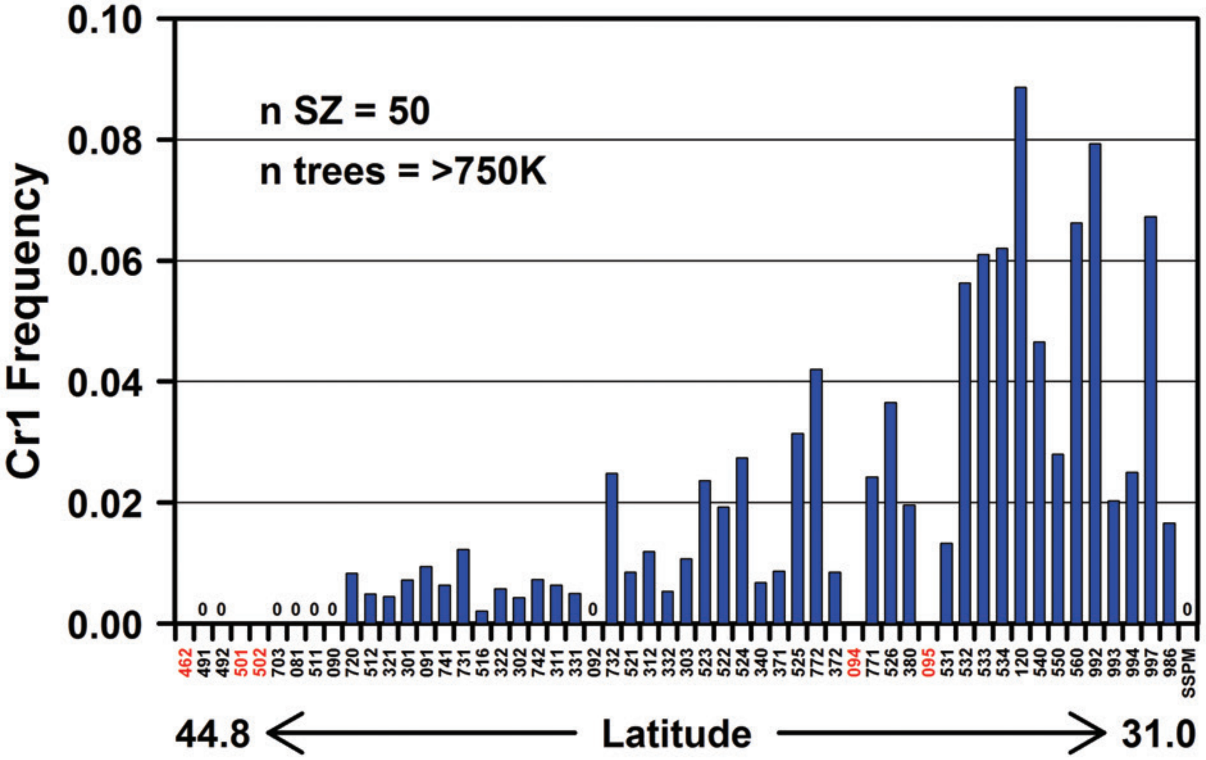

Figure 2—Frequency of Cr1 in different seed zones of sugar pine. (No data for zones colored red.)

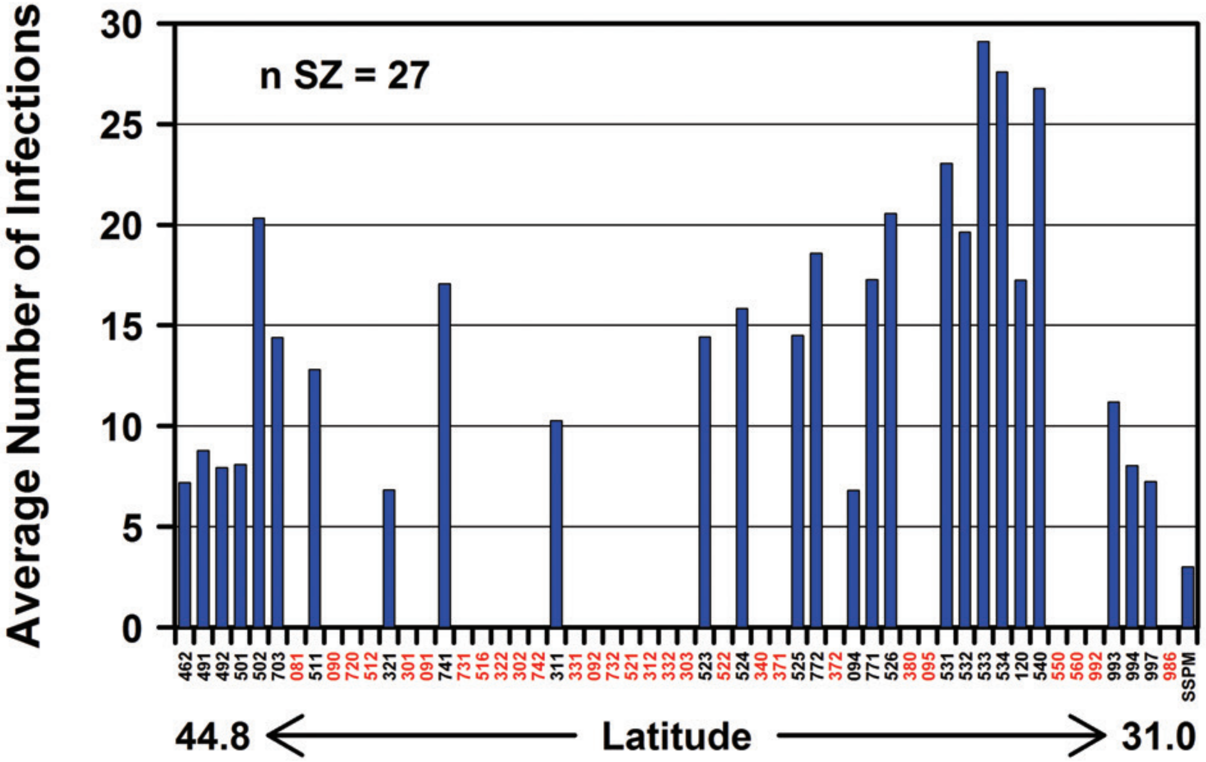

Figure 3—Average number of blister rust infections per tree in different seed zones of sugar pine. (No data for zones colored red.)

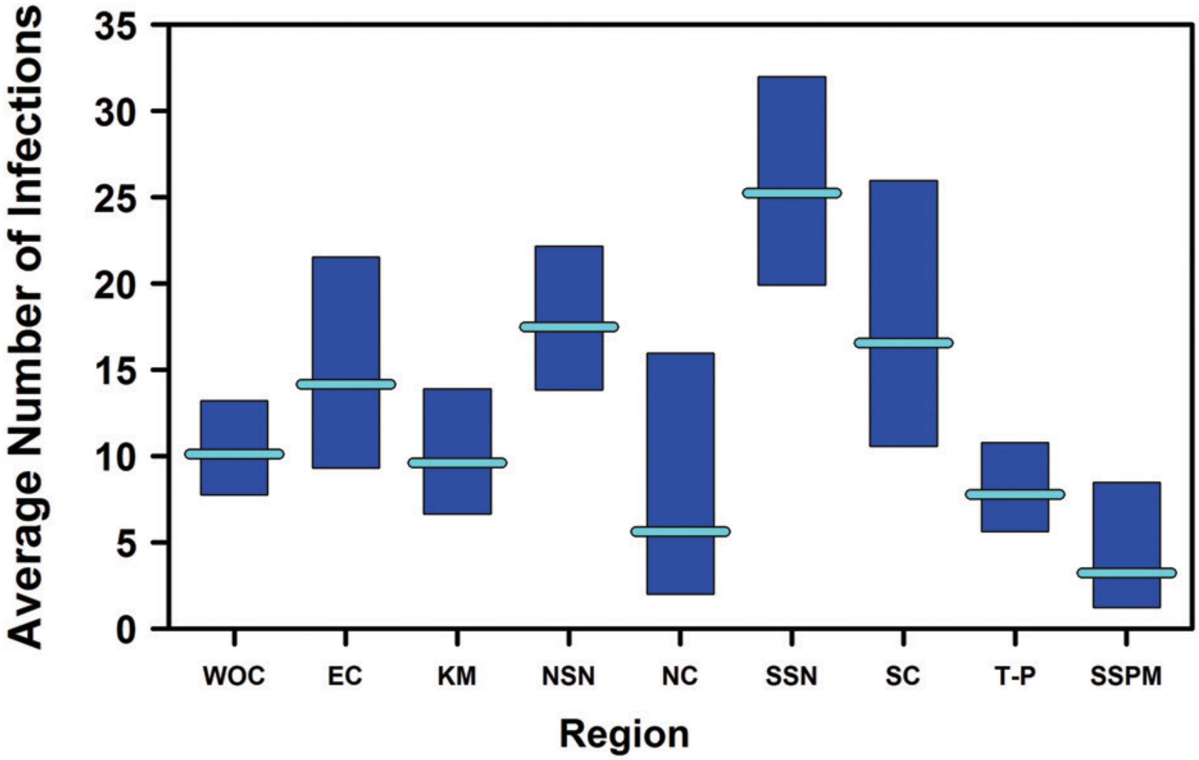

Figure 4—Average number (horizontal lines) and range (solid bars) of blister rust infections per tree in broad sub-regions of sugar pine, arrayed by latitude (WOC, western Oregon and Cascades; EC, eastern Cascades; KM, Klamath Mountains; NSN, northern Sierra Nevada; NC, north coastal; SSN, southern Sierra Nevada; SC, south coastal; T-P, Transverse and Peninsula Ranges; SSPM, Sierra San Pedro Martir.

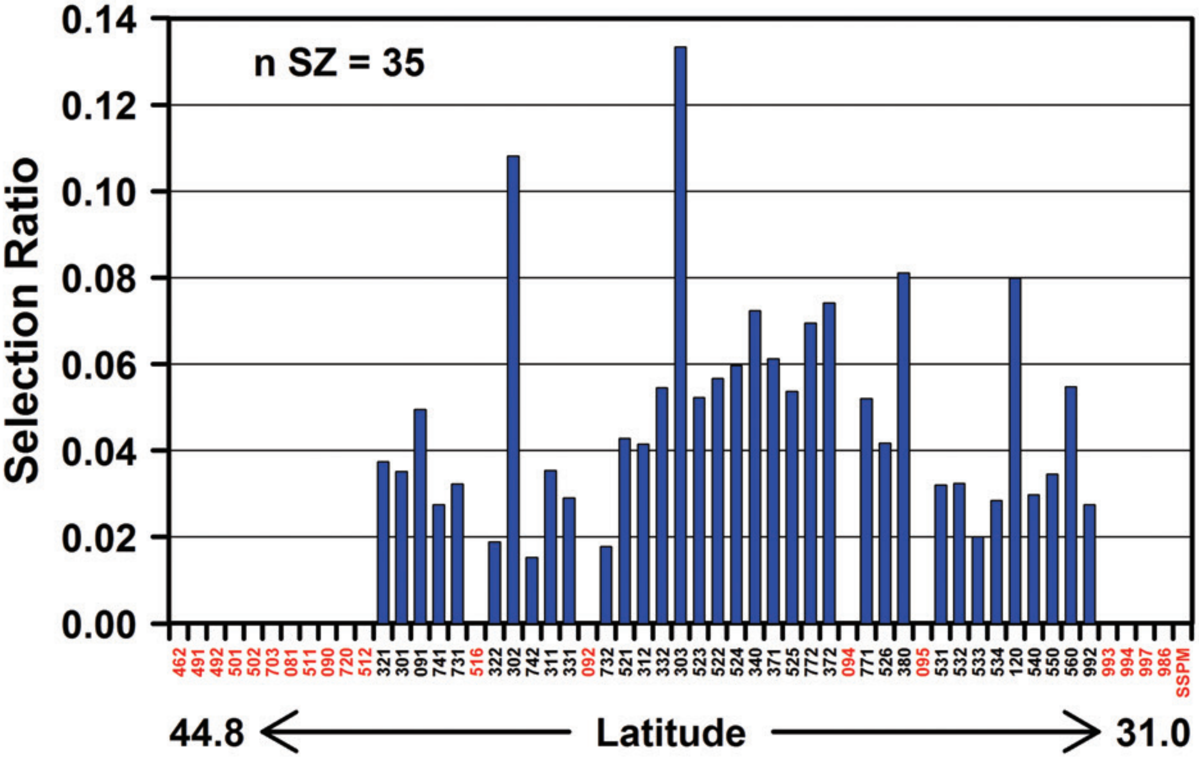

Figure 5—Selection ratio: average number of trees with no active infections (NAI), by seed zones of sugar pine. (No data for zones colored red.)

tandem selection of Cr1 with partial resistance can effectively restrict both rate and quantity of infection by all inocula. This would greatly lower the probability of infection by vcr1 (already rare in natural populations). The two resistances are mutually reinforcing, and developed together in synthetic populations could conceivably reduce the amount of disease to endemic levels in a few generations of selection and breeding.

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Blister Rust Resistance in Whitebark Pine (*Pinus albicaulus*)—Early Results Following Artificial Inoculation of Seedlings from Oregon, Washington, Idaho, Montana, California, and British Columbia Seed Sources

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Abstract—Whitebark pine (*Pinus albicaulis*) is very susceptible to white pine blister rust, caused by the fungal pathogen *Cronartium ribicola*. Operational programs to develop resistant populations of whitebark pine are underway. These programs rely on artificial inoculation of young seedlings and can evaluate hundreds of parent trees using seedling progeny in a relatively short time. The trial reported here was undertaken at Dorena Genetic Resource Center (Cottage Grove, Oregon, USA). Inoculation was very effective and produced a high incidence and severity of disease in the seedlings with 99.9 percent of seedlings exhibiting needle spots and averaging more than 125 needle spots per seedling 9 months post-inoculation (mpi), and more than 11 stem symptoms per seedling and over 77 percent of seedlings with stem symptoms approximately 15 mpi. Overall, 47 of the 225 families had 100 percent of seedlings with stem symptoms 15 mpi. In this summary, we examine the variation among seed sources in one measure of rust resistance, the percentage of seedlings with early stem infections. The results showed large differences among populations from Oregon, Washington, Idaho, Montana, and California (USA), and British Columbia (Canada). The highest levels of resistance were observed in some seed sources from Oregon and Washington, with some sources showing less than 60 percent stem infection. The two Idaho and Montana seed lots that had been rated moderately rust-resistant in previous testing elsewhere were among the most susceptible in this trial, with 92.4 percent of seedlings with stem symptoms approximately 15 mpi. This was very likely due to lower effective inoculation in the prior test.

Caution is warranted in extrapolating results to the field, especially when the effective inoculation level is relatively low and suboptimal, but the best candidates, to date, are likely to be those from trials such as this one with a very high effective level of inoculation. However, repeated testing of a subset of seed lots is warranted to improve confidence. Long-term field testing, with a susceptible control, is also essential to establish the relationship between the seedling screening assays as well as to examine durability of resistance. Field trials, with susceptible controls, have been established in Oregon and are just starting to show rust infection. Land managers have been notified of the highest ranking parents, and seed from these can be used in restoration efforts or to establish seed orchards. A synthesis of results underway from the full 5-year post-inoculation data from this trial and several other trials at Dorena Genetic Resource Center will provide further insights on genetic resistance to white pine blister rust.

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INTRODUCTION

Whitebark pine (*Pinus albicaulis*), a long-lived conifer in high elevation ecosystems of the western United States and Canada, is highly susceptible to white pine blister rust, caused by the nonnative fungal pathogen *Cronartium ribicola*. Developing populations of trees with genetic resistance to this pathogen has been an essential strategy for reforestation in species such as western white pine (*P. monticola*) and sugar pine (*P. lambertiana*) (King et al. 2010; Kinloch et al. 2012; McDonald et al. 2004; Sniezko 2006; Sniezko et al. 2014). In many areas, restoration of whitebark pine is being done or is being contemplated (Beck and Sniezko, this proceedings), and the use of rust-resistant whitebark pine is seen as the key to success in many areas. Several programs to develop resistant populations of whitebark pine in the United States and British Columbia (Canada) are underway, and similar plans are being contemplated in Alberta (Canada). Until relatively recently, little was known about the components of genetic resistance and their frequency in whitebark pine, but some detailed reports of results in the first few years post-inoculation are now available (Kegley et al. 2012; Sniezko et al. 2007). Many aspects of blister rust resistance in whitebark pine are similar to those observed in western white pine (see review in Sniezko et al. 2014).

Tens of thousands of whitebark pine seedlings have been evaluated for rust resistance at the U.S. Department of Agriculture, Forest Service, Dorena Genetic Resource Center (DGRC; Cottage Grove, Oregon, USA; 45.78°N, 122.96°W, 244.1 m above sea level). These resistance screening trials have provided insights into the levels of the natural genetic resistance, some of its components (Sniezko et al. 2011), and an indication of the geographic distribution of resistance, as well as some caveats on extrapolation of results. Each study is monitored for 5 years post-inoculation; however, in this overview we will focus on the first 15 months post-inoculation (mpi) for one of the large recent studies and present results on differences among seed sources in the percentage of seedlings with stem symptoms (normal cankers and bark reactions).

METHODS

This report provides a partial summary of early results from a seedling trial (SY2007R4) involving 225 half-sib families in which the level of effective inoculation was extremely high (99.9 percent needle spotting). The artificial inoculation used standard DGRC protocols, which have been used with success for greater than 20 years (see Sniezko et al. 2011 for some details). The trial included 14 half-sib seedling families previously rated as “highly susceptible” or “highly resistant” to serve as controls and to examine repeatability of results over years.

The trial was sown in 2007 and inoculated in September 2008, as 2-year-old seedlings. See Hamlin et al. (2011) for some background on the trial, including geographic sources for the 215 Oregon and Washington families; the additional families from Idaho and Montana (2), California (3), and British Columbia (5) were not included in the height and foliage color summary by Hamlin et al. (2011). The 18 Oregon and Washington populations are represented by families from different national forests, national parks, and Confederated Tribes of Warm Springs (1 to 42 families per population).

RESULTS AND DISCUSSION

The spore density used in artificial inoculation can elicit varying results, depending on the pre- and post-inoculation environment of seedlings and other factors. The effectiveness of the inoculation can be discerned by examining key factors such as the number of needle spots on a seedling, the percentage of seedlings with needle spots, and the number of stem infections on each seedling (most notably on susceptible controls). In this trial, the four susceptible control families had 96.7 to 100 percent of seedlings with stem symptoms and 9.3 to 18.6 stem infections per seedling. Figures 1, 2 and 3 show the high level of needle spots and cankering in this trial. At DGRC, over decades of trials, we have generally observed that more than 95 percent of seedlings develop needle spots for the various white pine species evaluated. In this whitebark

Figure 1—Whitebark pine seedling with hundreds of white pine blister rust needle spots, ~9 months post-inoculation.

Figure 2—Heavily cankered whitebark pine seedlings ~12 months post-inoculation.

Figure 3—Heavily cankered whitebark pine seedling (left) and canker-free seedling (right), from half-sib family from seed collected from a Wenatchee National Forest parent tree, ~13 months after artificial inoculation.

pine trial the artificial inoculation was extremely effective, with means greater than 125 needle spots per seedling and 99.9 percent of seedlings displaying needle spots approximately 9 months mpi. The level of needle spotting per seedling (the first symptom of blister rust infection noted after inoculation) varied dramatically among families (25.8 to 247.4) and populations (50.3 to 165.1) (Sniezko, unpublished data on file at DGRC). The trial averaged 11.3 stem symptoms per seedling and 77.5 percent of seedlings with stem symptoms ~15 mpi. Forty-seven of the 225 families reached 100 percent of seedlings ~15 mpi. This level of infection might correspond to a field site with very high levels of infection (many cankers per tree and a very high percentage of infected trees).

Families that show resistance in a trial such as the one reported here should be the best candidates for establishing whitebark pine plantings that will show the highest levels of field survival with exposure to the rust pathogen over the hundreds of years' lifespans of these ecologically important trees. However, field testing on sites of high rust hazard will be needed to confirm the relationship of the seedling rust-resistance testing and field resistance.

The 21 geographic sources in this trial varied greatly in the level of stem infections (fig. 4). There was also large variation among families within populations (Sniezko, unpublished data). Many of the sources with the highest level of resistance (based on low early stem infection percentage) appear to be from the Cascade Mountains of Oregon and Washington; those from eastern Oregon, the Interior West (Idaho and Montana), California, and British Columbia show much lower levels of rust resistance. Results from other studies have also suggested that the highest levels of resistance may be in the Pacific Northwest

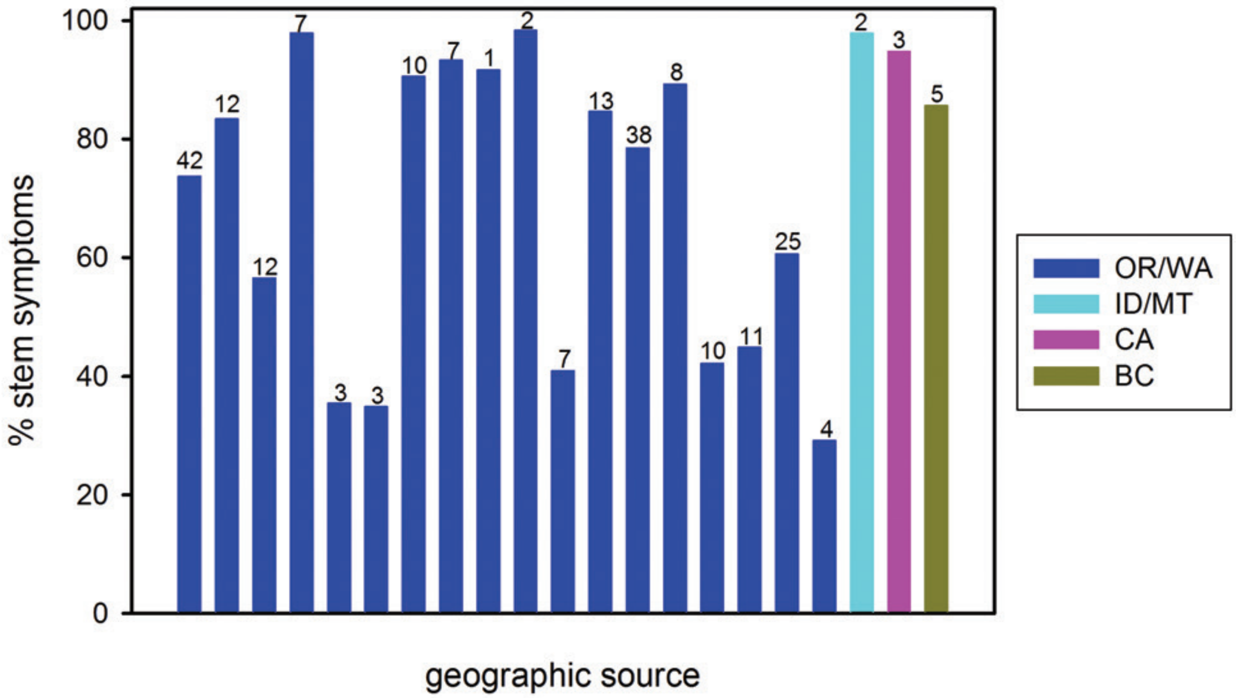

Figure 4—Genetic variation among seed sources in percentage of seedlings with early blister rust stem symptoms ~15 months post-inoculation. The number of half-sib families represented in each seed source mean is indicated above the bar (varies from 1 to 42). The 18 Oregon and Washington sources refer to individual National Forest, National Park and Confederated Tribes of Warm Springs boundaries from which the seedlots were collected.

populations (Kegley et al. 2012; Mahalovich et al. 2006; Sniezko et al. 2007). Hundreds of additional seedling families are now under test, and the results will help better delineate geographic patterns.

The high level of stem symptom-free percentage for some families and populations at the 15-month assessment suggests that the resistance could be similar to the major gene resistance found in other white pine species such as *P. monticola*, *P. lambertiana*, *P. flexilis*, and *P. strobiformis* (reviewed in Sniezko et al. 2014). However, other tests at DGRC indicate that the whitebark pine seedlings in the highest canker-free families do not exhibit the hypersensitive-like reaction in the needles observed in those four species (Sniezko et al. 2008; Sniezko, unpublished data). The frequency and the level of resistance in these whitebark pine families is higher than generally seen in much more extensive resistance testing of half-sib families of *P. monticola* and *P. lambertiana* (Kegley and Sniezko 2004; Sniezko, unpublished data); at least one half-sib family of *P. monticola* from the Cascades of Washington shows a relatively low percentage of seedlings with stem symptoms and is confirmed not to be from major gene resistance (see Sow #75 in table 1 in Sniezko et al. 2014). The high level of resistance shown in some of these open-pollinated whitebark pine families is comparable to or higher than that seen at DGRC in the Bingham F2 orchard seedlot of *P. monticola* (second generation of improved stock) (see Sow #71 in table 1 in Sniezko et al. 2014). This is very encouraging considering that whitebark pine was initially considered to be the most susceptible of the North American five-needle pine species (Hoff et al. 1980). Further summary and analyses of this whitebark pine trial and other trials of this species underway or recently completed at DGRC will provide greater elucidation of the type and frequency of blister rust resistance in whitebark pine.

Fourteen families represented in earlier trials at DGRC were included as controls in this trial. Of these 14, the 4 families with the highest percentages of seedlings with early stem infection in earlier trials also exhibited very high percentages of seedlings with early stem infection in this trial (100 percent for three of the families). In contrast, the 10 families showing a low-to-moderate level of early stem infection in a previous trial also showed a low-to-moderate percentage of early stem infections in this trial (40 to 65.3 percent).

The two families from the Interior West (Idaho and Montana) had previously been rated as moderately to highly resistant in an artificial inoculation trial elsewhere (Mahalovich et al. 2006), but appear to have been subjected to a much lower level of effective inoculation. These two families were among the most susceptible families in this trial (fig. 4) (Sniezko and Kegley 2015; Sniezko et al. 2011), with not only a very high early stem infection percentage (92.4 percent) and number of stem infections per seedlings (10.3) but also 98.1 percent mortality 3 years after inoculation. In a second trial (also inoculated in 2008), with a different geographic source of rust inoculum, the two families from the Interior West source again rated poorly for resistance (97.9 percent of seedlings with stem infections ~15 mpi). Ratings of families or seed sources for relative resistance levels may be influenced by the effective level of inoculation. A less effective inoculation may give some indication of relative resistance among sources, but may not be indicative of how these sources will survive on higher hazard field sites. It is more likely that a less effective inoculation would lead to designating seedling families as resistant when they in fact escaped inoculation due to poor spore coverage or physiological status of the seedling.

The presence of families and populations with relatively moderate levels of stem symptoms in this trial is encouraging given the highly effective inoculation. Land managers have been notified of the highest ranking parents, and seed from these can be used in restoration efforts or to establish seed orchards. A more comprehensive summary of all resistance traits compiled over the 5-year post-inoculation period for this trial and other trials at DGRC is underway.

Additional half-sib families from British Columbia are now being tested at DGRC (Murray and Berger, this proceedings), and some of these show promise in the early stages of the test. Hundreds of other selections are now in testing in both the Pacific Northwest and Interior West rust-resistance testing programs, and updates on trends and levels of rust resistance will be presented over time.

Extrapolating seedling testing results to field resistance over the life of long-lived whitebark pine needs to be done with caution. Results from trials with low effective inoculation should be used with caution or the trial repeated with a higher effective inoculation. At least a subset of seedling families should be included in multiple tests in different years to demonstrate repeatability of performance as well as to provide linkages between different trials. Long-term field trials with susceptible controls should be established to provide validation of the seedling screening results. In the Pacific Northwest region (Oregon and Washington) and British Columbia, field trials with known susceptible controls have been established in several areas to examine field resistance and durability of resistance (e.g., Beck and Sniezko, this proceedings; Murray and Berger, this proceedings).

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Blister Rust Inoculation Trials for Whitebark Pine (*Pinus albicaulis*) in the Canadian Kootenay Region

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Abstract—Controlled inoculations of whitebark pine (*Pinus albicaulis*) families in British Columbia (BC), Canada, took place in 2013 and 2014 at the Kalamalka Forestry Centre in Vernon. This effort has focused on 40 families from the Kootenay region with results (identification of disease-resistant families) expected over the next 5 years. Meanwhile, demand continues for further screening of whitebark pine from other regions in Canada. New funding from the BC Forest Genetics Council will permit work to continue. Our plan is to inoculate 40 additional families per year (2016–2018). Seedlings will be collected by multiple partners such as national and provincial parks and possibly mining companies.

INTRODUCTION

Whitebark pine (*Pinus albicaulis*) is a widespread and valuable tree species in western Canada that continues to suffer widespread mortality due to the introduced blister rust disease (agent: *Cronartium ribicola*). The decline appears to have increased during the last 20 years. In 2000, Campbell and Antos (2000) reported on 54 stands across the range in British Columbia (BC) and remarked that on average about 50 percent of whitebark pine was killed or infected by white pine blister rust. They noted the worst rates of rust infection were in the northwest (Smithers) and southeast (Fernie). A more recent survey of the Rocky Mountains in Canada and Montana (USA) (Smith et al. 2008) found mortality had increased from 26 percent (1996) to 61 percent (2004). Due to steep declines, the Canadian government declared whitebark pine as endangered under the Species at Risk Act in June 2012.

As many denuded populations have few surviving trees, their future is jeopardized. Several authors predict that whitebark pine will be locally extirpated where it is less favored and rust thrives (Krakowski et al. 2003; Smith et al. 2008; Tomback and Achuff 2010). Screening selected families for rust resistance is a critical component for maintaining whitebark pine (Environment Canada 2013; Schoettle and Sniezko 2007). To do this, artificially inoculating seedlings with *Cronartium ribicola* followed by post-inoculation assessments of seedling health and survival are useful for identifying disease-resistant families (Sniezko et al. 2011).

LAUNCH (2013)

During August 2013 an inoculation trial was performed at the Kalamalka Forestry Centre (KFC), Vernon, BC, operated by the BC Ministry of Forests, Lands and Natural Resource Operations. Seedlings representing 10 whitebark pine families from BC's Kootenay region were used. These same families were also submitted to the U.S. Department of Agriculture, Forest Service (USDA FS) facility, Dorena Genetic Resource Center

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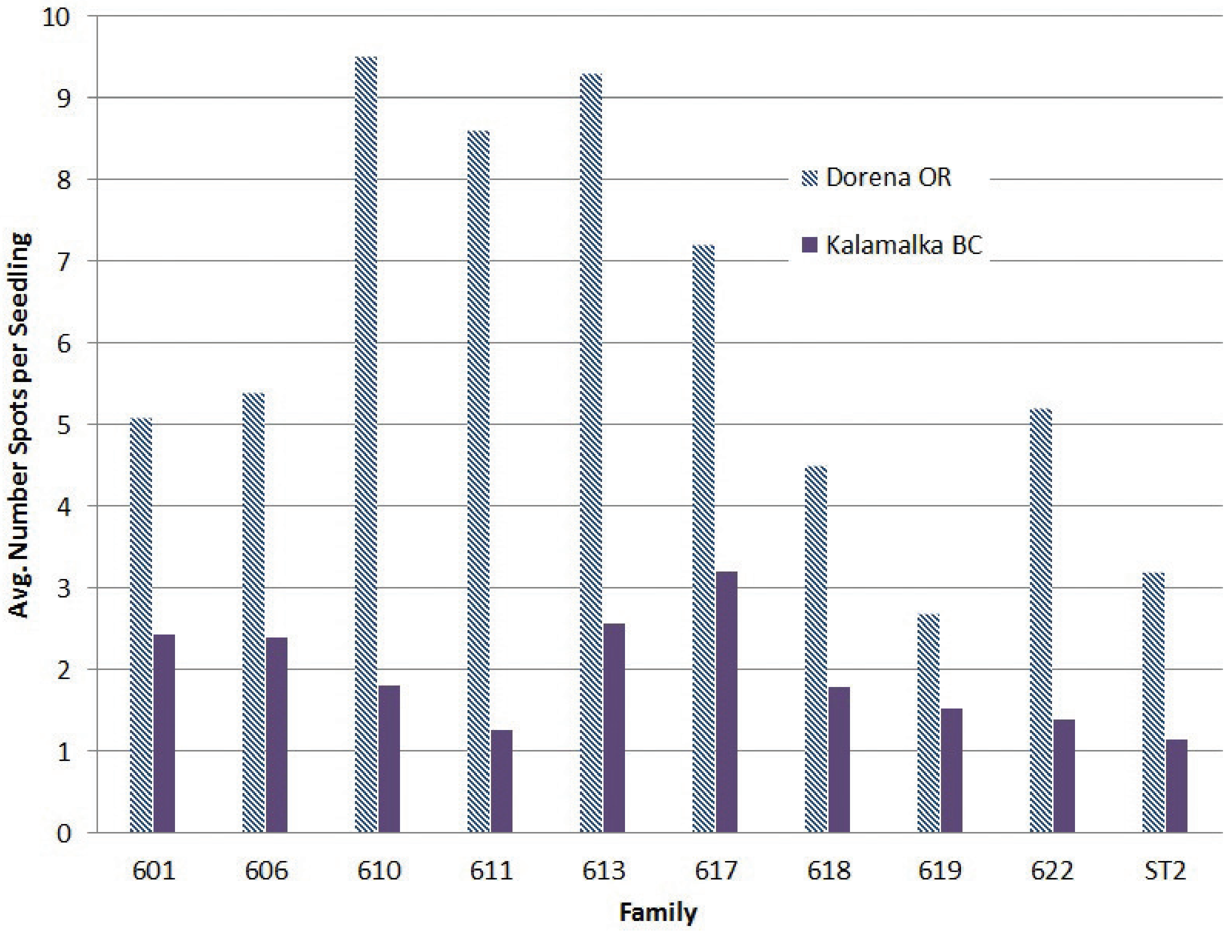

Figure 1—Spots counts observed on needles of inoculated seedlings at 9–10 months post-inoculation.

(DGRC), for tandem inoculations to provide a comparison of results. Each family consisted of 50 individuals per inoculation run (10 families × 50 seedlings × 2 runs = 1,000 seedlings). Our methods were adapted from the protocol in use by DGRC (Danchok et al. 2004). This included randomized blocks of seedlings and achieving a basidiospore load of 3,000 cm^{-2}.

The first run took place within a greenhouse where a plastic frame (polyvinyl chloride piping) formed an inoculation chamber. The second run was conducted in a growth chamber room. Temperatures were maintained near 20 °C and relative humidity was kept in the high 90s (percent). We achieved a target spore loading of about 3,000 spores cm^{-2} in our greenhouse run. However, there were no basidiospores produced in the growth chamber run; this was due to overmature telia on the *Ribes* leaves. Thus, these uninoculated seedlings were allocated to a field trial in Glacier National Park (BC).

During the following spring (2014) separate surveys were conducted by DGRC and Kalamalka personnel for needle spots and cankers associated with the inoculated seedlings. In comparing spot counts between KFC and DGRC, there is notable incongruence (fig. 1). A subsequent survey for cankers was conducted in November 2014 (14 months post-inoculation). Again, surveys indicated very low numbers of cankers on seedlings inoculated at Kalamalka (fig. 2). Although germination of spores was verified, we cannot explain the low incidence of symptoms. A variety of factors may have intervened, including additional biological agents, seedling physiological traits, and low virulence.

2014 AND BEYOND

Seedlings from an additional 30 Kootenay families were inoculated at KFC in August 2014. All inoculations were conducted in the chamber room with spore

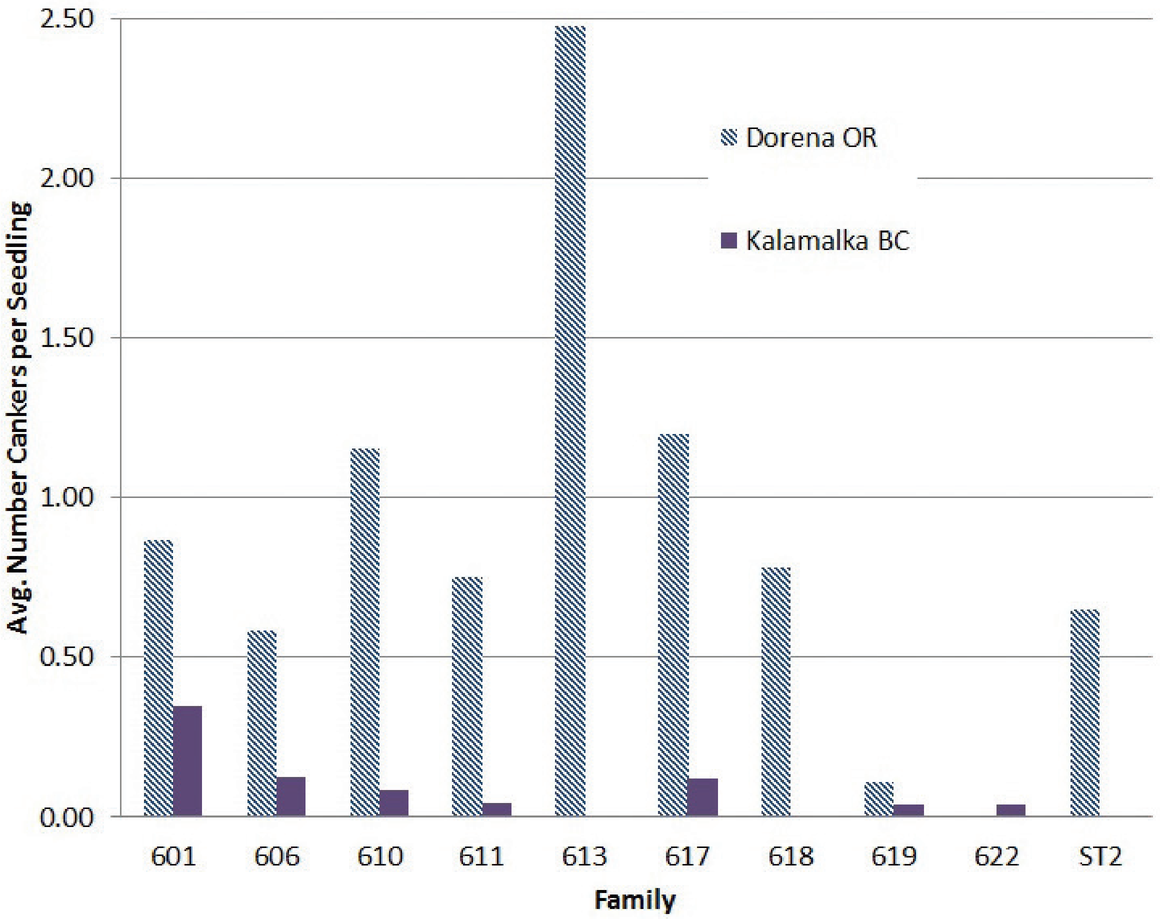

Figure 2—Canker counts observed on inoculated seedlings at 14 months post-inoculation..

loads averaging 4,400 cm^{-2}. In September 2014, three field screening trials were established to screen non-inoculated seedlings from the same 40 families in natural (uncontrolled) environments. About 50 seedlings from each family were planted at each site (fig. 3). During the same week in September, a fourth screening trial was established in Glacier National Park near Golden, BC. This trial used only 10 families (from the failed inoculation run of 2013).

Demand continues for screening additional families. New funding from the BC Forest Genetics Council will permit work to continue. In December, cones and seeds from 26 new families were submitted by Parks Canada. These seedlings will be ready for inoculating in 2016. Our plan is to perform controlled inoculations on up to 40 additional families per year until 2018. The number of families is likely to vary each year based on cone production and capabilities of cone collecting partners. These partners include national and provincial parks and possibly First Nations groups. In addition, coal and gold mining operations are expanding in whitebark pine habitat. The industry is expressing an interest in using whitebark pine for rehabilitation (Moody and Clason 2013).

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Figure 3—Planting a rust screening trial at Idaho Peak, above Slocan Lake, BC (September 2014).

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Variation in Blister Rust Resistance and Early Height Growth in Three Populations of Southwestern White Pine (*Pinus strobiformis*) and Implications for Management and Conservation

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Abstract—Southwestern white pine (SWWP, *Pinus strobiformis*), a long-lived conifer in Mexico and the southwestern United States, is very susceptible to the nonnative pathogen *Cronartium ribicola*, the cause of white pine blister rust. Due to the recent introduction of *C. ribicola* to the Southwest, the future viability of many populations of SWWP is in jeopardy. Fortunately, some early studies showed that there is at least a low frequency of genetic resistance in SWWP. In partnership with the U.S. Department of Agriculture, Forest Service (USDA FS), Southwestern Region Forest Health Protection and colleagues at Northern Arizona University, several trials to evaluate genetic resistance to the rust are now underway at the USDA FS Dorena Genetic Resource Center (Cottage Grove, Oregon, USA). We summarize here early results from a seedling inoculation trial involving 40 half-sib families from three populations in New Mexico. Sources of the three populations were the Lincoln, Cibola, and Santa Fe National Forests. Seventeen of the 20 selections from the Lincoln National Forest were from putatively canker-free trees present within a heavily infested stand; the three others were cankered trees and serve as susceptible controls. There is currently a low incidence of rust infection on the Cibola and Santa Fe National Forests, and the 20 parents selected there were considered random selections. Data collected in this experiment show differences between the tested SWWP populations in early height growth as well as resistance to *C. ribicola*. The Lincoln National Forest population was taller and more resistant to *C. ribicola* than the randomly collected populations from the Cibola and Santa Fe National Forests.

INTRODUCTION

Populations of southwestern white pine (SWWP, *Pinus stobiformis*), a long-lived conifer native to the American Southwest and Mexico, are increasingly threatened by white pine blister rust (WPBR) disease, caused by the nonnative fungal pathogen *Cronartium ribicola* (fig. 1). This disease was first reported causing damage to SWWP in the Sacramento Mountains of southern New Mexico in 1990 (Hawksworth 1990). Since then, the fungus has spread throughout central and southern New Mexico and the White Mountains of Arizona (Conklin et al. 2009; Fairweather and Geils 2011). On the Lincoln National Forest in southern New Mexico, incidence of infection has exceeded 80 percent in some areas (Conklin 1994, 2004), with many infected trees in advanced stages of decline. Some large mature trees had hundreds of cankers (fig. 2) and topkill (Conklin 2004).

Both major gene resistance and partial resistance have been previously documented in this species (Hoff et al. 1980; Kinloch and Dupper 2002; Sniezko et al. 2008), but relatively few families have been evaluated for partial resistance. In this common garden study

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Figure 1—Southwestern white pine (a) sapling with several branch cankers, (b) bole canker with aecia, and (c) large tree with many cankers and top kill from rust (photo credits: Mary Lou Fairweather (a & b); Betsy Goodrich (c)).

we examine variation in early height growth and resistance to WPBR among half-sib seedling families of SWWP originating from three populations in New Mexico: Cibola (CNF), Santa Fe (SFNF), and Lincoln (LNF) National Forests. The study is ongoing, and we report here only the early trends among the three forest populations in seedling growth, effectiveness of the artificial inoculation, and some elements of early resistance.

METHODS

Seed from 40 families of SWWP from the CNF, SFNF, and LNF were sown in March 2009 at the U.S. Department of Agriculture, Forest Service (USDA FS) Dorena Genetic Resource Center (DGRC; Cottage Grove, Oregon, USA). The seedlots were individual tree collections from 10 parent trees each from the CNF and the SFNF and from 20 parent trees on the LNF. Phenotypically canker-free SWWP surrounded by heavily infected individuals had been previously documented in the Bradford Canyon area of the LNF (Conklin et al. 2009). The parent trees from the LNF included 17 putatively resistant trees that did not exhibit observable cankering and 3 trees that had moderate-to-heavy cankering (to serve here as susceptible controls). The parent trees from the CNF and SFNF populations were in areas with little or no rust at the time of selection and represent random selections from those populations.

Seedlings were grown for two growing years, 1 year in supercell Cone-tainers™ (Ray Leach, Canby, Oregon) (164 cm3) in family blocks in the greenhouse,

Figure 2— "Discovery Tree"—the first infected *Pinus strobiformis* tree found in the Southwest, as it appeared in 1990. Branch 'flagging' from hundreds of blister rust cankers is from 5–6 year-old infections. The tree is located 2 miles NE of Cloudcroft, on the Lincoln National Forest (photo: Dave Conklin).

followed by transplanting to 0.9 m × 1.2 m × 0.3 m boxes outside for the second growing season (fig. 3). Twelve to 60 seedlings were available for each family (mean = 47), and seedlings were transplanted into family row plots in a randomized complete block design with 6 blocks and up to 10 seedlings per family per block. Seedlings were inoculated in September 2010 with basidiospores of *C. ribicola* (fig. 4) (see Kegley and Sniezko 2004 and Sniezko et al. 2008, 2011 for general protocols used in inoculations at DGRC). Mean inoculum density was 4,527 spores/cm^2; basidiospore germination was 98.7 percent. Both primary and secondary needles were present at the time of inoculation.

Seedlings were assessed in spring 2011 for second-year height (HT2), number of needle spots on secondary needles and primary needles, and number of stem symptoms (cankers or bark reactions). Additionally, the percentage of secondary needle necrosis caused by needle blight fungi and environmental factors was recorded for each seedling. Subsequent annual assessments were done in late fall and early winter of 2011, 2012, 2013, and 2014; presence and number of stem symptoms by type (normal canker or bark reaction), severity of stem symptoms, and mortality were recorded. Third-year height (HT3) was recorded in fall 2011; this was the new growth in the year following inoculation with rust, including any late-season lammas growth. The severity score quantified the cumulative impact of all the stem symptoms for a seedling at each assessment. It ranged from 0 to 9 (where 0 = no stem symptom; 1 to 4 = very small or nongirdling infection(s); 5 = at least one stem symptom encircling the diameter of the seedling, but little vertical extension; 6 to 8 = extensive cankering encompassing much of the seedling radially and vertically; 9 = seedling dead from rust). Here, we report on some assessments through winter 2011, using the four groups (populations) of families: (1) CNF, (2) SFNF, (3) LNF-R (from canker-free parents), and (4) LNF-S (three susceptible controls).

Statistical analyses were conducted by using SAS® 9.4 (SAS Institute Inc., Cary, North Carolina, USA). The analyses of variance (ANOVAs) for heights were based on family-plot means using PROC MIXED. Forests and families nested within forests were considered fixed effects; block effects were considered random. For HT3, the severity of rust was added to the model as an explanatory variable. For analyses on binomial responses such as mortality, a logistic regression model with logit link was fit using PROC GLIMMIX, with forests treated as fixed effects and random block effects.

RESULTS

There were statistically significant differences among the four populations and among families within populations for both second-year height (pre-inoculation, $P < 0.0001$ for both) and third-year height (1 year post-inoculation, $P < 0.0001$ for both). Seedlings from the LNF-R were on average taller (20.1 cm for the 17 resistant parents) than those from CNF (19.2 cm) or SFNF (18.2 cm) or those from the three susceptible parents on the LNF (18.5 cm) for HT2. Some of the height differences between families within a population were also visibly striking (fig. 5). In this trial,

Figure 3—Southwestern white pine (*Pinus strobiformis*) trial shortly after artificial inoculation with *Cronartium ribicola*.

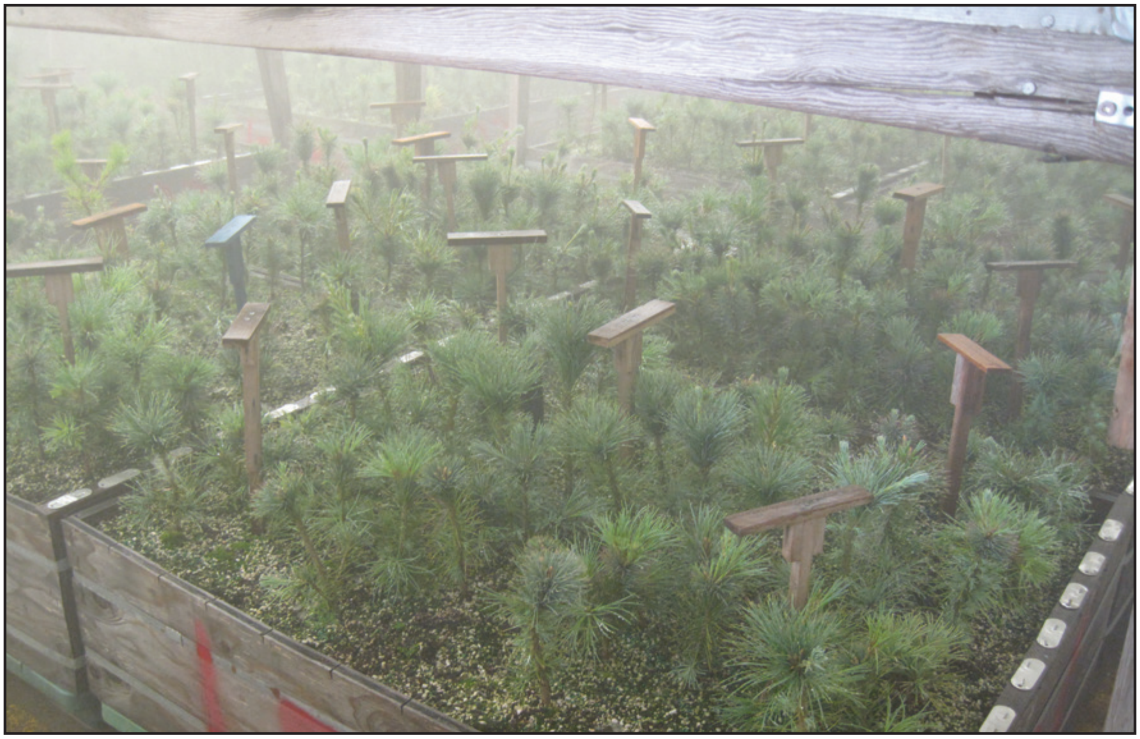

Figure 4—Southwestern white pine (*Pinus strobiformis*) in Dorena Genetic Resource Center 'fog' chamber just prior to artificial inoculation with *Cronartium ribicola*.

Figure 5—Large within-population variation in height growth after second growing season for two families from Cibola National Forest. Family ‘49’ is taller (row plot on left, mean 2nd year height = 23.5cm) and the much shorter Family ’45’ (row plot on right, mean height = 17.7cm).

there was a significant negative correlation between third-year height and the severity of rust infection at the time of inspection ($P = 0.002$). Seedlings with a one-unit higher canker severity score were on average 0.9 cm shorter (95-percent confidence interval from 0.3 to 1.4 cm shorter).

The inoculation of the seedlings with *C. ribicola* was very effective in this experiment, with 97.3 and 99.6 percent of seedlings exhibiting characteristic needle spots on the primary and secondary needles, respectively (fig. 6). The mean number of needle spots per seedling on secondary needles was 40.9 at 9 months post-inoculation (mpi).

Stem symptoms, cankers, and bark reactions appeared relatively quickly; 56.9 percent of seedlings had stem symptoms 9 mpi and 87.0 percent had stem symptoms at 15 mpi (fig. 7). At second assessment (~15 mpi), the seedling progenies from the LNF-R averaged 75.7 percent with stem infections versus 94.1, 98.0, and 90.7 percent with stem infections for those from the CNF, SFNF, and LNF-S, respectively; the mean number of stem infections per family was 11.2 (ranging from 2.9 to 20.7 for the 40 half-sib families). Mean severity of rust ~15 mpi varied from 3.9 for LNF-R to >6.0 for the other three populations in this study.

The percent secondary needle necrosis present in early spring 2001 in this study varied significantly for the four groups of seedling families studied ($P < 0.0001$), with 54, 67, 59, and 68 percent of seedlings affected on the LNF-R, LNF-S, CNF, and SFNF, respectively. The mean percentage of secondary tissue affected overall was 8.2 percent. Only 10.4 percent of seedlings had more than 20 percent of secondary tissue affected.

Figure 6—Southwestern white pine (*Pinus strobiformis*) seedling from Santa Fe National Forest (Sow #59) with hundreds of needle spots after blister rust inoculation; this family had 41 needle spots per seedling and 94.4 percent of seedlings with stem symptoms (and 13 stem symptoms per seedling) at 9 and 15 months post-inoculation, respectively.

After accounting for forest differences, there was no evidence of a relationship between either the probability of a seedling exhibiting needle necrosis or the percentage of secondary tissue affected and the percentage of seedlings cankered ($P = 0.54$ and $P = 0.90$, respectively).

Eight percent (150 of the 1,872) of the trees in the trial were killed by *C. ribicola* over the 15-mpi monitoring period reported here. This mortality varied significantly by population ($P < 0.0001$), from 2.5 percent for LNF-R, to 7.5 percent for LNR-S and 12.5 percent for both CNF and SFNF. One family from CNF had 36 percent rust-related mortality at this early stage.

DISCUSSION

Differences in early height growth were found between the populations and may suggest a need for establishing breeding zones within this species. Other trials are now underway to examine in more detail the differences in some adaptive traits in this species (Goodrich et al., this proceedings). The rapid development of cankers during the first growing season after inoculation affected normal season growth, the lammas growth at the end of the growing season, or both; seedlings with the highest severity ratings for rust showed the most impact to growth in the 15 months following inoculation.

Figure 7—Southwestern white pine seedling from Cibola National Forest family (Sow #50) with numerous stem cankers 14 months after inoculation; this family had 58 needle spots per seedling (9 months post-inoculation) and 94.8 percent of trees with stem symptoms and 17 stem infections per seedling, at ~15 months post-inoculation.

The overall frequency of canker-free seedlings was low (<6 percent) at 15 mpi in the seedling families from the parental selections from the SFNF and CNF, populations which originated from stands with little or no blister rust present. A low frequency of canker-free trees was also found in several earlier seedling inoculation studies of SWWP resistance to WPBR in the United States (Hoff 1980) and Germany (Stephan 1986). These data and the high infection (>80 percent) in some natural stands (Conklin 2004) confirm the high susceptibility of SWWP and the relatively low incidence of genetic resistance to WPBR in this species. Seedling families from the canker-free parent trees on the LNF had the lowest levels of early stem infection (mean = 75.7 percent) following artificial inoculation, but several families from this population had more than 90 percent of seedlings with stem symptoms. The parent trees from LNF are in an area of very high incidence and severity of rust infections, and 17 of the 20 selections tested were canker-free at the time of seed collection. Efficacy of selection was expected to be higher in this population than that from the other two forests given the presence of *C. ribicola* within the LNF for decades before selection, and this holds true for the early component of resistance reported here. A smaller, earlier trial of progeny from other parent trees from LNF also indicated a relatively high frequency of resistance, including a relatively low percentage of trees with stem symptoms (42.5 percent) 39 mpi (Sniezko et al. 2008). The lower level of stem symptoms in the earlier trial may be due to the different families utilized, a higher effective inoculation in the trial reported in this paper, or a combination thereof. In previous seedling testing at the USDA FS Institute of Forest Genetics (Placerville, California), progeny of the 17 canker-free LNF parents showed no evidence of 1:1 segregation for major gene resistance (D.A. Conklin, personal communication, 2008). The primary needles (which are more susceptible than secondary needles) present on the seedlings in this test may have led to somewhat higher stem infection than if only secondary needles had been present. Regardless, selections from the canker-free LNF parents were more resistant to WPBR in this trial than those from the other two geographic areas. Field trials to confirm the actual level and durability of resistance are needed before extrapolating any seedling assay such as this one to long-term field results.

This trial is still ongoing, but large differences among families and populations in resistance to WPBR are apparent. Analyses are currently underway, and in later summaries we will discuss the family and seed source variation in additional rust-resistance traits over the 5-year post-inoculation assessment period. In this study we observed necrosis on the secondary needles for each of the seedling families. The data indicated that although a moderate frequency of needle necrosis caused by an unknown incitant was present in each of the seedling families, it affected only a small percentage of the secondary needle tissue on most seedlings. The incitant probably had very little impact on cankering incidence, as we did not observe any association between percent necrosis and percent cankered. An additional 158 families from throughout the U.S. portion of the range of SWWP were sown in 2014 for blister rust inoculation in 2015, and this will provide a broader estimate of the frequency and distribution of resistance to *C. ribicola* in this species.

Field trials to validate resistance observed in seedling inoculation trials and to examine its durability are planned. Seed collections for gene conservation have begun, and studies of genetic variation in adaptive traits are underway (Goodrich et al., this proceedings). Other management options for mitigating the effect of WPBR in natural stands have been discussed elsewhere (Conklin et al. 2009). Some of the high-hazard sites in the Southwest may necessitate planting of resistant seedlings, but much can also be done by favoring white pine in silvicultural treatments. Although thinning of stands can decrease the likelihood of stand-replacing fire and thereby conserve possible resistant/tolerant white pines, it also increases the understory sunlight and in some areas, especially when combined with underburning, may lead to an increase in populations of *Ribes* species, the alternate host for *C. ribicola*. Fortunately, there are some forested areas in the Southwest with little to no presence of susceptible *Ribes* species (M.L. Fairweather email to Sniezko, Feb. 12, 2015; Geils et al. 1999; Looney 2012; van Arsdel et al. 1998).

Southwestern white pine is very susceptible to white pine blister rust, and the frequency of naturally occurring resistance is low. Overall forest management goals, as well as an understanding of the natural resistance present in SWWP, will have to be considered if the region's white pine resources in many areas are to remain viable. Given the relatively recent introduction of WPBR to the Southwest, it is imperative that we identify and quantify resistance in this species, including the frequency of occurrence over the landscape. Based on permanent plot monitoring, it is clear that retaining SWWP on high-hazard sites will be difficult without resistant planting stock. The first rust-resistant parents have now been identified, and seed can be collected from them to use for reforestation and restoration. These selections should also be grafted into orchards or clone banks to ensure retention of these valuable resistant parents.

ACKNOWLEDGMENTS

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Persistence of Major Gene Resistance in Western White Pine (*Pinus monticola*) in British Columbia

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White pine blister rust, a devastating disease caused by the rust pathogen *Cronartium ribicola*, has been attacking and killing western white pines (*Pinus monticola*) for more than 100 years since its introduction to the Canadian province of British Columbia (BC) (Hunt 2009). There is a single dominant (major) gene for resistance (MGR) that produces a hypersensitive infection spot similar to that observed in resistant reactions in other plants attacked by rust pathogens. It was first found in a limited geographic area of southern Oregon, USA, known as the Champion Mines (Kinloch et al. 1999). Resistant Champion Mines clones were established at Dorena, Oregon, in the mid-1960s; since then, however, the pathogen has circumvented this resistance at Dorena and other areas in southern Oregon (Kinloch et al. 2004). This MGR resistance was imported to BC in seed collected from Dorena. Resistance came from seedlots from two geographic areas: (1) open-pollinated (OP) Champion Mines clones, and (2) OP Washington State, USA clones. Seedlings were planted into nine root disease sites in 1986 and 1987 throughout BC. Details of the plantation design using these 2 pine sources and 12 other provenances can be found in Hunt (1987) and Meagher and Hunt (1998). The first seedlot was also planted into three coastal and three interior provenance trials in 1988 (see Meagher and Hunt 1998) and the second seedlot into three demonstration plantations in 1994. These trees have been examined for growth and blister rust periodically over the years. At the previous IUFRO rust meeting held in OR in 2001, we (Hunt et al. 2004) reported that this resistance appeared to be persistent. In this report we provide a 2014 update.

The growth of the Washington trees was consistently superior to that of the Champion Mines-derived trees. At age 10 to 12, it was apparent in several of the plantations that about 50 percent of the trees in each of these two seedlots were rust resistant. Seed was collected from a sample of these canker-free trees. By using the protocol of inoculating 50 seedlings/parent as recommended by Bro Kinloch, it was determined that 50 percent, or all, of their offspring produced hypersensitive needle reactions indicative of heterozygous and homozygous MGR resistance, respectively. The MGR resistance within the Washington trees was attributed to pollination at Dorena from Champion Mines clones, because none of these trees was homozygous resistant. Rust surveys and observations in 2013 and 2014 of six root disease sites continued to demonstrate that there was no apparent failure of MGR resistance (table 1; trees missing or those killed by other agents were omitted from the dataset, and MGR and neighboring geographic sources were pooled). The six provenance sites have not been formally inspected since 2001. The demonstration plantation

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Table 1—Blister rust infection on pooled sources of western white pine across seven plantation sites.

	Canker-free (%) white pine from four pooled sources[a]			
Site	**Pool #1**	**Pool #2**	**Pool #3**	**Pool #4**
Marl, 51°31 W117°25	87	89	88	53
Tisdall, 52^{0}14 W120^{0}54	85	84	62	73
Beaver FR, 54°3 W122°10	75[b]	75[b]	11[b]	75[b]
Busk, 49°38 W117°9	63	24	7	0
Sharpe, 55°07 W127°21	56	12	0	4[b]
Alberni, 49°14 W124°44	52	12	6	0
Smallwood, 49^{0}30 W117^{o}26	40[c]	31	4	0

[a] Pooled sources: #1 MGR, #2 BC coast, #3 BC interior/Idaho/Montana, and #4 California/Warner Mts.

[b] Small sample size.

[c] After blister rust killed many young trees, drought appeared to preferentially kill the large survivors. In the MGR group this meant loss of canker-free trees and a reduction in the sample size to 30.

at Woss (northern Vancouver Island) was inspected in 2007. Washington sources at the other two plantations (Texada Island and southern Vancouver Island) were inspected in 2011 for rust status prior to scion collection. In each of these three plantations, the Washington source was least cankered at about 50 percent.

In BC, most cankers were located close to the ground (Hunt 1982, 1998) so the three demonstration plantations have largely grown through that young age when they were most susceptible to attack. To continue monitoring for the persistence of MGR resistance in BC, younger plantations should be inspected. More recently, the BC Forest Service has imported three seedlots from Dorena for operational use. We have periodically monitored one of these younger plantations and confirmed hypersensitive needle spots by inoculating offspring from canker-free parents. A 2013 survey of a 2002 trial using these three lots on three coastal sites, averaged 64, 66, and 70 percent canker-free trees per lot compared to 27, 29, and 30 percent canker-free trees for non-MGR selections. To date there is no evidence of MGR failure in BC.

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Comparative Look at Rust Infection and Resistance in Limber Pine (*Pinus flexilis*) and Rocky Mountain Bristlecone Pine (*P. aristata*) Following Artificial Inoculation at Three Inoculum Densities

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INTRODUCTION

Limber pine (*Pinus flexilis*) and Rocky Mountain bristlecone pine (*Pinus aristata*) are important components of forest ecosystems in the southern Rocky Mountains, United States. Both species are susceptible to white pine blister rust caused by the fungal pathogen *Cronartium ribicola*. However, very little is known about the relative resistance of the two species. In this study, we used artificial inoculation of seedlings to compare the relative susceptibilities of these two species. The objective of the inoculation test was to compare the performance of seedlings from bulk seedlots of limber pine and bristlecone pine exposed to three inoculum densities (low, medium, and high). This information will provide guidelines for inoculum density levels for future trials of these species and a first look at the extent of resistance present in the general population and its efficacy under exposure to increasing levels of inoculum.

METHODS

The seedlings were grown by the Colorado State Forest Service Nursery in Fort Collins, Colorado, USA from bulk seedlots collected from parent trees in the Rollinsville, Colorado, area. Limber pine seedlings were 3 years old and bristlecone pine 2 years old when transported by covered truck on August 14, 2003, to the U.S. Department of Agriculture, Forest Service, Dorena Genetic Resource Center (DGRC; Cottage Grove, Oregon, USA). Seedlings were transported and inoculated in 30-cell Styroblocks™ (Beaver Plastics, Acheson, Alberta, Canada). A randomized complete block design with three replications of three treatments for the two species was used for inoculation and outplanting. Thirty limber pine seedlings were used in each block-by-treatment combination for a total of 90 seedlings per treatment. Twenty-four bristlecone pine seedlings were used per treatment block for a total of 72 seedlings per treatment. The limber pine seedlings were grown in removable containers placed in Styroblocks, and individual seedlings were randomly assigned to the treatment blocks. Seedling numbers were equalized (30 seedlings per treatment block) among the blocks. Limber pine seedlings were also spaced out (15 seedlings per Styroblock) to increase the likelihood of infection. The bristlecone seedlings, however, were directly planted into the Styroblocks, so an entire Styroblock was randomly assigned to a treatment block.

Trees were inoculated on September 18, 2003, with naturally infected *Ribes* leaves placed above the trees (*Ribes* species are the main alternate host). The

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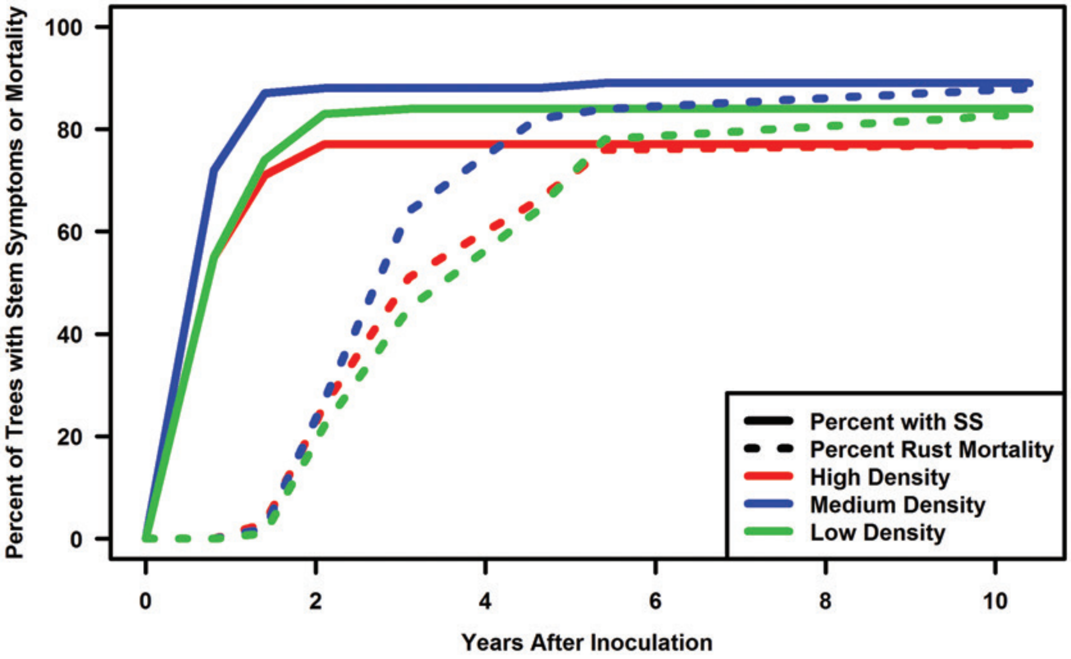

Figure 1—Limber pine seedlings with stem symptoms and mortality following inoculation by *C. ribicola* at three inoculum spore densities.

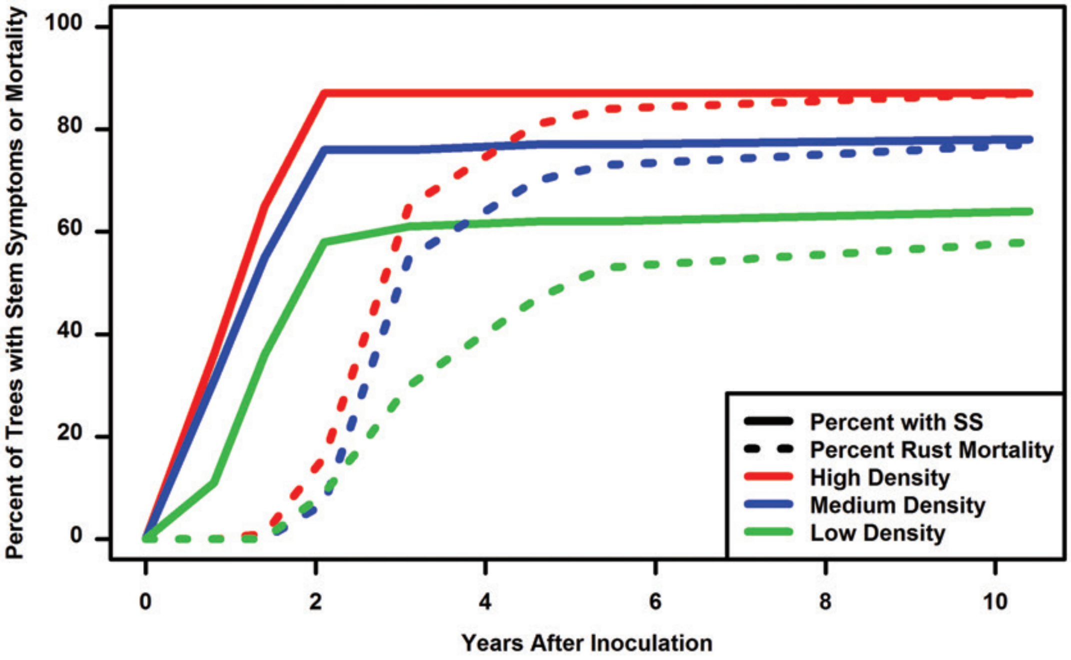

Figure 2—Bristlecone pine seedlings with stem symptoms and mortality following inoculation by *C. ribicola* at three inoculum spore densities.

infected *Ribes* leaves were collected from forest stands in Oregon and Washington (USA) and represent non-vcr2 sources. Treatments consisted of three target inoculum densities: low (3,000 basidiospores cm^{-2}), medium (6,000 basidiospores cm^{-2}), and high (9,000 to 10,000 basidiospores cm^{-2}). At DGRC, 3,000 spores cm^{-2} is the density used in operational inoculations of western white pine (*P. monticola*); 6,000 spores cm^{-2} is the density used in inoculation trials of sugar pine (*P. lambertiana*). When the targeted inoculum density was reached, the *Ribes* leaves were removed from that treatment. The actual inoculation densities achieved were somewhat higher than the target (3,260, 8,227, and 12,053 spores cm^{-2}) (table 1). Basidiospore germination was monitored at inoculation and was at least 90 percent for all treatments except the rep one high density, which was 52 percent. Seedlings were transplanted in October 2003 to three standard DGRC outdoor planting boxes (0.9 m wide × 1.2 m long × 0.3 m high). One box was used per species replication, and there were three contiguous rows of 10 limber pine and 8 to 9 bristlecone pine seedlings per treatment. Seedlings were assessed for needle lesions (spots) twice during the first year and for stem symptoms yearly for 5 years and then at 10 years.

RESULTS

At the first-year assessments, we noted that inoculum spore density did not affect measures of infection on limber pine as much as Rocky Mountain bristlecone pine (table 2). The effect of inoculum density could be noted in bristlecone pine after 2 years, but limber pine was so susceptible that the low spore density caused as much infection as the high concentration (figs. 1 and 2). We also found that averaged over all spore densities at 1 year post-inoculation, limber pine exhibited a much higher percentage (61 percent) of stems with symptoms than bristlecone pine (26 percent). By 10 years post-inoculation, 83 percent of limber pine and 76 percent of bristlecone pine seedlings exhibited stem symptoms, with little change from the 5-year assessment (figs. 1, 2, 3–5; table 2). There was little or no change in percentage of stems infected after year 2, so we assume there was little to no natural infection from spores in the DGRC area between year 1 and 10. Aecia production occurred by year 3 on many seedlings (figs. 5 and 6). On average, 83 percent of limber pine and 74 percent of bristlecone pine seedlings were dead 10 years post-inoculation, and the temporal dynamics of mortality were similar (figs. 1 and 2).

At the first assessment, 10 months post-inoculation, limber pine showed a much higher incidence of needle spots per tree (15 to 23; fig. 6) compared to the 1 to 2 spots per tree on Rocky Mountain bristlecone pine at the three inoculum densities (table 2). The percentage of seedlings with spots on secondary needles was also much higher in limber pine (mean of 92 percent), with 89 percent of seedlings exhibiting needle spots at the low inoculum density, 98 percent at medium density, and 90 percent at high density. In Rocky Mountain bristlecone pine the mean was 35 percent of seedlings with needle spots, with 23 percent at low inoculum density, 42 percent at medium density, and 45 percent at high density. Rocky Mountain bristlecone pine seedlings had a fairly high percentage of primary needles with needle spots (34 to 69 percent over the three densities), whereas limber pine had only 3 to 7 percent of seedlings with primary needle spots. This difference is most likely because the limber pine seedlings were 3 years old and had relatively few primary needles present compared to the 2-year-old bristlecone pine seedlings.

SUMMARY AND DISCUSSION

The bulk seedlots from the Rollinsville, Colorado, area produced Rocky Mountain bristlecone and limber pine seedlings that were very susceptible to white pine blister rust. The levels of inoculum density utilized influenced infection levels in bristlecone but not limber pine. The lowest inoculum density level used here (~3,260 spores cm^{-2}) may be adequate in future trials for limber pine, but Rocky Mountain bristlecone pine is likely to require higher inoculum densities.

Over all inoculum densities, limber pine had a higher percentage (61 percent) of stems infected than bristlecone (26 percent) 1 year after inoculation, but by year 5 this was narrowed to 83 and 75 percent, respectively, indicating that latent canker development (a possible partial resistance trait) was more common in bristlecone pine (table 2). Both species had latent infections developing after those present 1 year post-inoculation. The 26-percent survival by bristlecone and 17-percent survival of limber pine at year 10 may indicate a source of resistance in the source population as seen by subsequent tests (Schoettle et al. 2011, 2014). However, only a few inoculated seedlings with stem infections survived the duration of the trial, indicating limited occurrence of bark resistance.

The canker-free seedlings in limber pine may be predominantly due to major gene resistance (Schoettle et al. 2014). Major gene resistance has been noted in limber pine (Schoettle et al. 2014) and would not generally be influenced by inoculum density, but this type of

Table 1—Mean inoculation time, inoculum density, and basidiospore germination percentage (± 1 standard error) for three treatments of bristlecone and limber pine.

Treatment	Duration (h)	Mean inoculum density	% basidiospore germination
Low	7.1 ± 0.3	3260 ± 155	94.7 ± 2.6
Medium	11.4 ± 1.4	8227 ± 590	95.0 ± 1.5
High	11.3 ± 0.1	12053 ± 670	81.0 ± 14.7

Table 2—Average number of spots and stem symptoms per tree and percentages of pine seedlings with stem symptoms and killed by white pine blister rust infections.

Inoculum density	Year one needle spots/tree		Year one stem symptoms/tree		% seedlings with stem symptoms / % mortality					
					year one		year five		year ten	
	limber	bristlecone	limber	bristlecone	limber	bristlecone	limber	bristlecone	limber	bristlecone
low	15	1	3.3	0.2	55/ 0	11/ 0	84/ 78	62/ 53	84/ 83	64/ 58
Medium	23	1	7.2	0.6	72/ 0	31/ 0	89/ 84	77/ 73	89/ 88	78/ 77
High	19	2	4.2	0.9	55/ 0	36/ 0	77/ 76	87/ 84	77/ 77	87/ 87

Figure 3—Limber pine seedling 1 year after inoculation with needle spots.

Figure 4—Limber pine seedling 1 year after inoculation with stem symptoms and needle spots.

Figure 5—Limber pine with aecia 3 years after inoculation.

Figure 6—Bristlecone pine 3 years after inoculation with aecia on the stem.

resistance has not been confirmed in bristlecone pine. The increasing level of infection in bristlecone pine with increasing inoculum density may suggest that partial resistance is present and can be at least somewhat eroded at very high inoculum densities. Future trials should investigate even higher levels of inoculum density to ascertain whether some level of resistance is maintained in Rocky Mountain bristlecone pine under extreme pathogen pressure. The inoculum levels used at DGRC may need adjustment depending on the seedling culture (seedling physiological state) and local environment pre- and post-inoculation. Primary needles are more susceptible than secondary needles. Nursery protocols that provide young seedlings with predominantly secondary needles, which are likely to give the best correspondence with field resistance, should be developed.

Artificial inoculation trials to examine blister rust resistance using seed from individual parent tree collections (half-sib seedlots) has begun for seedlings of both limber pine and Rocky Mountain bristlecone pine (Schoettle et al. 2011, 2014; Sniezko et al. 2011, 2016). Such trials will help delineate the level and type of partial resistance, as has been noted in other species (Sniezko et al. 2014). Field trials of these two species to validate resistance from seedling trials, like those established for *P. monticola*, *P. lambertiana*, and whitebark pine (*P. albicaulis*), are needed. Several small trials have recently been established (see Schoettle et al., this proceedings, *Southern Rockies Rust Resistance Trial*). If confirmed in other tests, the level of resistance found in the bulk seedlots used in this study is encouraging, and the information can be used by land managers for restoration efforts.

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Southern Rockies Rust Resistance Trial

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The nonnative pathogen *Cronartium ribicola*, the cause of the lethal white pine blister rust (WPBR) disease, is spreading through limber pine (*Pinus flexilis*) and Rocky Mountain bristlecone pine (*P. aristata*) forests of the southern Rocky Mountains (USA). An integrated regional program—the Proactive Strategy—is characterizing the infestation and gaining ecological and genetic knowledge of these less well studied ecosystems. This knowledge will provide the science foundation for early interventions to mitigate the development of ecological impacts to the high mountain headwater ecosystems (Burns et al. 2008; Schoettle and Sniezko 2007; Schoettle et al., this proceedings, *The Proactive Strategy: Preparing the Landscape for Invasion by Accelerating the Evolution of Resistance*). Identifying and developing planting material with genetic resistance to WPBR, and understanding its field performance, is essential for managers to sustain these forests into the future.

The Southern Rockies Rust Resistance Trial (SRRRT) was begun in 2013 to field verify genetic resistance to WPBR identified during seedling inoculation screening tests of limber and Rocky Mountain bristlecone pines conducted in collaboration with the U.S. Department of Agriculture, Forest Service (USDA FS) Dorena Genetic Resource Center (DGRC; Cottage Grove, Oregon, USA) and the Institute of Forest Genetics (IFG; Placerville, California, USA) (Schoettle et al. 2011, 2014). This field test will assess if the resistance frequencies and disease symptom phenotypes that develop under natural growing and inoculation conditions at SRRRT are similar to those observed for the same families under the controlled inoculation tests of young seedlings in a different environment and with a different geographic source of the pathogen. Growing conditions, rust hazard, and inoculum sources can affect the expression of disease resistance and susceptibility, so field verification increases restoration deployment guidance and the potential for restoration success.

An existing administrative site on the Pole Mountain Unit of Laramie Ranger District, Medicine Bow National Forest that was used as a USDA FS nursery in the past was revitalized for this project. Seed from previously tested resistant and susceptible individual-tree collections (i.e., families) was sown March 22–23, 2012, and seedlings grown at the Colorado State Forest Service Nursery (Fort Collins, Colorado). Thirteen limber pine families and 11 Rocky Mountain bristlecone pine families are included in the study and represent seed sources from throughout the southern Rocky Mountains (fig. 1). Administrative approval and site preparation were completed in 2012 and 2013. More than 700 seedlings were outplanted August 26–28, 2013, and another 700 seedlings planted May 27–28, 2014 (figs. 2 and 3). White pine blister rust is common in the limber pine forest in and around the SRRRT site, providing a natural source of inoculum to

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In: Schoettle, Anna W.; Sniezko, Richard A.; Kliejunas, John T., eds. 2018. Proceedings of the IUFRO joint conference: Genetics of five-needle pines, rusts of forest trees, and Strobusphere; 2014 June 15–20; Fort Collins, CO. Proc. RMRS-P-76. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 245 p.

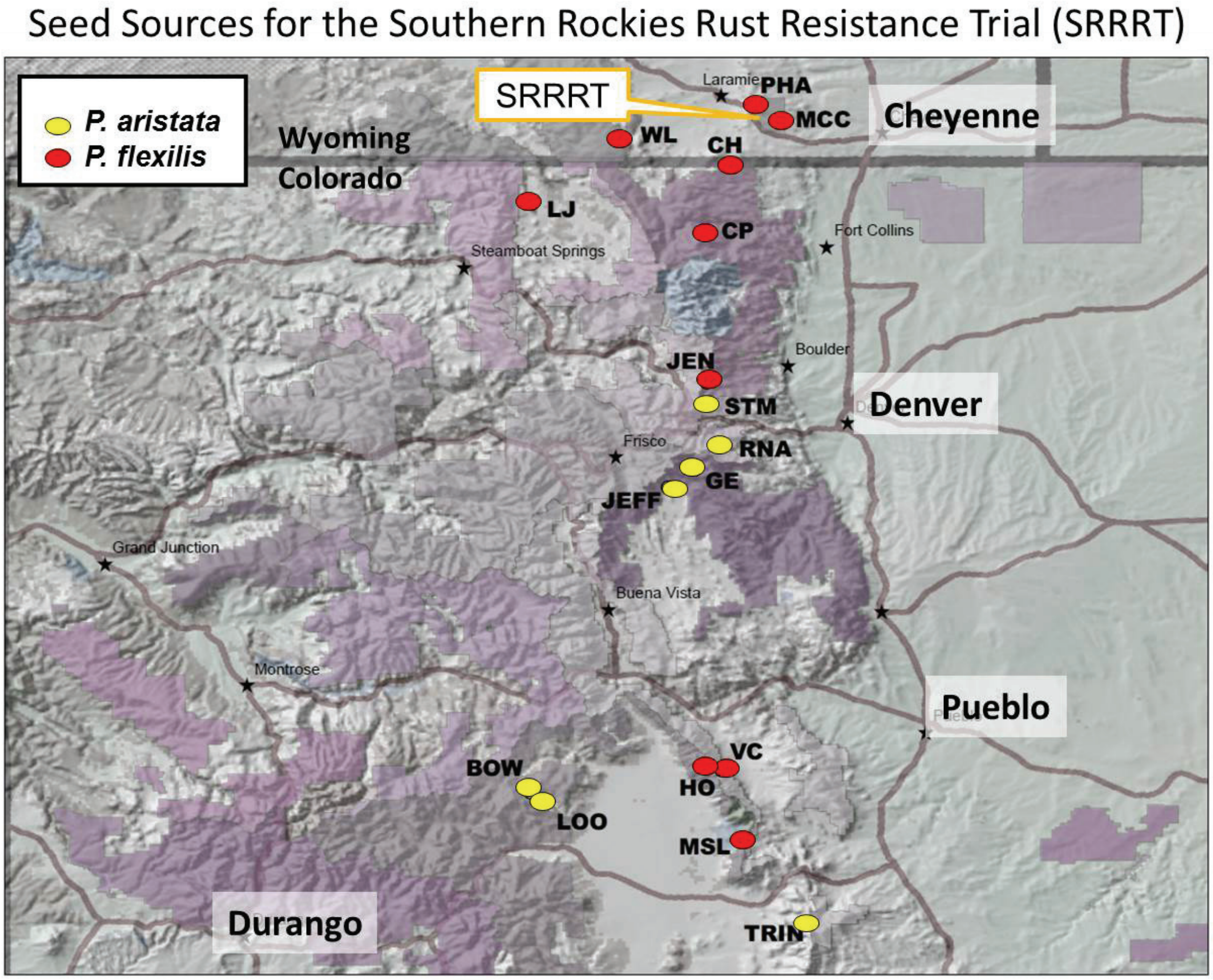

Figure 1—Source locations of the WPBR-resistant and susceptible families in the southern Rocky Mountains and the location of the SRRRT site. Yellow and red symbols denote source locations for bristlecone and limber pine families, respectively.

the seedlings; ponderosa pine (*P. ponderosa*) is also a common forest species in the area. The seedlings will be periodically assessed for signs and symptoms of WPBR over the next 10 years. This project was funded largely by the USDA Forest Service Rocky Mountain Region, Regional Office and is a cooperative effort between the Medicine Bow National Forest, Forest Health Protection, Rocky Mountain Research Station, and Colorado State University. Wyoming Conservation Corps provided a volunteer field crew that was instrumental in completing site preparation, and citizen and University of Wyoming volunteers assisted with seedling plantings.

Additional field trials of southern Rockies seed sources have been established at Happy Camp (California) with previously tested families of both species: Rocky Mountain bristlecone pine planting in 2006 and limber pine in 2012. The Happy Camp planting site is a component of the Pacific Southwest Region's Sugar Pine Resistance Program and offers exposure to the virulent strain of the *C. ribicola* (vcr1) that can overcome Cr1 complete resistance in sugar pine (*P. lambertiana*). Additional previously tested *P. flexilis* seedlings from the southern Rocky Mountains have also been planted in 2008 in an area of the Deschutes National Forest (Oregon).

All of these plantings, and those at SRRRT, include families that showed resistance in seedling inoculation testing at DGRC or IFG as well as families shown to be highly susceptible to *C. ribicola*. The susceptible families are monitored to detect exposure of the seedlings to rust spores to confirm that both resistant and

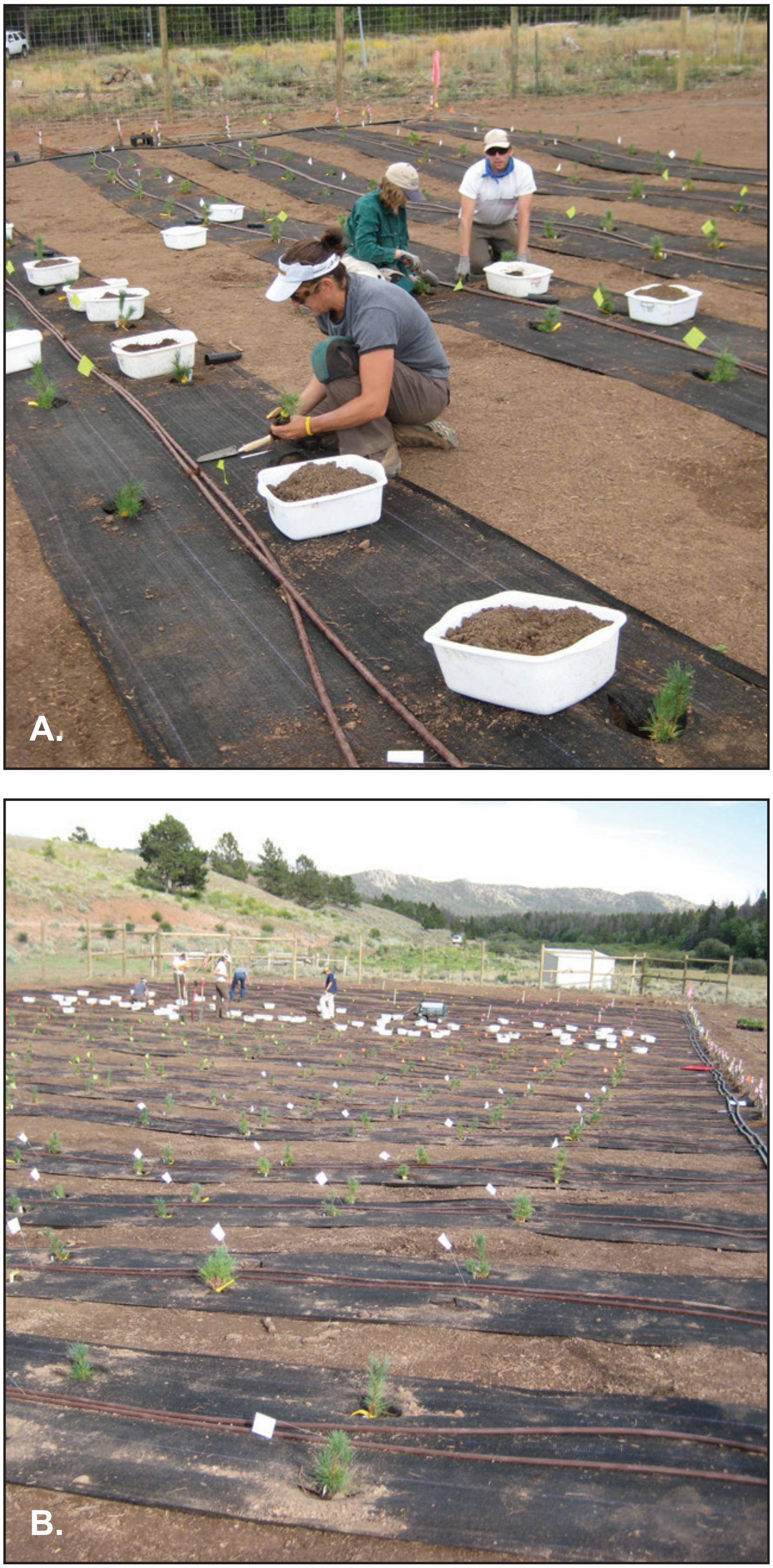

Figure 2—A. Planting seedling on the site. B. Overview of planting area.

Figure 3—Overview of the SRRRT site.

susceptible seedlings are being challenged over time. The resistant limber pine families have shown predominantly major gene resistance (Schoettle et al. 2011, 2014). Resistance in Rocky Mountain bristlecone pine is less well characterized at this point (Jacobi et al., this proceedings; Schoettle et al., this proceedings, *Patterns of Resistance to White Pine Blister Rust in Rocky Mountain Bristlecone Pine (*Pinus aristata*)*).

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Sugar Pine (*Pinus lambertiana*) Partial Resistance Heritability Study

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The health and regeneration of sugar pine (*Pinus lambertiana*), a major species in the mountain forests of California (USA), has been substantially affected by an exotic pathogen, *Cronartium ribicola*, which causes white pine blister rust. Seedlings from trees with major gene resistance (MGR) are being used to increase field survival in plantations. Partial resistance (PR) due to multiple genes is considered to be more durable than MGR, providing protection to sugar pines exposed to a virulent form of blister rust. In 2006, a field study was established on the Klamath National Forest to examine PR traits and their inheritance in progeny from 128 families whose parents were known to produce rust-susceptible (FR) or PR progeny as well as parents having PR traits but without information about their progeny. The crossing scheme was a mixed-factorial diallel; the field design was randomized incomplete blocks with noncontiguous single-tree plots. Trees were planted at close spacing, irrigated, and naturally exposed to blister rust over multiple years from onsite *Ribes* species, the alternate hosts. Data on mortality from rust, number of rust cankers, and canker types were collected from 2010 to 2013. Progeny from PR parent(s) had a substantially lower percentage of trees that died from blister rust (8 to 53 percent with three to one PR trees in a family) when compared to progeny from FR parents (64 to 99 percent with one to two FR parents in a family). Progeny of PR parents also had a higher percentage of trees that were clean of rust (6 to 23 percent) and with no active infections (17 to 58 percent) compared to progeny from FR parents (0 to 2 percent clean of rust; 0 to 10 percent with no active infection). Progeny from other selected parents, based on their PR phenotypes, performed more like progeny from one PR parent or intermediate between one and two PR parents, depending on the trait examined. Quantitative analyses from 2-year data revealed that 58 and 78 percent of the variance in PR inheritance was additive (compare with Kinloch et al. 2012). Further analyses are needed on more parents and to examine components of heritability. Seed orchards with trees having MGR, PR, or both traits have been established for the western Sierra Nevada and are in development for northern California forests.

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Patterns of Resistance to *Cronartium ribicola* in Rocky Mountain Bristlecone Pine (*Pinus aristata*)

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The core distribution of Rocky Mountain bristlecone pine (*Pinus aristata*) extends from central Colorado into northern New Mexico with a disjunct population in northern Arizona (USA). Populations are primarily at high elevations and often define the alpine treeline, but the species is also found in open mixed-conifer stands with ponderosa (*P. ponderosa*) or pinyon (*P. edulis*) pines. On dry, exposed sites the stands are open and bristlecone is commonly the only species present. The combination of the pine's adaptive traits with infrequent disturbance has enabled trees on these sites to attain ages of more than 2,500 years. These same traits and conditions will inevitably hinder the ability of bristlecone pine to adapt to novel anthropogenic stresses such as climate change and infection by the nonnative pathogen (*Cronartium ribicola*) that causes the lethal disease white pine blister rust (WPBR). Infection of bristlecone pine by *C. ribicola* was first documented in the field in 2003 in south-central Colorado (Blodgett and Sullivan 2004). These threats and the species' unique aesthetic and ecological roles make bristlecone pine a species of conservation interest, and we have begun implementing the Proactive Strategy (Schoettle 2004).

The frequency of genetic resistance to WPBR will affect the trajectory of bristlecone pine populations in the future. Here we report results from two of our studies of rust resistance for bristlecone pine families (Schoettle et al. 2011). Seeds collected in 2001 from 184 individual trees across 11 sites throughout Colorado were sown in 2002. The 3-year-old seedling families were inoculated with *C. ribicola* at Dorena Genetic Resource Center (Cottage Grove, Oregon, USA) in 2005. Seeds for an additional smaller test were sown in April 2009 and seedlings inoculated with *C. ribicola* in September 2009. Needle infection lesions (spots) were easily identified on needles of the seedlings inoculated at a young age (2009 trial), but were less obvious on needles of the seedlings inoculated when older (2005 trial). However, WPBR stem symptoms developed on many of the older seedlings in the 2005 trial for which infection spots were not observed, suggesting that needle lesions are not a good early measure of infection in older bristlecone pine seedlings. Bristlecone pine expresses multiple WPBR-resistant phenotypes, including canker-free seedlings, seedlings with partial bark reactions, seedlings developing stem infections later or showing longer survival with stem infections, and seedlings developing stem infections with infections becoming inactive over time; the frequency of each varied geographically.

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Genetic Resistance to *Cronartium ribicola* in Limber Pine (*Pinus flexilis*)

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Limber pine (*Pinus flexilis*) is being threatened by the lethal disease white pine blister rust (WPBR) caused by the nonnative pathogen *Cronartium ribicola*. The types and frequencies of genetic resistance to the rust in native populations before invasion are likely to determine the potential success of restoration or proactive measures.

The first extensive inoculation trials using individual tree seed collections from more than 100 limber pine trees confirm that genetic segregation of a stem symptom-free trait to blister rust is consistent with inheritance by a single dominant resistance gene (*Cr4*) and the resistance allele appears to be distinct from the *Cr2* allele in western white pine (*P. monticola*) (Schoettle et al. 2014). The frequency of the *Cr4* allele across healthy and recently invaded populations in the southern Rocky Mountains was unexpectedly high (5.0 percent, ranging from 0 to 13.9 percent), and northern Colorado (USA) appears to be a hotspot. Other tests by us and others suggest that the *Cr4* allele may be absent in populations in the U.S. northern Rockies and California. An ongoing inoculation trial of 74 limber pine families confirms the low frequency of bark reaction and resistance mechanisms for slow canker development.

Genetic resistance is present in limber pine populations and occurs at frequencies that are detectable even in populations that have not yet been invaded by the pathogen (i.e., sampling without the benefit of putative resistance phenotypic selection in the field). The limber pine seed trees that our trials reveal to have the *Cr4* allele will serve as monitors for the evolution of the *vcr4* virulent allele in *C. ribicola* (Schoettle et al., in press). To date, the *Cr4* trees in populations sampled here and exposed to WPBR for more than 20 years remain disease-free. The combination of low stand densities and open canopies of southern Rocky Mountain limber pine stands and the lack of a strong relationship between proximity to *Ribes* and disease incidence in limber pine suggest long-distance transport of *C. ribicola* spores in these ecosystems. The atmospheric mixing of spores from multiple *C. ribicola* populations over extensive landscapes may reduce localized selection pressure on *C. ribicola* and, therefore, slow the proliferation of virulence. However, relying on *Cr4* alone to sustain limber pine populations into the future in the presence of increasing WPBR pressure is not recommended and investigations to identify other more durable types of resistance to WPBR in limber pine are underway. The identification of *Cr4* and other types of resistance in native populations of limber pine early in the invasion process in this region provides useful information for predicting near-term impacts and structuring long-term management strategies (Schoettle and Sniezko 2007).

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Blister Rust Resistance in Whitebark Pine (*Pinus albicaulis*) at Crater Lake National Park

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Abstract—Whitebark pine (*WBP, Pinus albicaulis*) has been declining at Crater Lake National Park (CLNP) in southern Oregon, USA for several decades due to infection with white pine blister rust, caused by the pathogen *Cronartium ribicola*. This decline has been exacerbated in the past decade by significant levels of additional mortality caused by the mountain pine beetle (MPB, *Dendroctonus ponderosae*). In 2003, CLNP initiated a partnership with the Dorena Genetic Resource Center (DGRC) to collect WBP cones from phenotypically rust-resistant trees within the park and test progeny of these parent trees for genetic resistance to blister rust. As part of this effort: (1) seed is collected from candidate trees and seedlings are infected via artificial inoculation to assess their resistance and rate the parents' rust resistance; (2) trees identified as rust resistant are protected from MPB attack by application of verbenone; (3) extra seed is available for use in (i) genetic trials to gather further information on adaptive genetic variation in progeny of CLNP parent trees, (ii) ex situ gene conservation, and (iii) restoration plantings at CLNP; and (4) information is made available to the public on the status of WBP at CLNP. Through fall 2013, seed collections have been made from 101 parent trees, and 63 of these are currently in rust resistance trials at DGRC. Half of the seedling families show low-to-moderate levels of resistance. CLNP implemented a collection tree monitoring program in 2012 that tracks the fate of trees with progeny in rust resistance screening trials on an annual basis; results from monitoring can be directly tied to those from resistance screening trials and restoration outplantings. Thirty-one percent of trees with progeny showing the highest level of rust resistance are now dead; all were killed by MPB attack.

It is imperative to keep efforts going to identify new rust-resistant trees, protect trees with known or potential rust resistance, and preserve their genetic resources before they are lost. Seedlings from trees with known resistance to blister rust have been planted in restoration outplantings at CLNP. Family identity has been retained for the seedlings in these plantings to permit more information to be learned about the genetic variation present in the population of WBP trees at CLNP and its influences on traits such as survival, growth, and rust resistance. Small quantities of seed from most of the resistant parent trees are currently being stored for gene conservation and potential restoration use.

In: Schoettle, Anna W.; Sniezko, Richard A.; Kliejunas, John T., eds. 2018. Proceedings of the IUFRO joint conference: Genetics of five-needle pines, rusts of forest trees, and Strobusphere; 2014 June 15–20; Fort Collins, CO. Proc. RMRS-P-76. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 245 p.

The Definitive Guide for White Pine Blister Rust Resistance in White Pine Species of Western North America (beta 2014.0 version)—a Tree Breeder's Perspective

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The nine native species of white pines in the United States (four of which also occur in Canada) are all highly susceptible to white pine blister rust caused by the fungal pathogen *Cronartium ribicola*. Research and applied programs in the United States and Canada to develop resistant populations of white pine species have been underway for more than 50 years. The regional program based at the U.S. Department of Agriculture Forest Service's Dorena Genetic Resource Center (DGRC; Cottage Grove, Oregon, USA) has the most experience and continuity and has been active for over 50 years. The DGRC has evaluated rust resistance in families of eight of the nine species of white pines that are native to the United States, for an array of resistance responses in seedling inoculation trials (and seedlings for the remaining species, foxtail pine [*Pinus balfournia*], are slated for inoculation in fall 2015). Inoculations of seedlings of more than 10,000 families have been completed over this time. A subset of these families has been tested several times in different years or with different geographic sources of rust and differing inoculum densities, or a combination thereof. Another subset of these families—western white pine (*P. monticola*), sugar pine (*P. lambertiana*), and whitebark pine (*P. albicaulis*)—has been used to established field trials to validate the results of seedling screening and to monitor the durability of the different types of resistance (Sniezko et al. 2012). The extensive experience gained through this large series of trials has helped refine the understanding of the types of resistance and their frequencies (Kegley and Sniezko 2004; Sniezko et al. 2008, 2011, 2012, 2014). These screening trials have focused on both major gene resistance (present in four of the species; see Schoettle et al. 2014) and other types of resistance apparent in the 5 years of observations following each artificial inoculation (Kegley and Sniezko 2004; Sniezko et al. 2011, 2012, 2014).

In this talk, we will present some results from both inoculation trials and field tests to provide insights into the current state of knowledge of frequency, levels, and types of rust resistance present (using western white pine, whitebark pine, southwestern white pine [*P. strobiformis*], and sugar pine as examples) in our North America species. Results from the latest control crosses of western white pine will be used to help assess the potential level of resistance that may be possible in the next generation of improvement. A detailed overview of the genetic resistance present, using western white pine as an example, has recently been published (Sniezko et al. 2014).

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Performance of Blister Rust-Resistant Eastern White Pine (*Pinus strobus*) Hybrids With Himalayan Blue Pine (*P. wallichiana*) in Field Trials

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Abstract—Introducing white pine blister rust (caused by *Cronartium ribicola*) resistance genes to eastern white pine (*Pinus strobus*) from Eurasian five-needle pine species is a breeding strategy used in Ontario, Canada, to help regenerate eastern white pine at sites with high hazard ratings. Among Eurasian five-needle pine species, Himalayan blue pine (*P. wallichiana*) was identified as a desirable resistance gene donor because some of its hybrids with *P. strobus* demonstrated heritable and strong blister rust resistance as well as improved adaptation and growth after backcrossing to *P. strobus*. Data from field trials provided evidence of the effectiveness of this breeding approach and a resistance screening protocol that evaluates resistance by using the post-inoculation survival rate of 6-month-old seedlings.

At a field site near Sault Ste. Marie, Ontario, where greater than 90 percent of previously planted eastern white pine trees died of blister rust or had rust cankers at a plantation age of 10 years, 3.7 percent of replanted hybrid progeny from eight top-ranking open-pollinated families (7.0 percent for all hybrids) developed stem or branch cankers, compared with 13.3 percent of commercial eastern white pine controls 4 years post-trial establishment. Furthermore, none of the 263 trees propagated (by using rooted cuttings) from seedlings that survived blister rust infection had developed stem or branch cankers in the same trial. In another field trial, hybrid progeny of *P. strobus* with *P. wallichiana*, which had about a 75-percent *P. strobus* pedigree, demonstrated a growth rate and resistance to weevil (superfamily Curculionoidea) attack that were similar to those of pure eastern white pine at a trial age of 9 years. Because somatic embryogenesis (SE) is operationally applicable for vegetative propagation of eastern white pine and its hybrids, clonal deployment of SE emblings from elite hybrid backcrosses carrying up to a 94 percent *P. strobus* pedigree may be an appealing strategy to forest products companies and others for regenerating white pine at sites, such as those in northern Ontario, where eastern white pine is often decimated by blister rust.

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Field-Level Resistance of Western White Pine (*Pinus monticola*) to White Pine Blister Rust Assessed in Progeny Trials Planted in Coastal British Columbia

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Abstract—Partial resistance of western white pine (*Pinus monticola*) to the invasive pathogen *Cronartium ribicola* (white pine blister rust) was detected in controlled inoculation experiments, which found variation in resistance among half-sib families of parents selected for resistance in natural stands. Information from these experiments was used to select parent trees for breeding. Breeding was conducted on some of the parent trees in natural stands, but mainly on grafts in seed orchards. The resulting F1 crosses were deployed in four series of field progeny tests (16 test sites) in coastal British Columbia, Canada between 2001 and 2006. Approximately 100 full-sib cross families were established per series. In addition to providing a population for recurrent selection, the F1 full-sib design will allow some genetic analysis. Included in these field trials were a series of diallels created at the U.S. Department of Agriculture, Forest Service Dorena Genetic Resource Center (Cottage Grove, Oregon, USA), which will help this genetic analysis.

The field tests have been assessed for blister rust infection and height growth. Some preliminary genetic analysis results will be presented. One finding is that selfed families had higher levels of resistance, but a lower growth rate, suggesting the possibility that recessive genes may be involved in partial resistance. Although not explicitly part of the F1 program, major gene resistance has also been deployed in British Columbia. Families with major gene resistance do well against blister rust on coastal sites in British Columbia although certain families appear more susceptible to other fungal pathogens such as *Dothistroma pini* (cause of Dothistroma needle blight). We present these results and discuss some of the implications for the breeding and orchard programs in British Columbia.

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Genetic Architecture of Quantitative *Melampsora larici-populina* Leaf Rust Resistance in Poplars (*Populus* spp.)

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Poplar (*Populus* spp.) leaf rusts caused by *Melampsora* species are considered among the most crucial sanitary problems threatening poplar stands worldwide. Several major resistance factors specific to poplar rusts have been identified, each of them discovered by using a single *Melampsora* species. Most of them are inherited from *Populus* species that did not co-evolve with the pathogen. The origin and function of such "exapted" resistance are thus called into question.

The purpose of this presentation is to compare the genetics of exapted and co-adapted resistance to *M. medusae* f. sp. *deltoidae* (*Mmd*) and to *M. larici-populina* (*Mlp*), respectively. To achieve this, resistance quantitative trait locus (QTL) against *Mlp* was studied in two pedigrees. The first, an F2 *P. trichocarpa* × *P. deltoides* family, was previously evaluated for resistance to *Mmd*, and a major gene for resistance, *Mmd1*, inherited from the *P. trichocarpa* grandparent, was mapped. The second pedigree was an F1 *P. nigra* family.

In the F2 pedigree, 17 QTLs were detected that are involved in the 3 components of quantitative resistance to the 5 *Mlp* strains inoculated in laboratory. These QTLs explain between 3 and 30 percent of the phenotypic variance. None of these QTLs has a broad-range effect. No major resistance factor against *Mlp* was identified in this pedigree, showing that *Mmd1* is not functional against *Mlp* strains tested.

In the *P. nigra* family, 11 QTLs explaining between 2 and 60 percent of the phenotypic variation observed after inoculation with the two *Mlp* strains were detected. All except one of these QTLs were trait- or strain-specific. A major QTL inherited from the most resistant parent and located at the end of LG I explained more than 60 percent of uredinia size variation after inoculation with one *Mlp* strain and showed a moderate effect after inoculation with the other strain. Genomic mining of the QTL region revealed a cluster of 25 NB-LRR genes. Those results confirm in *P. nigra* the gene-for-gene model suggesting specific interactions between QTLs for quantitative resistance and *Mlp* strains.

The results presented will be compared and discussed together with results of previous studies on *Melampsora*/Salicaceae pathosystems.

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White Pine Blister Rust Hazard Rating for 265 Sites in Southern Oregon, USA

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Abstract—Sugar pine (*Pinus lambertiana*) and western white pine (*Pinus monticola*) are two important long-lived conifer species in forests of southwestern Oregon (USA) and elsewhere. Both of these white pine species have been in decline for decades due largely to the presence of the nonnative white pine blister rust, caused by the fungal pathogen *Cronartium ribicola*. Many land managers are interested in keeping these white pine species present in forest ecosystems or in managed forests, but the very high susceptibility of these species to the blister rust and the lack of information rating the landscape for rust hazard have prevented their full use. This study provides an overview of the rust infection levels at 265 sites in southwestern Oregon during the 1980s, including the frequency of cankered trees at each site and maps showing the geographic distribution patterns of the differing levels of infection. This information will assist land managers in plans for deploying seed from the blister rust resistance programs. All except one site had infected white pine species, and the mean percentage of trees with cankers over the 265 sites was 43.9 percent, with the average number of cankers per tree ranging from 0 to >11 per site. The mapping of the levels of infection showed some areas of notable low and high infection. Cankers tended to be within 2.4 m of the ground, suggesting that early branch pruning of planted trees might increase survival. This extensive one-time survey of sites is valuable in helping delineate areas of lowest and highest infection levels, but other data suggest that such surveys may underestimate the level of infection and should be complemented by a subset of permanent plots. With the widespread presence and impact of the rust, the continued development and use of genetically resistant sugar pine and western white pine seed will be vital in retaining or restoring these species to desirable levels in southwestern Oregon.

INTRODUCTION

Sugar pine (SP, *Pinus lambertiana*) and western white pine (WWP, *Pinus monticola*) are long-lived conifers that are important components of forest ecosystems in southwestern Oregon (USA). Both of these pines are very susceptible to the nonnative fungal pathogen *Cronartium ribicola*, cause of white pine blister rust (WPBR). Detailed knowledge of the geographic distribution of the rust and its intensity can be an important aid to managers developing restoration or reforestation prescriptions with these species.

The Medford District of the U.S. Department of the Interior, Bureau of Land Management (BLM) contains nearly 405,000 ha of forest land in southwestern Oregon, including many areas with sugar pine, and to a lesser extent, western white pine. In the mid-1980s, BLM personnel completed a WPBR hazard survey of 265 sites in southwestern Oregon. The objective of the survey was to document how common the rust was in this area and to provide information on relative levels of site hazard for rust over this area. This is the largest and most intensive WPBR rust hazard survey

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of an area for sugar pine, and perhaps any five-needle pine species. The information provided here will serve as a valuable tool for land managers in southwestern Oregon for use in determining where five-needle pine species may be used for reforestation and restoration, and where genetically tested resistant trees should be used for forest development projects.

In this paper, we quantify rust hazard over the landscape for the 265 locations surveyed. Hazard is quantified by hazard rating, as discussed by Geils et al. (1999) and Burns et al. (2008):

> *Forest disease outbreaks are described in the epidemiological terms of rates of spread and intensification, incidence, severity, damage, and ecological impacts. Hazard rating provides a means for combining these attributes into a relative index or classification for comparing disease potential among sites. Rust epidemiology is strongly influenced by genetics, abundance, and proximity of hosts and by microclimate… (Geils et al. 1999: 2).*
>
> *Hazard can be quantified several different ways, such as the proportion of infected trees, percent mortality, or the # and severity of cankers. By delineating rust hazard zones, managers can focus on white pine management in those areas where successful management is more likely to be heavily impacted (Burns et al. 2008: 9).*

We present hazard rating data in separate maps using proportion of trees affected, number of cankers per tree, and rust index.

METHODS

The areas examined in this project were portions of southwestern Oregon, primarily BLM's Medford District forest lands, and some adjacent U.S. Department of Agriculture, Forest Service (USDA FS) and private timber company lands (fig. 1). All BLM sites selected were from the Medford District forest inventory database of young stands with a component of either SP or WWP. Generally, sites were included only if they had a minimum of 50 trees and ranged in size from 8 to 10 ac (3 to 4 ha), up to 100 ac (40.5 ha). Due to various factors, 71 of the 265 sites had fewer than 50 trees sampled (lowest number was 25), and 6 sites had more than 50 trees sampled (1 site had 100 trees sampled). Sites were evaluated over a several-year period in the mid-1980s. Of the 265 sites, 244 sites contained a component of sugar pine, and 21 had western white pine present. The scatter of sites in this study provides a fairly systematic and representative coverage of sites in this area with white pine species. Wild/natural regeneration trees occurred on 255 sites, and another 10 sites had been previously planted (in the mid-to-late 1960s) with seedlings from putatively rust-resistant (RR) parent trees (RRSP and RRWWP). Between 67 and 2,780 trees were planted at the RR sites.

For the 255 naturally regenerated stands, field crews generally inspected 40 to 50 trees per site using a random path method (i.e., walk 50 paces and look left and then assess the nearest tree). However, in some sites where white pines were at very low frequency, most trees in sight were selected to achieve the objective of a 40- to 50-tree count. Dead trees were sampled if they fell into the random scheme (same as for live trees) provided that mortality was recent, and the number of cankers could be assessed. The 10 RRSP/RRWWP sites were sampled in a slightly modified manner to predominantly include only trees (marked or tagged) of the RR plot, but some control or natural trees were included in some sites.

For individual sample trees, measurements included age, height, number of cankers (main stem and branches), presence/absence of cankers on the main stem, and height of the highest canker. Tree age was approximated by counting whorls. For each site, aspect and elevation were noted, and township, range, and section were used to obtain latitude and longitude at the center of each section for mapping purposes.

Basic site criteria were that the trees should be at least 10 years old and generally less than 6 m in height to allow them to be potentially exposed to rust infection for a number of years, and to allow for proper identification and counting of cankers. Sites with steep slope allowed for taller trees to be assessed. Sites where pines were being suppressed by overstory or brush were not chosen for assessment.

The collected individual tree data for each site were used to calculate percentage of infected trees (PI). Rust index (RI), where RI = number cankers per 1,000 needles per year, was calculated by using a program

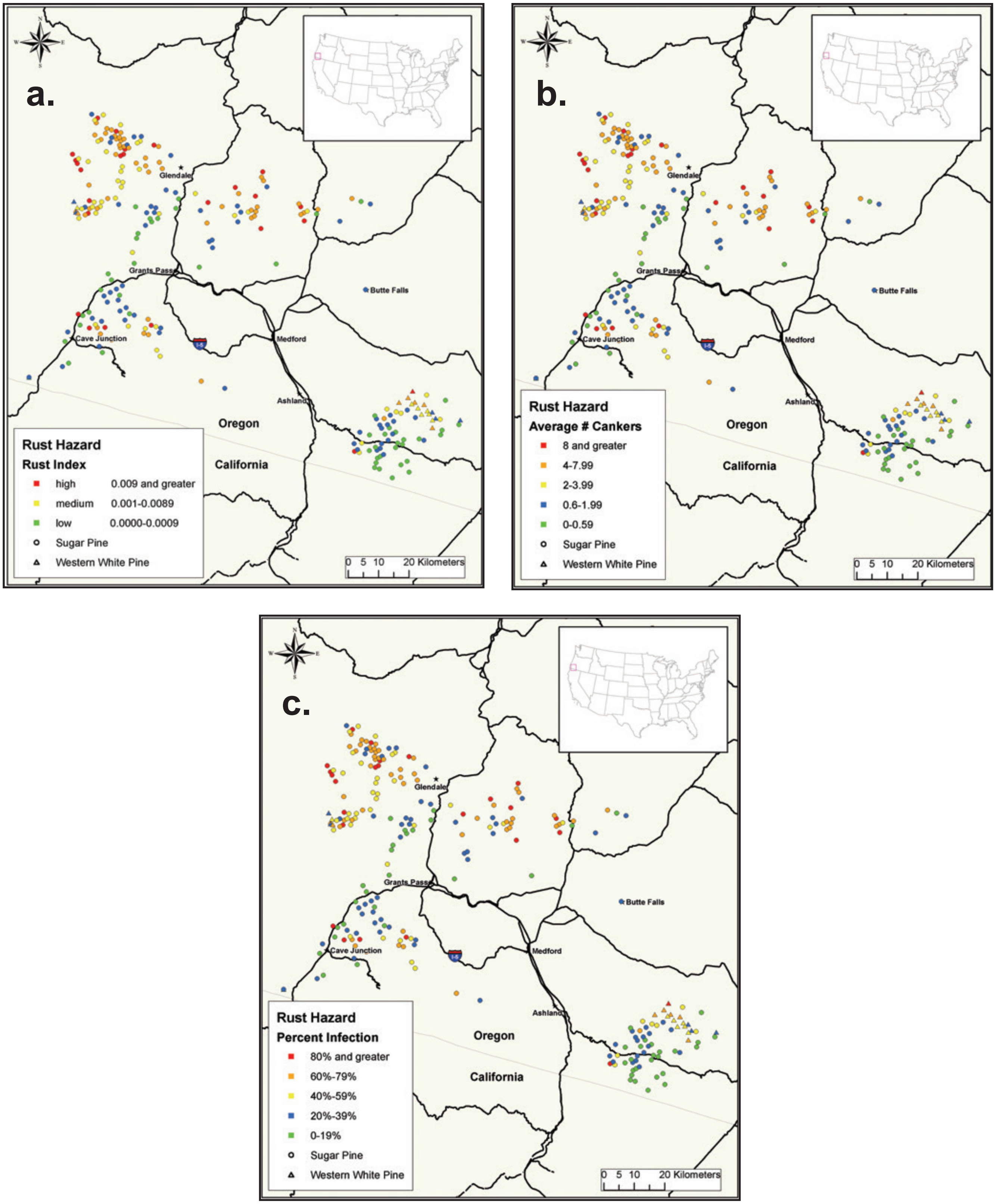

Figure 1—Geographic distribution of lowest to highest rust hazard sites, classified by (a) rust index, (b) average number of cankers per tree, and (c) percentage of trees infected. Sugar pine (*Pinus lambertiana*, 235 sites represented) and western white pine (*Pinus monticola*, 20 sites represented) are shown with filled circles and triangles, respectively. The map area is shown in the inset. Sites with improved stock were excluded. Datum used was North American Datum 1983; projection used was Albers Equal Area Conic. Maps were generated by Jennifer Christie.

Figure 2—Relationship between tree height and age for 265 stands surveyed for rust hazard on BLM's Medford District.

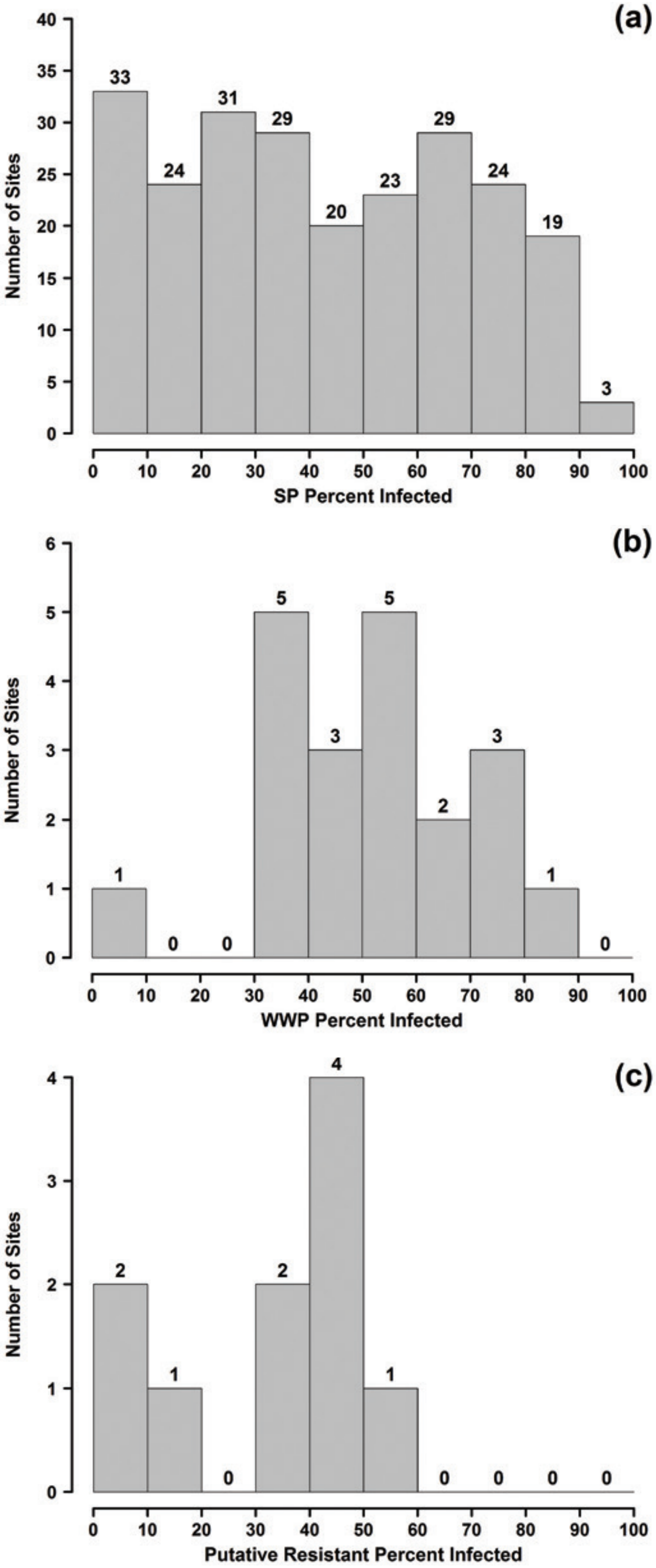

Figure 3—Distribution of percent infected with white pine blister rust for (a) sugar pine stands, (b) western white pine stands, and (c) sites planted with seedlings from putatively resistant parents.

developed earlier for western white pine (Hagle et al. 1989). Only the 255 naturally regenerated sites were used to create maps. For mapping rust hazard, rust index was divided into three groups, as follows:

Rust Hazard:

High: *Rust index ≥0.009 (~>70 percent of trees with cankers)*

Medium: *Rust index 0.001 to 0.009 (~50 percent of trees with cankers)*

Low: *Rust index up to 0.001 (~25 percent of trees with cankers)*

Rust index is a measurement of a tree's or site's rust infection level and therefore susceptibility to additional blister rust infections. According to the USDA FS Forest Health Technology Enterprise Team [n.d.], "The blister rust index, a measure of white pine blister rust infection level or potential for a site, area, or stand, drives the model. The initial value of the blister rust index can be provided by the user, calculated from current infections provided with inventory data, or calculated based on *Ribes* populations (white pine blister rust-*Cronartium ribicola*-is heteroecious and its alternate host is *Ribes*)."

For comparison, we also show maps of the relative rust hazard based on number of cankers per tree (using five categories) and percentage of trees with cankers (five

Table 1—Summary information for 255 natural (n.s.) and 10 planted (RR) stands of sugar pine (*Pinus lambertiana*) and western white pine (*Pinus monticola*) assessed for blister rust in southwest Oregon.

Type of site	Number of sites	Measure	Elevation (m)	Average age	Average height (m)	Percent infected	Average number of cankers
SP n.s.	235	Ave	930	29.9	3.8	43.6	2.06
		Min	340	13	1.8	0	0.00
		Max	1,890	65	10.4	94	11.50
SP RR	9	Ave	1,000	19.2	3.3	37.2	1.70
		Min	680	17	2.1	6	0.16
		Max	1,250	23	4.3	60	3.41
WWP n.s.	20	Ave	1,470	24.9	3.5	51.8	2.20
		Min	640	15	1.8	4	0.06
		Max	1,740	57	7.3	86	5.04
WWP RR	1		1,100	20	4.3	16	0.46

categories). Mean plot data and calculated variables (such as rust index) generated after the survey were used in creating the map and graphs for this summary.

Statistical analyses were conducted using R version 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria). Natural log transformations were applied to the average number of cankers per site and the rust index values, and a logit transformation was applied to the percent infected data. Very low levels of infection (0 to ~5 percent) were rounded to 0.0000 on the rust index scale, so the seven sites with these values were dropped from linear regression analyses involving rust measures. The 10 sites with improved stock were also removed from all linear regression analyses because inference is desired for naturally regenerated stands.

RESULTS

Most trees surveyed here were less than 7 m tall, but the age of trees varied from about 12 years to more than 50 years old (fig. 2). There is strong evidence of an association between the average height and the average age of trees in the naturally regenerated stands (Pearson's correlation = 0.225, $P = 0.0003$). It is estimated that the mean height at a site is 0.97 m greater for each 10-year increase in average age (95-percent confidence interval from 0.44 m to 1.49 m or greater). Of the 265 sites surveyed, 264 had one or more trees with blister rust cankers; the mean across sites was 43.6 percent infection and 2.1 cankers per tree for the 235 SP natural stand sites (table 1; figs. 3, 4). The number of cankers per tree per site ranged from near 0 to >11 (fig. 4).

Percent infection and number of cankers per tree are strongly related to rust index (fig. 4). Rust index (as well as infection percentage and number of cankers per tree) varied widely among sites (figs. 1, 3, 4). The mean height of the highest canker was less than 1.8 m (6 ft) for most sites (fig. 5).

The natural logarithm of rust index is strongly associated with both the logit of percent infection (Pearson's correlation = 0.890, $P < 0.0001$, fig. 6a) and the natural logarithm of the average number of cankers per tree (Pearson's correlation = 0.930, $P < 0.0001$; fig. 6b). The mean rust index changes from low hazard to medium hazard and from medium hazard to high hazard at 28.5 and 78.0 percent infected trees, respectively. Similarly, the mean rust index changes from low hazard to medium hazard and from medium hazard to high hazard at an average of 0.58 and 4.85 cankers per tree, respectively.

There is strong evidence of an association between the average height of the highest canker in naturally regenerated stands with nonzero rust index and the average age of the stand (Pearson's correlation = 0.639, $P < 0.0001$, fig. 7). For each 10-year increase in the

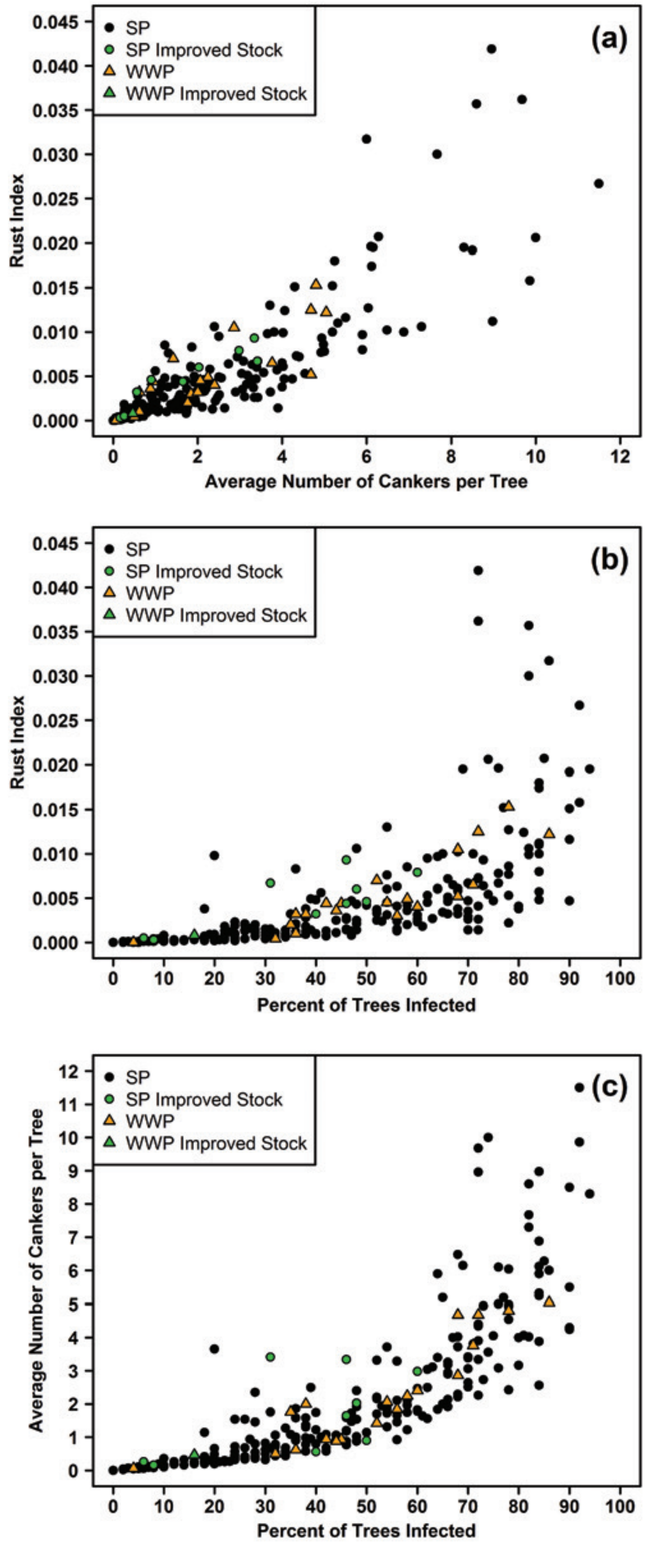

Figure 4—Relationship between (a) average number of cankers/tree and rust index (RI), (b) percent infected and RI, and (c) percent infected and average number of cankers/tree.

average age, the mean height of the highest canker is estimated to be 0.42 m higher (95-percent confidence interval from 0.36 m to 0.49 m higher). There is also strong evidence of an association between the percentage of trees infected at a site and the average age of the site (*P* = 0.008). Younger stands in general had a greater percentage of trees infected; the odds of infection were on average 52 percent greater at a site for each 10 years younger (95-percent confidence interval from 30 percent to 78 percent greater).

DISCUSSION AND SUMMARY

The data summarized here and elsewhere (Goheen and Goheen 2014; Sniezko et al. 2012, 2013) indicate that the risk of blister rust is ubiquitous in the portion of southern Oregon in this study. However, the site rust hazard varies from low to relatively high (fig. 3). Some geographic clumping of low and mid-to-high hazard areas occurs (fig. 3). Land managers can use this information to guide the use of genetically rust-resistant stock, branch pruning, and restoration options.

Based on past observations, most of the trees with lower bole infections are expected to die. The impact of blister rust reported here is likely to be an underestimate due to the one-time survey of the stands. The survey excludes most trees that have previously died from rust, and obviously does not account for future infections. Other studies (Sniezko et al. 2012, 2013) suggest the impacts could be much greater over time. Further, the criteria for stand selection may have excluded areas of highest rust hazard due to low frequency of surviving WWP or SP in those areas.

On many sites, rust-resistant stock or pruning of branches, or both, will be needed to help maximize survival and keep these species as viable components in forest ecosystems or managed plantings. Sugar pine and western white pine will continue to decline in southwestern Oregon without the utilization of seedlots with genetic resistance to blister rust. The BLM and USDA FS manage seed orchards for sugar pine and western white pine and are involved in breeding and resistance testing to further increase the level of resistance (Sniezko et al. 2014; Waring and Goodrich 2012). Most of the cankers noted are generally within 2.4 m of the ground (fig. 5), suggesting that branch

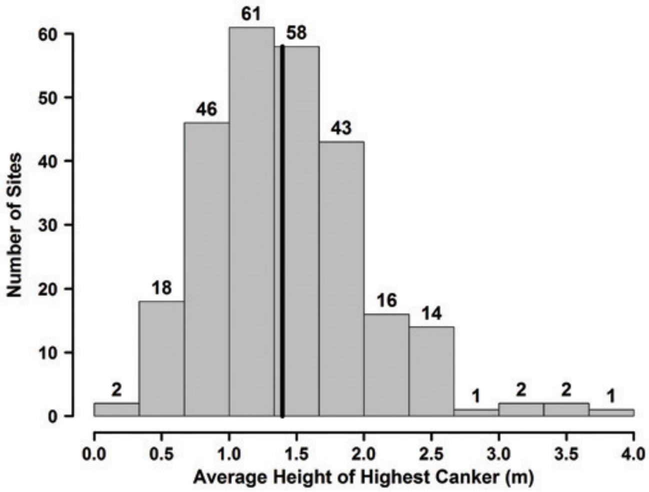

Figure 5—Frequency histogram denoting the average height of the highest canker at each of the 264 sites with a canker present. The vertical black line shows the overall average.

pruning (and removing needles attached directly to the main bole) could potentially be effective in any reforestation or restoration plantings in these areas. Several studies to examine efficacy of pruning are now underway. Note that this would help survival for current trees on the landscape, but not the ensuing generations. Only an increase in durable resistance will provide that ongoing benefit.

The maps and data summarized here provide a planning tool to land managers wishing to retain these species in forest ecosystems, restore these species, or use them in managed forests. The value of such maps has been touted elsewhere:

> *Hazard maps provide a tool for management planning at forest and project scales, a basis for stratifying landscapes for damage and impact surveys, and a foundation for additional epidemiological and ecological studies (Geils et al. 1999: 2).*

From a practical standpoint, the number of cankers per tree or percentage of trees with cankers provides a useful rating of site rust hazard when rust index is not calculated.

The effect of a changing climate on the incidence and intensity of blister rust infection is unknown. The data for this study were collected decades ago. However, recent studies suggest that blister rust continues to be very active in southwestern Oregon (Sniezko et al. 2012, 2013). Permanent plots would provide a better tracking of the impacts of blister rust dynamics, and would complement a large-scale one-time survey such as this.

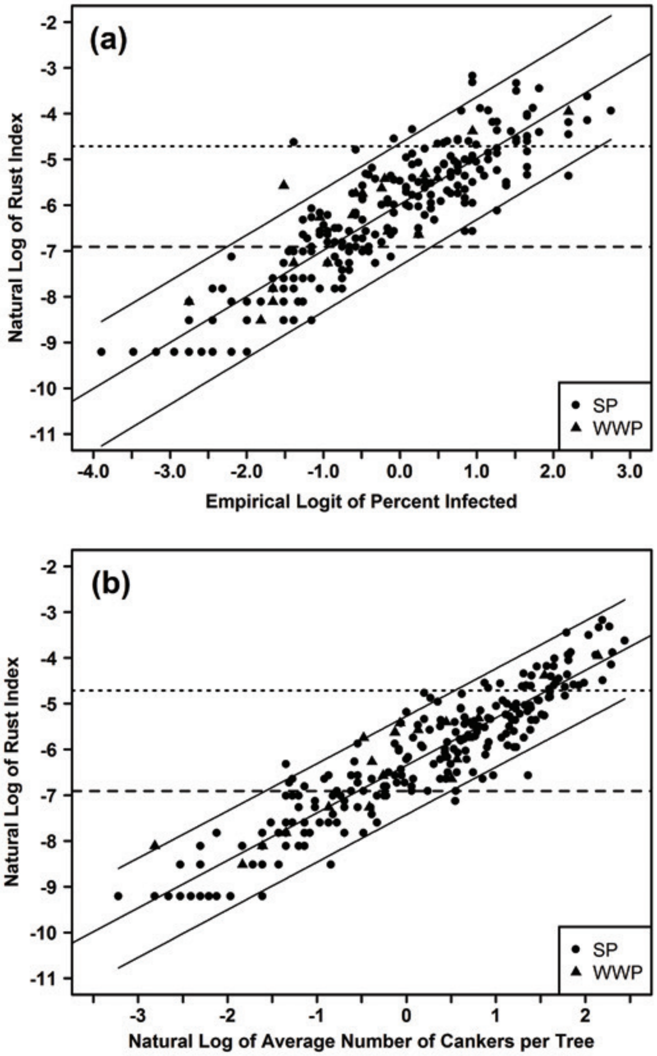

Figure 6—Fitted regression line and 95% prediction intervals for estimating the natural logarithm of rust index from (a) the logit of percent infected and (b) the natural logarithm of the average number of cankers per tree. The dashed line is the boundary between low and medium rust hazards, while the dotted line is the boundary between medium and high hazards.

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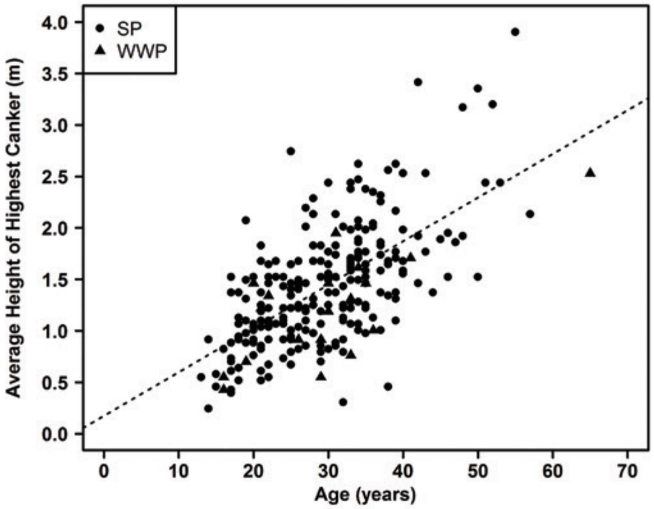

Figure 7—Relationship between the average height of the highest canker and the average age of the stand for natural stands with nonzero rust indices. The linear least squares regression line is shown with a dotted line.

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Genetic Variability in *Puccinia psidii* as Revealed in Commercial Plantations of *Eucalyptus* Species

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Abstract—The climates of central and southern regions of São Paulo state (Brazil) are suitable for pathogens such as *Puccinia psidii*, which causes a common and serious disease in eucalyptus plantations less than 2 years old. We studied the genetic variability of *P. psidii* in commercial plantations of *Eucalyptus* species by random amplified polymorphic DNA molecular markers. All 56 isolates of *P. psidii* analyzed showed high polymorphism and there was no evidence of preferential infection in different plant tissues. In the analysis of pooled pustules according to the clone of origin, we observed the formation of two groups, one with isolates from susceptible clones and the second group only with isolates from resistant clones, indicating that isolates infecting resistant clones are more genetically similar than isolates infecting susceptible clones.

INTRODUCTION

Puccinia psidii was originally described from common guava (*Psidium guajava*) in eastern Brazil in 1884. It became notorious for its host jumps to nonnative Eucalyptus, first observed in Brazil in the 1940s. It caused substantial economic damage to large Brazilian plantations in the 1970s (Coutinho et al. 1998). The wide host range of the rust fungus and its potential threats to many native or introduced species in Myrtaceae has become a major concern to many countries, including New Zealand, Australia, South Africa, and Brazil, where Myrtaceae species are the major components of the forests and ecosystem (Coutinho et al. 1998; Glen et al. 2007; Tommerup et al. 2003).

The host jump in Brazil is currently cited as a prime example in the literature illustrating the potential danger for host jumps following anthropocentric movement of potential hosts (Slippers et al. 2005; Wingfield et al. 2001). The probability of a new race is particularly high when a particular resistance gene is used extensively in breeding (Keiper et al. 2003).

Breeding programs are underway to improve resistance of eucalypts to *P. psidii*. In *E. grandis*, resistance is controlled by a locus of main effect, which makes this species useful in breeding programs (Junghans et al. 2003). However, based on the pattern of inheritance, slight changes in the pathogen's genetic structure can overcome this host resistance gene and render it ineffective (Graça et al. 2011).

The lack of information about the origin and genetic variation of the pathogen population has hindered choosing the best form of management aimed at reducing disease.

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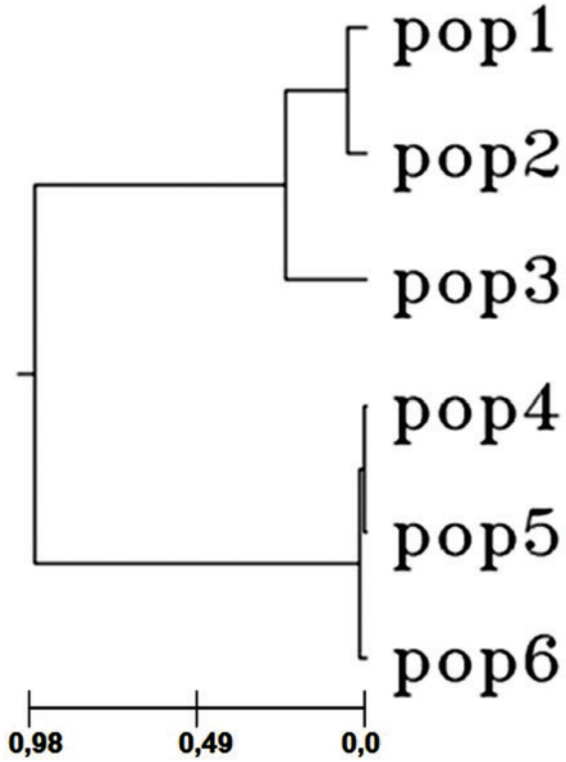

Figure 1—Cluster diagram based on genetic distance calculated from the presence/ absence of polymorphic markers across the pustules grouped by clone. Pop 1, 2, 3, 4, 5 and 6: name of each group of pustules in the clones.

Our aim is to determine the genetic relationships among *P. psidii* isolates from *Eucalyptus* species in Brazil with random amplified polymorphic DNA (RAPD) markers, allowing us to investigate whether the pathogen has undergone a genetic change that could enable it to break the barrier of plant resistance. Results of this research are expected to assist in developing effective control measures.

METHODS

Fungal Isolates

Single uredinial isolates of *P. psidii* were collected from six different Eucalyptus clones, totaling 56 pustules (table 1). These clones are located in the city of Jacarei, São Paulo (Brazil) and were provided by the Fibria SA breeding program.

DNA Extraction

Genomic DNA was extracted by using Chelex® (Sigma-Aldrich Co., St. Louis, Missouri, USA) resin. The protocol is to add to each sample 100 µl of 5 percent Chelex, then to soak the spores until the entire contents are homogenized, and to add 80 mg of proteinase K. Samples were placed in a water bath overnight at 65 °C. The next day, the samples were exposed to a temperature of 95 °C water bath for 15 minutes, removed, and centrifuged for 1 minute at 14,000 rpm. The supernatant was transferred to new tubes.

RAPD Genotyping

We genotyped 56 isolates with the primers OPAD-01, OPM-16, OPM-10, OPX-06 by Operon Technologies (Olive Branch, Mississippi, USA). Each 13-µL polymerase chain reaction (PCR) contained 10 ng total genomic DNA, 0.8 mM of dNTP, 1923 mM of MgCl2, 0.36 percent of bovine serum albumin (BSA), 1 unit of Taq DNA polymerase (Invitrogen®; Thermo Fisher Scientific, Waltham, Massachusetts, USA), 0.3 mM of primer, and autoclaved deionized water. The PCR reaction was performed in a PT 100™ thermocycler (MJ Research, Waltham, Massachusetts) with one cycle at 95 °C for 3 minutes, followed by 40 cycles at 92 °C for 1 minute, 35 °C for 1 minute, and 72 °C for 2 minutes; a final elongation step of 5 minutes at 72 °C; and finally a holding step at 10 °C. The PCR products were separated by using a 2.5 percent agarose gel.

Data Analysis

A binary matrix recording specific RAPD fragments as present (1) or absent (0) was generated for each genotype, and the data obtained plotted on a Microsoft® Excel® spreadsheet.

Analysis of diversity and genetic distance was performed with the software PopGene 1.32. A dendrogram was generated based on UPGMA analysis of array-based genetic distance of Nei (1978).

Table 1—Plant location of *Puccinia psidii* pustules collected from six *Eucalyptus* sp. clones obtained from crosses between susceptible and resistant trees of *E. grandis* located in Jacarei-SP, Brazil, from Fibria SA breeding program.

Sample Number	Description
1	Clone A, Plant 1, Leaf 1, Tree top
2	Clone A, Plant 1 ,Leaf 2 , Pock 1 ,Tree top
3	Clone A, Plant 1, Leaf 2 ,Pock 2 ,Tree top
4	Clone A ,Plant 1, Leaf 3 ,Tree bottom
5	Clone A, Plant 1, Leaf 4 , Tree bottom
6	Clone A, Plant 2, Leaf 1, Tree top
7	Clone A, Plant 2, Leaf 2, Tree bottom
8	Clone A, Plant 2, Leaf 3, Pock 1, Tree bottom
9	Clone A, Plant 2, Leaf 3, Pock 2, Tree bottom
10	Clone A, Plant 2, Leaf 4, Tree top
11	Clone B, Plant 1, Leaf 1, Pock 1, Tree top
12	Clone B, Plant 1, Leaf 1, Pock 2, Tree top
13	Clone B, Plant 1, Leaf 1, Pock 3, Tree top
14	Clone B, Plant 1, Leaf 1, Pock 4, Tree top
15	Clone B, Plant 1, Leaf 2, Pock 1, Tree top
16	Clone B, Plant 1, Leaf 2, Pock 2, Tree top
17	Clone B, Plant 1, Leaf 2, Pock 3, Tree top
18	Clone B, Plant 1, Leaf 2, Pock 4, Tree top
19	Clone B, Plant 1, Leaf 2, Pock 5, Tree top
20	Clone B, Plant 1, Leaf 3, Pock in the stem, Tree top
21	Clone B, Plant 2 ,Leaf 1, Pock in the petiole, Tree top
22	Clone B, Plant 2, Pock in the stem, Tree top
23	Clone B, Plant 3, Leaf 1, Pock 1, Tree top
24	Clone B, Plant 3, Leaf 1, Pock 2, Tree top
25	Clone C, Plant 1, Leaf 1, Tree top
26	Clone C, Plant 1, Leaf 2, Tree top
27	Clone C, Plant 1, Leaf 3, Pock 1, Tree top
28	Clone C, Plant 1, Leaf 3, Pock 2, Tree top
29	Clone C, Plant 1, Leaf 3, Pock 3, Tree top
30	Clone C, Plant 2, Leaf 1, Tree top
31	Clone C, Plant 2, Leaf 2, Pock in veins, Tree top
32	Clone C, Plant 4, Leaf 1, Pock in veins, Tree top
33	Clone D, Plant 1, Leaf 1, Pock 1, Tree top
34	Clone D, Plant 1, Leaf 1, Pock 2, Tree top
35	Clone D, Plant 1, Leaf 2, Pock 1, Tree top
36	Clone D, Plant 1, Leaf 2, Pock 2, Tree top
37	Clone D, Plant 2, Leaf 1, Pock in the petiole, Tree top
38	Clone D, Plant 2, Leaf 2, Tree top
39	Clone D, Plant 2, Leaf 3, Pock in the petiole, Tree top
40	Clone D, Plant 2, Leaf 4, Pock in the petiole, Tree top
41	Clone D, Plant 3, Leaf 1, Tree top
42	Clone D, Plant 3, Leaf 2, Tree top
43	Clone E, Plant 1, Leaf 1, Tree top
44	Clone E, Plant 1, Leaf 2, Tree top
45	Clone E, Plant 1, Leaf 3, Pock 1, Tree top
46	Clone E, Plant 1, Leaf 3, Pock 2, Tree top
47	Clone E, Plant 1, Leaf 3, Pock 3, Tree top
48	Clone E, Plant 2, Leaf 1, Tree top
49	Clone E, Plant 2, Leaf 3, Pock 1, Tree top
50	Clone E, Plant 2, Leaf 3, Pock 2, Tree top
51	Clone E, Plant 2, Leaf 4, Tree top
52	Clone F, Plant 1, Leaf 1, Tree top
53	Clone F, Plant 1, Leaf 2, Tree top
54	Clone F, Plant 1, Leaf 3, Tree top
55	Clone F, Plant 1, Leaf 4, Pock 1, Tree top
56	Clone F, Plant 1, Leaf 4, Pock 2, Tree top

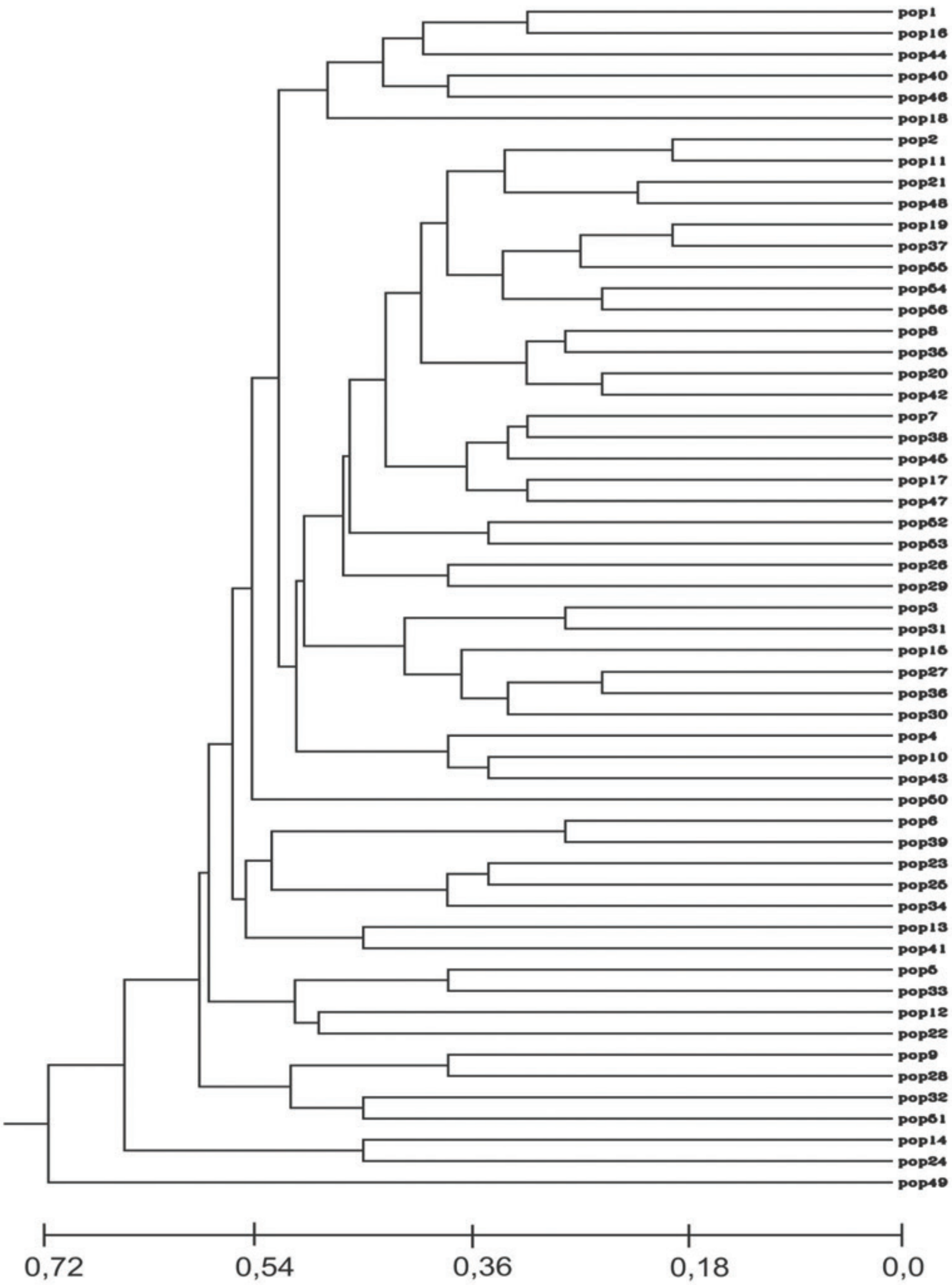

Figure 2—Cluster diagram based on genetic distance calculated from the presence or absence of polymorphic markers across the pustules not grouped by clone. Pop 1 until 56: name of each pustule.

RESULTS AND DISCUSSION

Two dendrograms were built, one with the pustules grouped by clone and another with the pustules separated without regard to the origin clone. In the dendrogram with pustules grouped by clone (fig. 1), we observed two main groups. Group I with isolates from pop1, pop2, and pop3 was subdivided into a cluster formed by pop1 and pop2 with a similarity coefficient of 0.1. Group II is an isolate of pop3. The two groups were correlated, with a similarity coefficient of 0.2. In group II, formed by pop4, pop5, and pop6, two clusters were observed with a few genetic polymorphisms (fig. 1). Note that the first group contains only susceptible clones and the second group, only resistant clones, which means that *Puccinia* isolates from resistant clones are genetically more similar than isolates from susceptible clones.

The analysis of pustules separately (unrelated to the clone of origin) showed high genetic diversity. We observed the formation of many groups with no apparent relationship between the pustules taken from the same clone forest (fig. 2). There was no relationship between the pustule positions along the tree, showing no fungus preference to infect a specific tree tissue. This high diversity was expected because the fungus is native to Brazil.

These results open a favorable perspective to the development of specific fungus markers for each *Eucalyptus* clone. These and similar results can provide a basis for choosing new strategies for improving plant resistance.

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Competing Interests: The study presented in the manuscript is a collaboration between the University of São Paulo State and Fibria Pulp and Paper. The collaboration did not involve any financial agreement between the two parties. Juliana Érika de Carvalho Teixeira Yassitepe was employed by Fibria at the time of the study, but is no longer with the company. This does not alter the policies on sharing data and materials.

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Genetic and Genotypic Diversity of *Puccinia psidii*—The Cause of Guava/Eucalypt/Myrtle Rust—And Preliminary Predictions of Global Areas at Risk

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INTRODUCTION

Puccinia psidii (Basidiomycota, Uredinales) is a biotrophic rust fungus that was first reported in Brazil from guava (*Psidium guajava*) in 1884 (Winter 1884) and later from nonnative eucalypt (*Eucalyptus capitelata* and *Corymbia citriodora*, syn. *Eucalyptus citriodora*; Joffily 1944). Considered to be of Neotropical origin, the rust has also been reported to infect diverse myrtaceous hosts elsewhere in South America, Central America, the Caribbean, Mexico, the United States (California, Florida, and Hawaii), Japan, Australia, China, and most recently South Africa and New Caledonia (Carnegie et al. 2010; Giblin 2013; Kawanishi et al. 2009; MacLachlan 1938; Marlatt and Kimbrough 1979; Mellano 2006; Pérez et al. 2011; Roux et al. 2013; Uchida et al. 2006; Zambino and

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Table 1—Geographic origin and host of confirmed *Puccinia psidii* genotypes.

Origin	**Host**	**Cluster**[a]
Australia	*Pilidiostigma glabrum*	Pandemic
	Backhousia oligantha	Pandemic
	Rhodomyrtus psidioides	Pandemic
Brazil	*Eucalyptus* spp.	Eucalypt
	Eugenia uniflora	Guava
	Myrciaria cauliflora	Guava
	Psidium guajava	Guava
	Psidium guineenese	Guava
	Syzygium cumini	Guava
	Syzygium jambos	Eucalypt
Costa Rica	*Callistemon lanceolatus*	Pandemic
	Syzygium jambos	Pandemic
Jamaica	*Pimenta dioica*	Allspice
	Syzygium jambos	Pandemic
Mexico	*Syzygium jambos*	Pandemic
Paraguay	*Eucalyptus grandis*	Eucalypt
Puerto Rico	*Syzygium jambos*	Pandemic
Uruguay	*Psidium guajava*	Guava
	Eucalyptus grandis	Eucalypt
	Eucalyptus globulus	Eucalypt
	Myrrhinium atropurpurea	Guava
United States	*Eugenia koolauensis*	Pandemic
	Melaleuca quinquenervia	Pandemic
	Metrosideros excelsa	Pandemic
	Metrosideros polymorpha	Pandemic
	Myrcianthes fragrans	Pandemic
	Myrtus communis	Pandemic
	Rhodomyrtus tomentosa	Pandemic
	Syzygium jambos	Pandemic

[a] (1) Allspice = collections from *Pimenta dioica* from Jamaica; (2) Pandemic = collections from diverse hosts from Australia, the Caribbean, Mexico, and the United States; (3) Eucalypt = collections from *Eucalyptus* spp. and *Syzygium jambos* from Brazil, Paraguay, and Uruguay; (4) Guava = collections from *Psidium guajava*, *Psidium guineense*, *Syzygium cumini*, *Myrciaria cauliflora*, *Eugenia uniflora*, and *Myrrhinium atropurpureum* var *octandrum* from Brazil and Uruguay.

Nolan 2011; Zhuang and Wei 2011). Given the rate at which the pathogen is spreading and its wide host range, the objectives of this study are to determine how much genetic diversity exists within populations across the species' putative native range as well as areas of recent introduction, evaluate possible pathways of spread, and predict the geographic area that is climatically suitable for the species to determine areas at risk of invasion.

METHODS

Six microsatellite loci were scored for *P. psidii* samples derived from diverse hosts and geographic locations (table 1). Samples were assigned to genetic clusters using a Bayesian genetic clustering algorithm implemented in STRUCTURE v2.3.4 (Pritchard et al. 2000). Posterior probabilities were estimated for $K = 1$ to $K = 12$ assuming an admixture model.

Table 2—Summary of genetic variation among clusters of *Puccinia psidii*.

	Allspice[a]	**Pandemic**	**Eucalypt**	**Guava**
Alleles per locus	2.50 (0.43)	3.50 (0.67)	2.33 (0.21)	4.83 (0.60)
Effective alleles per locus	2.26 (0.35)	2.91 (0.45)	1.97 (0.25)	3.40 (0.39)
Private alleles	4	9	1	11
Shannon's index	0.78 (0.18)	1.07 (0.16)	0.70 (0.12)	1.35 (0.12)
Number of MGs	5	9	6	6
Observed heterozygosity	0.77 (0.16)	1.00 (0.00)	0.69 (0.19)	0.61 (0.13)
Expected heterozygosity	0.49 (0.10)	0.62 (0.05)	0.44 (0.08)	0.69 (0.04)
Fixation index	-0.62 (0.16)	-0.67 (0.13)	-0.39 (0.31)	0.14 (0.16)

[a] Clusters of Allspice, Pandemic, Eucalypt, and Guava are described in Table 1.

STRUCTURE analyses were performed with clone-corrected data (unique multilocus genotypes, MGs). Fifty thousand burn-in generations were employed for each of 10 replicate runs of 1,000,000 generations of the Markov chain Monte Carlo (MCMC) sampler for each *K*. The optimal value of *K* was inferred using the method of Evanno et al. (2005). Population genetic analyses of all samples grouped by genetic cluster were performed using GenAlEx v6.501 (Peakall and Smouse 2006). To examine relationships among MGs, a minimum spanning network was estimated using the genetic distance measure of Bruvo et al. (2004). Bioclimatic modeling was performed using all documented rust occurrences and reduced sets of the eucalypt, pandemic, and guava biotypes coupled with 19 bioclimatic variables in MaxEnt (Maximum Entropy Species Distribution Modeling) v3.3.3K. Cross-validation was used to verify results among 10 replicate runs. Quantum GIS was used to create the final output in a cumulative format according to prediction probability.

RESULTS AND DISCUSSION

Among the *P. psidii* samples collected from Australia, Brazil, Costa Rica, Jamaica, Mexico, Paraguay, Puerto Rico, Uruguay, and the United States, 26 unique MGs were found. A Bayesian clustering analysis and a minimum spanning network revealed four major genetic clusters among the MGs: (1) Allspice = collections from *Pimenta dioica* from Jamaica; (2) Pandemic = collections from diverse hosts from Australia, the Caribbean, Mexico, and the United States; (3) Eucalypt = collections from Eucalyptus species and *Syzygium jambos* from Brazil, Paraguay, and Uruguay; and (4) Guava = collections from *Psidium guajava*, *Psidium guineense*, *Syzygium cumini*, *Myrciaria cauliflora*, *Eugenia uniflora*, and *Myrrhinium atropurpureum* var. *octandrum* from Brazil and Uruguay (table 1; figs. 1 and 2).

All loci were polymorphic, with 5 to 11 alleles detected per locus. Genetic diversity was highest within the guava cluster, followed by the pandemic, the allspice, and finally the eucalypt clusters (table 2). Similar to previous attempts to assess the geographic invasive potential of the species (Booth and Jovanovic 2012; Booth et al. 2000; Elith et al. 2013; Glen et al. 2007; Kriticos et al. 2013; Magarey et al. 2007), the model using all records of occurrence projects many areas throughout the world as having some degree of probability of suitable climate for *P. psidii* (fig. 3). Models using reduced sets of the eucalypt, pandemic, and guava biotypes show predictions of different distributions (figs. 3 and 4). Inoculation tests can also help to assess threats posed by different pathogen biotypes. For example, Silva et al. (2014) showed that the eucalypt biotype was highly virulent on Hawaiian ʼōhiʼa lehua (*Metrosideros polymorpha*) plants (fig. 5), and Zauza et al. (2010) showed that diverse myrtaceous species from Australia varied greatly in their resistance/susceptibility to one race of the eucalypt biotype.

When bioclimatic modeling is coupled with inoculation tests, it suggests that different biotypes pose different risks to various global regions, should they be introduced. This work builds on previous research that shows that diversity must be considered when assessing the invasive threat posed by this pathogen

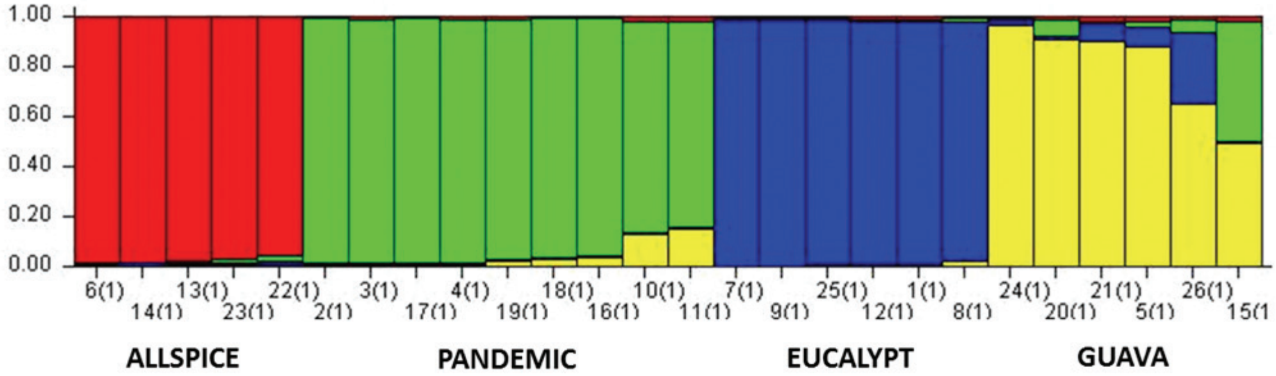

Figure 1—A Population structure inferred using a Bayesian clustering algorithm implemented in STRUCTURE at $K = 4$ (estimated ln probability = -418.9).

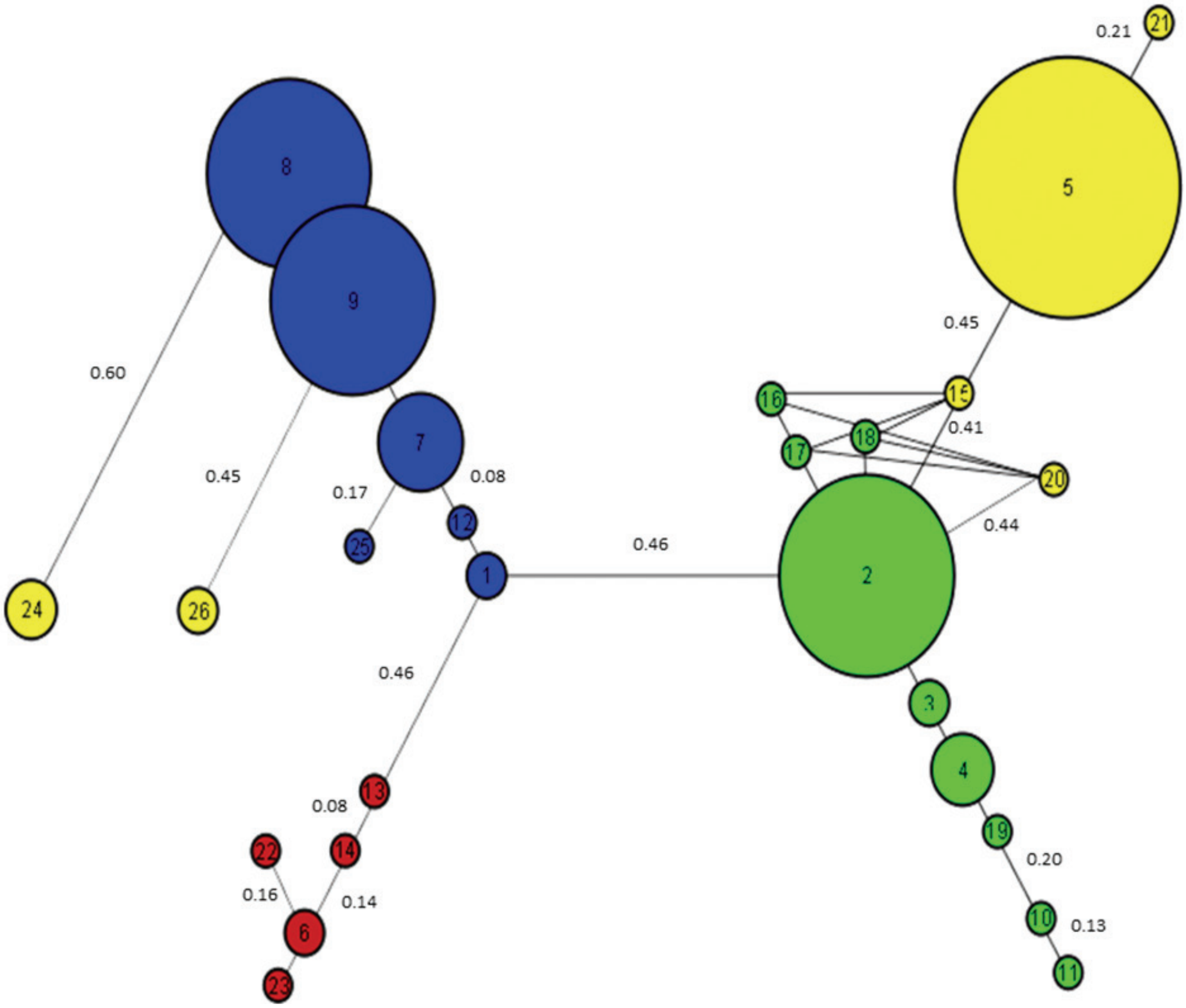

Figure 2—Minimum spanning network of multilocus genotypes represented by red for collections from *Pimenta dioica* (Jamaica), blue for collections from *Eucalyptus* spp. and *Syzygium jambos* (Brazil, Paraguay, and Uruguay), green from the pandemic cluster (which includes collections from diverse hosts from the Australia, the Caribbean, Mexico, and United States), and yellow from other hosts (collections from *Psidium guajava*, *Psidium guineense*, *Syzygium cumini*, *Myrciaria cauliflora*, *Eugenia uniflora*, and *Myrrhinium atropurpureum* var *octandrum*). Sizes of circles are proportional to MG frequency and connections are labeled with Bruvo genetic distances if different from 0.06, which corresponds to one mutational step at one locus. Loops in the network indicate multiple tied minimum spanning trees.

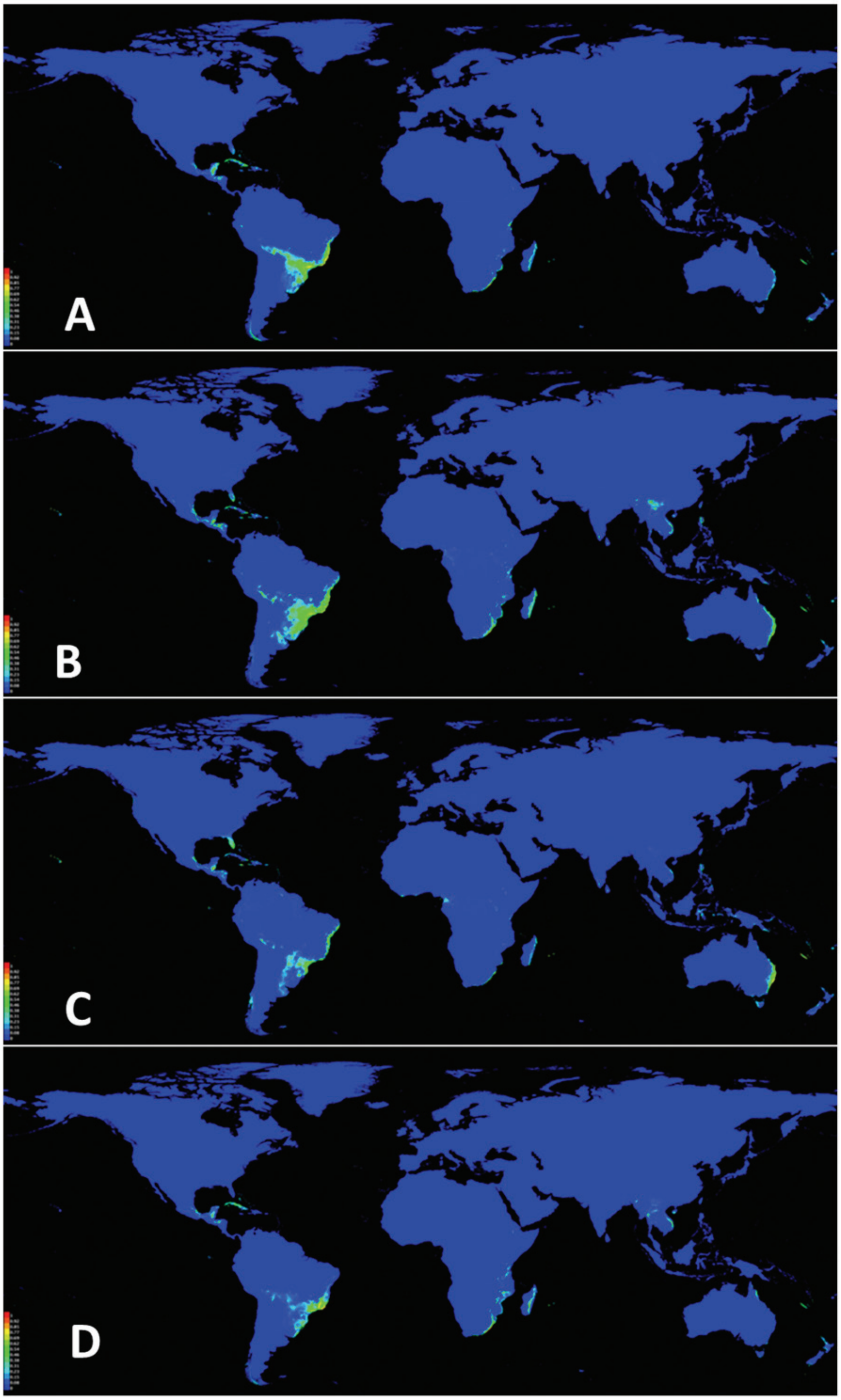

Figure 3—Maxent models of suitable climate space (potential distribution) for *Puccinia psidii* based on 19 global bioclimatic variables derived from the WorldClim (worldclim.org) database for A all biotypes (245 occurrence points); B eucalypt biotype (88 occurrence points); C pandemic biotype (89 occurrence points); and D guava biotype (65 occurrence points).

Figure 4—Assessment of Brazilian strain virulence to Hawaiian ʼŌhiʻa (*Metrosideros polymorpha*) (Silva et al. 2014). The Brazilian *Puccinia psidii* eucalypt biotype was highly virulent on Hawaiian ʼōhiʻa plants.

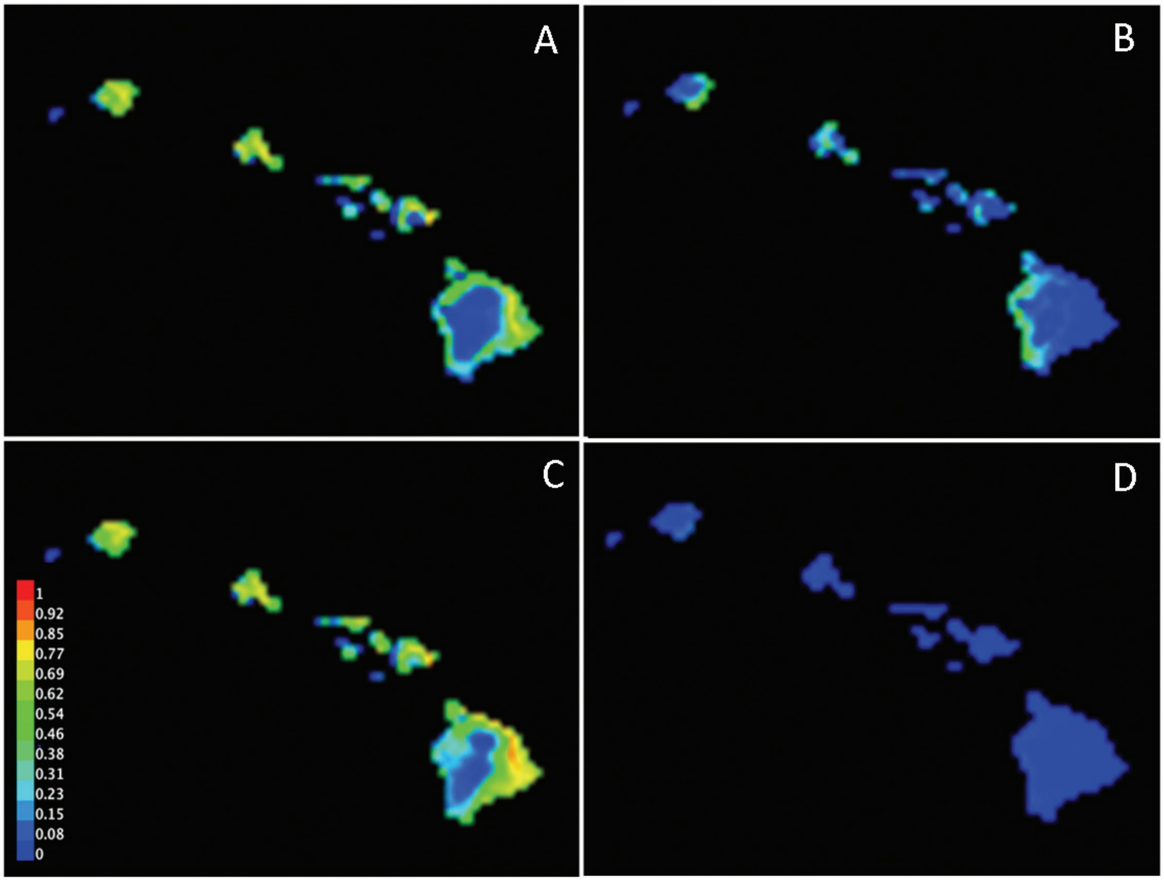

Figure 5—Maxent models of suitable climate space (potential distribution) for *Puccinia psidii* in Hawaii based on 19 global bioclimatic variables derived from the WorldClim (worldclim.org) database for A all biotypes; B eucalypt biotype; C pandemic biotype; and D guava biotype.

to myrtaceous hosts worldwide (Graça et al. 2013). Future studies that include *P. psidii* genotypes from additional geographic areas will allow a better prediction of global geographic areas that are climatically suitable for *P. psidii* and for subspecies-level genetic clusters, which will provide a more robust assessment for pathways of spread and areas at risk of invasion.

ACKNOWLEDGMENTS

Eric Pitman was instrumental in the bioclimatic modeling efforts.

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Cronartium Rusts Sporulate on a Wide Range of Alternate Hosts in Northern Europe

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INTRODUCTION

Cronartium flaccidum is a serious pathogen of two-needle pines, especially of *Pinus sylvestris*, in northern Fennoscandia (Samils et al. 2010). The rust spreads via a number of alternate hosts in natural forests and gardens, among which species in genera *Melampyrum, Pedicularis*, *Vincetoxicum*, and *Paeonia* are the most important known ones (Gäumann 1959; Kaitera et al. 1999). In northern Europe, the most important species is *Melampyrum sylvaticum* (Kaitera et al. 2005), while in southern Europe the respective species is *Vincetoxicum hirundinaria* (Gäumann 1959). *Cronartium ribicola* is a common pathogen on five-needle pines in arboretums, botanical gardens, and parks in northern Europe (Gäumann 1959; Kaitera et al. 2013). The rust spreads mainly via *Ribes* (Kaitera and Nuorteva 2006).

METHODS

To test the susceptibility of known and potential alternate hosts to *C. flaccidum* and *C. ribicola*, 109 plant species in 18 families were inoculated using various Fennoscandian spore sources in 2008–2013. The inoculations were performed in the greenhouse and in the laboratory, and plants were also exposed to natural inoculum in the field (e.g., Kaitera et al. 2015).

RESULTS AND DISCUSSION

Over the years, 45 plant species in 13 families supported uredinial or telial sporulation of *C. flaccidum* on tested leaves, while *C. ribicola* sporulated on 16 species or cultivars in 5 families (Kaitera and Hiltunen 2011, 2012; Kaitera et al. 2012, 2015). Among the tested species, hemiparasites were highly susceptible to both rusts. All of the tested genera of family Orobanchaceae included species that were susceptible to *C. flaccidum* in the greenhouse and in the laboratory. Some of these species were also infected when exposed to natural inoculum in the field (Kaitera et al. 2015).

Cronartium ribicola sporulated on species in genera such as *Bartsia* (fig. 1), *Castilleja* (fig. 2), *Pedicularis* (fig. 3), *Mentzeli*a (fig. 4), *Loasa* (fig. 5), *Tropaeolum* (fig. 6) and *Ribes* (figs. 7–9). *Cronartium flaccidum* sporulated on species in genera such as *Melampyrum* (figs. 10–13), *Pedicularis* (figs. 14–17), *Rhinanthus* (figs. 18–19), *Euphrasia* (figs. 20–23), *Loasa* (figs. 24–25), *Bartsia* (fig. 26), *Impatiens* (figs. 27–28), *Vincetoxicum* (fig. 29), *Asclepias* (fig. 30), *Apocynum* (fig. 31), *Nicotiana* (fig. 32), *Swertia* (fig. 33), *Veronica* (figs. 34–38), *Nemesia* (figs. 39–40), *Paeonia* (figs. 41–45), *Tropaeolum* (fig. 46), *Verbena* (figs. 47–48), *Saxifraga* (figs. 49–50), *Castilleja* (fig. 51) and *Myrica* (fig. 52).

In: Schoettle, Anna W.; Sniezko, Richard A.; Kliejunas, John T., eds. 2018. Proceedings of the IUFRO joint conference: Genetics of five-needle pines, rusts of forest trees, and Strobusphere; 2014 June 15–20; Fort Collins, CO. Proc. RMRS-P-76. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 245 p.

In conclusion, a high number of previously unknown alternate hosts can support rust sporulation under laboratory and greenhouse conditions, as well as in the field, and therefore, may also spread rust epidemics under favorable conditions in natural forests.

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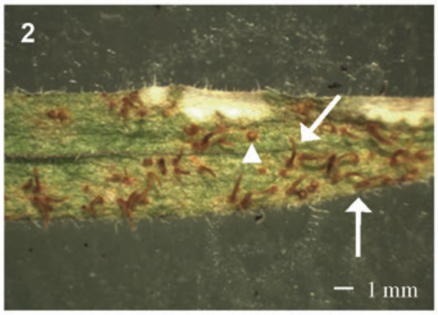
Figure 1–52—(1) Uredinia (white arrow heads) and telia (white arrows) of *Cronartium ribicola* (nos. 1-9) and *C. flaccidum* (nos. 10–52) on abaxial leaf surface of the plants.



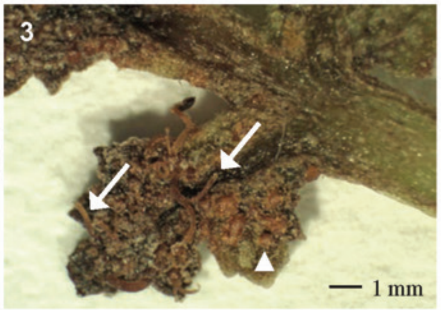
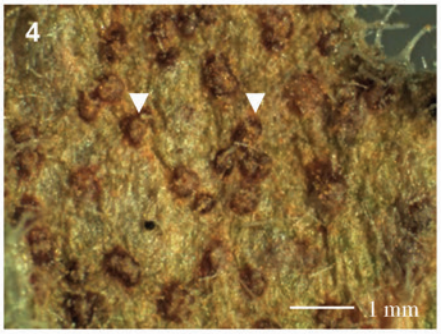
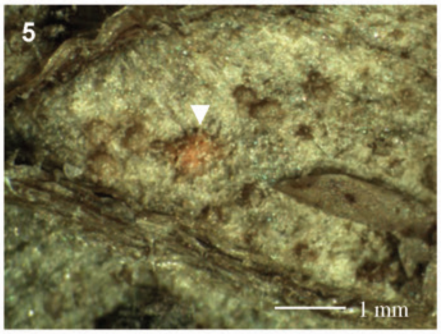


Figure 1—Uredinia of *Cronartium ribicola* on *Bartsia alpina*.
Figure 2—Uredinia and telia of *C. ribicola* on *Castilleja sulphurea*.
Figure 3—Uredinia and telia of *C. ribicola* on *Pedicularis palustris* ssp. *palustris*.





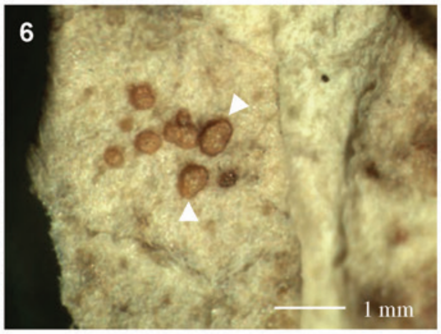
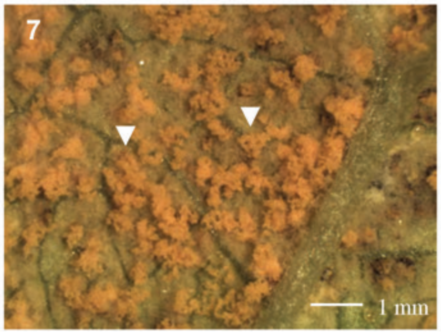
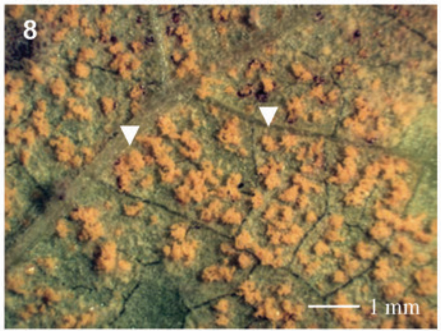


Figure 4—Uredinia of *C. ribicola* on *Mentzelia lindleyi*.
Figure 5—Uredinia of *C. ribicola* on *Loasa triphylla*.
Figure 6—Uredinia of *C. ribicola* on *Tropaeolum majus*.





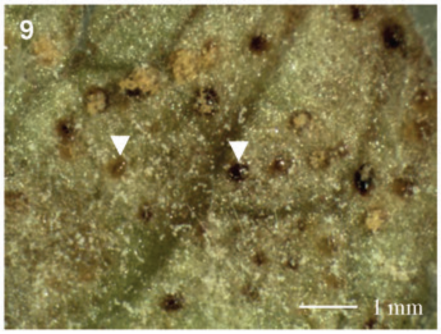


Figure 7—Uredinia of *C. ribicola* on *Ribes nigrum* 'Mortti'.
Figure 8—Uredinia of *C. ribicola* on *Ribes spicatum* subsp. *spicatum.*
Figure 9—Uredinia of *C. ribicola* on *Ribes laxiflorum*.

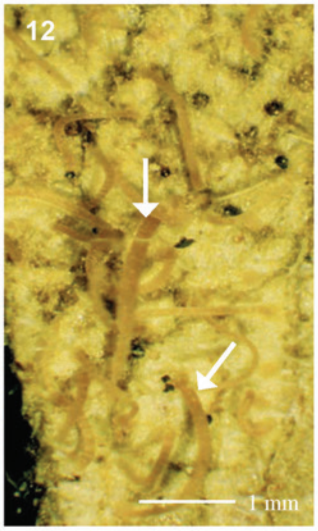
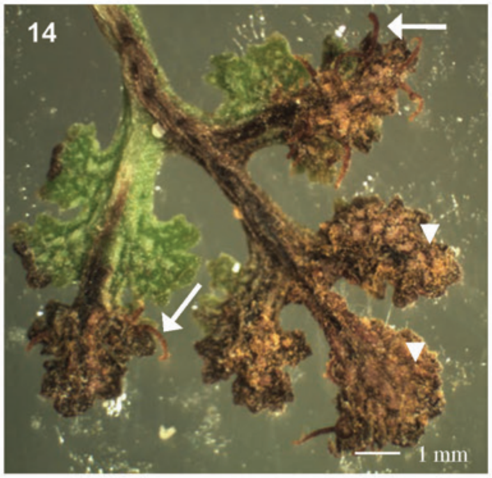


Figure 10—Telia of *Cronartium flaccidum* on *Melampyrum sylvaticum*.
Figure 11—Telia of *C. flaccidum* on *Melampyrum pratense*.
Figure 12—Telia of *C. flaccidum* on *Melampyrum nemorosum*.
Figure 13—Telia of *C. flaccidum* on *Melampyrum arvense*.



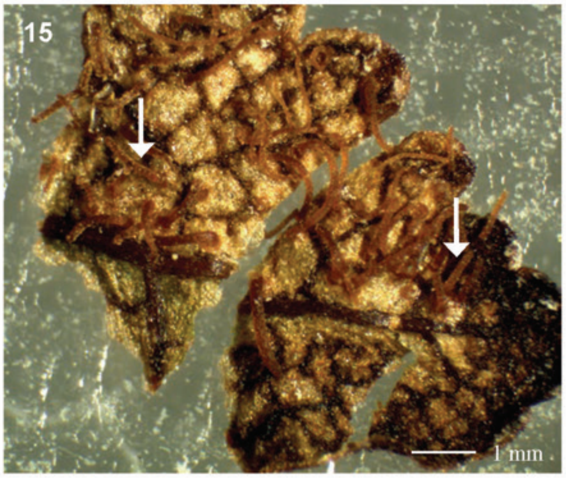
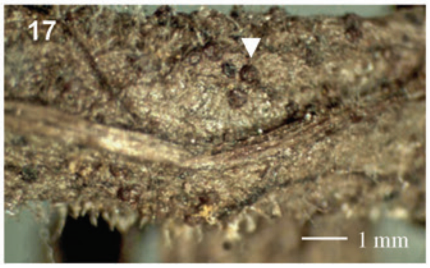
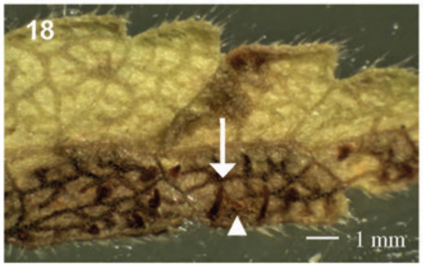


Figure 14—Uredinia and telia of *C. flaccidum* on *Pedicularis palustris* ssp. *palustris*.
Figure 15—Telia of *C. flaccidum* on *Pedicularis sceptrum-carolinum*.
Figure 16—Telia of *C. flaccidum* on *Pedicularis groenlandica*.





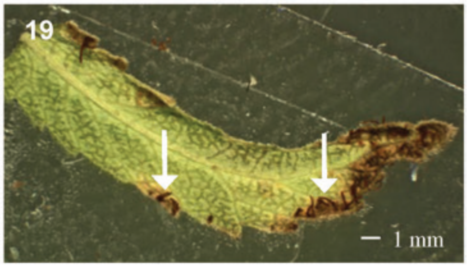
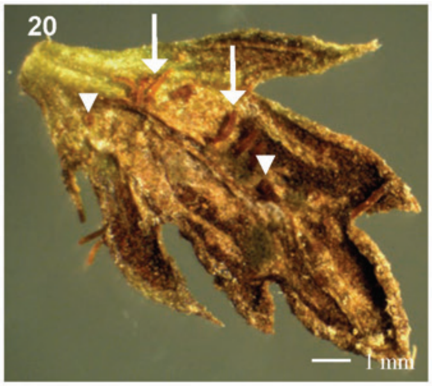
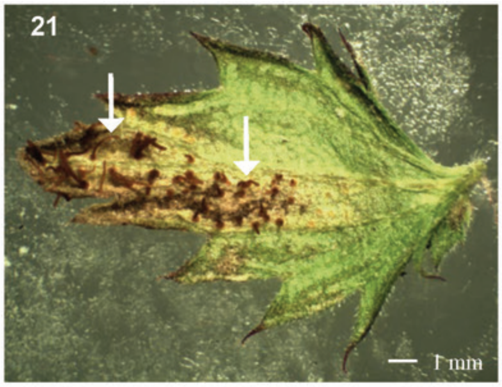


Figure 17—Uredinia of *C. flaccidum* on *Pedicularis dolichorrhiza*.
Figure 18—Uredinia and telia of *C. flaccidum* on *Rhinanthus minor*.
Figure 19—Telia of *C. flaccidum* on *Rhinanthus aestivalis*.





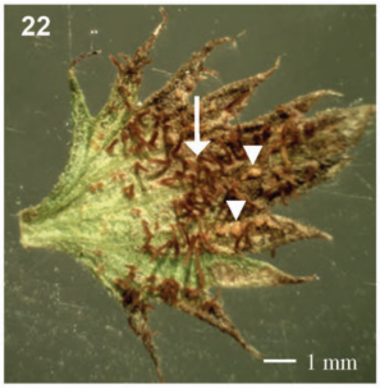
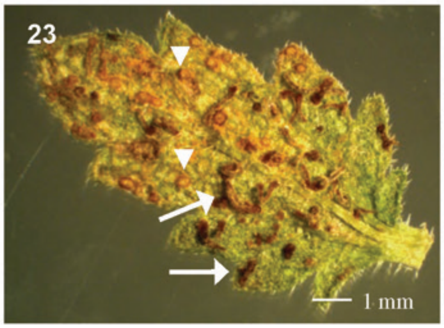
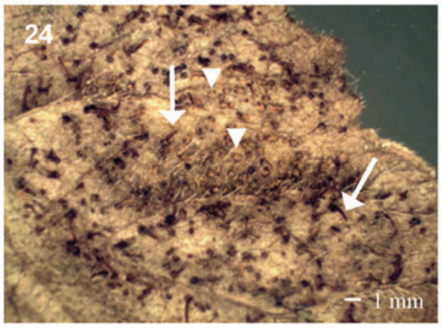


Figure 20—Uredinia and telia of *C. flaccidum* on *Euphrasia minima*.
Figure 21—Telia of *C. flaccidum* on *Euphrasia brevipila*.
Figure 22—Uredinia and telia of *C. flaccidum* on *Euphrasia stricta*.





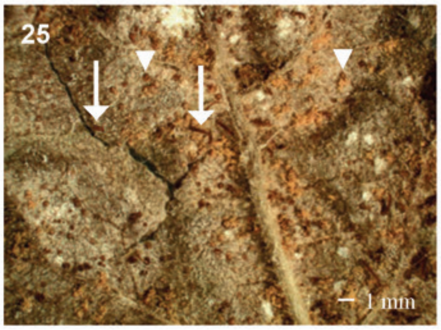
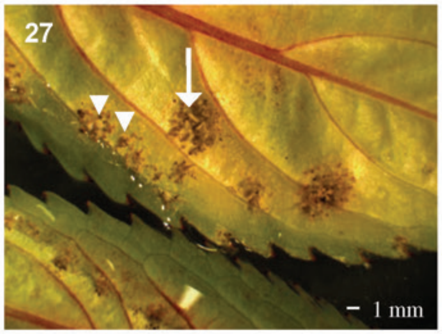


Figure 23—Uredinia and telia of *C. flaccidum* on *Euphrasia officinalis*.
Figure 24—Uredinia and telia of *C. flaccidum* on *Loasa tricolor*.
Figure 25—Uredinia and telia of *C. flaccidum* on *Loasa triphylla*.



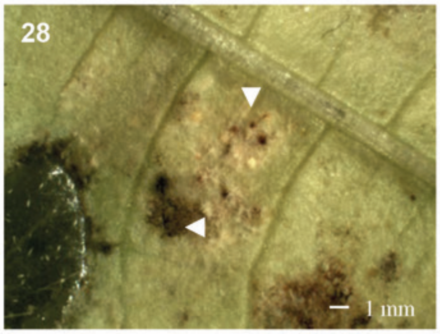
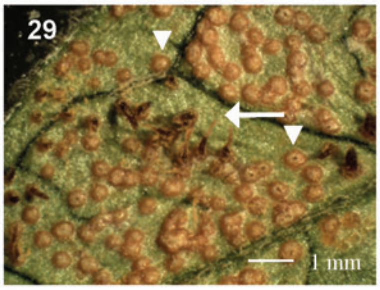
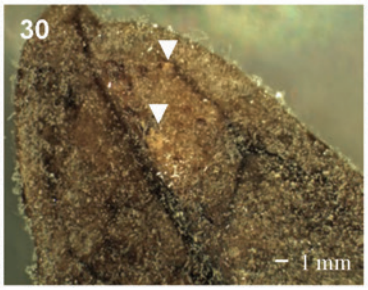


Figure 26—Uredinia and telia of *C. flaccidum* on *Bartsia alpina*.
Figure 27—Uredinia and telia of *C. flaccidum* on *Impatiens balsamina*.
Figure 28—Uredinia of *C. flaccidum* on *Impatiens glandulifera*.





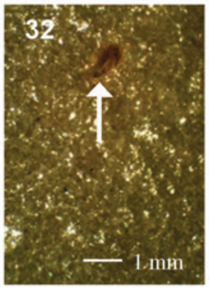
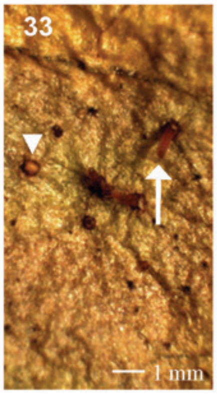
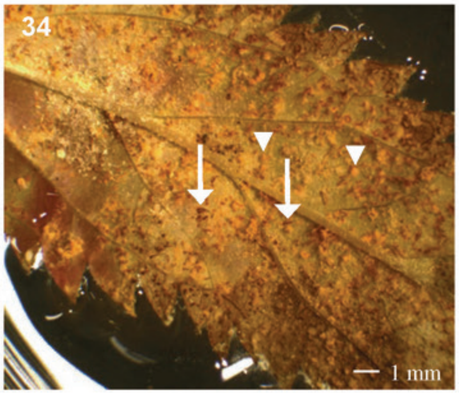
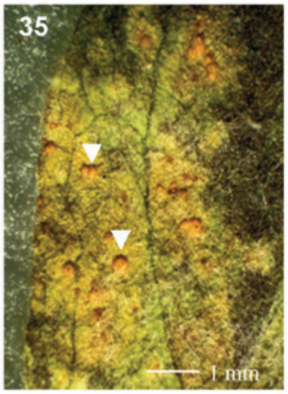


Figure 29—Uredinia and telia of *C. flaccidum* on *Vincetoxicum hirundinaria*.
Figure 30—Uredinia of *C. flaccidum* on *Asclepias incarnata*.
Figure 31—Uredinia of *C. flaccidum* on *Apocynum cannabinum*.
Figure 32—Telium of *C. flaccidum* on *Nicotiana rustica*.







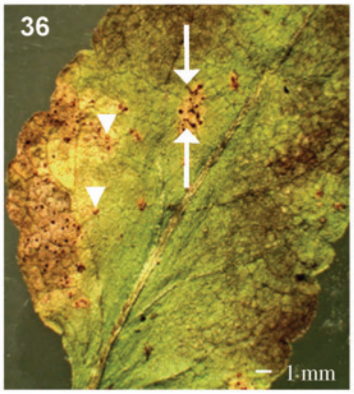
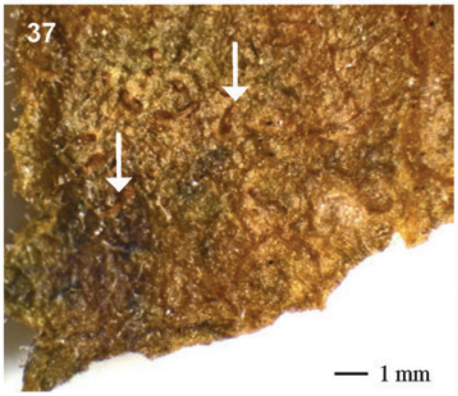
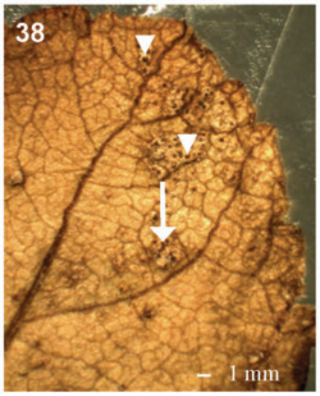
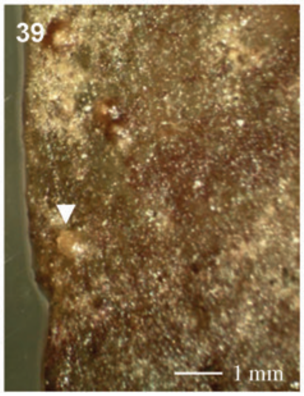


Figure 33—Uredinia and telia of *C. flaccidum* on *Swertia fedtschenkoana*.
Figure 34—Uredinia and telia of *C. flaccidum* on *Veronica longifolia*.
Figure 35—Uredinia of *C. flaccidum* on *Veronica incana*.
Figure 36—Uredinia and telia of *C. flaccidum* on *Veronica krylovii*.







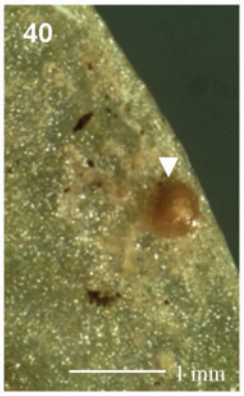
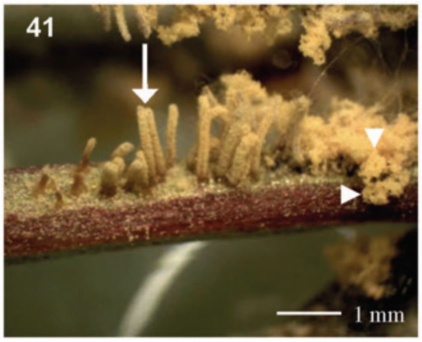
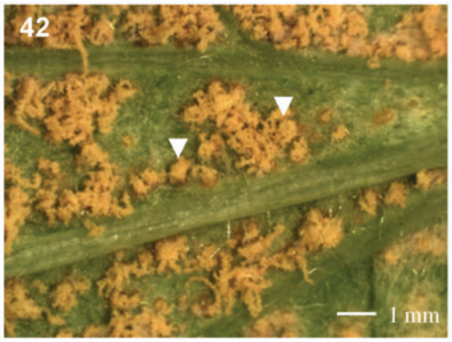


Figure 37—Telia of *C. flaccidum* on *Veronica daurica*.
Figure 38—Uredinia and telia of *C. flaccidum* on *Veronica grandis*.
Figure 39—Uredinia of *C. flaccidum* on *Nemesia versicolor*.
Figure 40—Uredinium of *C. flaccidum* on *Nemesia strumosa*.





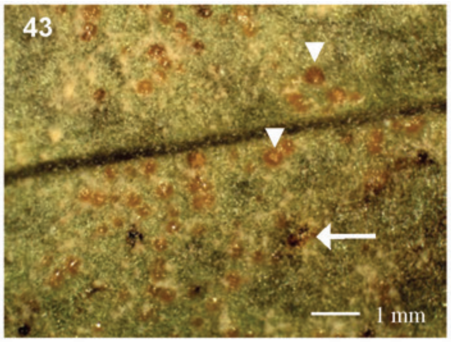
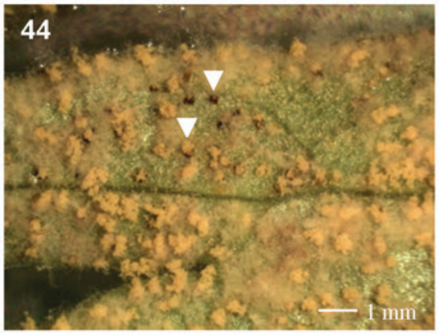
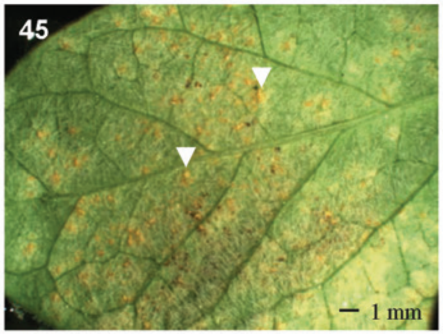


Figure 41—Uredinia and telia of *C. flaccidum* on *Paeonia tenuifolia*.
Figure 42—Uredinia of *C. flaccidum* on *Paeonia officinalis*.
Figure 43—Uredinia and telia of *C. flaccidum* on *Paeonia lactiflora*.





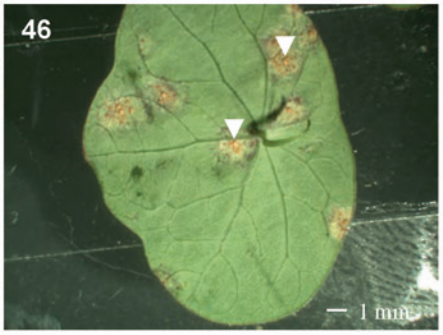
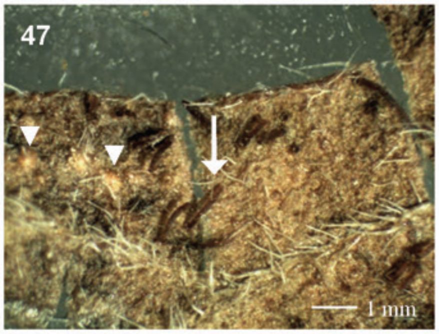


Figure 44—Uredinia of *C. flaccidum* on *Paeonia anomala*.
Figure 45—Uredinia of *C. flaccidum* on *Paeonia obovata*.
Figure 46—Uredinia of *C. flaccidum* on *Tropaeolum majus*.



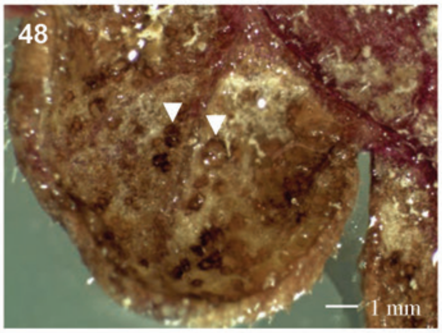
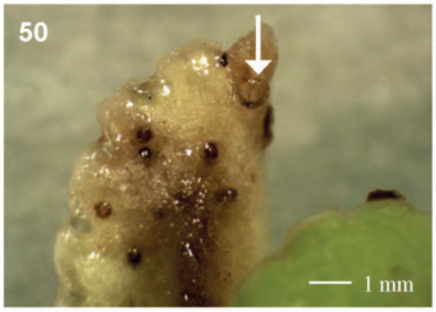
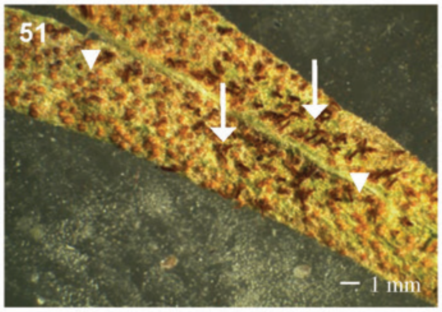


Figure 47—Uredinia and telia of *C. flaccidum* on *Verbena canadensis*.
Figure 48—Uredinia of *C. flaccidum* on *Verbena x hybrida*.
Figure 49—Uredinia of *C. flaccidum* on *Saxifraga exarata*.





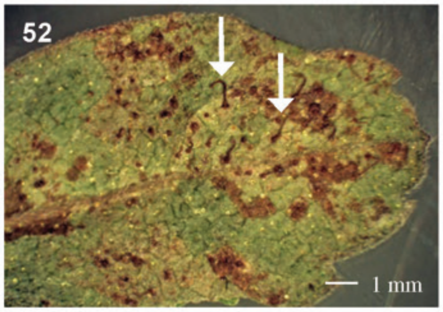


Figure 50—Telia of *C. flaccidum* on *Saxifraga hostii*.
Figure 51—Uredinia and telia of *C. flaccidum* on *Castilleja miniata*.
Figure 52—Telia of *C. flaccidum* on *Myrica gale*.

White Pine Blister Rust Confirmed on Limber Pine (*Pinus flexilis*) in Rocky Mountain National Park

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On August 24, 2009, while working in Rocky Mountain National Park (Estes Park, Colorado, USA) on the Conserving Limber Pine Genetic Diversity for Possible Future Restoration at Rocky Mountain National Park project (see Schoettle et al. 2011, 2013, in press), a crew from the U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station (RMRS) noticed a small limber pine (*Pinus flexilis*) with a dead top along Highway 36 near the Many Parks Curve (fig. 1). The tree was about 2 m tall and 7 to 10 cm in diameter at breast height (d.b.h., 1.37 m) and occurred at about 3,000 m in elevation. The UTM coordinates for the tree are E445370, N4471762 (WGS84, Z13N). The signs on the main stem were consistent with symptoms caused by the canker fungus *Cronartium ribicola*, which causes white pine blister rust (WPBR). Mature trees and seedlings in the area around the infected tree were surveyed on September 11, 2009. One branch in the upper crown of a mature tree at 3,020 m in elevation resembled WPBR because of the extensive chewing by squirrels (Family Sciuridae). The tree was flagged and its UTM coordinates are E445230, N4471698 (WGS84, Z13N). No other suspected cankers were found. Similar surveys of mature and young trees were conducted at each of 17 research sites within the eastern side of the park and no other suspected WPBR infections were observed.

Ribes inerme, a susceptible alternate host for *C. ribicola*, is present along Hidden Valley Creek in the Beaver Ponds area, suggesting that this area could serve as a source of basidiospores to infect limber pine up slope if the rust were present in the area. No evidence of rust infection of the *Ribes* plants in the Beaver Ponds area was seen during a 2009 inspection.

These observations provided strong evidence for the presence of WPBR in Rocky Mountain National Park. Plans were made to revisit the area in early summer 2010 in an attempt to observe the bright orange aecia that offer a phenotypic identification of the disease.

On June 22, 2010 RMRS and Rocky Mountain National Park employees surveyed the Many Parks Curve and Beaver Ponds areas of the park. Both areas were walked with global positioning system devices and an attempt was made to survey all limber pine within the area for signs or symptoms of WPBR by using binoculars or inspecting smaller trees closely. At Many Parks Curve, two crews of two people each walked parallel to the road for about 1.3 km and surveyed more than 289 limber pine trees and 95 limber pine seedlings (<1.34 m in height) over 2.6 ha (fig. 1). No WPBR infections were found. The limber pine tree above the road in the Many Parks Curve area found in 2009 with a possible canker (fig. 1) had no

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In: Schoettle, Anna W.; Sniezko, Richard A.; Kliejunas, John T., eds. 2018. Proceedings of the IUFRO joint conference: Genetics of five-needle pines, rusts of forest trees, and Strobusphere; 2014 June 15–20; Fort Collins, CO. Proc. RMRS-P-76. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 245 p.

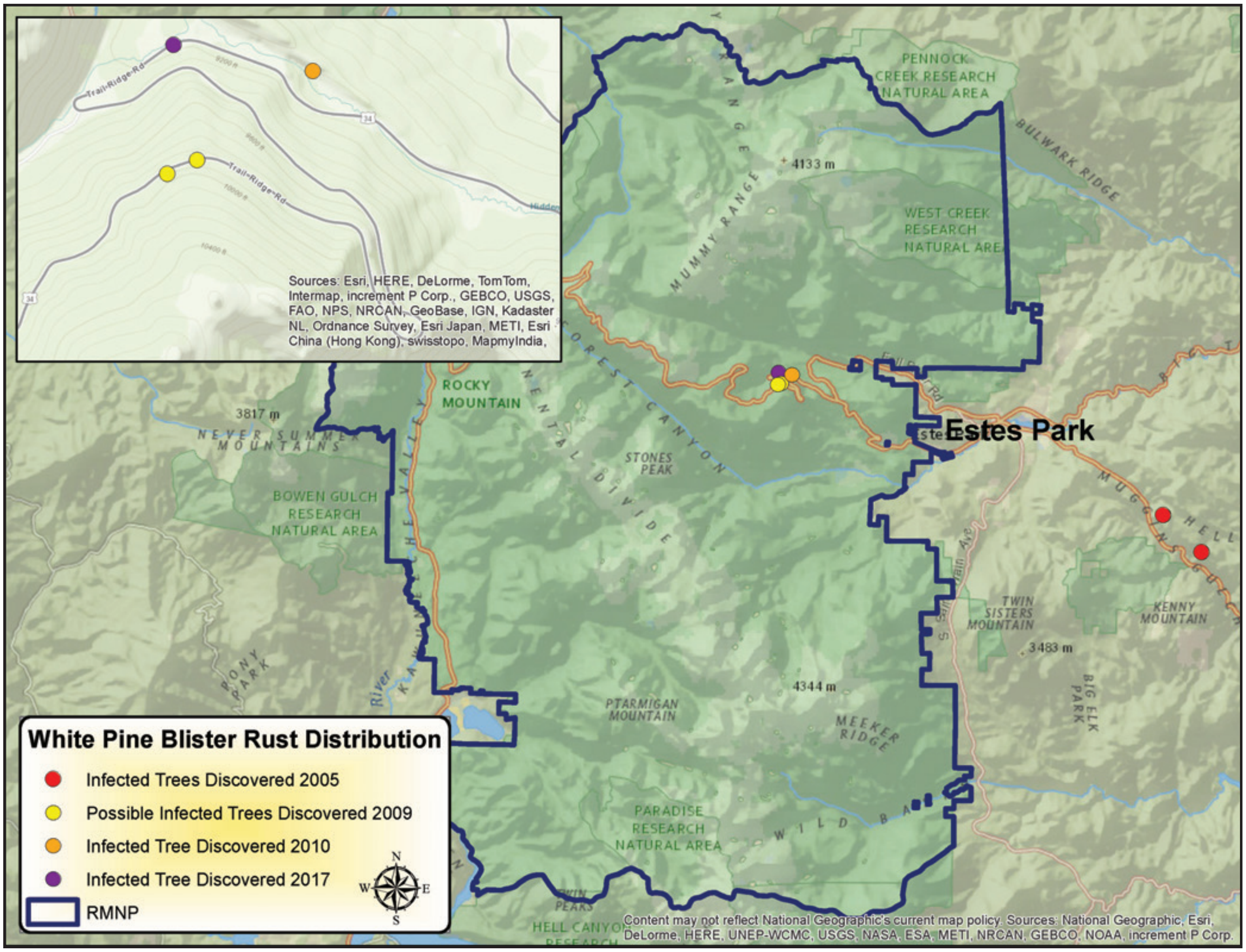

Figure 1—White pine blister rust survey areas of Many Parks Curve and Beaver Ponds in Rocky Mountain National Park. A limber pine possibly infected with *Cronartium ribicola* was found above Trail Ridge Road in Many Parks area in July 2009 and a confirmed white pine blister rust canker found in the Beaver Ponds area in 2010 and another up the road in 2017.

sporulation, suggesting that the infection was successfully removed the previous summer. The second tree found in 2009 with the squirrel chewing also had no evidence of sporulation.

At Beaver Ponds, one crew of three people walked about 1.2 km and surveyed 78 mature limber pine trees and 25 limber pine seedlings (<1.34 m in height) between the creek and the ridgeline over 1.8 ha (fig. 1). One mature limber pine tree was found with a *C. ribicola* sporulating canker (figs. 1 and 2). The tree was tagged with a round metal tag (#4149); the UTM coordinates for the tree are E 445914, N 4472182 (WGS84, Z13N), at an elevation of 2,786 m. The tree is within 200 m of the stream in a riparian area with substantial canopy cover by limber and lodgepole (*P. contorta*) pines as well as some Engelmann spruce (*Picea engelmannii*). The d.b.h. was 28 cm. The single canker was 25 cm long on a branch about 1.25 m above the ground (fig. 3). Approximately 9 cm of the branch had aecial blisters at both the proximal and distal ends of the canker; the proximal sporulating region was within 20 cm of the main tree bole. The aecial blisters had already ruptured (fig. 4). A small dead twig was present in the center of the canker and a needle on that twig, when alive, was most likely the tissue that was initially infected. With further dissection of the cankered branch, our best estimate is that infection may have occurred 4 to 6 years before 2010. No other cankers were detected in the immediate area or on the infected tree. This is the first confirmed case of WPBR in Rocky Mountain National Park.

The cankered branch was removed. Annual inspections in early summer have been made in this area and no additional infections have been identified (C. Cleaver, Colorado State University, and A. Schoettle, personal communication, 2015). *Cronartium ribicola* was detected, however, on *R. inerme* in the area in fall 2015 (K. Burns, personal communication, 2015). Because WPBR is difficult to identify in the early stages of an outbreak, it is possible there are more infected trees in the area.

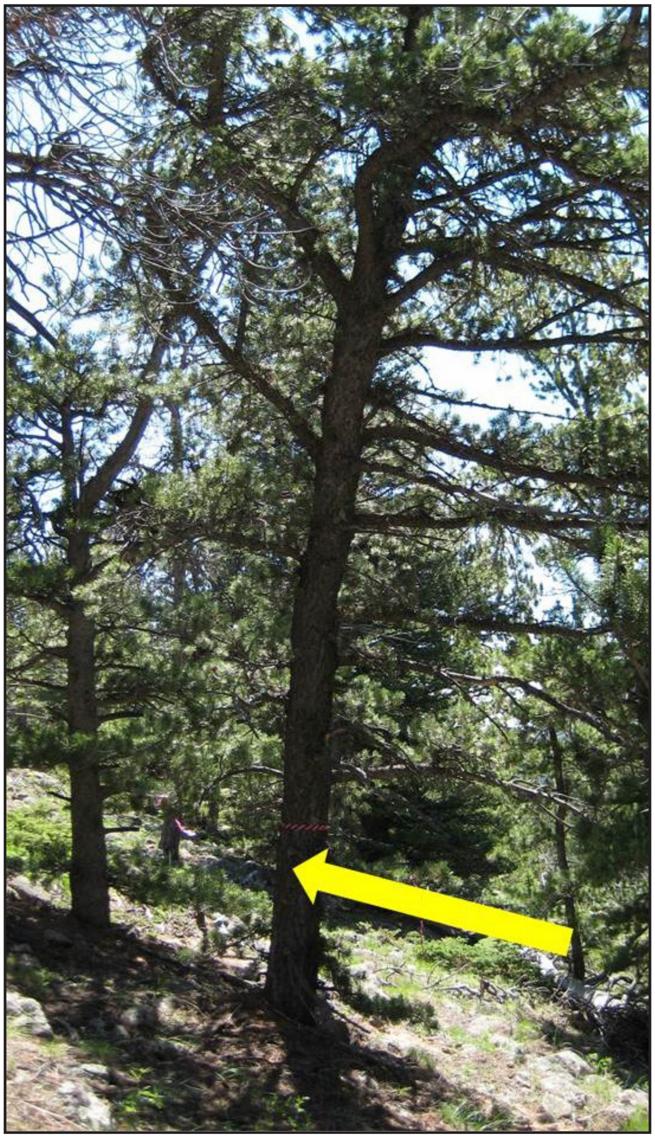

Figure 2—The limber pine tree found infected with white pine blister rust in the Beaver Ponds area of the Rocky Mountain National Park. The cankered branch is the lowest branch on the left (yellow arrow).

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Figure 3—The distal end of the canker with the aecia is on the left and the proximal end of the canker, also with aecia, is on the right. Overall canker length is 25 cm. The tree bole is to the right.

Figure 4—Proximal sporulating end of the canker (ruler included for scale). Note that the aecial blisters have already ruptured and begun releasing the orange aeciospores.

Efficacy of Early Pruning to Reduce the Incidence of White Pine Blister Rust on Sugar Pine (*Pinus lambertiana*)

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INTRODUCTION

Sugar pine (*Pinus lambertiana*) is a five-needle pine native to the mixed-conifer forests of the Sierra Nevada and Cascade Ranges of western North America. Sugar pine has great aesthetic, ecological, and economic value and is among the largest of all pines. Like other five-needle pines, sugar pine is susceptible to white pine blister rust (WPBR), caused by the nonnative pathogen *Cronartium ribicola*. Although tree-breeding efforts have been the most successful strategy against WPBR, artificial pruning by removing the lowest branches (where infections are most common) can potentially enhance survival of five-needle pines exposed to WPBR. Artificial pruning has been used successfully in WPBR management programs for western white pine (*Pinus monticola*), although most pruning is not done until trees are 10- to 15-year-old saplings. Earlier pruning against WPBR cankers and pruning on sugar pine have received less attention. As part of a larger restoration effort involving several Federal agencies, personnel from the U.S. Department of Agriculture, Forest Service, Dorena Genetic Resource Center (DGRC; Cottage Grove, Oregon, USA) established monitoring plots to measure the efficacy of early pruning on the incidence and impact of WPBR on planted sugar pine seedlings.

METHODS

Ten planting sites with moderate-to-high WPBR pressure were selected in the southern Cascade Range of Oregon, near the Little River on the Umpqua National Forest. For each site, sixteen 202-m^2 plots were established, cleared of woody vegetation, and planted with improved and unimproved sugar pine seedlings. Twenty-four families of containerized seedlings were grown at the DGRC and outplanted in fall 2000. These families comprised eight resistant families (SB orchard), eight F1 orchard families, and eight demonstrated nonresistant families (Wild). Each plot was planted with the same mix of 24 seedlings, one representative per family per plot.

Using a paired plot design, half of the plots at each site were randomly selected for the pruning treatment, and the other half remained untreated as controls. All trees in the treated plots were pruned following the fourth growing season (2003, when they were 1.2 to 1.5 m in height) and every 4 years thereafter. Whole branches were removed, starting at the ground, leaving about 50 percent canopy remaining. Periodic pruning will continue until the trees are pruned to a height of 3 m. Surveys began in the fall of the fourth year and continued every 2 to 4 years through 2012. Data collected

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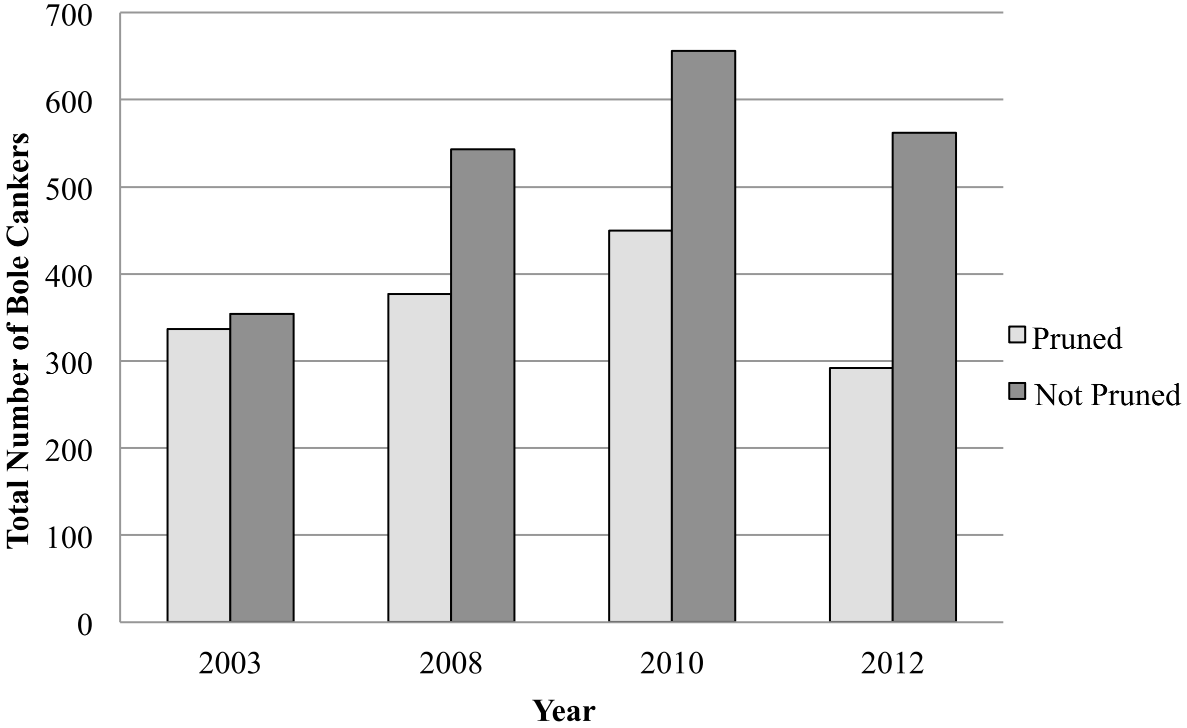

Figure 1—The total number of bole cankers on live trees for each year recorded.

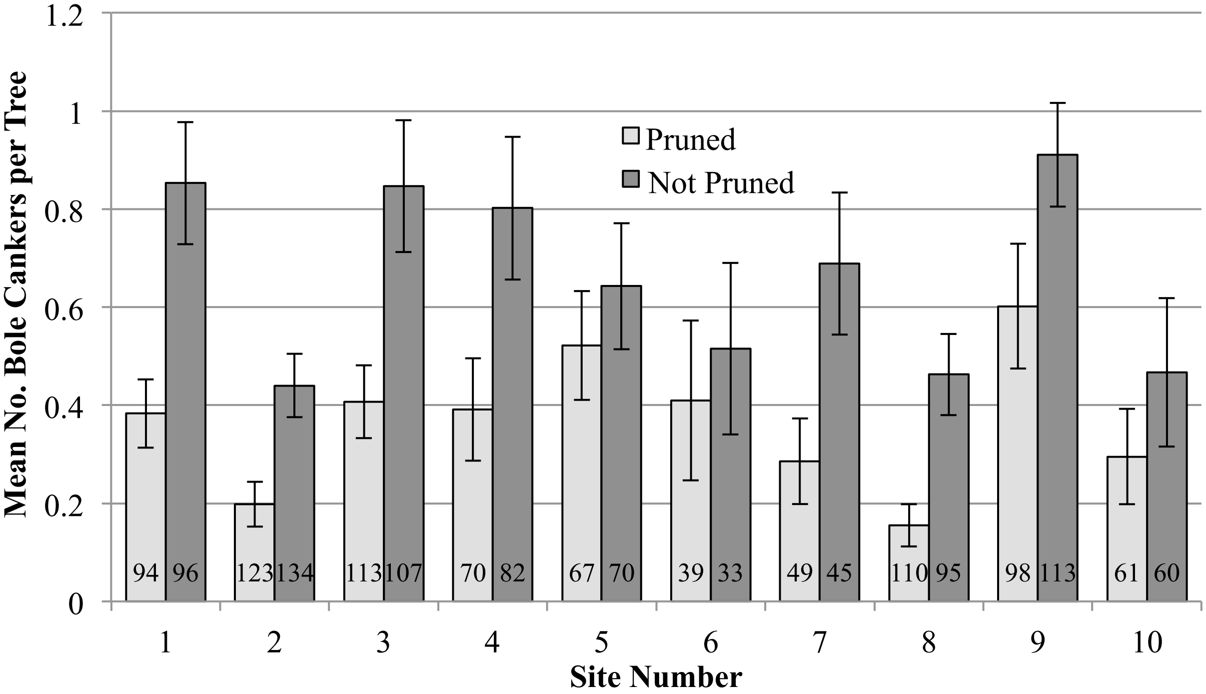

Figure 2—The mean number of bole cankers on live trees per site in 2012. Error bars indicate ± standard error of the mean. Numbers inside bars indicate the total number of live trees on the site indicated.

during the surveys include: mortality (and cause if evident), number and location of branch cankers, number and location of bole cankers, percentage of the bole circumference girdled by cankers, and other comments. The number of branch cankers on trees in treatment plots was recorded prior to pruning.

RESULTS AND DISCUSSION

Cumulative percent mortality on all sites was surprisingly similar between pruned and unpruned plots for the years recorded; in fact, cumulative mortality in 2012 was identical, 55 percent, for pruned and unpruned plots. Similarly, the percentage of trees that were killed by WPBR differed little between pruned and unpruned plots; 57 and 59 percent of mortality, respectively, was attributed to WPBR. WPBR was the primary mortality agent although all of the plots contained trees that suffered from animal and insect damage as well. The average height of trees in 2012 was 3.1 m on pruned plots, and 3.4 m on unpruned plots. Height growth of trees was highly variable within and between plots, but appeared to be minimally affected by early pruning. Between 2004 and 2012, the average increase in height of trees was 1.9 m (±0.9 m SD) on the pruned plots and 2.3 m (±1.1 m SD) on unpruned plots.

Overall, more total cankers were observed on untreated, control plot trees than on treated, pruned plot trees. As of 2012, trees in unpruned plots contained almost three times as many branch cankers as the pruned plots (2,147 and 785, respectively). This result is confounded by the pruning treatment that removes lower branches and reduces potential infection area. Analysis accounting for this reduction in lower branches and resistance capacity may reveal more subtle differences between treatment, family resistance, or both.

The most promising result thus far has been the reduction of bole (stem) cankers on trees in pruned plots. As of 2012, there were 292 bole cankers on trees on the pruned plots, compared to 562 bole cankers on unpruned plots (fig. 1). Trees on the pruned plots have had lower numbers of bole cankers consistently throughout the study (table 1). The average number of bole cankers recorded per tree has also been substantially less on pruned plots (fig. 2). In addition, pruning appears to reduce the percentage of trees with bole cankers regardless of seedling type (fig. 3). As of 2012, 16 percent of the F1 orchard families on pruned plots had bole cankers, compared to 31 percent on unpruned plots. Although early pruning has not yet resulted in a significant reduction in WPBR-caused mortality, the lower number of bole cankers on these trees is very promising. It is likely that we will not see the full benefits of pruning for several more years. Surveys are expected to continue through 2020.

Table 1—Total number of bole cankers in each site according to treatment[a] and year.

	2003		2008		2010		2012	
Site No.	P	NP	P	NP	P	NP	P	NP
1	14	12	54	66	67	99	36	81
2	12	8	17	44	27	73	24	59
3	6	8	42	94	33	98	47	90
4	21	38	35	40	23	37	27	65
5	31	62	33	62	40	63	35	45
6	152	142	48	49	36	64	16	17
7	60	53	59	78	76	74	14	31
8	3	6	5	22	7	32	17	44
9	7	14	53	60	108	92	59	102
10	31	13	31	28	33	24	18	28
Mean	34	36	38	54	45	66	29	56

[a] P - Pruned (treated) plots. NP – Unpruned (control) plots

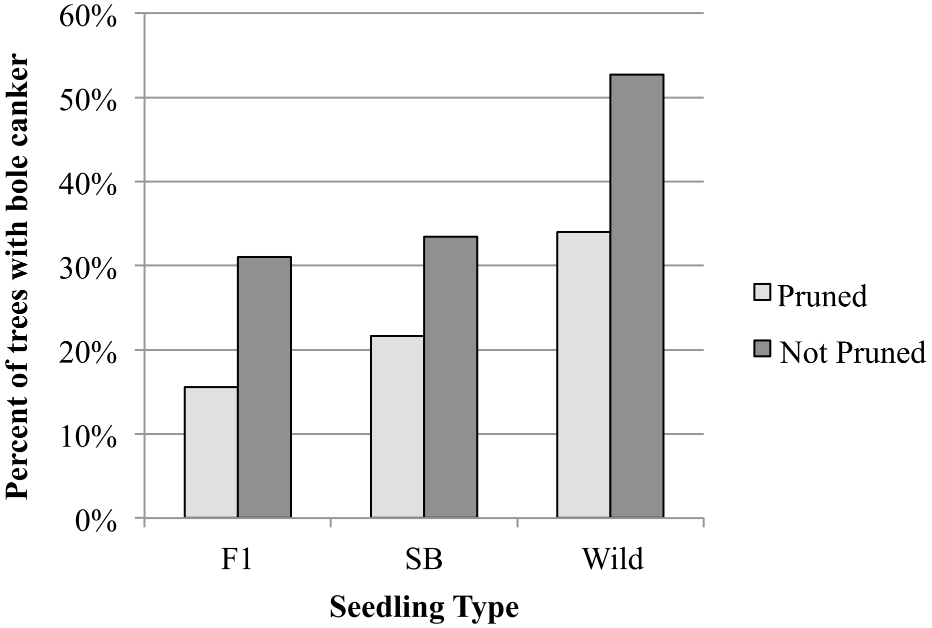

Figure 3—The percent of live trees with at least one bole canker by seedling type. F1 – F1 DGRC orchard families. SB – OP resistant families. Wild – screened susceptible families. All families are open-pollinated progeny of phenotypic selections.

Comparative Genomics of the *Cronartium* Genus

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Abstract—The obligate basidiomycete *Cronartium ribicola*, causal agent of white pine blister rust, has been responsible for the decline of five-needle pines across the North American landscape. This nonnative pathogen, originally from Asia, was first introduced into eastern North America from Europe. Multiple introductions of infected plant material from Europe have been recorded on the Eastern seaboard, whereas a single introduction has been described in the Pacific Northwest region. By decimating the natural stands of western white pine (*Pinus monticola*), *C. ribicola* has played a significant role in shifting the dominant tree species in the northwestern United States. We have sequenced the *C. ribicola* genome and two other rust pathogens using next-generation sequencing technology. *Cronartium comandrae*, a related rust species native to North America, is found on two- and three-needle pines and causes the comandra blister rust disease. Both of these rust pathogens are heteroecious, requiring two different plant hosts to complete their life cycle. In contrast, *Peridermium harknessii*, responsible for the western gall rust disease on two- and three-needle pines, is autoecious, requiring only one host for its survival. Through sequence analyses of *C. ribicola* and comparative studies with the related rust pathogens, we aim to explore and understand the underlying factors that determine the differences among these three rust species at the genomic level. This will help answer some fundamental questions about the biology, host preference, and etiology of each pathogen and its evolutionary significance.

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Colonization History, Host Distribution, and Landscape Features Shape White Pine Blister Rust Populations

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Abstract—White pine blister rust, caused by *Cronartium ribicola* (Basidiomycota, Pucciniales), was introduced into North America at the beginning of the 20th century on pine seedlings imported from Europe and has had serious economic and ecological impacts on white pines. We applied a population and landscape genetics approach to understand the patterns of introduction and colonization as well as population structure and migration of *C. ribicola*. We characterized 1,341 samples of *C. ribicola* from 76 geographic locations in North America using 31 single nucleotide polymorphisms and evaluated the effect of landscape features, host distribution, and colonization history on the population structure. We identified eastern and western genetic populations in North America that are strongly differentiated. Eastern populations have genetic diversity two to five times higher than western ones, which can be explained by the repeated accidental introductions of the pathogen in northeastern North America as opposed to the single documented introduction in western North America. These distinct genetic populations are maintained by a barrier to gene flow that corresponds to a region where host connectivity is interrupted. Furthermore, additional cryptic spatial differentiation was identified in western North America that corresponds to landscape features, such as mountain ranges. These results highlight the importance of monitoring this invasive alien tree disease to prevent admixture of the eastern and western populations where different pathogen races occur.

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The World Turned Upside Down: White Pine Blister Rust Pathogen Goes Down to Go Up

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Abstract—*Cronartium ribicola*, the fungal pathogen that causes white pine blister rust, infects pines through the needles and progresses to the stem, where cankers typically develop and seedlings or trees are killed after the canker girdles the tree. An alternate host, usually species of *Ribes* in North America, harbors the spore stage (basidiospores) that infects pines; there is thought to be no pine-to-pine infection via spores. However, in this report we present evidence of pine-to-pine transmission of white pine blister rust via root grafting. At Dorena Genetic Resource Center (DGRC; Cottage Grove, Oregon, USA), 2-year-old seedlings of North American white pine species are inoculated with *C. ribicola* to evaluate genetic resistance. After inoculation, the seedlings are assessed for up to 5 years for the different components of resistance. Most stem infections appear within 2 to 3 years following artificial inoculation, and mortality often occurs within a few years after appearance of stem symptoms (cankers). Some trees remain canker-free after artificial inoculation, due either to resistance or occasionally being an “escape.” Canker-free trees in some of the trials have been followed casually for up to 15 years.

In several of these trials (notably in western white pine, *Pinus monticola*), we have noted new cankers developing at the base of several trees that were previously canker-free, more than 8 years following inoculation. Such cankers could have resulted from new infections from ambient local rust basidiospores. New infections attributed to local inoculum have been observed sporadically, occurring on upper branches in surviving trees. In trees with new basal cankers, however, there was no foliage at the base of the tree to permit normal infection through needles. Alternatively, the cankers could be latent or slow-developing infections on resistant trees. A third explanation to consider is that the new cankers result from transmission of the rust fungus from the root system of one seedling to an adjacent tree via root grafting and manifest as a canker at the base of the previously non-cankered tree. The frequency of cankers resulting from putative root-grafting was 3.0 percent (46/1,539) and 1.5 percent (33/2,214) in the two trials summarized to date. The seedlings at DGRC are planted at close spacing, permitting a near-optimum scenario in which to observe this phenomenon. The excavations of roots of several surviving newly cankered trees have revealed root grafting between the live trees and adjacent rust-killed individuals. Histology work is now underway to examine the roots for presence of white pine blister rust. At least in these resistance trials, if root grafting occurs, it could potentially bypass some resistances in previously canker-free trees.

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Widening the Landscape: The Role of Ornamental and Wild Trees in the Spread and Adaptation of *Melampsora larici-populina* Leaf Rust

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Abstract—Poplar rust, caused by *Melampsora larici-populina*, has become the primary disease affecting poplars (*Populus* spp.) in northern Europe after several cultivars selected for complete resistance became susceptible following pathogen adaptation. Although understanding pathogen adaptation requires working at the genetic and molecular levels both in the cultivated host and in the pathogen, landscape-level study of all other partners of the interaction is also needed to understand the process.

We will present the results from a 3-year project aimed at elucidating the role of wild poplars in the adaptive potential of the pathogen to an exotic and noncommercially deployed resistance gene. *Melampsora larici-populina* isolates were sampled in different environments with different selective pressures. We found that up to 13 percent of the isolates collected on wild Lombardy poplar (*P. nigra*) trees in environments where the resistance gene is absent were able to defeat the gene. This finding demonstrates that wild host populations act as a reservoir for pathogen adaptation.

We will also present initial results from an ongoing project where ornamental poplar and larch (*Larix* spp.) (aecial host) trees have been recorded, mapped, and characterized in urban and rural areas outside the natural range of sympatry between alternate hosts. This project aims at elucidating how scattered trees outside the forest influence the timing and rate of rust epidemics.

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Adaptation of Poplar Rust to the Poplar Varietal Landscape

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The breakdown of host resistance to plant pathogens is of critical concern in agriculture, forestry, and the management of natural systems. Evolution of the fungal poplar rust pathogen *Melampsora larici-populina* has been shown to have been dramatically influenced by the deployment of resistance genes in commercial poplar (*Populus* spp.) plantations, with pathogen populations inundated by virulent genotypes (Xhaard et al. 2011). The deployment and subsequent breakdown of resistance genes in poplar plantations provide an experimental system for understanding the dynamics of pathogen evolution in response to resistance breeding, which will be critical to formulating effective management strategies in the future.

We describe a combined retrospective and prospective approach, integrating population genomics, landscape epidemiology, and evolution of life history traits of the poplar rust fungus. First, the records of poplar genotypes deployed across France over the last 17 years, along with genotypes of *M. larici-populina* collected across France, have been used to make overlaying maps of host and pathogen resistance and virulence genotypes. We demonstrate that the virulence landscape continues to be dominated by the sweep described by Xhaard et al. (2011); regions heavily planted with poplar resistance type R7 are still dominated by the corresponding virulence type, along with virulence types that "hitchhiked" across the landscape during the original sweep. These genotypes persist, despite a reduction in planting of R7, and a near-absence of resistance types corresponding to the hitchhikers. In addition, the beginnings of a new sweep are emerging in regions in which a recently overcome resistance type (R8) has been more widely planted. We also describe plans to forecast the ability of the pathogen to evolve resistance to the future deployment of quantitative resistance types. To accomplish this objective, we will use the information learned from objective 1, and experimental tests of tradeoffs in pathogen aggressiveness, reproduction, and spread in response to quantitative resistance (resistance measured by degree). These forecasts will provide a framework for the future management of poplar and other forest plantations, and will also provide testable hypotheses with which to continue to improve understanding of host–pathogen co-evolution.

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Impact of White Pine Blister Rust on Resistant and Previously Immune Cultivated *Ribes* and Neighboring Eastern White Pine (*Pinus strobus*) in New Hampshire, USA

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White pine blister rust (WPBR; causal agent: *Cronartium ribicola*) has been a threat to both forest resources and agricultural commodities since its introduction to North America in the early 1900s. Eastern white pine (*Pinus strobus*) is the most economically important timber species in New Hampshire (USA). From 1917 to 1970 *Ribes* throughout the State was eradicated to protect the timber industry. After the lifting of the Federal Quarantine Act (USDA Bureau of Entomology and Plant Quarantine Federal Domestic Plant Quarantine number 63 [1938]) restricting *Ribes* and the more recent availability of immune and resistant *Ribes* cultivars, several States in the Northeast amended their restrictions to allow planting of selected *Ribes* cultivars. In New Hampshire, 19 WPBR-resistant or immune *Ribes* cultivars were available for planting. In 2011, WPBR was reported for the first time on immune black currant (*Ribes nigrum* cv. Titania) in Connecticut. The objectives of this study were to evaluate the impact of WPBR on cultivated resistant and immune Ribes cultivars and neighboring white pines.

To accomplish our objectives, 42 sites with cultivated *Ribes* were surveyed in late July through September 2013. At each site, incidence and severity were assessed on up to five plants per *Ribes* cultivar. Heavily infected leaves of each cultivar were collected for polymerase chain reaction (PCR) analyses conducted at Cornell University. The same methods were used to assess WPBR incidence and severity of wild *Ribes* found in the vicinity. Samples from immune black currants infected with WPBR from New Hampshire were used to inoculate immune *Ribes* cultivars from the Canadian Clonal Genebank in Harrow, Ontario. In addition, the following data were collected for the 12 nearest eastern white pines within 300 m of cultivated *Ribes*: WPBR incidence, diameter at breast height (1.37 m above the ground), distance, and cardinal direction from cultivated *Ribes*.

Two hundred and fifty-five plants of 19 *Ribes* cultivars and 445 white pines were evaluated. 'Titania' was the most frequently planted *Ribes* cultivar. Incidence of WPBR ranged from 0 to 60 percent and from 0 to 100 percent for immune and resistant *Ribes* cultivars, respectively. As expected, mean WPBR severity on resistant *Ribes* was limited to less than 6 percent of leaf area whereas WPBR severity on previously immune *Ribes* was greater, 14 percent of leaf area. All infected *Ribes* cultivars were PCR-positive for *C. ribicola*. Samples from New Hampshire successfully infected immune *Ribes* cultivars from the Canadian Clonal Genebank. It was more likely to find infected pines neighboring infected immune black currants (probability = 0.18) than neighboring infected resistant *Ribes* (0.09), or WPBR-free *Ribes* (0.02). Results from this study led to the removal of the previously immune black currant cultivars 'Consort,' 'Coronet,' 'Crusader,' and 'Titania' from the New Hampshire approved list for planting. The breakdown of WPBR immunity poses a threat to the white pine resource and to cultivated *Ribes* production.

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Origin and Distribution in Canada of the *Cronartium ribicola vCr* Race, Virulent on Immune Black Currant (*Ribes nigrum*) Cultivars Derived from Hunter's Selections

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Abstract—Black currant (*Ribes nigrum*) breeding programs were undertaken by different countries as a mean to control pests and pathogens, including *Cronartium ribicola*, the agent responsible for white pine blister rust (WPBR). This rust pathogen requires currants as its alternate telial host to complete its life cycle and infect five-needle pines, often causing mortality. The *R. ussuriense Cr* dominant resistance gene was introgressed into *R. nigrum* to generate the first immune black currant cultivars, called Hunter's selections. Three of these cultivars ('Coronet,' 'Crusader,' and 'Consort') were commercially released in the 1950s, and further bred to generate the more recent *C. ribicola*-immune cultivars such as 'Titania,' 'Tisel,' and 'Blackcomb.' Breakdown of the *Ribes Cr* resistance gene was reported in Connecticut in 2011. The same year, the *vCr* race was observed on immune *Ribes* cultivars in Quebec. Aecidiospores carrying the *vCr* gene were sampled on eastern white pine (*Pinus strobus*) cankers in spring 2012 and 2013. In late summer 2013, v*Cr* stains were present in fields of black currant producers in Quebec, New Brunswick, Nova Scotia, and Prince Edward Island, but absent in southern Ontario (Canada). Genetic analyses suggest the *vCr* race is not from a new introduction, but rather resulted from either a novel mutation or DNA recombination in a North American strain.

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Frequency and Distribution of Virulence in *Cronartium ribicola* to Two Simply Inherited Resistance Genes in Sugar Pine (*Pinus lambertiana*) and Western White Pine (*P. monticola*) in California, USA

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Abstract—For the last two decades, we have been documenting in California (USA) the frequency of virulence in the blister rust pathogen *Cronartium ribicola* to simply inherited genes for complete resistance in sugar pine [*Pinus lambertiana (Cr1)*] and western white pine [*P. monticola (Cr2)*]. At the Happy Camp Outplant Site in the north, seedlings of both species have been planted extensively for silvicultural and research purposes. At Mountain Home Demonstration State Forest in the southern Sierra Nevada, sugar pine is a major component of the giant sequoia- (*Sequoiadendron giganteum*) dominated forests. At both sites, blister rust infection occurs at high frequency because of near-optimal climatic conditions and an abundance of *Pinus* and *Ribes* hosts. Our test protocol requires suspending telia-bearing *Ribes* leaves, collected in early fall from several sites at each field location, over resistant (R_) and susceptible (rr) juvenile seedlings of each pine host. We inoculate pines for 72 hours in the dark in dew chambers under optimal conditions for infection at the U.S. Department of Agriculture, Forest Service, Institute of Forest Genetics in Placerville. As infection appears, we monitor resistant and susceptible needle-spot reactions, observe their development over 6 months, and confirm onset of definitive stem infection. We report here results from the mid-1990s through spring 2014. At both sites, virulence in *C. ribicola* has increased in frequency and become more widespread.

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Stem Rust Diseases of *Pinus kesiya* in Vietnam

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Surveys of two stem rust diseases of *Pinus kesiya* were implemented in Da Lat, Lam Dong province, in the Central Highlands of Vietnam. Symptoms of the first disease, a gall rust, are very similar to those of *Cronartium quercuum* in North America. Our surveys found many *Quercus cambodiensis* trees severely infected by the uredinial and telial states of a *Cronartium* species, later identified by microscopic characteristics as *Cronartium orientale*, which is native to eastern Asia. The aecial state of *C. orientale* occurs on various two-needle pines. We tentatively identified the gall rust on *P. kesiya* as *C. orientale*. We conducted inoculation experiments to demonstrate host alternation by *C. orientale*.

The gall rust of *P. kesiya* is very common in both forest plantations and natural stands in Lam Dong province. Disease incidence and severity vary among survey areas. In cases of high disease severity, almost all branches of a pine tree have galls, and exhibit poor form due to the death of many branches and stems. Disease incidence and severity are lowest in young plantations, reach the greatest levels in 5- to 10-year-old plantations, and are lower again in 15-year-old plantations. Main stems that have galls are very brittle.

The second rust found during surveys was a blister rust, with symptoms much like those produced by *C. flaccidum* and *Endocronartium* species, although these species have not previously been recorded in Vietnam. The blister rust attacks stems and branches of the pine. The period of aeciospore production is longer than 3 months. The germination type of aeciospores differed from that of *Endocronartium*, which has a pine-to-pine life cycle. The microscopic characteristics of the aecial states are also identical to those of *C. flaccidum*. By comparisons with *C. flaccidum* from Japan and Russia based on nucleotide sequences of the D1/D2 region of 28S rDNA, the blister rust on *P. kesiya* was identified as *C. flaccidum*. This species is a host-alternating species, but no suspected alternate hosts have been found in Da Lat. A pine-to-pine biological form of this species is known in Europe. To clarify the life cycle in Da Lat, an artificial inoculation experiment with aeciospores onto seedlings of *P. kesiya* was conducted in Hanoi. However, the seedlings remained uninfected for 2 years after the inoculation. Further studies on the life cycle are needed.

The damage by the two rusts to young pine plantations is very severe. Infected trees were very susceptible to attack by bark beetles (*Dendroctonus* spp.) and the longhorned beetle *Monochamus alternatus*, a vector of the nematode *Bursaphelenchus* species, which causes pine wilt disease. Measures for minimizing disease impacts based on integrated pest management methods are discussed.

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Barcoding the Rust Fungi of Germany

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The German Barcode of Life (GBOL) project is a large-scale DNA barcoding initiative to assess the biodiversity of animals, fungi, and plants of Germany. Here we introduce the subproject focusing on rust fungi (*Pucciniales, Basidiomycota*). This is the only group of fungi represented in the initial 3.5-year phase of the project.

The goal of the rust project is a complete DNA-barcode sequence library for the roughly 500 rust species reported for Germany. The DNA barcode database will link the extensive information available on morphology, ecology, and host range studies with barcoding data linked to individual specimens. Rust fungi are exceptionally well studied in Central Europe. A significant number of cryptic species may be recovered; alternatively, several species described from different hosts may need to be merged. Further, the full life cycles and host relationships, particularly of some host-alternating species, await elucidation. These data will allow identification of rust specimens in museum collections, which to date could not be identified because of missing discriminatory spore states.

Most DNA samples are extracted from herbarium specimens of the fungus collections preserved at the Natural History Museum Karlsruhe in Karlsruhe Germany, but targeted collecting of additional specimens will also be necessary. In the first phase of the project, primer selection and optimization were found to be essential for successful generation of DNA barcodes. The internal transcribed spacer (ITS) rDNA region and the large subunit of the rDNA are suitable common fungal barcode markers. To facilitate high-throughput barcoding, methods have been developed for semiautomatic DNA extraction from herbarium specimens and polymerase chain reactions in 96 well plates.

Currently the following groups are extensively DNA-barcoded: *Uromyces pisi* s.l., an especially complicated complex of species, and the genera *Melampsora* and *Coleosporium*. An example of an as-yet unidentified *Coleosporium* species on invasive *Solidago* (goldenrod) is presented.

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Geographic Distribution of *Cronartium flaccidum* and *Peridermium pini* in Sweden and Finland

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Abstract—Extensive attacks of blister rust on Scots pine (*Pinus sylvestris*) are widespread across northern Sweden, especially in Norrbotten and Västerbotten Counties. The first reports of damage came in 2002, and an inventory in 2008 showed that 130,000 ha, or 34 percent of young pine forests in this region, were infested. A follow-up inventory in 2012 did not indicate any decrease in rust attacks. What induced the sudden epidemic is not known. The blister rust on Scots pine is caused by two forms of the fungus: the macrocyclic host-alternating *Cronartium flaccidum*, and *Peridermium pini* (syn. *Endocronartium pini*), which spreads clonally from pine to pine. *Melampyrum sylvaticum* is considered to be the most important alternate host of *C. flaccidum* in the northern parts of Sweden and Finland, but several other plants from various families are also suitable alternate hosts. The two fungi were recently suggested to belong to the same species, as molecular marker data show little overall genetic differentiation between the fungi. It is possible to separate the two forms by using molecular markers because *P. pini* is characterized by totally homozygotic aeciospores and *C. flaccidum* aeciospores contain heterozygous loci.

To study the population genetic structure and geographic distribution of *C. flaccidum* and *P. pini*, aeciospore samples were collected from eight different locations in Sweden and five locations in Finland in 2011. In total, 220 samples were analyzed by using seven microsatellite markers. Our results showed that *C. flaccidum* dominates the fungal populations in the epidemic areas in northern Sweden. We could not detect any distinct geographic separation between *C. flaccidum* and *P. pini*, although specific locations or areas were dominated by one or the other form. Populations of *C. flaccidum* in the epidemic areas in northern Sweden were genetically similar to the neighboring populations in northern Finland.

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Monitoring Trends in White Pine Blister Rust Infection in the Greater Yellowstone Ecosystem

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Abstract—Due to the significant role of whitebark pine (*Pinus albicaulis*) in the Greater Yellowstone ecosystem (Wyoming, Montana, and Idaho, USA) and recent declines of mature, cone-bearing trees, monitoring trends in the health and status of whitebark pine populations is an informative component for current and future management of the species. Since 2004, the Interagency Whitebark Pine Monitoring Program has been documenting whitebark pine health across this ecosystem with data compiled from 176 permanent transects in 150 randomly selected pure and mixed whitebark pine stands. Specific long-term monitoring objectives for this program include estimating (1) the rate at which white pine blister rust (causal agent: *Cronartium ribicola*) infection is changing over time, (2) severity of infection and the transition of infection from what is regarded as less harmful to infections considered more lethal, (3) trends in and influences on mortality, and (4) recruitment of understory constituents. From 2004 to 2011, each of the 176 transects was surveyed twice for the presence of blister rust and three times for mortality. Using these cumulative data, we have completed the first statistical analysis examining step-trends in blister rust infection, transition of blister rust severity, and overall mortality. This monitoring affords a better understanding of the overall condition of the ecosystem and allows managers to gain knowledge, to make informed decisions, and to work more effectively with other agencies and individuals for the benefit of parks, other Federal lands, and privately held resources.

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The Proactive Strategy: Preparing the Landscape for Invasion by Accelerating the Evolution of Resistance

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Restoration of forests devastated by nonnative invaders often dominates the attention of forest managers and their actions. However, taking a broader view of the invasion beyond the crisis areas reveals opportunities where proactive management can alter the outcome of the invasion in threatened areas. Proactive management moves past the idea of protecting the hosts from exposure to the established nonnative invader and shifts toward facilitating naturalization by preparing the landscape to sustain critical ecosystem function into the future in the presence of the invader (Schoettle and Sniezko 2007).

Increasing the frequencies of durable resistance or tolerance traits within tree populations is accepted as a promising avenue for the coexistence of native tree species and nonnative pathogens (King et al. 2010). Therefore the objective of proactive intervention is to position the ecosystem to facilitate the evolution of pathogen resistance in the tree populations upon invasion. Diversifying the age class structure by stimulating regeneration in the healthy forest will provide a larger population size for resistance selection upon invasion and simultaneous selection in both the younger and older cohorts (Schoettle and Sniezko 2007). Accelerating the generation time and natural selection process through silvicultural treatments will reduce the ecological consequences of mortality in any one cohort and increases the potential for development of durable resistance within the population while maintaining broad genetic diversity. Planting seedlings or seed from seed trees that have heritable resistance before pathogen invasion will provide additional time for these individuals to mature and contribute to gene flow before the overstory is heavily affected upon invasion. Screening for resistance and planting early, rather than after the stand is heavily infested, reduces the window of time when forest regeneration may be limited by seed availability.

Early attention to threatened but not yet infected species or stands also provides opportunity for gene conservation. Seed, pollen, or tissue collections can be made from the full genetic diversity of the population before the potential bottleneck caused by high pathogen-induced mortality. In the case of *Pinus aristata*, rangewide seed and tissue samples have now been archived before population impacts by *Cronartium ribicola*, and working collections are available for research collaborations.

The Proactive Strategy has been adopted for *P. flexilis*, *P. aristata*, *P. longevea*, and *P. strobiformis* in the southern Rocky Mountains, the Southwest, and portions of the Great Basin (which covers parts of six western States) for the white pine blister rust pathosystem (Goodrich et al., *Pinus strobiformis Gene Conservation and Genecology*, this

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proceedings; Schoettle et al. 2011, 2013) and for healthy *Chamaecyparis lawsoniana* in stands at risk for impacts from *Phytophthora lateralis*. It has been incorporated into the National Strategic Framework for Invasive Species Management (2013) (http://www.fs.fed.us/foresthealth/publications/Framework_for_Invasive_Species_FS-1017.pdf).

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White Pine Blister Rust Infection Dynamics Over 25 Years in Sugar Pine (*Pinus lambertiana*) Progeny Tests on Six Sites Rated as Low, Mid, and High Rust Hazard in Southern Oregon, USA

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Abstract—Sugar pine (SP, *Pinus lambertiana*), western white pine (WWP, *P. monticola*), whitebark pine (*P. albicaulis*), and limber pine (*P. flexilis*)—the four white pine species native to Oregon (USA)—are very susceptible to white pine blister rust (WPBR), caused by the fungal pathogen *Cronartium ribicola*. High infection and mortality have been documented on some sites, but few or no long-term evaluations of the dynamics of rust infection over time have been made. We report here on rust infection over approximately 25 years for six SP trials established in southwestern Oregon. The sites had been rated for rust hazard by using protocols developed for WWP, and the hazard for the sites ranged from low to high. Open-pollinated seedling progeny of phenotypic parent tree selections were established at the sites (36 to 49 families per site), with 31 families in common. The families included some highly susceptible, some with low levels of partial resistance, and some with major gene resistance (MGR).

Performance at 5 and 10 years after planting was consistent with the hazard ratings; all sites showed rust infection, and the sites rated as high hazard showed the highest incidence of stem infection (cankers). Little mortality was observed at age 5, but the high hazard sites experienced moderate to high levels of mortality by age 10. By age 15, infection levels on the low-to-moderate hazard sites reached high levels; but mortality levels were still relatively low, whereas mortality on the two high hazard sites was high. At the latest assessment, 23 to 27 years post-planting, all sites showed high levels of infection (81.4 to 94.8 percent) and mortality (61.9 to 93.4 percent), with the low-to-moderate hazard sites lagging only slightly behind the high hazard sites. Excluding nonrust mortality and averaged over all 6 sites, only 8.9 percent of the 8,340 trees were canker-free and only 22.4 percent were alive. Approximately 60 percent of the surviving trees were cankered (with the percentage varying by site, from 21.2 to 77.0 percent), and mortality is expected to increase. A notable number of cankered trees seemed to be restricting the radial growth of the canker, suggesting that some form of partial resistance may be operating. The families with MGR had the lowest infection at all sites. Most of the cankers were relatively low in the tree, which suggests that branch pruning could be an effective strategy to enhance survival at least on some sites. Hazard rating for WPBR in southern Oregon has value, but these data indicate that it may not be a static value; perhaps "wave" years or changes in rust hazard (for example, due to adjacent stand management or fire, which potentially increases the number of alternate hosts) can affect the long-term predictive ability of current rust hazard rating protocols.

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White Pine Blister Rust Infection in Whitebark Pine (*Pinus albicaulis*) Seedlings Over a Wide Range of Inoculum Density Levels in an Artificial Inoculation Trial

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Abstract—Whitebark pine (*Pinus albicaulis*) is a wide-ranging high elevation conifer of forest ecosystems in the western United States and Canada. It is highly susceptible to white pine blister rust (WPBR), caused by the nonnative fungal pathogen *Cronartium ribicola*. Whitebark pine has been in decline in many forest ecosystems for decades, with WPBR being a major causative agent. In Canada, it has been listed as an endangered species, and in the United States it has been proposed for listing under the Endangered Species Act. Gene conservation efforts have begun and programs to identify parent trees of whitebark pine genetically resistant to WPBR are underway. In WPBR testing, seedling progeny of candidate parent trees are artificially inoculated with *C. ribicola* basidiospores to determine the level of genetic resistance conveyed by each parent. Progeny of hundreds of parent trees have now been evaluated for resistance. Thus far, no complete resistance (a hypersensitive-like response in the needles that precludes stem infection in the absence of a virulent strain of rust) such as that which has been found in four other species of white pines native to the western United States has been found in whitebark pine. However, in rust resistance screening, the whitebark pine seedling families show a range of partial resistance from very highly susceptible to moderately resistant. In most inoculation trials, the seedling progeny are usually inoculated with a single spore density (usually ~3,000 spores cm^{-2}, at Dorena Genetic Resource Center, Cottage Grove, Oregon, USA) to help determine the relative rankings of the families. Little is known about how varying spore density levels might affect the level of resistance shown in this species. We report on results from a trial where 2-year-old seedlings from 7 families (2 susceptible, 5 resistant) were inoculated (in September 2011) with 12 spore densities ranging from 50 to 15,400 spores cm^{-2}. Inoculation was very successful, with 99.1 percent of seedlings showing needle spots. Averaged over all inoculum levels, 80.8 percent of seedlings had cankers; the two susceptible families averaged 97.9 percent of seedlings with cankers, whereas 73.9 percent of the seedlings in the five resistant families were cankered. In the most resistant family, only 59.6 percent of the seedlings had cankers. Through December 2013, overall mortality was 59.3 percent. Higher spore densities applied during artificial inoculation resulted in increased levels of needle spots, severity of stem symptoms, percentage of trees with stem symptoms, and mortality percentage.

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Cytological Analyses Reveal Variations in Nuclear Content Along the Urediniosporic Infection Cycle of *Hemileia vastatrix* and other Rust Fungi

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The recent completion of genome sequencing for some rust fungi has contributed to suggest a link between biotrophic specialization and genome size expansion. The measurement of genome sizes for a selection of rust fungi has revealed some of the largest genomes among fungi, with nine rust species with haploid genomes between 300 and 780 Mbp. In particular, *Uromyces appendiculatus*, *Phakopsora pachyrhizi*, *U. transversalis*, and *Hemileia vastatrix* genomes were measured as 652, 716, 746, and 772 Mbp, respectively. Moreover, flow cytometric analysis of *H. vastatrix*-infected coffee (*Coffea* spp.) leaf samples consistently yielded profiles compatible with the occurrence of three different nuclear contents (n, 2n, and 4n). The n and 2n populations contained relatively similar numbers of nuclei, whereas the 4n population clearly represented a minority. Pre-sporulating and sporulating (urediniosporic) coffee leaf infections presented these three nuclei populations. The n population was absent from both resting urediniospores and germinating urediniospores but reappeared in appressoria. These findings are compatible with the occurrence of karyogamy and meiosis prior to sporulation, although parasexuality phenomena cannot be ruled out. In fact, although several orthologs of meiosis-specific genes are present in the *H. vastatrix* genome, only one of these was identified in *H. vastatrix* expressed sequence tag (EST) libraries obtained from germinating urediniospores, appressoria, and pre-sporulating infected leaves. Some karyogamy-related genes were identified both in the genome and in the EST libraries. Moreover, the occurrence of three different nuclear contents was also verified in infected host samples (at the urediniosporic stage, just before sporulation) for at least *Phakopsora pachyrhizi*, *Puccinia pelargonii-zonalis*, *U. appendiculatus*, and *U. fabae*, in contrast with the absence of the n population in resting and germinating urediniospores. This finding suggests that this phenomenon may be common across the Pucciniales.

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Discovery of Mycovirus Community in White Pine Blister Rust Ecosystems by Rust Transcriptome Profiling

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In North America, the nonnative rust fungus *Cronartium ribicola* kills immature native five-needle pine trees and indirectly predisposes maturing trees to secondary attack by insects and other fungi, which may result in severe mortality. Since the early 1900s when *C. ribicola* arrived, environmental factors, individual trees, the rust fungus, and insects (for example, mountain pine beetle [*Dendroctonus ponderosae*]) have all interacted to dramatically change forested ecosystems across North America. To date our white pine blister rust (WPBR) research has generated genomics data from the WPBR ecosystem, including transcriptomes and single nucleotide polymorphisms (SNPs) from healthy western white pines (WWPs, *Pinus monticola*), whitebark pines (WBPs, *P. albicaulis*), and infected white pines (down to 60 percent of transcripts from tree tissues, up to 40 percent of transcripts from *C. ribicola*), and from *C. ribicola* strains at different stages of its life cycle by RNA-sequencing technology. Transcriptome analyses have identified many expressed genes derived from mycoviruses. Even though the genomic information from organisms other than white pine and *C. ribicola* accounted for about 1 percent of the total sequence data, this eco-genomics strategy showed complex interactions of multiple organisms in the WPBR ecosystem. Bioinformatics analysis indicates that there are multiple mycoviral double-stranded RNAs (ds-RNAs) in *C. ribicola*, the presence of which was further supported by ds-RNA extraction and gel electrophoresis analysis. We observed variation in mycoviral sequences (including presence and absence) across *C. ribicola* strains collected from British Columbia (Canada) and Oregon (USA). Kinloch et al. (2004) provided evidence and suggested cytoplasmic inheritance of *C. ribicola* virulence (both vcr1 and vcr2), so our discovery of novel mycoviral ds-RNAs as cytoplasmic genetic factors in *C. ribicola* may open new avenues for WPBR management. We are investigating rust mycoviruses to develop cytoplasmic markers for epidemiological diagnosis and study of biodiversity. Progress on this and our related work will be further discussed.

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Research on the Rust Hyperparasite *Cladosporium tenuissimum*: Status and Prospects

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The use of biological agents to control plant diseases is an attractive approach, especially at a time when managers and other stakeholders seek to limit the impact of synthetic pesticides. Further, chemical pesticides cannot be used in natural habitats because of their expense and potential negative effects on ecosystems.

Mycoparasites are viewed as effective and environmentally friendly tools for the management and control of many important rust pathogens. Several species of the genus *Cladosporium* are mycoparasites of rust fungi. Of these, *C. tenuissimum* strongly inhibits its important rust agents in the genera *Melampsora*, *Cronartium*, *Peridermium*, *Puccinia*, and *Uromyces*. It is also effective against nonrust fungal pathogens and oomycota. It suppresses disease by various means such as mechanical action, enzymatic action, and the production of metabolites that have antifungal properties. It reduces the number of rust propagules and their longevity, and hence curtails the diseases these rusts cause in a controlled environment (greenhouse).

Despite all these positive indications, several factors have so far made it difficult to use *C. tenuissimum* as a conventional biofungicide. Among these complicating factors are the complexity and variability of the habitat in which the mycoparasite occurs, the response of the other partners of the three-way interaction (the host and the parasite), the mode of application of the mycoparasite (its formulation and its combination with other control measures), and variations in mycoparasite virulence. In field tests the effectiveness of the fungus did not reach the level required to be commercially used for crop protection.

The situation might be completely different, however, in the prevention and control of human diseases. Here there are high hopes that a way will be found to use this mycoparasite, or the metabolites it produces, to protect human beings against disease. Cladosporols A–E, the family of metabolites that the fungus produces in vitro, strongly inhibited in vitro the growth of several *Candida* species, and it has recently been demonstrated that some of these metabolites are also effective antitumor agents, inhibiting the proliferation of cancer cells. The possible use of these molecules as therapeutic tools opens unexpected and promising avenues of research.

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In Vitro Culture of the Alder (*Alnus* spp.) Pathogen *Melampsoridium hiratsukanum*: Practical Aspects, Problems, and Future Prospects

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Abstract—The exotic alder (*Alnus* spp.) rust fungus *Melampsoridium hiratsukanum* has recently spread throughout several European countries. In Italy, it has been found heavily parasitizing alder leaves at the end of summer in several alpine valleys. Here the fungus is very aggressive on gray alder (*A. incana*) growing in riparian formations, and in thickets at the upper elevations of the forest, where decline and mortality of trees as a result of the strong parasitic action of this fungus could seriously compromise the stability of slopes and river banks. A better understanding of this micro-organism, and particularly a way to cultivate it axenically, would be a great step forward, as it would enable new and hitherto unexplored aspects of this still largely unknown rust agent to be elucidated. The in vitro axenic culture would be essential to advance cellular, developmental, and molecular research on the fungus, as well as to explore in vitro its nutrition, physiology, and interaction with its host(s).

Here we report on the growth trials that have successfully obtained the growth of saprophytic mycelium of *M. hiratsukanum* on some synthetic media in the laboratory. Urediniospores were used as an inoculum source for mass spore seeding. Axenic cultures were obtained on several media having a variety of organic and inorganic constituents, demonstrating that the nutritional requirements of the fungus are relatively non-specific. We report on the main obstacles in obtaining axenic growth of the rust fungus and on practical ways to eliminate unwanted microbial contaminants and to obviate other drawbacks. We also give some useful hints for obtaining stable and sustained growth. Implications for future research may include new characters (for example, morphology-based and molecular-based) to improve fungus taxonomy and to elucidate in vitro its host range in a simplified, controlled system.

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Eucalyptus grandis Anomaly Related to Expression of Defense Genes

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In collaboration with the Suzano Papel and Celulose SA company, we identified an anomaly derived from a full-sib population in a controlled cross between two *Eucalyptus grandis* individuals. Abnormal seedlings died in a few months and showed significant phenotypic differences ($P < 0.05$), including seedling height, seedling stem-base diameter, and individual leaf area, compared with normal seedlings. Previous genetic analysis of the full-sib progeny showed a Mendelian segregation of 3 (normal): 1 (abnormal) ($\chi^2 = 2.32$, $P > 0.1$), suggesting that the lethality phenotype is controlled by a recessive allele (Tambarussi 2006). To identify the genes associated with the anomaly detected, we compared the transcriptional profiles of leaf, stem, and root tissue of normal and abnormal plants by RNA-seq technology. RNA-seq analysis showed the most differentially expressed genes were upregulated in the anomalous plants in all tissues analyzed. Most of these upregulated genes belong to functional groups of defense response and metabolism. Functional and metabolic analysis also demonstrated gene ontology and pathways involved in defense response upregulated in the abnormal plants.

Our results suggest that this lethality is associated with inappropriate activation of the immune system, similar to the phenomenon of hybrid necrosis. Hybrid necrosis is a deleterious genetic incompatibility often associated with negative epistatic interactions (Bateson-Dobzhansky-Muller model) or single-locus incompatibility. Due to some characteristics such as rapid nucleotide sequence evolution and high copy number, response genes have a predisposition to cause genetic incompatibility. Therefore, three gene families differentially expressed (thaumatin, Bet v1, and chitinase class I) were considered as candidates for causing the negative interactions. Research is underway to identify the locus by using high-resolution mapping with the molecular markers derived from the candidate families of which the genes are differentially expressed between normal and lethality plants.

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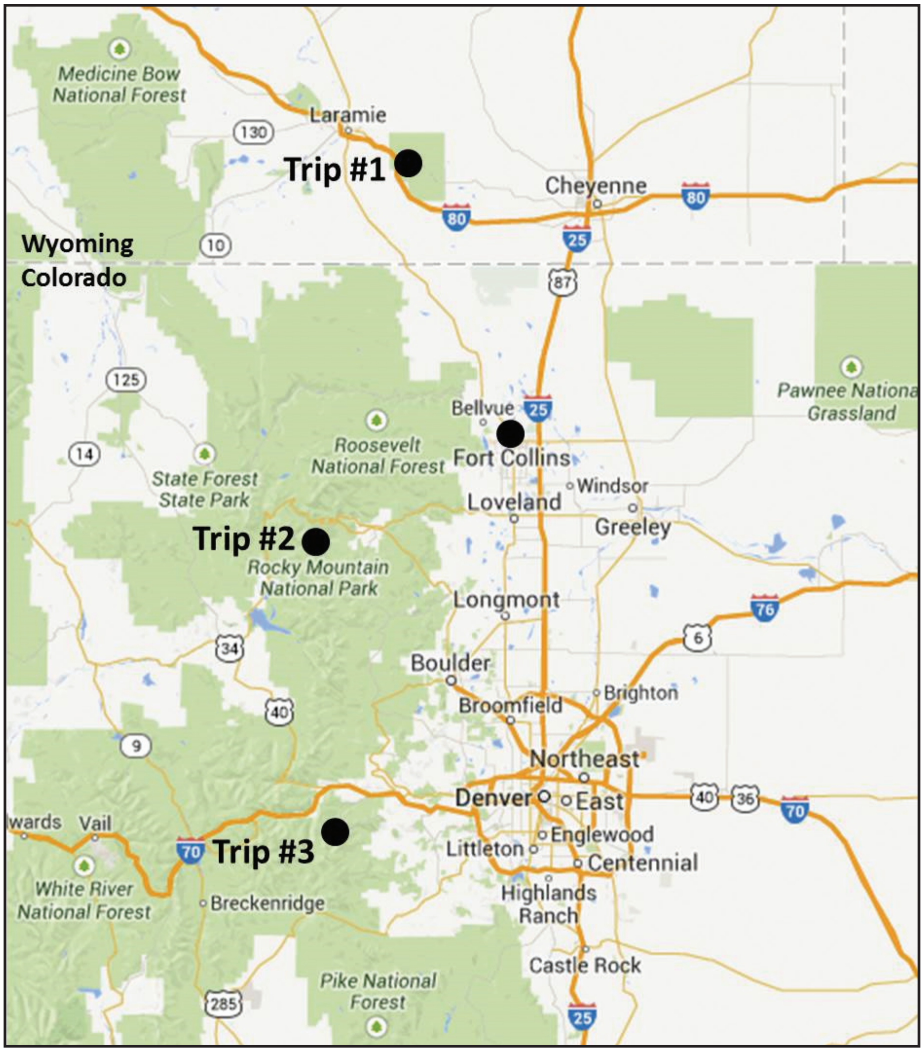
Appendix A—Field Trips

Trip 1—Tuesday, June 17, all day: Southern Rocky Mountains Forested Ecosystems

Native forested landscapes of southern Wyoming challenged by rusts, bark beetles, and other disturbances. Tour several *Pinus flexilis* research installations. Presenters include: Dennis Knight, William Jacobi, Kelly Burns, Christy Cleaver, Sara Alberts, Anna Schoettle, and Frank Romaro.

Trip 2—Thursday, June 19, all day: Rocky Mountain National Park

All-day field trip to Rocky Mountain National Park to visit a treeline *Pinus flexilis* stand and other locations with spectacular mountain views. Presenters include: Jeff Connor, Diana Tomback, Bob Cain, and Anna Schoettle.



Trip 3—Friday, June 20, all day: Mount Goliath Research Natural Area and Mount Evans Ecosystems

Optional all-day field trip to Mount Evans (a "14er") west of Denver with a stop at the Mount Goliath Research Natural Area and a short walk on the Walter Pesman Alpine trail through ancient *Pinus aristata* at treeline. Presenters include: Jeremiah Hyslop, Anna Schoettle, and Diana Tomback.

Trip 4—Wednesday, June 18, mid-day: USDA Agricultural Research Service, National Center for Genetic Resources Preservation

Genetic resources (plants and animals) are preserved using state-of-the-art technology that often involves cryogenics. Presenters include: Christina Walters, Patricia Conine, and Chris Richards.

Appendix B—Event Participant List (updated March 2018): Genetics of Five-Needle Pines and Rusts in Mountain Landscapes. June 15 – 20, 2014

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Appendix C—IUFRO Joint Conference - Genetics of 5-Needle Pines, Rusts of Forest Trees & Strobusphere Program

IUFRO Joint Conference - Genetics of 5-Needle Pines, Rusts of Forest Trees & Strobusphere (6/13/14)

PROGRAM

Sunday June 15 **At the Hilton**

1600-1900 Registration in Atrium

Set up posters

1700-2030 **Welcome Social and Poster Session**

Monday June 16 **At the Hilton - One Session**

				duration
0700-0800	REGISTRATION			
	Joint White Pine & Rust Group Session (all day)			
0745-0820	Introduction & Welcome	Foster/Schoettle/Hamelin/Neale/Sniezko		35
	TOPIC: Genomics			
	Moderator 1: Jill Wegrzyn			
0820-0845	1 Keynote	**D. Neale**	Genome Sequencing in Conifers: Implications for Breeding and Gene Resource Management	25
0845-0910	2 Keynote	**R.C. Hamelin**, S. Brar, C.K.M. Tsui, B Dhillon, M. Bergeron, D.L. Joly and Y.A. El-Kassaby	Colonization History, Host Distribution and Landscape Features Shape White Pine Blister Rust Populations.	25
0910-0935	3 Keynote	**J. Davis**	Genomic Warfare in the Woods: Scaling Fusiform Rust Resistance from Molecules to Landscapes	25
0935-0945	Q&A	Discussion		10
0945-1000	4	B. Petre, S. Hacquard, A. Persoons, N. Saveleva, E. Tisserant, E.Morin, C. Delaruelle, J. Petrowski, M. Pernaci, B. Fabre, S. De Mita, F. Halkett, A. Hecker, N. Rouhier, P. Frey, and **S. Duplessis**	Genomics, Transcriptomics and Effectoromics of the Poplar Leaf Rust Fungus *Melampsora larici-populina*	15
1000-1015	5	Zhen-Zhen Hao, Yan-Yan Liu, Liu Liu, **Xiao-Xin Wei** and Xiao-Quan Wang	Evolution and Biogeography of section Quinquefoliae (subgenus *Strobus* , *Pinus*)	15
1015-1025	Q&A	Discussion		10
1025-1040	Break	View Posters		15
	TOPIC: Genetic Resistance - all species			
	Moderator 2: Bruce Moltzan			
1040-1055	6	**V. Jorge,** A. Dowkiw, R. El-Malki, V. Segura, V. Guérin, P. Poursat, P. Faivre-Rampant, A. Rae, G. Taylor, and C. Bastien	Genetic Architecture of Quantitative *Melampsora larici-populina* Leaf Rust Resistance in Poplars	15
1055-1110	7	**R. Reich** and S. John	Ten Year Results of a Comandra Blister Rust Screening Trial in the Central Interior of British Columbia	15
1110-1125	8	**R.A. Sniezko** and A. Kegley	The Definitive Guide for White Pine Blister Rust Resistance in White Pine Species of Western North America (beta 2014.0 version) – A Tree Breeders Perspective	15
1125-1140	9	R.A. Sniezko, **B. Kinloch**, D.P. Savin, R. Danchok, and A. Kegley	Provenance Variation in Blister Rust Resistance in Sugar Pine	15
1140-1155	Q&A	Discussion		15
1155-1300	Lunch	Included		65

	TOPIC: Adaptations & Landscape Variation			
	Moderator 3: Andrew Eckert			
1300-1325	10	**B. Heinze** and K. Holzer	Present and Possible Past Distribution of *Pinus cembra* in the Austrian Alps – The Ups and Downs of Climate Change?	25
1325-1340	11	K. J. Hayden, C. Xhaard, B. Fabre, F. Halkett, and **P. Frey**	Adaptation of Poplar Rust to the Poplar varietal landscape	15
1340-1355	12	P.J. Vogan and **A.W. Schoettle**	Associations Between Complete Resistance to White Pine Blister Rust and Abiotic Stress Tolerances in Limber Pine (*Pinus flexilis* James)	15
1400-1410	Q&A	Discussion		10
1410-1425	13	**M.V. Warwell**	Genecology and Phenotypic Evolution of Whitebark Pine under Warm-Dry Climate: joint analysis of 12-year survival and growth of 49 populations in common gardens.	15
1425-1440	14	**A. Bontemps**, J. Wright, R. Sniezko, D. Savin, and J. Schmitt	Using Historical Provenance Test Data to Understand Tree Responses to a Changing Climate in Sugar Pine, *Pinus lambertiana*	15
1440-1455	15	**D.F. Tomback**, L.M. Resler, G.P. Malanson, E.K. Smith-McKenna, S.C. Blakeslee, and J.C. Pyatt	White Pine Blister Rust Alters Facilitation Interactions at Treeline: Implications for Treeline Communities and Response to Climate Change	15
1455-1505	Q&A	Discussion		10
1505-1520	Break	View Posters		15
	TOPIC: Western Wildland Environmental Threat Assessment Center Sponsored Session: Patterns of WPBR disease, impacts and risk across the landscape – Implications on decision making for conservation of whitebark pine and other 5 needle pines			
	Moderator 4: Holly Kearns			
1520-1530	16	**Holly Kearns**	Session Introduction	10
1530-1545	17	F.J. Krist, A.J. McMahan, **E.L. Smith**	White Pine Blister Rust, Mountain Pine Beetles, and their Potential Impact on White Pines: A National Assessment from the National Insect and Disease Risk Map	15
1545-1600	18	**A. J. McMahan** and E.L. Smith	Spatial Modeling of Past and Future Losses of White Pines of the Western U.S.	15
1600-1615	19	**C. Cleaver**, W. Jacobi, K. Burns, and R. Means	Limber Pine Stand Conditions after White Pine Blister Rust and Mountain Pine Beetle Caused Mortality in the Central and Southern Rocky Mountains	15
1615-1730	20	**Bob Means** (BLM); **Dan Reinhart** (NPS); **Peter Achuff** (Canada); **Amy Nicholas** (FWS); **Sara Alberts** (USFS)	Panelist Presentations and Discussion with Audience Participation	75
1800-2030	**POSTER SESSION**	ATRIUM --- Cash bar, light appetizers, posters (Dinner on your own)		150

Tuesday **June 17** **Field Trip**

0730-2000 **FIELDTRIP to Southern Wyoming; dinner at Avogadro's Number in Fort Collins (included); Group Photo**

Wednesday **June 18** **At the Hilton - CONCURRENT SESSIONS**

PINES	**CONCURRENT SESSIONS (a) 5-needle pines, (b) Rusts**			
	TOPIC: Gene Conservation & Restoration			
	Moderator 5: Bill Libby			duration
0745-0800		Announcements		
0800-0830	21 Keynote	**W.S. Dvorak**	The Efficacy of *Ex Situ* Conservation of the Mexican and Central American White Pines, *Pinus ayacahuite* and *Pinus chiapensis*, in Developing Countries	30
0830-0845	22	**P. H. Hai**	Study of Occurences of *Pinus dalantensis* De Ferré for Gene Conservaiton in Vietnam	15
0845-0855	Q&A	Discussion		10
0855-0910	23	**A. Moreno-Letelier**, A. Ortiz-Medrano, D. Piñero, and T.G. Barraclough	Genetic andEecological Divergence in *Pinus flexilis -Pinus strobiformis -Pinus ayacahuite* Species Complex: Clues to Understanding Speciation in Pines	15
0910-0925	24	**C. Wehenkel**, C.Z. Quiñones-Pérez, S.L. Simental-Rodríguez, C.A. López Sanchez	Genetic Structure in *Pinus strobiformis* Engelm. on the Sierra Madre Occidental, Mexico	15
0925-0940	25	**K.M. Waring** and B.A. Goodrich	*Pinus strobiformis* Gene Conservation, Adaptive Traits and Climate Change	15
0940-1055	Q&A	Discussion		15
1055-1010	Break	View Posters		15
	TOPIC: Gene Conservation & Restoration, continued			
	Moderator 6: Bill Jacobi			
1010-1025	26	**D. Politov**, Y. Belokon, M. Belokon, E. Mudrik, T. Poliakova, E. Petrova, and S. Goroshkevich	Population and Evolutionary Genetic Studies of 5-Needle Pines in Russia	15
1025-1040	27	**M. F. Mahalovich** and D.L. Foushee	*Pinus albicaulis* Engelm. Genetic Restoration Program for the Inland West (USA): a first generation of improvement	15
1040-1055	28	G. Man and **B. Moltzan**	Forest Health Protection Gene Resource Conservation a Lasting Legacy	15
1055-1105	Q&A	Discussion		10
1110-1345	FIELDTRIP	Instructions and head over to NCGRP		30
		NCGRP tours and Lunch		105
		Return to Hilton		20
	TOPIC: Rust Resistance Trials, Field Performance & Genetic Variation			
	Moderator 7: James Jacobs			
1345-1400	29	**P. Lu**	Performance of Blister Rust Resistant Eastern White Pine Hybrids with Himalayan Blue Pine in Field Trials	15
1400-1415	30	**C. Cartwright**, N. Ukrainetz, and M. Murray	Whitebark Pine Field Screening for Blister Rust Resistance: Early results.	15
1415-1430	31	**J. N. King**	Field Level Resistance of Western White Pine to White Pine Blister Rust Assessed in Progeny Trials Planted in Coastal British Columbia	15
1430-1445	32	**M. P. Murray**	Blister Rust Inoculation Trials for Canadian Whitebark Pine	15
1445-1500	33	S.U. Han, H.S.Lee, J.M. Park and **K.S. Kang**	Genetic Relationship between Reproductive Morphology and Growth Characteristics of *Pinus koraiensis*	15

1500-1515	Q&A	Discussion		15
1515-1535	Break	Poster viewing		20
	TOPIC: Strobusphere Special Session			
	Moderator 8: David Neale			
1535-1550	34	**A.J. Eckert**, B.M. Lind, C.J. Friedline, E. Hobson, J.W.R. Zinck, O.P. Rajora, D.B. Neale, D. Vogler, and P.E. Maloney	The genetic architecture of local adaptation and the genomic exploration of rugged evolutionary landscapes within species of *Pinus* subgenus *Strobus*	15
1550-1605	35	**J.L. Wegrzyn**, P.J. Martinez-Garcia, C.A. Loopstra, K. Mockaitis, R. Famula, and D.B. Neale	Sequencing the sugar pine transcriptome	15
1605-1620	36	**J.B. Mitton**, J. Wegrzyn, S. L. Stowell, M. Mitter, L. Kueppers and D. Neale	Genetic Resources for Limber Pine and a Survey Focusing on Potentially Adaptive SNPs	15
1620-1635	37	**J.A. Gruhn**, B.A. Schaal	Using Genomics Tools to Assess the Phylogeography of Whitebark Pine	15
1635-1645	Q&A	Discussion		10
1645-1700	38	**A. Vázquez-Lobo**, C. Vangestel, P.J. Martínez-García, J.L. Wegrzyn, I. Calic, J.D. Liechty, and D.B. Neale	Identifying the Genetic Basis of Partial Resistance on Sugar Pine (*Pinus lambertiana*) Under White Pine Blister Rust Infection (WPBR).	15
1700-1715	39	**S. Nadeau**, N. Isabel, J. Godbout, M. Lamothe, M.-C.Gros-Louis, and K. Ritland	Patterns of Population Structure and Environmental Associations Across the Range of Two Ecologically and Economically Important Five-Needle Pine Species	15
1715-1730	40	**J.-J. Liu** and R.A. Sniezko	Genomics Study of Western White Pine Genetic Resistance Against White Pine Blister Rust	15
1730-1745	41	**H. S. Ginwal** and P. Chauhan	Cross Species Transferability of Microsatellites, and Genetic Diversity in Himalayan Five Needle Pine (*Pinus wallichaia* A.B. Jacks) and Chir pine (*P. roxburghii* Sarg.) Forests	15
1745-1800	Q&A	Discussion		15
1800-1830		Overall Strobusphere discussion		
1830-1900	**Business meetings**	Dinner on your own	take posters down	

RUSTS	**CONCURRENT SESSIONS (a) 5-needle pines (b) Rusts**			
	TOPIC: Epidemiology & Population Genetics			
	Moderator 9: Pascal Frey			
0745-0800		Announcements		
0800-0820	42	**A. Dowkiw**, E. Voisin, V.Dievart, T. Servouse, M. Berteloot, V. Guérin, T. Bourgeois, A. Andrieux, and P. Frey	Widening the Landscape: The Role of Ornamental and Wild Trees in the Spread and Adaptation of *Melampsora larici-populina* Leaf Rust	15+5
0820-0840	43	**P. Tanguay**	Origin and Distribution in Canada of the *Cronartium ribicola* vCr Race, Virulent on Immune Blackcurrant Cultivars Derived from Hunter's Selection	15+5
0840-0900	44	**I.A. Munck**, P. Tanguay, J. Weimer, K. Lombard, S. Villani, K. Cox	Impact of White Pine Blister Rust on Resistant and Previously Immune Cultivated Ribes and Neighboring Eastern white pine in New Hampshire	15+5
0900-0920	45	**B. Samils**, P. Barklund, J. Kaitera, and J. Stenlid	Geographic Distribution of *C. flaccidum* and *P. pini* in Sweden and Finland	15+5
0920-0940	46	**A. Ross-Davis**, N. Klopfenstein, and 24 others	Genetic and Genotypic Diversity of *Puccinia psidii* - The Cause of Guava/Eucalypt/Myrtle Rust - and Preliminary Predictions of Global Areas at Risk	15+5
0940-1000	47	**J. Kaitera**, R. Hiltunen, B. Samils, and J. Hantula	*Cronartium* Rusts Sporulate on a Wide Range of Alternate Hosts in Northern Europe	15+5
1000-1020	Break	View Posters		20
	TOPIC: Resistance			
	Moderator 10: Salvatore Moricca			
1020-1040	48	**D.R. Vogler**, A.D. Mix, D. Burton, D. Davis, R. Westfall, and B.B. Kinloch Jr.	Frequency and Distribution of Virulence in *Cronartium ribicola* to Two Simply-Inherited Resistance Genes in Sugar Pine and Western White Pine, Respectively, at Locations in Northern Siskiyou County and in the South Central Sierra Nevada of California	15+5
1040-1100	49	**R.S. Hunt**, M. Murray, R. Reich, D. Rusch, A. Woods, S. Zeglen	Survival of MGR Resistance in Western White Pine in British Columbia	15+5
1100-1345	FIELDTRIP	Instructions and Head over to NCGRP		30
		NCGRP tours and Lunch	Christina Walters; Pat Conine	115
		Return to Hilton		20
	TOPIC: Genetics, Genomics & Barcoding			
	Moderator 11: Sebastien Duplessis			
1345-1405	50	**M. Pernaci**, S. De Mita, B. Fabre, A. Andrieux, F. Halkett, and P. Frey	On the Road to Mapping QTLs of Morphological and Aggressiveness Traits in the Poplar Rust Fungus	15+5
1405-1425	51	**B. Dhillon** and R. Hamelin	Comparative Genomics of the *Cronartium* genus	15+5
1425-1445	52	S. Tavares, A.S. Pires, H.G. Azinheira, A.P. Ramos, C. Bispo, C. Andrade, A. Loureiro, D. Schmidt, T. Link, R.T. Voegele, R. Gardner, R. Abranches, M.C. Silva, J. Loureiro, **P. Talhinhas**	Cytological Analyses Reveal Variations in Nuclear Content along the Urediniosporic Infection Cycle of *Hemileia vastatrix* and Other Rust Fungi	15+5
1445-1505	53	**M. Scholler**, L. Beenken, M. Lutz, W. Maier, A. Tahir and M. Thines	Barcoding the Rust Fungi of Germany	15+5
1505-1535	Break	Poster viewing		30
	TOPIC: Etiology & Management			

	Moderator 8: Richard Hamelin			
1535-1555	54	**P.Q. Thu**, S. Kaneko and M. Imazu	Stem Rust Diseases of *Pinus kesiya* in Vietnam	15+5
1555-1615	55	**S. Moricca**, B. Ginetti, A. Lupo, A. Ragazzi and G. Assante	Research on the Rust Hyperparasite *Cladosporium tenuissimum* : Status and Prospects	15+5
	Topic: Host - Disease Climate Interactions on Landscapes			
	Moderator 12: Diana Tomback			
1620-1635	56	**W.B. Monahan**, T. Cook, F. Melton, J. Connor, B. Bobowski, and F. Krist	Forecasting Climate and Disease Impacts on Limber Pine in Rocky Mountain National Park	15
1635-1650	57	**J.E. Major**, A. Mosseler, J. Malcolm and M. Cambell	Comparative Photosynthetic Aadaptive Traits in Four Pinus species (*P. strobus, P. resinosa, P. banksiana, P. rigida*) to CO2 x Moisture Stress Factorial	15
1650-1705	58	**E. Shanahan**, K.M. Irvine, C. Hollimon, K. Legg, R. Daley, Greater Yellowstone Whitebark Pine Monitoring Working Group	Monitoring Trends in White Pine Blister Rust Infection in the Greater Yellowstone Ecosystem	15
1705-1720	59	**D.M. Davíd-Chavez**, R.T. Belote, Matt Dietz, and G.H. Aplet	Climate Change Alters Distribution of Whitebark Pine (*Pinus albicaulis*) with Respect to Land Management Status: are there Implications for Adaptation?	15
1720-1735	Q&A	Discussion		15
1830-1900	**Business meetings**	Dinner on your own	Take posters down	

Thursday **June 19** **Field Trip**

0730-2130 **FIELDTRIP to Rocky Mountain National Park; dinner at the Stanley Hotel, Estes Park (included)**

Friday **June 20** **Optional Field Trip**

0700-2000 **Optional FIELDTRIP to Mount Goliath and Mt Evans; light dinner at Barrels & Bottles Brewery in Golden (included)**

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