Proceedings of the IUFRO 2019 Joint Conference Genetics of Five-Needle Pines and Rusts of Forest Trees

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Jodie Krakowski, Ward Strong, and Richard A. Sniezko (compilers)

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Jodie Krakowski
Independent consultant
Crowsnest Pass, Alta.

Ward Strong
B.C. Ministry of Forests
Vernon, B.C.

Richard A. Sniezko
United States Department of Agriculture Forest Service
Cottage Grove, Oreg.
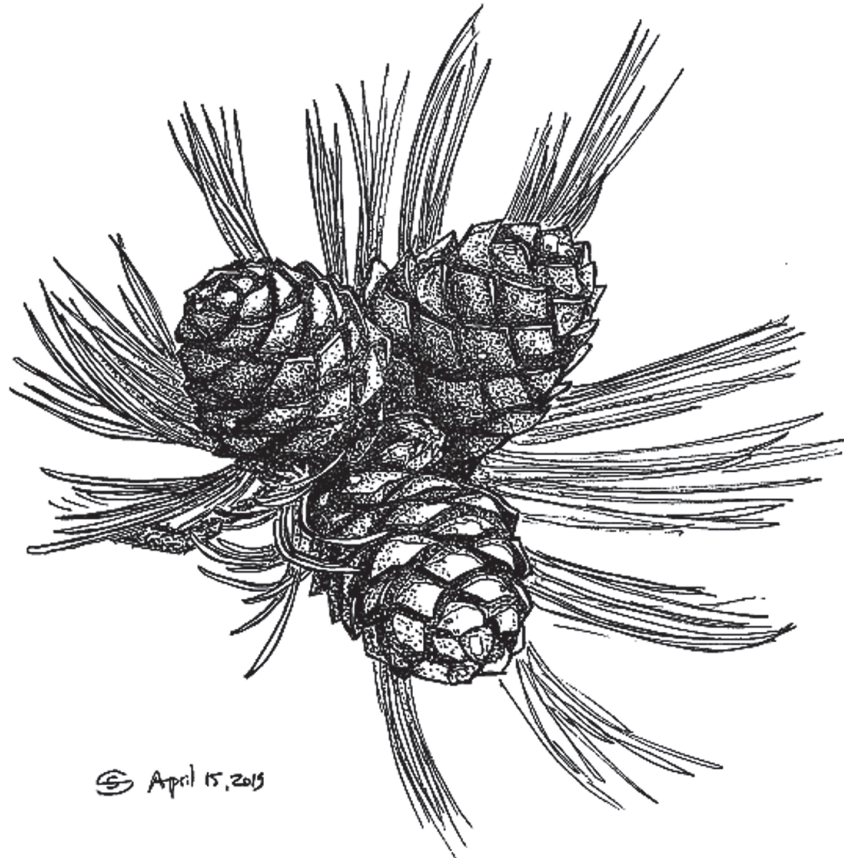
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IUFRO 2019
Joint Conference
Genetics of Five-Needle
Pines & Rusts of Forest Trees

July 22–26, 2019 • Invermere • British Columbia

Proceedings

GS April 15, 2019

CONTENTS

The IUFRO 2019 Joint Conference on Genetics of Five-Needle Pines and Rusts of Forest Trees was designed to highlight the synergies and alignment in current research and operational applications of two key divisions of the International Union of Forest Research Organizations (IUFRO). At this meeting, Division 2.02.15 focussed on screening and genetics programs for resistance to white pine blister rust (*Cronartium ribicola*); Division 7.02.05 updated the current state of knowledge of research on rusts (including blister rust) of forest trees.

The following describes the key focus of each division; additional information is available by accessing the URLs.

IUFRO Division 2.02.15 – Breeding and genetic resources of five-needle pines
https://www.iufro.org/science/divisions/division-2/20000/20200/20215/

The Working Party on Breeding and Genetic Resources of Five-Needle Pines is concerned with research co-operation and exchange of information on all aspects of genetic research on five-needle pines. This includes provenance testing and genetic resources, breeding, species hybridization, clonal propagation and testing, tissue or cell culture, cell and molecular genetics, and genetics of host–pathogen interactions. Increasingly, though, we are using this knowledge to address issues related to climate change, land use pressure, and conservation.

IUFRO Division 7.02.05 – Rusts of forest trees
https://www.iufro.org/science/divisions/division-7/70000/70200/70205/

Our Working Party aims to bring together scientists and investigators who are working on tree rusts. Our goal is to foster scientific discussion and exchanges related to tree rust epidemiology, biology, host–pathogen interactions, resistance, control and management, and genomics. Our Working Party meets approximately once every 4 years 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.

CONFERENCE ORGANIZATION AND SPONSORS

Conference Organization

Program . . . . .Richard Sniezko, Pascal Frey, Philippe Tanguay, Anna Schoettle, Bruce Moltzan
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Local arrangements . . . . Randy Moody, Marnie Duthie-Holt, Barb Gass
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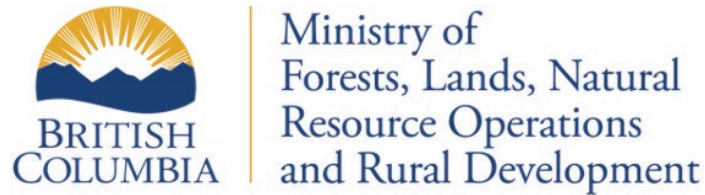
- Copper Point Resort

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- Western Forestry and Conservation Association

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- Yellow Point Propagation Ltd

Conference Website
- https://westernforestry.org/past-conferences/iufro-2019-joint-conference-genetics-of-five-needle-pines-rusts-of-forest-trees

Main Conference Program

July 23, 2019		
08:15–08:30	Welcome and IUFRO overview	Ward Strong, Richard Sniezko, Pascal Frey
Session 1a: White Pine Blister Rust Resistance Moderator #1: Jeremy Johnson		
08:30–09:00	**1 (Plenary)**: Breeding for white pine blister rust resistance—50+ years of experience at Dorena Genetic Resource Center	Richard Sniezko
09:00–09:20	2: Blister rust distribution, trends, and resistance screening in southern British Columbia's endangered whitebark pine	Michael Murray
09:20–09:40	3: Results from long-term field trials in British Columbia: resistance of western white pine to white pine blister rust	Nick Ukrainetz
09:40–10:00	4: Genomic approaches to dissecting lodgepole and jack pine interactions with *Cronartium harknessii*, the causal agent of western gall rust	Janice Cooke
10:00–10:30	Morning break	
Session 1b: White Pine Blister Rust Resistance Moderator #2: Richard Sniezko		
10:30–10:50	5: Screening whitebark pine for white pine blister rust resistance in British Columbia	Ward Strong
10:50–11:10	6: Identifying range-wide patterns of genetic resistance to save a species threatened by a non-native disease and climate change	Jeremy Johnson
11:10–11:30	7: The influence of genetics, defensive chemistry, and the fungal microbiome on disease outcome in white pines	Lorinda Bullington
11:30–11:50	8: Implementing a bioinformatics-based targeted sequencing strategy for R-genes in the *Cronartium ribicola*–white pine pathosystem	David Oline
11:50–12:05	**Discussion #1**: Durability of white pine blister rust resistance: What is the evidence? What is needed? Where do endophytes fit in? Physiological trade-offs: how important?	Panel: Richard Sniezko and others
12:05–13:30	Lunch	
Session 2: Genomics of Rust Resistance Moderator #3: Nick Ukrainetz		
13:30–14:00	**9 (Plenary)**: Genomics of quantitative resistance to white pine blister rust in sugar pine (*Pinus lambertiana*)	David Neale
14:00–14:20	10: Fusiform rust resistance gene discovery in *Pinus taeda* using RNAseq bulked segregant analysis and two-way testcross QTL mapping	Fikret Isik
14:20–14:40	11: Uncovering the natural physiological variation driving lodgepole pine resistance to western gall rust	Nadir Erbilgin
14:40–14:55	**Discussion #2**: Genomics and molecular tools—ready for prime time? How can they help us in the next 5 years?	Panel: Dave Neale, Janice Cooke, Fikret Isik, Jun-Jun Liu, Nick Ukrainetz
14:55–15:25	Afternoon break	
Session 3: Hard Pine Stem Rusts Moderator #4: Janice Cooke		
15:25–15:45	12: Age-related resistance to hard pine stem rusts in the Central Interior of British Columbia	Richard Reich
15:45–16:05	13: Potential trade-offs in growth and environmental resilience of lodgepole pine in Alberta	Eden McPeak
16:05–16:25	14: What do we know about hemiparasitic plant hosts of hard pine stem rusts?	Sybille Haeussler
Session 4: British Columbia Overview—White Pines		
16:25–16:45	15: Five-needle pines in British Columbia—status and distribution	Randy Moody
16:45–17:10	Poster speed talks	
19:00–21:00	Reception and poster session	

	July 24, 2019	
07:30–08:30	Concurrent IUFRO business meetings	
08:45–15:30	**Field trip**	
	• Ponderosa pine regeneration stock type—site prep. trial	Nick Ukrainetz, Marie Vance
	• Whitebark pine planting site	Charlie Cartwright
	• Whitetail whitebark pine permanent sample plot, seed production area, provenance trial	Don Pigott, Randy Moody
	• Long-term forest health monitoring plot, whitebark pine harvest retention, limber pine	Don Pigott, Randy Moody
19:00–23:00	Banquet and entertainment	

	July 25, 2019	
Session 5a: Rusts of Forest Trees Moderator #5: Bruce Moltzan		
07:30–08:30	Concurrent IUFRO business meetings	
08:30–09:00	**16 (Plenary)**: Pine rusts never sleep: deciphering the biology and epidemiology of white pine blister rust with genomics	Richard Hamelin
09:00–09:20	17: Interspecific hybridization in *Melampsora* rusts of poplars: a mechanism of emergence of new species	Pascal Frey
09:20–09:40	18: Evaluating the presence and introgression of the hybrid forest pathogen *Cronartium × flexili*	Kiah Allen
09:40–10:00	19: Genotypic diversity and reproductive biology of *Thekopsora areolata*, the causal agent of cherry spruce rust in Norway spruce seed orchards	Åke Olson
10:00–10:30	Morning break	
Session 5b: Rusts of Forest Trees Moderator #6: Philippe Tanguay		
10:30–10:50	20: Morpho-anatomical characteristics of *Eucalyptus urophylla* leaves inoculated and not inoculated with *Austropuccinia psidii*	Edson Luiz Furtado
10:50–11:10	21: Alien rust fungi in Switzerland	Valentin Queloz
11:10–11:30	22: The effect of the alien rust fungus *Melampsoridium hiratsukanum* on riparian alder formations in the eastern Italian Alps	Salvatore Moricca
11:30–11:45	**Discussion #3**: Rusts of forest trees in the genomics era	Panel: Richard Hamelin, Phil Tanguay, Bruce Moltzan, Salvatore Moricca, Janice Cooke, Pascal Frey
11:45–13:15	Lunch	
Session 5c: Rusts of Forest Trees Moderator #7: Scott Kolpak		
13:15–13:35	23: *Pinus cembra*'s last stands? Small and isolated populations of stone pine in the Eastern Alps in past and current climate change	Berthold Heinze
13:35–13:55	24: Evolution and speciation of the Chinese white pines	Xiao-Xin Wei
13:55–14:15	25: Whitebark pine restoration strategy for the U.S. Forest Service Pacific Northwest Region	Andy Bower
14:15–14:35	26: Progress and challenge in tree breeding of Korean pine (*Pinus koraiensis*) in South Korea	Kyu-Suk Kang
14:35–14:55	27: Clonal variation of strobilus and seed production in a 1.5-generation seed orchard of *Pinus koraiensis*	Yang-Gil Kim
14:55–15:25	Afternoon break	
Session 5d: Rusts of Forest Trees Moderator #8: Michael Murray		
15:25–15:45	28: Field testing of range-wide populations of whitebark pine in British Columbia	Charlie Cartwright
15:45–16:05	29: Pines (including white pine) versus spruce: comparative fitness in an elevated CO_2 × soil moisture stress factorial	John Major
16:05–16:25	30: Identification of *Phytophthora* species associated with western white pine mortality in British Columbia tree seed orchards	Nicolas Feau
16:25–16:45	31: Chemical and anatomical defences of lodgepole pine relate to infection by western gall rust, *Endocronartium harknessii*	Jennifer G. Klutsch
16:45–17:00	**Discussion #4:** Climate change and white pines – what is the future?	
17:00–17:10	Final wrap-up and farewell	

	Extended field trip itinerary	
	July 26, 2019	
08:30	Leave Copper Point	
09:00	Parks Canada Sinclair Canyon site	Gregg Walker
11:00	B.C. Ministry of Forests, Lands, Natural Resource Operations and Rural Development assisted migration adaptation trial (B.C. Min. FLNRORD AMAT) Spillimacheen	Nick Ukrainetz
12:00	Lunch	
12:30	B.C. Min. FLNRORD AMAT site with low survival and nearby lodgepole pine with western gall rust	Ward Strong
14:00	Drive to Golden	
15:00	Louisiana-Pacific: value added (plywood and laminated veneer lumber) mill in Golden	Scott King
17:00	Arrive at Golden Village Inn	
	July 27, 2019	
8:00	Leave Golden	
9:00	Lake Louise Ski Resort tour, interpretation and education programs	Jennie Bishop, Doug Macdonald
12:00	Lunch at Lake Louise Ski Resort	
13:00	Drive to Saskatchewan Crossing	
14:00	North Saskatchewan River bridge: limber pine forest, restoration project	
15:00	Alberta whitebark pine and limber pine recovery program, limber pine provenance trial (Anna Schoettle)	Jodie Krakowski
17:30	Arrive at The Crossing Resort	
	July 28, 2019	
08:30	Leave Saskatchewan Crossing	
09:15	Arrive at Columbia Icefield Discovery Centre	
12:00	Lunch at the Discovery Centre	
13:00	Drive to Peyto Lake	
14:15	Hike into Peyto Lake	
15:30	Drive to Canmore	
17:00	Arrive at Canmore Inn & Suites	
	July 29, 2019	
08:30	Leave Canmore	
09:00	Arrive at Banff, those who want to visit	
11:00	Arrive at Invermere, those who do not visit Banff	
12:00	Leave Banff	
14:00	Arrive at Invermere, tour finished	

1. Plenary: Breeding for White Pine Blister Rust Resistance—50+ Years of Experience at Dorena Genetic Resource Center

Richard A. Sniezko[1*] and Angelia Kegley[1]

1 United States Department of Agriculture Forest Service, Dorena Genetic Resource
   Center, Cottage Grove, Oreg.
*Presenting and corresponding author: richard.sniezko@usda.gov

ABSTRACT

The nine species of white pines that are native to the United States or Canada are highly susceptible to white pine blister rust caused by the fungal pathogen *Cronartium ribicola*. Resistance breeding is considered to be the best tool to help ensure that our future forests will continue to have white pine species present. The U.S. Department of Agriculture Forest Service's Dorena Genetic Resource Center has been involved in resistance evaluation and breeding for more than 50 years and has evaluated all nine species, to varying extents, with an emphasis on species occurring in the west. Seed orchards have been established for several species, while collections from genetically resistant parent trees in the forests are being used for some species for restoration and reforestation. Genetic resistance exists in the white pines, but the type of genetic resistance and its level varies by species. Major gene resistance has been characterized in four species (sugar pine [*Pinus lambertiana*], western white pine [*P. monticola*], southwestern white pine [*P. strobiformis*], and limber pine [*P. flexilis*]), while all nine species appear to have some degree of quantitative disease resistance. The lessons learned through the resistance breeding program, including from the assessment of long-term field trials, provide guidance on rust hazard and the durability, stability, and usability of resistance; potential opportunities for the future; and guidance to land managers about using resistance. Some misconceptions from the past about major gene resistance, virulence, and level of resistance are also discussed. Lessons learned over this period can also provide valuable inputs to applied resistance programs for other pathogens or pests.

2. Blister Rust Distribution, Trends, and Resistance Screening in Southern British Columbia's Endangered Whitebark Pine

Michael P. Murray[1*] and Randy Moody[2]

1 Forest Pathologist, B.C. Ministry of Forests, Lands, Natural Resource Operations and Rural Development, Nelson, B.C.
2 President, Whitebark Pine Ecosystem Foundation of Canada, Kimberley, B.C. whitebarkrandy@gmail.com
*Presenting and corresponding author: michael.murray@gov.bc.ca

ABSTRACT

In 2020, long-term forest health monitoring of endangered whitebark pine (*Pinus albicaulis*) enters its 21st year in British Columbia. The Province of British Columbia maintains 19 long-term forest health monitoring plots. Results indicate a continuous decline in healthy trees in southeast British Columbia due primarily to white pine blister rust (*Cronartium ribicola*) and mountain pine beetle (*Dendroctonus ponderosae*). Along with this provincial network of monitoring plots, dozens of ancillary surveys have been conducted and monitoring transects have been established at additional sites. Combined data indicate that both blister rust infection and whitebark pine mortality rates differ across the pine's range in southern British Columbia. In the southeast corner of the province, most live trees are infected, whereas the west Chilcotin region supports very low incidences of infection. Overall whitebark pine mortality rates due to all forest health agents appear to be highest in southeast British Columbia, where more than 80% of standing trees at many sites are dead. Highly affected stands in southeast British Columbia may provide the best opportunities for selecting rust-resistant parents for recovery efforts.

SUMMARY

Whitebark pine (*Pinus albicaulis*) was listed as Endangered under the Canadian *Species at Risk Act* in 2012. It is the only tree listed in Western Canada. The primary agent of mortality is white pine blister rust caused by the introduced fungus *Cronartium ribicola*. During the last two decades, whitebark pine has also been under increasing pressure from mountain pine beetle (*Dendroctonus ponderosae*) and changing fire regimes. This tree is a renowned source of habitat for a variety of wildlife, including grizzly bears (*Ursus arctos*) and Clark's Nutcrackers (*Nucifraga columbiana*), which both consume the large, nutrient-rich seeds. Because this high-mountain pine species tends to thrive in remote, inaccessible sites, our understanding of its distribution and associated stand health remains weak.

In 2020, long-term forest health monitoring of endangered whitebark pine in British Columbia enters its 21st year. British Columbia may be the first jurisdiction in the world to set up a system to gauge trends specifically in whitebark pine. The first monitoring plot was installed on Blackcomb Mountain by Coast

Area Forest Pathologist Stefan Zeglen in 2000. Since then, more plots have gradually been added throughout the whitebark pine's expansive range, which extends across the southern two-thirds of the province. The 19 plots are typically about 50 × 50 m in area. Trees are tagged with unique identifiers and are assessed for diameter, height, vigour, and forest health agents approximately every 5 years. All trees, regardless of species, are surveyed. The most common associates are sub-alpine fir (*Abies lasiocarpa*), Engelmann spruce (*Picea engelmannii*), and lodgepole pine (*Pinus contorta* var. *latifolia*). This small collection of plots is known as the provincial whitebark pine forest health monitoring system.

Four plots, located in southeast British Columbia, were established beginning in 2003. The most recent repeat surveys of these plots occurred in 2015. Results indicated an overall decrease in the proportion of healthy trees (Figure 1). The percentage of living trees (>1.37 m tall) infected with blister rust varied from 1 to 46% based on plot location. The proportion of trees that were killed by blister rust since 2003 ranged from 5 to 24%. The most dramatic effect on health occurred
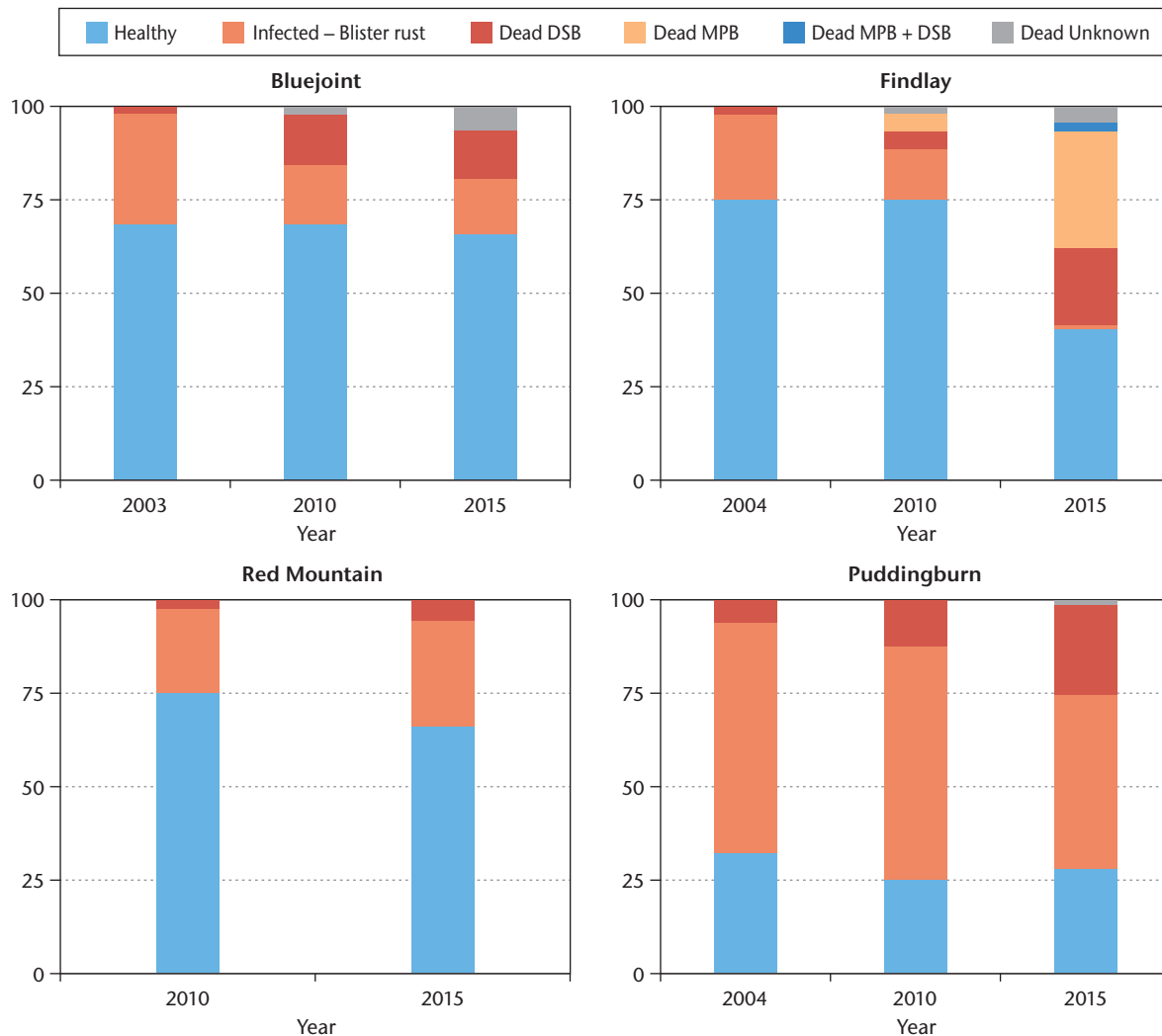
FIGURE 1 *Proportional percent of health status at four locations in southeast British Columbia. The two most common forest health agents are white pine blister rust (DSB) and mountain pine beetle (MPB); Unknown refers to mortality from unknown sources.*

between 2013 and 2014 when a mountain pine beetle epidemic killed approximately one-third of the trees in the Whitetail Mountain plot. An epidemic also occurred on Puddingburn Mountain; however, the trees in that plot are at a higher elevation and are smaller than the trees in neighbouring decimated stands; thus, they were bypassed by the beetles.

In addition to the provincial whitebark pine forest health monitoring system, health data were collected at numerous locations around the range of whitebark pine in British Columbia (Figure 2). These data were often collected opportunistically while other fieldwork was being conducted at the site. Tallies of dead, healthy, and infected trees were recorded. These surveys are useful for quickly identifying and comparing forest health conditions with those at other locations.
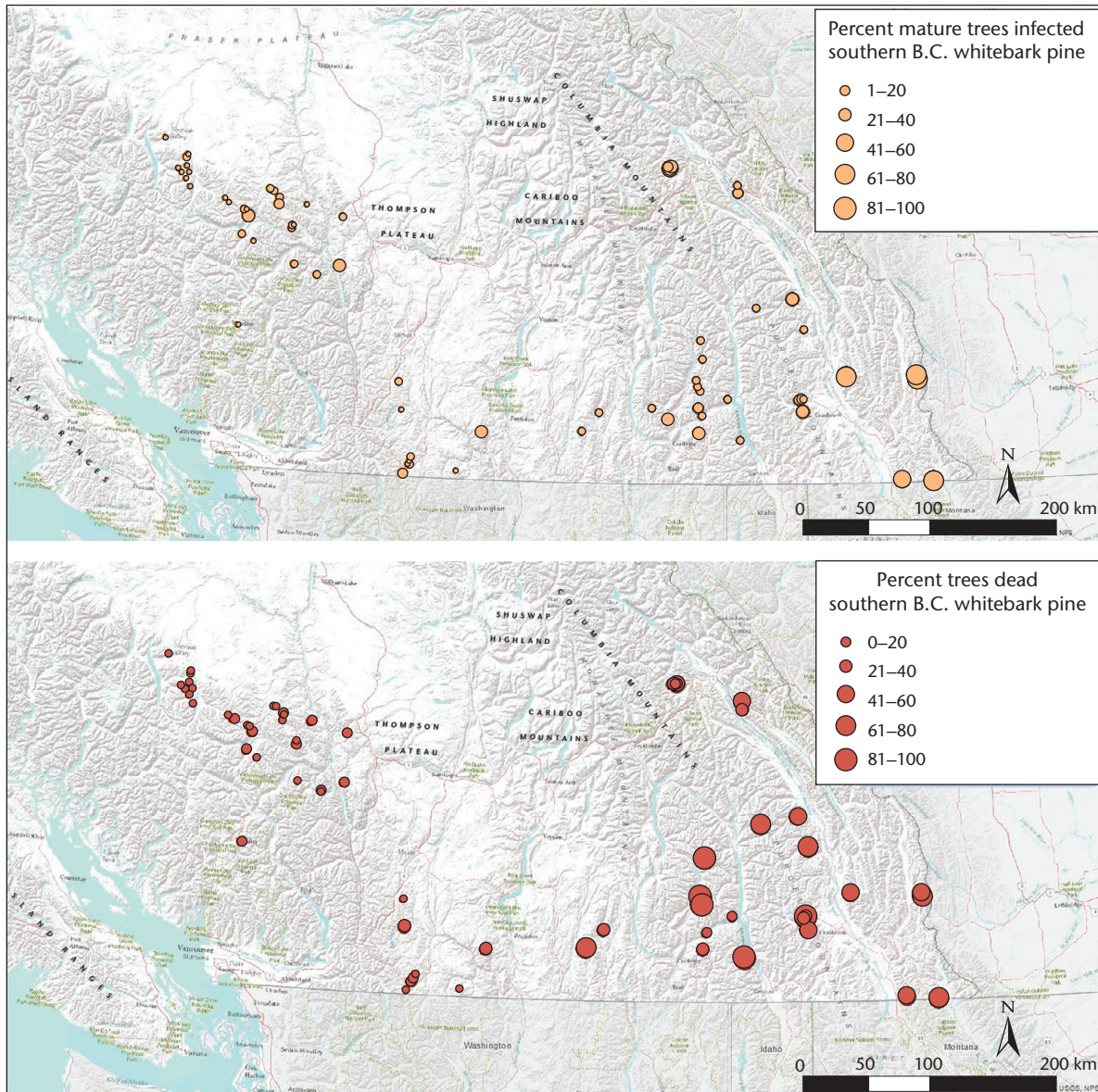
FIGURE 2   *Incidence of white pine blister rust (top) and whitebark pine tree mortality (bottom) based on field inventories (surveys and monitoring transects) conducted between 2008 and 2018. Dead trees are due primarily to blister rust and/or mountain pine beetle infection.*

Where pre-planning was possible, permanent transects were installed. While these one-time observations provide the same useful information as other types of surveys, periodic repeated inventories provide valuable monitoring of trends in forest health (Tomback et al. 2005).

Surveys began in 2008 and indicate that blister rust is present at all surveyed locations in southern British Columbia (Figure 2). In southeast British Columbia, most live trees are infected, whereas the west Chilcotin region supports the lowest incidence of infection. We suggest that the lower incidence in the west Chilcotin region is due to climatic conditions that are unfavourable for rust transmission (colder and drier). The highest incidence of blister rust occurs in the Continental Divide area near the U.S. border. At Crown Mountain, near the Crowsnest Pass, 100% of trees are infected. Our results reflect the findings of Shepherd et al. (2018), who found, based on a network of permanent monitoring plot remeasurements (Tomback et al. 2005), that infection rates were accelerating in southern British Columbia. Combining our data with the separate data set maintained by Parks Canada (Shepherd et al. 2018) would further enhance our understanding of whitebark pine forest health.

Our field efforts also focussed on determining the proportion of whitebark pine trees that were dead. Overall mortality due to all forest health agents appears to be highest in the Selkirk Mountains near Nelson, where more than 80% of standing trees are dead at most sites surveyed (Figures 2 and 3). Higher-elevation stands appear to be affected primarily by blister rust and mountain pine beetle. Lower-elevation stands are typically in mixed-conifer communities where increasingly dense canopies, likely due to lack of fire, add stress to whitebark pine. Thus, in addition to blister rust and mountain pine beetle, competition appears to be contributing to whitebark pine mortality. Also, at lower elevations in British Columbia, whitebark pine trees are prone to timber harvesting effects. Under current harvesting practices with variable retention, if not felled during operations, most whitebark pine trees retained in harvest units appear to fall over within 10 years post harvest (unpubl. data).

The high infection and mortality rates of whitebark pine in southeastern British Columbia are alarming due to the ecological and recovery obstacles presented by such declines; however, these scenarios may also present opportunities in that a high level of selection pressure has been applied to stands in which blister rust is the primary agent of decline. In these stands, trees from the healthiest cohort are suitable candidates for rust screening programs because they may be demonstrating some level of rust resistance.

Most installations were remeasured in 2019, and additional remeasurements of the provincial whitebark pine forest health monitoring system were made in 2020; these remeasurements will provide an update on the most current status of whitebark pine in the province and on rates of infection and mortality. The 2019 data were not included in this summary, and an updated provincial rust analysis will be forthcoming in 2020–2021.

FIGURE 3   *Mortality rates of whitebark pine in British Columbia are highest in the Selkirk Mountains. Photo taken near Slocan City, B.C. (photo credit: B.C. Ministry of Forests, Lands, Natural Resource Operations and Rural Development).*

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Tomback D.F., R.E. Keane, W.W. McCaughey, and C. Smith. 2005. Methods for surveying and monitoring whitebark pine for blister rust infection and damage. Whitebark Pine Ecosys. Found., Missoula, Mont.

3. Results from Long-Term Field Trials in British Columbia: Resistance of Western White Pine to White Pine Blister Rust

Sarah Leroux,[1][**] Nicholas Ukrainetz,[1][*] John King,[2] David Ponsford,[2] Vicky Berger,[3] and Mike Carlson[3]

1 Forest Improvement and Research Management Branch, B.C. Ministry of Forests, Lands, Natural Resource Operations and Rural Development, Vernon, B.C.
2 Forest Improvement and Research Management Branch, B.C. Ministry of Forests, Lands, Natural Resource Operations and Rural Development, Victoria, B.C., retired
3 Forest Improvement and Research Management Branch, B.C. Ministry of Forests, Lands, Natural Resource Operations and Rural Development, Vernon, B.C., retired

*Presenting author: michael.murray@gov.bc.ca

**Corresponding author: Sarah.Leroux@canada.ca

ABSTRACT

White pine blister rust (*Cronartium ribicola*) has greatly affected the distribution of western white pine (*Pinus monticola*) on the British Columbia landscape by causing widespread mortality, especially in young trees. As well, foresters have limited their use of western white pine in reforestation because of the risk of mortality and plantation loss due to blister rust. In response, forestry companies and government have invested in breeding programs to increase rust resistance in seedlings used for reforestation. We present the results of long-term field tests on blister rust resistance, including realized gain trials of seedlots that are representative of British Columbia seed orchards and F1 progeny tests. Based on realized gain field trials, survival of white pine seedlings from existing orchards is expected to be between 59% and 75% in the presence of blister rust. The results from F1 progeny tests indicate that future orchards will produce seedlings with expected survival greater than 80%, which is much higher than that expected from wild seedlots (approximately 30% survival). The success of the white pine breeding programs in British Columbia is due to the good relations and collaboration between the B.C. Ministry of Forests, Canadian Forest Service, U.S. Department of Agriculture Forest Service, and forest industry. For example, many trees in local seed orchards originate from the screening and breeding programs in Oregon and Idaho. Confidence in the resistance of western white pine to blister rust has increased in recent years in British Columbia, which has contributed to an increase in planting from 328 000 seedlings in 2011 to 1.58 million seedlings in 2017.

4. Genomic Approaches to Dissecting Lodgepole and Jack Pine Interactions with *Cronartium harknessii*, the Causal Agent of Western Gall Rust

Chandra McAllister,[1] Marion Mayerhofer,[1] Joshua Miller,[1] Rhiannon Peery,[1] Tod Ramsfield,[2] Andy Benowicz,[3] Deogratias Rweyongeza,[3] Rong-Cai Yang,[1] Patrick Lenz,[4] and Janice Cooke[1*]

1 University of Alberta, Department of Biological Sciences, Edmonton, Alta.
2 Natural Resources Canada, Canadian Forest Service, Northern Forestry Centre, Edmonton, Alta.
3 Alberta Agriculture and Forestry, Forestry Division, Alberta Forest Management Branch, Edmonton, Alta.
4 Natural Resources Canada, Canadian Forest Service, Canadian Wood Fibre Centre, Québec, Que.
*Presenting and corresponding author: janice.cooke@ualberta.ca

ABSTRACT

Lodgepole pine (*Pinus contorta*) is susceptible to *Cronartium harknessii* ([J. P. Moore] E. Meinecke), the causative agent of western gall rust. The goal of this project is to discover genomic signatures for *C. harknessii* resistance in lodgepole pine. We conducted resistance screens with *C. harknessii*–inoculated seedlings that represented 76 lodgepole pine half-sib families, and performed detailed phenotyping on approximately 20–24 seedlings per family. In parallel, gall incidence indices were determined for 35 half-sib families of mature lodgepole pine planted on multiple sites. Thirty-three of the 35 mature tree families were the same as those phenotyped in the seedling resistance screen, and good correlations were found between seedling and mature tree gall incidence data. In total, 1920 individuals representing 62 seedling families and 35 mature tree families were genotyped using a pine Affymetrix Axiom 50K single nucleotide polymorphism array. Following a number of strict data quality assessment analyses, 1085 individuals from the seedling resistance screen and 667 individuals from the mature tree trials have been selected for genome-wide association studies and genomic selection, using approximately 20K robust, bi-allelic, high-resolution single-nucleotide polymorphisms. In parallel, we are conducting transcriptomic analyses of pines inoculated with *C. harknessii* to better understand lodgepole pine molecular responses to this biotrophic pathogen. Outcomes from this research will be used to develop genomic markers and statistical models that predict western gall rust resistance, with the long-term aim of integrating these genomic tools into lodgepole pine breeding programs.

5. Screening Whitebark Pine for White Pine Blister Rust Resistance in British Columbia

Ward Strong[1*] and Michael Murray[2]

1   Research Scientist, B.C. Ministry of Forests, Lands, Natural Resource Operations and Rural Development, Vernon, B.C.
2   Forest Pathologist, B.C. Ministry of Forests, Lands, Natural Resource Operations and Rural Development, Nelson, B.C.
*Presenting and corresponding author: ward.strong@gov.bc.ca

ABSTRACT

Whitebark pine (*Pinus albicaulis*), a keystone subalpine species in British Columbia, is threatened by mountain pine beetle (*Dendroctonus ponderosae*), fire, and white pine blister rust (*Cronartium ribicola*). To stem the mortality caused by white pine blister rust, we have established a screening program to identify genetically resistant individuals and deploy their offspring across the landscape. Parent trees are rust-free individuals selected from areas with high rust mortality. Fifteen to 50 progeny from each of 40–70 parent trees are screened annually. In August, when *Cronartium* telia have formed on the secondary host (*Ribes* species), 2-year-old seedlings are placed in a growth chamber. *Ribes* leaves that are at least 50% covered with telia are suspended on wire mesh above the seedlings. The chamber is maintained at approximately 16°C and 100% relative humidity until a target spore load of 3000–5000 spores/cm² is achieved. Then we maintain 20°C and 100% relative humidity for spore germination and stomatal penetration. Plants are then moved to a greenhouse. The following May, needle spots are counted; in June, seedlings are planted out to raised beds in the field. For the next 4–5 years, plants are scored for vigour, lesion incidence and size, and mortality.

Our program started with 11 families in 2013; to date, we have screened 147 families and 5482 seedlings. We have selected 13 parents with heritable resistance, from which scion were collected and 237 ramets were grafted. These ramets will eventually be deployed in a seed orchard. Currently, we are collecting scion of all parent trees during cone collection, not just from resistant parents after progeny screening. We have grafted 938 ramets from 105 parents so far. These grafts will be stored in a gene conservation clone bank. Those that show resistance will be moved to a seed orchard.

6. Identifying Range-Wide Patterns of Genetic Resistance to Save a Species Threatened by a Non-Native Disease and Climate Change

Jeremy Johnson,[1,2][*] Richard Sniezko,[2] Hunter Mackin,[3] Evan Heck,[2] Megan Lewien,[2] Darren Brons,[2] Shannon Mccoy,[2] Sara Fraser,[2] Angelia Kegley,[2] Gerald Page,[4] Marja Haagsma,[4] Christopher Still,[4] and Kristen Waring[1]

1 School of Forestry, Northern Arizona University, Flagstaff, Ariz.
2 U.S. Department of Agriculture Forest Service, Dorena Genetic Resource Center, Cottage Grove, Oreg.
3 Department of Biology, University of Oregon, Eugene, Oreg.
4 Department of Forest Ecosystems and Society, Oregon State University, Corvallis, Oreg.
*Presenting and corresponding author: jeremy.johnson@nau.edu

ABSTRACT

Southwestern white pine (*Pinus strobiformis*), a large, long-lived conifer that is native to the United States and Mexico, is very susceptible to white pine blister rust (caused by the non-native fungal pathogen *Cronartium ribicola*). *P. strobiformis* has a major gene that has been discovered at low frequency in some populations and imparts complete resistance (major gene resistance) to the disease but may be overcome in the future by virulent strains of the pathogen. Quantitative disease resistance has also been documented in *P. strobiformis*. Although resistance has been found to occur in southwestern white pine, little is known about the type, frequency, and geographic pattern of resistances across the species' range. As part of a large collaborative and interdisciplinary study, we present early results from a range-wide assessment of 446 families from 104 populations of major gene and quantitative disease resistance. Seedlings were artificially inoculated with *C. ribicola* spores and were scored for disease symptoms and patterns of growth. All populations show high rates of infection; however, early results indicate that there is a significant ($P < 0.05$) relationship between the probability of canker development and geographic origin of maternal parent. Moreover, we identified variation in quantitative disease resistance at low frequencies (7–11%) in the species and have identified several major gene resistance families. By making selections from families with identified resistance, we can test their durability, stability, and usability in field trials. These selections can then be used in proactive management of *P. strobiformis* stands that have been identified as likely to experience the arrival of white pine blister rust. These results will provide guidance on refining genetic conservation efforts and future seed collections for reforestation and restoration.

7. The Influence of Genetics, Defensive Chemistry, and the Fungal Microbiome on Disease Outcome in White Pines

Lorinda Bullington,[1][*] Beau Larkin,[2] Emily Martin,[1] and Richard Sniezko[3]

1 Research Scientist, Molecular Ecology and Bioinformatics, MPG Ranch, Missoula, Mont.
2 Forestry and Conservation, MPG Ranch, Missoula, Mont.
3 U.S. Department of Agriculture Forest Service, Dorena Genetic Resource Center, Cottage Grove, Oreg.
*Presenting and corresponding author: LBullington@mpgranch.com

ABSTRACT

Hundreds of asymptomatic fungi live inside white pine tissues, and recent studies suggest that these fungi can influence the frequency and severity of pathogens such as *Cronartium ribicola*, the causal agent of white pine blister rust. We used molecular methods to characterize the foliar fungal community in white pine seedlings from 21 half-sibling seed families, sourced throughout the Pacific Northwest, in a common garden. We investigated endophyte communities before and after experimental inoculation with *C. ribicola* and compared community composition in susceptible versus resistant seedlings to determine which endophytes are most likely to influence disease severity. We also explored the defensive chemistry in needle tissue of these same seedlings in relation to the fungal community and overall disease severity. Seedling family identity most influenced the fungal endophyte community in white pines. There were also shifts in fungal endophyte communities in response to infection by *C. ribicola*. Seed family identity also appears to influence tree defensive chemistry in needle tissue, with foliar terpenes showing varying concentrations in resistant and susceptible white pine seedlings. In addition, both terpenes and individual endophytic taxa correlated with quantified characteristics of disease severity. These results suggest that the resistance in natural white pine populations may be a combination of genetics, fungal endophytes, and terpene composition in needle tissue, where initial interactions between endophytes, the host, and *C. ribicola* occur. We plan to test this hypothesis in a greenhouse experiment this fall.

8. Implementing a Bioinformatics-Based Targeted Sequencing Strategy for R-genes in the *Cronartium ribicola*–White Pine Pathosystem

David Oline[1*]

1 Biology Program, Southern Oregon University, Ashland, Oreg.
*Presenting and corresponding author: olined@sou.edu

ABSTRACT

Based on current models of plant immune system function, we hypothesize that dominant major gene resistances to specific pathogens are due to R-genes in the nucleotide-binding site leucine-rich repeat (NBS-LRR) families. A bioinformatics-informed targeted sequencing strategy can (1) test candidate genes for correlation of specific alleles with known resistance phenotypes, and (2) create a resistance gene profile for individuals or populations. We are beginning to implement this strategy by BLASTing transcriptomes assembled from the abundant data at the NCBI Sequence Read Archive for resistance gene analogs matching candidate resistance genes. From these, we design polymerase chain reaction (PCR) primers to amplify different families of related alleles as widely as possible within *Pinus*, followed by pooling PCR products for high-throughput nanopore sequencing. We have begun testing this strategy using the *Cronartium ribicola*–white pine pathosystem on known resistant and susceptible individuals of both *Pinus monticola* and *P. albicaulis*. Resistance gene analogs similar to candidate genes for *Cr1*, *Cr2*, and *Cr4* are found in most pine transcriptomes but can be very diverse and consist of multiple isoforms. Initial data from PCR of genomic DNA suggest a high number of pseudogenes for many NBS-LRR genes. Despite this additional complexity, these focussed data on families of R-genes may provide insight into the history and evolution of disease resistance.

9. Plenary: Genomics of Quantitative Resistance to White Pine Blister Rust in Sugar Pine (*Pinus lambertiana*)

Amanda R. De La Torre,[1] Matthew Weiss,[2] Richard Sniezko,[3] Daniela Puiu,[4] Steven L. Salzberg,[5] and David B. Neale[6]*

1 Assistant Professor of Forest Genomics, School of Forestry, Northern Arizona University, Flagstaff, Ariz.
2 Graduate student, School of Forestry, Northern Arizona University, Flagstaff, Ariz.
3 U.S. Department of Agriculture Forest Service, Dorena Genetic Resource Center, Cottage Grove, Oreg.
4 Bioinformatician, Department of Biomedical Engineering, Computer Science and Biostatistics and Center for Computational Biology, Johns Hopkins University, Baltimore, Md.
5 Professor, Department of Biomedical Engineering, Computer Science and Biostatistics and Center for Computational Biology, Johns Hopkins University, Baltimore, Md.
6 Professor, Department of Plant Sciences, University of California at Davis, Davis, Calif.
*Presenting and corresponding author: dbneale@ucdavis.edu

ABSTRACT

White pine blister rust (*Cronartium ribicola*) is an exotic, devastating, fungal disease that is causing great ecological and economical damage in five-needle pines in North America. Efforts to mitigate the effects of white pine blister rust in sugar pine (*Pinus lambertiana*) have relied on the development of genetic resistance; however, selection of resistant individuals can take up to 20 years. This project aims to identify genes that confer quantitative resistance to white pine blister rust, and to use this information to develop the basis for genomic-based breeding in sugar pine. We tested for genome-wide associations between a set of 130K single-nucleotide polymorphisms obtained from whole-genome resequencing, and white pine blister rust disease phenotypes and presence of the major gene for resistance in a large number of individuals broadly distributed across the species' natural range. In addition, we are building high-resolution quantitative trait locus mapping using a previously established two-generation full-sib cross. Population structure was determined using principal component analysis in Adegenet. A Bayesian cluster analysis using fastSTRUCTURE was also conducted and involved 10 independent runs of K = 2–10. The most frequent ΔK from these 10 runs was used to determine the number of clusters within the population (K = 4). In total, 154 single nucleotide polymorphisms were significantly associated with the measured traits. Of these, 27 were from coding regions of the sugar pine genome, with complete annotations found for 19 of them.

10. Fusiform Rust Resistance Gene Discovery in *Pinus taeda* Using RNAseq Bulked Segregant Analysis and Two-Way Testcross QTL Mapping

Eddie Lauer[1] and Fikret Isik[1*]

1 North Carolina State University, Cooperative Tree Improvement Program, Raleigh, N.C.

*Presenting and corresponding author: fisik@ncsu.edu

ABSTRACT

Fusiform rust disease, caused by the fungus *Cronartium quercuum* f. sp. *fusiforme*, has long been recognized as the largest threat to pine farming in the southern United States. The objective of this research is to discover and map Fr resistance genes in *Pinus taeda* using bulk-segregant analysis and high-density genotyping. We used high-density artificial inoculations of basidiospores from multiple pathogen isolates (100 000 spores per mL) to challenge 1200 progeny from each of two full-sib families in August 2018. In family Pita4, the disease incidence was 25%, whereas it was 67% in family Pita9, which suggests that there are two distinct modes of inheritance in the two genetic backgrounds. In each family, eight bulked samples of 50 individuals each were produced: three non-diseased bulks, three diseased bulks, and two random bulks (before inoculation). RNA libraries of the diseased and non-diseased bulks will be created and sequenced using an Illumina HiSeq2500 instrument. The 100bp reads will be aligned against custom reference transcriptomes derived from PacBio RNAseq data from the random bulks of each family. Disease symptoms will be associated with variants discovered from RNAseq analysis. Additionally, 1000 individually labelled samples from each family will be genotyped with Pita 50K single-nucleotide polymorphism array in August 2019 to associate the disease incidence with the single nucleotide polymorphism markers using a high-density genetic map.

11. Uncovering the Natural Physiological Variation Driving Lodgepole Pine Resistance to Western Gall Rust

Nadir Erbilgin,[1*] Harleen Kaur,[1] and Jennifer Klutsch[1]

1 Department of Renewable Resources, University of Alberta, Edmonton, Alta.
*Presenting and corresponding author: erbilgin@ualberta.ca

ABSTRACT

Western gall rust, caused by the fungal pathogen *Endocronartium harknessii*, is one of the most prominent tree diseases of lodgepole pine (*Pinus contorta* var. *latifolia*) forests in Alberta: it causes significant tree mortality and growth loss. Infection by the rust usually starts when trees are young, and it remains present in the infected trees as they grow. There are large variations in susceptibility and resistance of mature lodgepole pine trees to the western gall rust in nature. However, the mechanisms underlying lodgepole pine resistance to the rust are unknown. Likewise, why young trees are more vulnerable to the pathogen, relative to older trees, needs to be investigated. We investigated three aspects of plant physiology that are critical for pathogen resistance in lodgepole pine: cell wall thickness, cell wall chemistry (lignin), and secondary compounds (terpenes and phenolics). Our goal is to characterize the differences in cell wall structure and cell wall chemistry between young and mature pine trees as well as between putatively resistant and susceptible phenotypes of lodgepole pines. Our long-term goal is to evaluate whether epigenetics of mature trees can be incorporated into acquiring resistance to the rust in pine seedlings in Alberta. The information obtained for cell wall structure and cell wall chemistry will likely result in the identification of possible physiological mechanisms that can be incorporated into the selection of rust-resistant lodgepole pine phenotypes in Alberta.

12. Age-Related Resistance to Hard Pine Stem Rusts in the Central Interior of British Columbia

Richard W. Reich[1*]

1 College of New Caledonia, Prince George, B.C.
*Presenting and corresponding author: reichr1@cnc.bc.ca

ABSTRACT

Lodgepole pine (*Pinus contorta* var. *latifolia*) is a major commercial species in British Columbia, and is highly susceptible to several different fungal pathogens. The hard pine stem rust causal agents *Cronartium comandrae, C. coleosporioides,* and *Peridermium harknessii* are common in the interior of British Columbia. This presentation focusses on the temporal aspect of susceptibility, using material of wide-ranging physiological age, in relation to resistance phenotype, as well as site factors related to spatial aspects of infection. Although selection for resistance through tree breeding is widely accepted as the most feasible control option, understanding the various factors that influence phenotypic resistance may result in better selection of resistance phenotypes. We explore a replicated field trial, featuring 50-year-old (physiological age) ramets from 25 clones from a resistance-phenotyped orchard, in an attempt to quantify the relative significance of physiological age-related resistance. These results are contrasted with replicated full-sibling (physiological age 0) progeny trial results at the same locations. The effects of site factors that may confound the results, such as the presence of false toadflax (*Geocaulon lividum*) and cow-wheat (*Melampyrum lineare*), are compared to those in other well-documented trials in the central interior of British Columbia. On-site climate monitoring results are used to evaluate the relative suitability of conditions for infection, and the possible role of climate-based stress on infection.

13. Potential Trade-Offs in Growth and Environmental Resilience of Lodgepole Pine in Alberta

Eden McPeak[1*] and Barb R. Thomas[1]

1 Faculty of Agricultural, Life and Environmental Sciences, Renewable Resources Department, University of Alberta, Edmonton, Alta.
*Presenting and corresponding author: emcpeak@ualberta.ca

ABSTRACT

In an effort to increase the growth and yield of commercial timber species, such as lodgepole pine (*Pinus contorta* var. *latifolia*), height growth alone has been selected for in tree breeding programs in Alberta. Although these programs are still in the early stages of selection, there is the potential for a trade-off between selection for height and a reduced ability of selected trees to withstand abiotic (drought) and biotic (disease) stress. A loss of resilience to withstand such stressors in improved stock deployed on the land base could have detrimental consequences for reforestation in the province, particularly in a changing climate. In a growth chamber study, we measured the effect of drought and western gall rust (*Cronartium harknessii*) infection on the growth and ecophysiology of a seed orchard versus a wild seedlot of lodgepole pine from the Region C breeding program in central Alberta.

14. What Do We Know about Hemiparasitic Plant Hosts of Hard Pine Stem Rusts?

SYBILLE HAEUSSLER[1*]

1 Adjunct Professor, University of Northern British Columbia, Smithers, B.C.
*Presenting and corresponding author: Sybille.Haeussler@unbc.ca

ABSTRACT

Secondary (telial) plant hosts of *Cronartium* fungi are understudied parts of these rust pathosystems. Many telial hosts are root hemiparasites—plants that partially depend on resources extracted via roots of neighbouring plants. I review the taxonomy, life histories, and cultural and ecological values of these fascinating plants. Interdependencies among *Cronartium* fungi, their primary (aecial) hosts, hemiparasitic telial hosts, and their host plants are a case study in biocomplexity, with unpredictable dynamics. It is unclear whether forest practices that reduce abundance of hemiparasitic hosts will reduce tree stem rusts, and what treatments are warranted. The following are some research questions: (1) How does *Cronartium* infection affect the dynamics and ecophysiology of host–hemiparasite–pollinator relationships? (2) How important is long-distance dispersal of basidiospores to aecial hosts, and consequently, how important are landscape versus local patterns of hemiparasite abundance? (3) How do population dynamics of short-lived Orobanchaceae influence pine infection rates that become apparent years later?

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INTRODUCTION

Tree stem rusts, caused by fungi of the genus *Cronartium*, are globally important forest pathogens that cause economic and ecological damage to forests throughout the Northern Hemisphere. *Cronartium* fungi are heteroecious and have complex life cycles consisting of four to five spore stages that alternate between two vascular plant hosts. Pine trees (*Pinus* spp.) typically serve as the primary (aecial) host species, and are commonly killed or badly damaged by the stem rust. Many different angiosperm species from various plant families serve as secondary (telial) hosts. Damage to telial hosts is normally restricted to deciduous foliage;

this part of the *Cronartium* pathosystem is less studied because most of these angiosperms are not economically important. The *Cronartium* pathosystem has an unusual feature: many telial host species are hemiparasitic plants that derive water, nutrients, and carbohydrates by attaching themselves to roots of neighbouring plants. *Cronartium* rusts also have autoecious forms (*Peridermium* spp. [syn. *Endocronartium*], not considered here) that spread directly from pine to pine without a telial host.

Not all species of *Cronartium* have hemiparasitic plants as hosts. Telial hosts of *C. quercuum* are members of the Fagaceae; those of *C. comptonia* belong to Myricaceae. There is also no evidence of an association between hemiparasitic plants and *Cronartium* in the Southern Hemisphere. In the Northern Hemisphere, however, several species (or ecotypes) of *Cronartium* are strictly associated with hemiparasitic telial hosts: two species, *C. ribicola* and *C. pini* (syn. *C. flaccidum*), can sporulate on autotrophic and hemiparasitic telial hosts (Table 1).

TABLE 1   *Species of* Cronartium *associated with hemiparasitic plants[a]*

Cronartium species	Primary hosts[b]	Hemiparasitic telial hosts[c]	Autotrophic telial hosts[c]	Geographic distribution
C. appalachianum	*Prunus virginiana*	*Buckleya distichophylla*	n/a	Eastern United States
C. arizonicum	*Pinus ponderosa* (hard)	*Castilleja* spp.	n/a	Arizona
C. pini (synonym *C. flaccidum*)	*Pinus sylvestris, P. mugo, P. nigra,* other hard *Pinus* spp.	*Melampyrum* spp., *Pedicularis* spp., Also *Euphrasia* spp., *Nemesia* spp., *Odontites* spp., *Rhinanthus* spp., *Siphonostegia* spp., *Bartsia alpina*. Also *Castilleja* spp. in controlled environment	*Vincetoxicum* spp., *Paeonia* spp. Also reported in *Apocynum cannabinum, Asclepias* spp., *Cynanchum* spp., *Delphinium* spp., *Gentiana* spp., *Grammatocarpus* spp., *Hyoscyamus niger, Impatiens* spp., *Loasaceae* (several genera), *Myrica* spp., *Nicotiana rustica, Papaver rhoeas, Ruellia* spp., *Saxifraga* spp., *Schizanthus* sp., *Swertia* sp., *Tropaeolum* sp., *Verbena* spp., *Veronica* spp.	Europe, northern Asia
C. comandrae	*Pinus* spp. (hard)	*Comandra umbellata, Geocaulon lividum*	n/a	Canada, United States
C. coleosporioides	*Pinus* spp. (hard); also reported on *P. flexilis* (soft)	*Castilleja* spp., *Melampyrum lineare, Pedicularis* spp., *Rhinanthus* spp. Also reported on *Euphrasia* spp., *Lamourouxia* spp., *Orthocarpus* spp.	n/a	North America to Central America
C. kamtschaticum (likely an ecotype of *C. ribicola*)	*Pinus pumila, P. sibirica*; also reported on *P. strobus* (soft)	*Castilleja* spp., *Pedicularis* spp.	n/a	Eastern Russia, Japan
C. ribicola	*Pinus* spp. (soft)	*Castilleja* spp., *Pedicularis* spp. Also reported on *Bartsia* sp., *Euphrasia* sp. in controlled environment	*Ribes* spp. Also reported on *Asclepias* sp., *Loasa* sp., *Mentzelia* sp., *Nasa* sp., *Tropaeolum* sp. in controlled environment	Temperate Northern Hemisphere

a Kaitera and Hiltunen 2012; Kaitera et al. 2012, 2015, 2018; CABI/EPPO 2019; Farr and Rossman 2019.
b "Hard" refers to subgenus *Pinus*, yellow pines; "soft" refers to subgenus *Strobus*, white pines.
c Sporulation under wild (field) conditions has not been demonstrated on all taxa listed.

Is the relationship between *Cronartium* and hemiparasitic plants an evolutionary coincidence, or is there something special about hemiparasites that makes them competent telial hosts? Either way, this extended network of plants, fungi, and their multiple interacting hosts is a textbook case of biological complexity, which illustrates why forest management problems require a comprehensive systems or ecosystems approach.

The B.C. Ministry of Forests, Lands, Natural Resource Operations and Rural Development, Resource Practices Branch, seeking to reduce damage from hard pine stem rusts, contracted me in 2017 to synthesize literature and data on the ecology and management of telial host species of *Cronartium comandrae* and *C. coleosporioides*, all of which are root hemiparasites from two unrelated plant families (Haeussler 2018). Often, the best information on the ecology of these hemiparasites was obtained from research on their close relatives in Europe or Asia. For this IUFRO Symposium Proceedings, I have prepared a short version of that review, and expanded its scope to consider the full range of hemiparasitic plants associated with *Cronartium* rusts worldwide.

WHAT, AND WHO, ARE HEMIPARASITIC PLANTS?

Hemiparasitic plants have evolved to be partially, but not fully, dependent on the resources (water, nutrients, carbohydrates) supplied by other plants. All parasitic angiosperms, including fully dependent holoparasites, have circular structures on their roots (haustoria) that clasp the roots or stems of neighbouring plants (Heide-Jørgensen 2008). Tracheid cells within the haustorium penetrate the tissues of the host plant, creating a continuous vascular connection that allows the plant to absorb water and chemicals from its host. Hemiparasites have chlorophyll in their leaves and are able to photosynthesize, but their host-dependence varies considerably. Some tap into the hosts' phloem to receive carbohydrates; others extract water, nutrient ions, and dissolved acids via the xylem only (Hibberd and Jeschke 2001; Phoenix and Press 2005). Carbohydrate transfer may depend on local circumstances: plants growing in shade may depend more on carbohydrates from hosts than plants of the same population that are growing in sunlight, where water is limiting. Root systems of hemiparasites are small: the extent of root development indicates how much the hemiparasite depends on its hosts for water and nutrients. Likewise, the amount of green tissue suggests the degree to which a hemiparasite receives carbohydrates from its hosts. Some hemiparasites are protected from herbivores by secondary defensive compounds (alkaloids, phenols, etc.) that are absorbed from their hosts (Adler and Wink 2001; Schädler et al. 2005).

Hemiparasitic plants should not be confused with myco-heterotrophic or mixotrophic plants such as orchids (Orchidaceae), wintergreens, and monotropes (Ericaceae). These plants, formerly mistaken for saprophytes, are not true parasites (Heide-Jørgensen 2008) because they obtain food and water indirectly via mycorrhizal fungi connected to other plants. Such plants are not hosts of *Cronartium* fungi.

Parasitism in plants has evolved independently several times (Heide-Jørgensen 2013). Of the 12 plant Orders that include parasites, root hemiparasites occur in four distantly related plant Orders (Laurales, Zygophyllales, Santalales, and Lamiales), but telial hosts for *Cronartium* fungi occur only in the Santalales and Lamiales.

In Santalales (Figure 1), *Comandra umbellata* (bastard toadflax) and *Geocaulon lividum* (false toadflax), both in the Comandraceae, are telial hosts for *Cronartium comandrae* (Comandra blister rust fungus), a deadly pathogen that occurs across Canada and the United States but is unknown in the Balkans of Europe, where *Comandra umbellata* ssp. *elegans* occurs. A rare hemiparasitic shrub, *Buckleya distichophylla* (piratebush, Santalaceae), is the telial host for *Cronartium appalachianum*, a lesser-known rust restricted to portions of South Carolina, Tennessee, and Virginia. The primary host for this rust is not a pine, but *Prunus virginiana* (choke cherry).

In Lamiales, all known hemiparasitic plants that act as telial hosts for *Cronartium* rusts are in the Orobanchaceae Family (formerly in Scrophulariaceae). Root hemiparasites that serve as telial hosts for at least four *Cronartium* rust species found in North America, Europe, and Asia (Table 1) occur notably in the Orobanchaceae genera *Pedicularis* (louseworts), *Castilleja* (paintbrushes), *Rhinanthus* (rattles), *Melampyrum* (cow-wheats), *Orthocarpus* (owl-clovers), and *Euphrasia* (eyebrights).
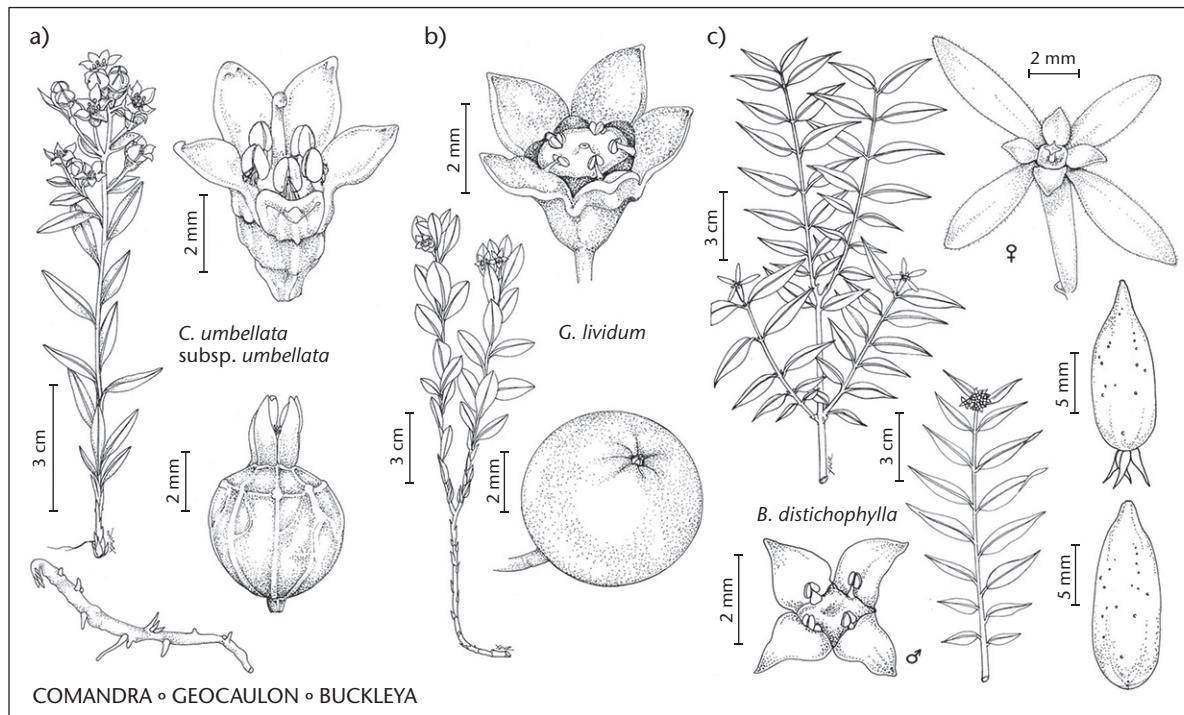
FIGURE 1 *Three hemiparasitic species in the Santalales Order are telial hosts for* Cronartium *rusts in North America. (a)* Comandra umbellata *(bastard toadflax) and (b)* Geocaulon lividum *(false toadflax) are hosts of* Cronartium comandrae*; (c)* Buckleya distichophylla *(piratebush) hosts* Cronartium appalachianum*. Courtesy of the Flora of North America Association, Yevonn Wilson-Ramsey.*

HEMIPARASITES IN THE *CRONARTIUM* LIFE CYCLE

Cronartium species are heteroecious parasitic fungi that require two hosts. Their complex life cycle typically involves the production of five morphologically distinct spore types. The first two stages—spermogonial (producing spermatia)

and aecial (producing aeciospores)—occur on the primary or aecial host, whereas the final three stages—uredial (producing uredinio[uredo]spores), telial (producing teliospores), and basidial (producing basidiospores)—occur on the secondary or telial host (Bergdahl and French 1976; van der Kamp 1993).

Aeciospores are released by the primary host in late spring or early summer, often coinciding with the release of pine pollen. These hardy spores are capable of travelling long distances in dry, windy weather. Under field conditions, aeciospores remain viable for several days to a few weeks and must be in contact with free water to germinate on leaves or stems of the telial host.

Upon germinating, aeciospores penetrate the epidermis of their host, forming yellow spots (uredia) that appear 10–20 days after germination. Under moist conditions, the uredial stage involves multiple generations in a single summer, with urediospores infecting other parts of the same plant or spreading to other telial host plants, thus building up the fungal population.

Several weeks after yellow uredial spots become visible on the telial host, yellow hair-like telia may burst through the leaf epidermis or from uredinia, typically on the underside of the leaf. The telia produce masses of thicker-walled teliospores. Aeciospore infection may lead to formation of the telial stage without uredinia production.

In mid to late summer, teliospores germinate to produce basidia, and after their germination, basidiospores. These dispersal spores enable the rust to travel from the secondary host to a primary host, thereby completing the life cycle. Basidiospores are fragile and sensitive to drying and high temperatures. Generally, they do not travel far, and infection rates are low. Infection episodes (wave years) are thought to be associated with slow-moving, saturated air masses at 10–20°C that persist for at least 6 hours (Jacobi et al. 1993, 2002).

Known telial hosts of *Cronartium* are herbaceous to semi-woody deciduous plants whose leaves or stems die annually. The fungus does not penetrate perennial stem bases, rhizomes, or roots; thus, unlike on the primary host, the infection is not perennial. There is also no evidence that *Cronartium* urediospores or teliospores can successfully overwinter. Telial hosts must therefore be newly infected in subsequent years. Furthermore, infection must alternate between the primary and secondary hosts. Infection cannot pass from telial host to telial host except during the uredial stage of a single growing season.

Within a genus, not all species are equally susceptible to *Cronartium* infection. In Finland, *C. pini* occurs frequently on *Melampyrum sylvaticum*, occasionally on *M. nemorosum* and *M. arvense*, but rarely on *M. pratense*. Kaitera and Witzell (2016) showed that the leaf content of secondary defensive chemicals differed greatly between *M. sylvaticum* and *M. pratense*. This may account for the difference in rust resistance. No studies have yet examined whether resistance to *Cronartium* is influenced by defensive compounds acquired from a host.

Fungal pathogens vary geographically and continually evolve. Although *C. pini* affects many species in Europe (Table 1), the local rust in some areas is able to infect only a few telial hosts. Host jumps are also common (Slippers et al. 2005). Hybridization (e.g., *Cronartium × flexili*) (Allen et al., these proceedings) or recombination of resident and recently arrived pathogens could result in new strains with life cycle attributes unlike those described above.

All hemiparasitic telial host plants of *Cronartium* fungi are root, not stem, hemiparasites. While some hemiparasites are host-specific, those described here tend to be opportunistic. Their hosts include conifers, hardwood trees, shrubs, graminoids, forbs, and more, including ectomycorrhizal, arbuscular mycorrhizal, ericoid mycorrhizal, and nitrogen-fixing plants (Warrington 1970). While some studies found a preference for the most vigorously growing neighbours (Gibson and Watkinson 1989) or for legumes (Ren et al. 2010), others reported that the hemiparasite attached to whatever it first encountered, including dead and decaying roots (Piehl 1965).

For hemiparasites, the primary advantage of parasitic attachment is the greater water and mineral supply resulting from an increase in the root-absorbing system (Heckard 1962). They are able to maintain open stomata and high transpiration rates under a hot, midday sun or in droughty soils when most plants close their stomata to maintain their internal water balance (Press et al. 1988). Most hemiparasites are either non-mycorrhizal (Lesica and Antibus 1986) or weakly arbuscular mycorrhizal (Li and Guan 2008).

The effect of hemiparasites on their hosts and on the dynamics of plant communities has been studied in agricultural settings (Smith and Cox 2014) and European grasslands and wildflower meadows managed for biodiversity conservation (Cameron et al. 2008). Host plants often have reduced biomass accumulation, and their reproductive output and metabolic processes such as photosynthesis may be disrupted (Cameron et al. 2006, 2008). Species included here commonly attach to roots of pine trees, but no publications were found that described the effects of this parasitism on the growth of pine hosts.

COMANDRACEAE LIFE HISTORIES

Comandra umbellata (bastard toadflax) and *Geocaulon lividum* (false toadflax) are elegantly simple perennial forbs (sub-shrubs) with horizontal rhizomes bearing erect, fertile shoots with simple alternate leaves and small flowers, and small roots that attach indiscriminately to nearby plant roots (Piehl 1965; Warrington 1970). They regenerate vegetatively by suckers and sprouts, and produce edible berries. They rarely reproduce sexually, but seeds stored in the soil will germinate after a severe disturbance, such as wildfire (Rowe 1983).

These two species differ markedly in their habitat requirements and distributions: *Comandra* favours open sites in warm, dry, temperate climates, often in the savanna-grassland ecotone, whereas *Geocaulon* is a late successional species of cold, moist boreal forest understoreys. Whereas *Comandra* usually resprouts vigorously after soil disturbance, fire, or herbivory, *Geocaulon* is disturbance-sensitive and recovers slowly. Together, these species cover almost the full range of Western Hemisphere pine forests, which accounts for the wide geographic distribution of *Cronartium comandrae*, although neither is present at subalpine to alpine elevations.

The 11 Orobanchaceae genera identified as *Cronartium* telial hosts (Table 1) include a variety of contrasting herbaceous life histories from showy, sun-loving, semi-woody rhizomatous perennials of moist meadows and roadsides that have tiny, wind-dispersed seeds (scarlet paintbrush [*Castilleja miniate*]) to tiny, inconspicuous annuals with large ant-dispersed seeds that persist in the understorey of pine forests (cow-wheat [*Melampyrum lineare*]). Orobanchaceae hosts range from aggressive invaders (yellow rattle [*Rhinanthus minor* ssp. *minor*]) to rare endemics (*Pedicularis ishidoyana*—in Korea, not confirmed as a *Cronartium* host). Common features include elaborate, colourful, tubular, nectar-filled flowers that are pollinated by insects or hummingbirds, dry capsule fruits, sexual reproduction as an essential mode of regeneration, and a preference for open, periodically disturbed habitats. Orobanchaceae hosts are common in almost all terrestrial biomes of Europe, Asia, and North America, and occur in a huge spectrum of habitats, including low-elevation deserts and grasslands, alpine meadows and tundra, Mediterranean scrub, temperate rainforest clearings, boreal forest understoreys, fens, bogs, estuaries, agricultural fields, roadsides, gravel pits, and urban gardens.

ECOSYSTEM SERVICES AND CULTURAL SIGNIFICANCE OF HEMIPARASITIC PLANTS

Hemiparasitic herbs have recently come to be viewed as "ecosystem engineers" because of their ability to reduce the competitive abilities of host plants and thus alter the structure and enhance the diversity of herbaceous plant communities (Cameron et al. 2006). This function has received attention mainly in semi-natural wildflower meadows and grasslands of Europe, where fertilizers and industrial emissions have led to species-poor degraded communities dominated by a few aggressive grasses. Deliberate introductions of hemiparasitic herbs, as well as mowing regimes that optimize the reproductive success of *Rhinanthus* and *Melampyrum* spp., are used to restore diverse herbaceous communities in Europe (Westbury et al. 2006; Blažek et al. 2016). Nutrient cycling by hemiparasitic plants is also recognized as an important ecosystem service (Demey et al. 2014). *Comandra* is valued for its role in reducing soil erosion in overgrazed or burned rangelands (Piehl 1965).

The functional role of hemiparasites as ecosystem engineers is less appreciated outside Europe. In North America, *Rhinanthus minor* is regarded mainly as an agricultural weed (e.g., Smith and Cox 2014); however, the ability of hemiparasites to enhance plant diversity by reducing competitive dominance could also be important in the wildflower meadows of Asia and the Americas. There, colourful displays of *Pedicularis, Castilleja* (Figure 2), and other Orobanchaceae are spiritually and aesthetically valued, and contribute economically to tourism. In the United States and China, *Castilleja* and *Pedicularis* have been studied from the perspective of pollinator diversity (Duffield 1972; Wang and Li 2005). An intriguing avenue of research examines the transfer of defensive chemicals from the roots of alkaloid-producing host plants to the roots and foliage of *Castilleja*. This can

FIGURE 2   *Hemiparasitic Orobanchaceae such as* Pedicularis bracteosa *(spikes, left) and* Castilleja miniata *(red inflorescence, right) help maintain floral diversity and pollinator health in wildflower meadows of the Northern Hemisphere, while also providing important medicinal, spiritual, aesthetic, and tourism values (photo: S. Haeussler).*

reduce herbivory and thereby enhance seed, nectar, and pollen production in *Castilleja* without damaging pollinators (Adler and Wink 2001).

Pedicularis species are revered medicinal herbs in Asia and among Indigenous peoples of the Americas, and are increasingly used therapeutically in Western societies. Phytochemical analyses have revealed a host of biochemical compounds, including phenols, flavonoids, and iridoids, that have antioxidant, antidiabetic, and immunomodulatory properties (Yatoo et al. 2017).

Comandraceae herbs have nutritious edible fruits that are eaten by wild animals, although they are not preferred by humans. They, and many of the Orobanchaceae, produce a delectable nectar that is a favourite children's snack in Indigenous and rural societies. *Geocaulon, Castilleja, Pedicularis,* and *Rhinanthus* are mostly unpalatable, whereas *Comandra* and *Melampyrum* are minor forage species for ungulates. Their palatability may be influenced by root uptake of toxic compounds from the host (Schädler et al. 2005).

HEMIPARASITE ABUNDANCE AND *CRONARTIUM* RUST OUTBREAKS

Prevalence of *Cronartium* rusts and damage to aecial hosts are not necessarily well correlated with the abundance of their telial hosts. In eastern North America, comandra rust and white pine blister rust are widely distributed and their telial hosts are common, but the rusts rarely reach the severe epidemic levels reported from western North America (Jacobi et al. 2002; Kinloch 2003). In central British Columbia field studies, correlations between *Cronartium* rust infection rates on

lodgepole pine and the proximity or abundance of telial hosts were strong on some sites but poor on others, notably for stalactiform rust (Figure 3) (Haeussler et al. 2017; Reich and John 2018).
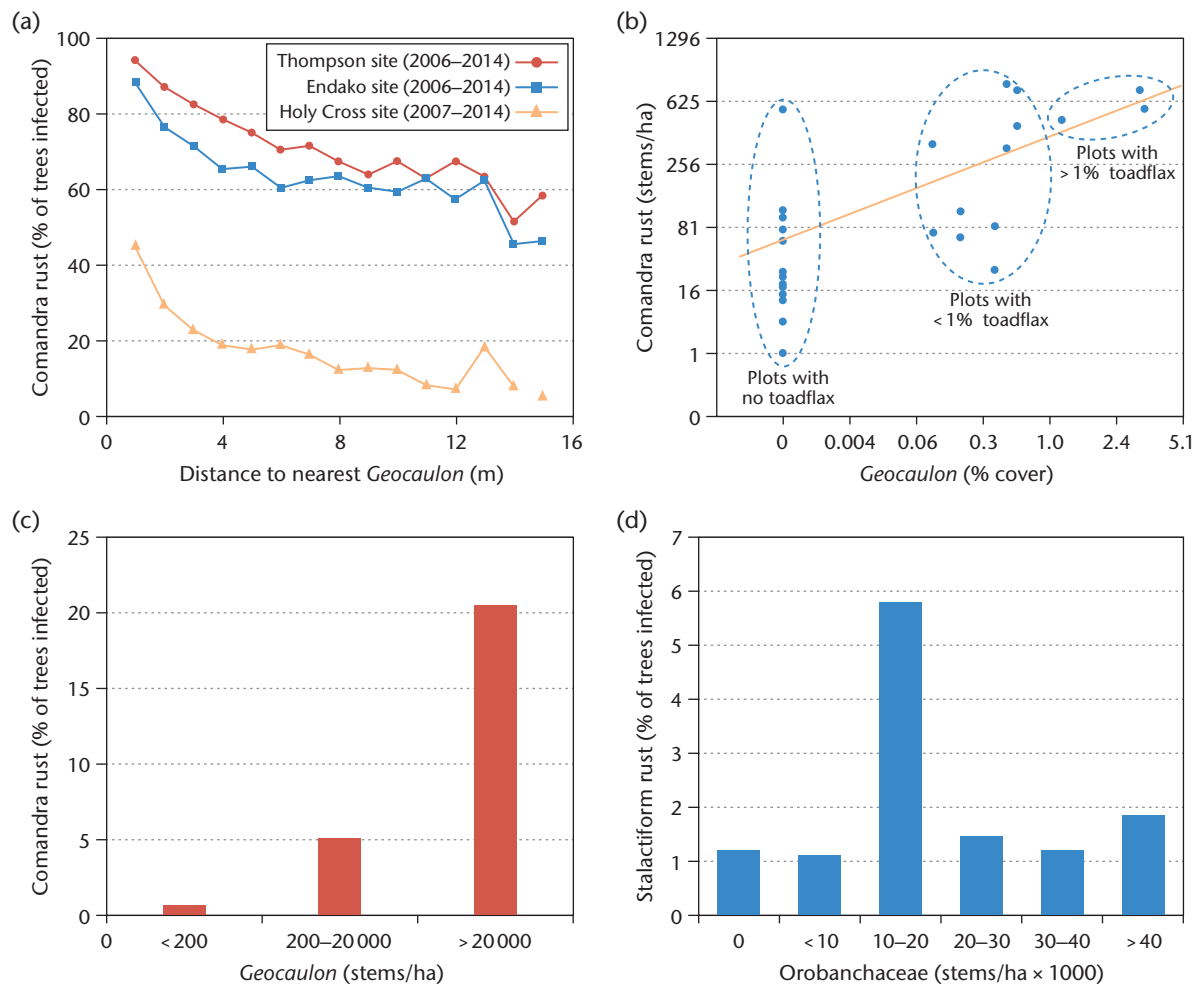
FIGURE 3   Cronartium *stem rust infection rates in central British Columbia shown in relation to (a) distance to nearest* Geocaulon *stem (after Reich and John 2018), (b)* Geocaulon *percent cover (A. Woods, data), (c)* Geocaulon *stem density, and (d) Orobanchaceae stem density (D. Rusch, data).*

Where telial hosts are abundant but infection rates are low, unsuitable weather or high host resistance are possible explanations (Reich and John 2018). For reverse situations, where high rates of rust infection occur on sites with few or no telial hosts, discrepancies may be due to mismatches in spatio-temporal scales of sampling. In dry or hot summer weather, infection rates are typically low and basidiospore travel distances are short, whereas under moist, warm conditions, massive clouds of viable spores are able to travel long distances (Jacobi et al. 2002; Kinloch 2003). Jacobi et al. (1993) developed a landscape-scale model for Wyoming and found good correlations between comandra rust infection rates on lodgepole pine and distances to the nearest *Comandra umbellata* (Jacobi et al. 1993). There, infected pines were separated from telial host populations by distances ranging from 1 to 10 km, much larger than spatial scales of the British Columbia studies (Figure 3).

Short-lived Orobanchaceae hosts such as *Rhinanthus minor, Castilleja miniate*, and Melampyrum lineare may differ in abundance between the year when infection occurred on young pine seedlings and the year when rust cankers were first noted on juvenile pine stems. European pine forests often occur in patchwork landscapes intermixed with hay meadows that are regularly mown or plowed. Short-lived soil seedbanks of *Rhinanthus* and *Melampyrum* in these fields vary substantially from year to year in response to weather and cultural practices such as the time of mowing (Blažek et al. 2016), which leads to large interannual variations in plant population numbers (de Hullu et al. 1985). Such fluctuations in population size have been linked to *Cronartium* epidemic waves (J. Kaitera, Forest Pathologist, Luke Natural Resources Institute Finland, Helsinki, Finland, pers. comm., 2019).

MANAGING HEMIPARASITE POPULATIONS TO REDUCE INCIDENCE OF PINE STEM RUSTS

For *Rhinanthus minor*, long considered an agricultural weed in Europe and still viewed as such in North America, a variety of mechanical and chemical control techniques have been tested, and changes in fertilization, mowing, and plowing practices have been implemented to reduce competition from this species (Westbury et al. 2006; Smith and Cox 2014). Management techniques to reduce damage associated with hemiparasitic *Cronartium* hosts are essentially unstudied. Knowledge of plant life histories and responses to natural and anthropogenic disturbances can suggest forest management approaches and silvicultural techniques that help reduce abundance of these hosts at local or landscape scales (Table 2). For example, literature and field data consistently show that *Geocaulon*

TABLE 2 *Example of predicted responses of false toadflax* (Geocaulon lividum) *abundance to forest condition or vegetation management activities[a]*

Forest condition or activity	Predicted response[b]	Scientific certainty[c]
Landscape composition	Old forest ++; agricultural – –	High; high
Logging practices	Clearcut – ; partial cut +	High; moderate
Tree species selection	Mixed conifer 0; broadleaves – –	Moderate; high
Pine tree density control	Spacing: dense – ; wide +	Moderate
Prescribed fire	Severity: low –; high – –	0–5 yr: high; > 5 yr: low
Mechanical site preparation	Severity: low –; high – –	0–5 yr: high; > 5 yr: low
Herbicide (glyphosate) treatment	Broadcast 0; targeted –	Low
Grazing	Cattle or sheep –	Moderate
Mowing	Single –; multiple – –	Moderate
Manual vegetation removal	Cutting – ; grubbing – –	Moderate
Sowing cover crop	Grasses –	Low
Soil amendment	N fertilizer –; wood ash –	Moderate; low

a Adapted from Haeussler 2018, Table 4.
b Abundance of hemiparasite: + increased, ++ greatly increased, 0 no change, – decreased, – – greatly decreased.
c Based on amount of supporting literature and unpublished British Columbia field data.

lividum is sensitive to, and recovers slowly after, fire. Thus, greater use of prescribed fire would likely reduce the abundance of this species in forest stands and landscapes.

The two comandra blister rust hosts (*Geocaulon lividum* and *Comandra umbellata*) are geographically segregated by climate, elevation, aspect, soil type, and slope position. False toadflax occupies cool, moist, shady, infertile locations, whereas bastard toadflax occupies warm, dry, sunny locations with nutrient-rich soils. Management actions to reduce risks of comandra blister rust infection at stand and landscape scales could target these species separately. These long-lived rhizomatous herbs will typically be established prior to site disturbance and are unlikely to aggressively invade new areas. Pre-treatment surveys can identify the location and density of these hosts, and plans can be developed in advance to ameliorate risks of infection of young pine stands.

Orobanchaceae rust hosts are very difficult to manage because there are so many species and morphological variants that occupy varied ecological niches and respond differently to management actions. Often multiple hosts co-occur in the same plant community or landscape (along with non-hemiparasite hosts, in the case of *Cronartium pini* and *C. ribicola*). In mountainous regions, Orobanchaceae hosts are typically most abundant on mountain tops and in valley-bottom grasslands, and additional populations can occur in agricultural fields, meadows, wetlands, and roadsides linking these areas. *Melampyrum* may also be present in forest and woodland understoreys. While some Orobanchaceae persist in relatively stable habitats (e.g., *Pedicularis* in bogs, *Melampyrum* in pine understoreys), other species (e.g., *Rhinanthus minor*) invade newly disturbed soils. Management of Orobanchaceae hosts thus requires balancing differences in niches, phenologies, and responses of multiple species and variants. In Canada and the northernmost United States, a further conflict may exist between managing forests to reduce incidence of Orobanchaceae hosts and managing to reduce incidence of *Geocaulon lividum*.

The history of *Ribes* eradication programs in North America (Kinloch 2003) provides important lessons for evaluating the prospects of successfully managing hemiparasitic *Cronartium* hosts. In western North America, the consensus is that attempts to control *Ribes* failed on multiple fronts. In eastern North America, some analysts agree that *Ribes* eradication significantly reduced white pine blister rust damage (Benedict 1981; Ostrofsky et al. 1988) and that local control can be successful when rigorously applied over a realistic target area. Kinloch (2003) concluded that small-scale *Ribes* eradication has a place in blister rust management when applied by practitioners who have intimate local knowledge, but that it, along with other silvicultural techniques, is likely to mitigate rather than eliminate the disease. Perspectives on the potential for, and scope of, hemiparasite management will depend on the geographic and cultural context, and will likely reflect organizational or individual preferences for a task-oriented versus an adaptive co-management style of forest management.

RESEARCH NEEDS: WHAT DO WE NOT KNOW ABOUT HEMIPARASITIC TELIAL HOSTS?

Research is needed on many fundamental questions related to the population dynamics, ecological and physiological relationships of hemiparasitic plants, and their pathogens and hosts. Questions of interest include the following:

1. How does *Cronartium* rust infection influence hemiparasite population dynamics, ecophysiology, and host–parasite–pollinator relationships? Do defensive chemicals from a host affect the hemiparasite's ability to resist or tolerate *Cronartium* infection? Do disease dynamics differ when hemiparasites are attached to a pine tree versus a non-pine host?

2. What is the relative importance of short-distance versus long-distance dispersal of basidiospores from hemiparasites to the aecial host in varied environments? Consequently, how does landscape composition, as opposed to stand composition, influence disease outbreaks?

3. Can we project how spore dispersal patterns in (2) will be affected by climate change and associated changes in disturbance and vegetation communities?

4. What are the population dynamics of short-lived Orobanchaceae, especially annual species such as cow-wheat and yellow rattle? Are they cyclical or do they display predictable dynamics in response to forest succession? Can local outbreaks of *Cronartium coleosporioides* (and Orobanchaceae-associated rusts) be predicted by monitoring Orobanchaceae populations?

5. Is small-scale targeted control of hemiparasitic hosts feasible and cost-effective for high-value stands such as seed orchards, or near tree nurseries? What are the best treatment options?

6. What are effective integrated forest and landscape management strategies for reducing the risks of damaging *Cronartium* disease outbreaks while maintaining the diversity and health of hemiparasite–pollinator ecological communities?

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15. Five-Needle Pines in British Columbia – Status and Distribution

Randy Moody[1*]

1 President, Whitebark Pine Ecosystem Foundation of Canada, Nelson, B.C.
*Presenting and corresponding author: whitebarkrandy@gmail.com

ABSTRACT

British Columbia is home to three species of five-needle pines: western white pine (*Pinus monticola*), whitebark pine (*P. albicaulis*), and limber pine (*P. flexilis*). Only western white pine is of merchantable value, although the other two species are of significant ecological importance. All three species are susceptible to white pine blister rust (*Cronartium ribicola*), but recovery efforts are underway. Due to the highly varied geography of British Columbia, unique habitat associations and a range in the incidence of blister rust infection occur across the province, resulting in a need for regionally based planning to ensure that these at-risk tree species are properly inventoried and documented during land management activities.

16. Plenary: Pine Rusts Never Sleep: Deciphering the Biology and Epidemiology of White Pine Blister Rust with Genomics

Richard C. Hamelin,[1][*] Kiah Allen,[1] Arnaud Capron,[1] Nicolas Feau,[1] Erika Dort,[1] and Philippe Tanguay[2]

1 Department of Forest and Conservation Sciences, University of British Columbia, Vancouver, B.C.
2 Natural Resources Canada, Laurentian Forestry Centre, Québec City, Que.
*Presenting and corresponding author: richard.hamelin@ubc.ca

ABSTRACT

White pine blister rust, caused by *Cronartium ribicola* J.C. Fisch (Basidiomycota, Pucciniales), was introduced into North America on pine seedlings imported from Europe at the beginning of the 20th century. This invasive tree pathogen has caused severe economic impacts and ecological effects on several North American white pine species. There is renewed interest in planting genetically resistant white pines, for both commercial uses and ecosystem restoration. But there are still many unanswered questions about the biology and epidemiology of this pathogen. In particular, the heteroecious nature of the rust fungi is still poorly understood. Genomics and transcriptomics can help us better understand the epidemiology, adaptation, hybridization, and interactions of *C. ribicola* with its hosts. Whole genome sequencing and analyses revealed that *Cronartium* fungi have a genomic profile that is distinct from other species, likely due to their obligate biotrophic lifestyle. Transcriptome comparison of rust infection revealed that *C. ribicola* expresses a different set of genes when it infects the telial (*Ribes* spp.) and aecial (white pine) hosts; it also uses a different gene set to infect the different tissue types in pine (needles versus leaves versus stems). Population genetic analyses have shown that eastern and western populations of the pathogen are strongly differentiated. However, western populations can, in some cases, hybridize with the native pine rust *C. comandrae* to form the hybrid offspring *C. × flexili*. Genetic analysis did not demonstrate evidence of introgression, thereby indicating that it likely does not facilitate gene flow between the introduced *C. ribicola* and the native *C. comandrae*. This could be due to a number of factors, including low relative fitness of the hybrid compared to the parental species. But the fitness of hybrid progeny and their effect on pines or other hosts remains to be investigated. These results highlight the importance of improving our understanding of the infection process and of monitoring this invasive alien tree disease.

17. Interspecific Hybridization in *Melampsora* Rusts of Poplars: A Mechanism of Emergence of New Species

Pascal Frey,[1*] Maria Marlin,[2] Héloïse Bastiaanse,[3] Axelle Andrieux,[1]
Andrew Groover,[4] Isabelle Henry,[5] Luca Comai,[5] and George Newcombe[2]

1 Institut National de la Recherche Agronomique (INRAE), Lorraine University, (UMR) Unité Mixte de Recherche, (IAM) Interactions Arbres-Microorganismes, Nancy, France
2 University of Idaho, College of Natural Resources, Moscow, Idaho
3 U.S. Forest Service, Pacific Southwest Research Station, Davis, Calif.
4 Department of Plant Biology, University of California at Davis, Davis, Calif.
5 Genome Center, University of California at Davis, Davis, Calif.
*Presenting and corresponding author: pascal.frey@inra.fr

ABSTRACT

Examples of disease outbreaks due to the introduction of exotic plant pathogens are numerous and well-known. Another increasing risk, which is very largely underestimated and poorly documented, is the accelerated evolution of pathogens resulting from genetic exchanges between native and exotic pathogens. This is particularly the case of interspecific hybridization between allopatric species, which may have new or increased pathogenicity (Brasier 1995). Two cases of interspecific hybridization have been documented within the genus *Melampsora*, which causes poplar rust. The *M.* × *medusae-populina* hybrid, discovered in 1991 in New Zealand, results from hybridization between the Eurasian rust fungus *M. larici-populina* (*Mlp*) and the North American rust fungus *M. medusae* f. sp. *deltoidae* (Spiers and Hopcroft 1994; Frey et al. 2005). Similarly, the *M.* × *columbiana* hybrid appeared in 1995 in the northwestern United States as a result of hybridization between *M. medusae* f. sp. *deltoidae*, which is native to the eastern United States, and *M. occidentalis* (*Moc*), which is native to the western United States (Newcombe et al. 2000, 2001). This hybrid presents symptoms on a range of hosts that extend throughout the ranges of the two parental species.

   We describe a new poplar rust hybrid that has resulted from hybridization between *Mlp* and *Moc*. It was discovered in 2017 on black cottonwood (*Populus trichocarpa*) trees in California, together with an *Mlp* isolate. Quantitative polymerase chain reaction dosage of *Mlp* versus *Moc* ITS indicated that the hybrid shows a typical 1:1 dosage. Furthermore, microsatellite analysis using 22 *Mlp*-specific loci showed that all the alleles found in the hybrid were present in the Californian *Mlp* isolate, and only one allele was retained at each locus in the hybrid, consistent with the hypothesis of a hetero-dikaryotic hybrid. Interestingly, the multilocus genotype of the Californian *Mlp* isolate was almost the same as that of an *Mlp* isolate collected in 1995 in Washington State during the first outbreak of *Mlp* in the United States (Newcombe and Chastagner 1993), which suggests a very strong founder effect.

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18. Evaluating the Presence and Introgression of the Hybrid Forest Pathogen *Cronartium × flexili*

Kiah Allen,[1*] Richard Hamelin,[1] and Nicolas Feau[1]

1 Department of Forest and Conservation Sciences, University of British Columbia, Vancouver, B.C.

*Presenting and corresponding author: kiahallan@hotmail.com

ABSTRACT

The recent discovery of a hybrid forest fungal pathogen, *Cronartium × flexili*, suggested to have arisen from sexual hybridization between the introduced *C. ribicola* and the native *C. comandrae*, was surprising because the parental species do not share hosts. Although the pathosystems of both parental species are well described, the effect of their hybridization is yet unknown. The purpose of this study is to determine the occurrence, level of hybridization, and introgression of *C. × flexili*. In total, 831 isolates from dikaryotic aecia of *C. ribicola* and *C. comandrae* were collected from 13 sites across British Columbia and Alberta, and were analyzed both genetically and morphologically over two sampling seasons. Genetic methods, including polymerase chain reaction (PCR), qPCR, and genotyping-by-sequencing were used to identify hybrid isolates. The results of the genetic analyses indicated that *C. × flexili* is either no longer prevalent in areas where it was previously found, or it occurs so infrequently that it has evaded detection. Two previously collected *C. × flexili* samples were examined using a fixed loci analysis and did not demonstrate evidence of introgression. Thus, when hybridization occurs between the parental *Cronartium* spp., first-generation hybrids are produced but are likely not fertile. This could be due to "hybrid breakdown" as explained by the Bateson–Dobzhansky–Muller incompatibility model, resulting in low relative fitness of *C. × flexili* compared to the parental species, or to variations in local climate factors influencing life cycle parameters, or to sexual incompatibility with the parental species.

19. Genotypic Diversity and Reproductive Biology of *Thekopsora areolata*, the Causal Agent of Cherry Spruce Rust in Norway Spruce Seed Orchards

Hernán Capador,[1] Berit Samils,[1] Juha Kaitera,[2] and Åke Olson[1*]

1 Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden
2 Natural Resources Institute Finland, University of Oulu, Oulu, Finland
*Presenting and corresponding author: Ake.olson@slu.se

ABSTRACT

Swedish forestry is sustained by planting approximately 200 million Norway spruce (*Picea abies*) seedlings every year. Most of these seedlings come from seed orchards, which have been established to transfer the genetic gain in growth and yield achieved through plant breeding since the 1940s. However, today there is a deficit of seeds from Swedish seed orchards because of irregular flowering and cone set, and pest and pathogen infections such as *Thekopsora areolata*— the causal agent of cherry spruce rust, a fungus that significantly reduces seed production in Fennoscandia. To investigate the reproduction mode and population structure of the pathogen, newly developed microsatellite markers and a hierarchical sampling strategy were used. From one location in Norway, one location in Finland, and five locations in Sweden, one aecium per infected cone was analyzed. In addition, multiple aecia per scale and cone were sampled at two locations in Sweden. The results showed overall high genotypic diversity. At the country and location levels, no genetic structure was found, which indicates high gene flow and random mating in *T. areolata*. At the cone and scale levels, multiple identical genotypes were found and non-random mating was observed. These results suggest that *T. areolata* has long-distance spore dispersal in Fennoscandia, with common recombination events and clonal or vegetative spread in cones and scales.

20. Morpho-Anatomical Characteristics of *Eucalyptus urophylla* Leaves Inoculated and Not Inoculated with *Austropuccinia psidii*

Edson Luiz Furtado,[1*] Celso Luis Marino,[2] Renata Ruiz Silva-Souza,[3] and Roberto Antonio Rodella[2]

1 School of Agriculture, São Paulo State University (unesp), Botucatu, Brazil
2 Institute of Biosciences, São Paulo State University (unesp), Botucatu, Brazil
3 Institute of Botany of São Paulo, São Paulo, Brazil
*Presenting and corresponding author: edson.furtado@unesp.br

ABSTRACT

Rust caused by *Austropuccinia psidii* occurs in young tissues of eucalyptus plants, and in the field, it affects individuals younger than 2 years old. Leaves on the branches change their anatomical characteristics according to different maturity stages. The objectives of this study were to evaluate morphological and anatomical differences in eucalyptus leaves in different phenological stages, propose a visual scale for classifying leaves according to their maturity stages, and verify resistance and anatomical changes in the leaves after inoculation. The first pairs of leaves were inoculated and compared to those of *Syzygium jambos* after the appearance of rust symptoms. A phenological scale was proposed to distinguish five stages of leaf development, considering morpho-anatomical characteristics such as shape, colour, and size. Anatomical analysis indicated that older leaves had a higher percentage of reinforced tissue such as sclerenchyma and collenchyma, greater blade thickness, and more cavities containing essential oils compared to younger leaves. In stages 1–3, leaves were susceptible to infection; in later stages, resistance was detected. Leaves of *S. jambos* that were evaluated 15 days after inoculation showed sporulation on both sides and slight modification of their anatomical constituents; in eucalyptus, sporulation also occurred on both sides, but the lacunar parenchyma presented low compression, and the upper palisade parenchyma had visible damage. Initially, inoculated spores presented changes in their size, which was also observed in spores obtained after sporulation.

1 INTRODUCTION

Austropuccinia psidii (G. Winter) Beenken is considered a devastating pathogen of eucalyptus in Brazil, causing severe damage to trees younger than 2 years old (Coutinho et al. 1998; Alfenas et al. 2004). It was first observed in guava (*Psidium guajava* L.) in 1884 and was later found in eucalyptus (*Eucalyptus citriodora* Hook) in 1944 in Brazil (Coutinho et al. 1998). Since then the fungus has spread to the Caribbean Islands and several other regions of the world, including South Africa (Coutinho et al. 1998), Florida (Marlatt and Kimbrough 1979; Rayachhetry et al. 1997), Hawaii (Uchida and Zhong 2006), Japan (Kawanishi et al. 2009), Australia (Carnegie et al. 2010), Hainan, China (Zhuang and Wei 2011), and most recently, South Africa and New Caledonia (2013), Indonesia (2016), and Singapore (2017).

Rust caused by *A. psidii* was first described by George Winter, in guava (*Pisidium guajava* L.) collected by Ernest H. G. Ule from Santa Catarina State, Brazil. This fungus belongs to the Family Sphaerophragmiaceae, Order Pucciniales, Class Pucciniomycetes, Phylum Basiodiomycota, and occurs in the Americas, from the southern United States to Argentina.

Structural mechanisms, which can be divided into pre-formed or constitutive mechanisms and post-formed mechanisms (i.e., originating after the pathogen–host contact), are important in providing the host with resistance to infection. These structural mechanisms include host production of biochemical resistance factors that are toxic to the pathogen or creation of adverse conditions that impair the development of the pathogen within the host. These substances occur at high concentrations in healthy tissues when they are pre-formed, and at low levels before infection when they are post-formed substances.

Members of the Myrtaceae family have protrusions in their leaf anatomical structure, which resemble the lenticels found in *Eucalyptus* and *Eugenia*; epidermis covered with wax in some *Eucalyptus* species; stomata on both leaf surfaces; and mesophyll showing isobilateral or central structure, which subsequently becomes a dorsiventral structure; hypodermis located below the epidermis may also occur in the beginning of leaf development in some *Eucalyptus* species (Metcalfe and Chalk 1957).

Studying leaf anatomy is important for conducting a comparative analysis among diverse hosts in different leaf stages so that a relationship between the resistance level and the amount of tissue that may constitute barriers to infection can be established.

2 MATERIALS AND METHODS

2.1 Definition of Leaf Maturity Stages Based on Morphological Evaluations

To study and define the maturity stages of eucalyptus leaves, 15 leaves from each of three *Eucalyptus grandis* trees were collected and analyzed. Leaf blade length and width were measured with a graphics tablet, leaf area was obtained with an area meter, and petiole diameter was determined with a digital caliper. The shape of the leaf blade and its apex and base were determined using the classification proposed by Hickey (1973); leaf colour was determined based on the *Exotical Horticultural Color Guide* (Graf 1970).

The data obtained were used to discriminate between five leaf maturity stages, which led to the elaboration of the differentiation grid shown in the Results section. Cluster analysis and principal components analysis were used to analyze data related to quantitative characteristics (Sneath and Sokal 1973).

2.2 Anatomical Study of Healthy Leaves

Healthy leaves of *Syzygium jambos* and *Eucalyptus urophylla* were collected from the arboretum of the Department of Plant Production, School of Agriculture, São Paulo State University (UNESP), Botucatu Campus, Brazil, and were evaluated.

Austropuccinia psidii manifests especially in the first pairs of leaves and organs in early development. Thus, three leaf stages were established for this anatomical study: the first stage (E1) was considered susceptible, the third stage (E3) was moderately resistant, and the fifth stage (E5) was resistant to rust, according to previously obtained results (unpubl. data).

Samples were collected from the middle third of the leaf blade, and after fixation in FAA 50 (formaldehyde + glacial acetic acid + 50% alcohol) for 48 hours, were stored in 70% alcohol (Johansen 1940). Leaf samples from the interveinal region and the midvein were embedded in glycol methacrylate resin according to the technique of Gerrits et al. (1991), then were transversally sectioned in a rotary microtome to 8mm thickness, and were stained with 0.05% toluidine blue, pH 4.7, and mounted on synthetic resin (O'Brien et al. 1964). For each leaf stage and each species, three replicates were taken so that more consistent results could be obtained. Leaf structures were drawn with a projection microscope, then the diverse tissues present in the structure were measured with a graphics tablet coupled to specific software.

For the interveinal region, the following traits were determined: percentages of upper (adaxial) and lower (abaxial) epidermis, upper and lower palisade parenchyma, and lacunar parenchyma, as well as leaf thickness and oil cavity area. For the midvein region, the following traits were determined: percentages of upper (adaxial) and lower (abaxial) epidermis, vascular bundle, collenchyma, sclerenchyma, and parenchyma.

The data underwent cluster analysis and principal components analysis (Sneath and Sokal 1973) to delineate anatomical differences between the studied species at different leaf stages, which could be related to susceptibility or resistance to rust.

2.3 Anatomical Analysis of Plants Inoculated with *Astropuccinia psidii*

2.3.1 Inoculum preparation Material containing fungal spores was collected from younger leaves of *S. jambos*. Uredospores from infected leaves of inoculum multiplier plants were removed with a scalpel and deposited on a Petri plate containing a solution of distilled water and a drop of Tween 20. The spore suspension was filtered into a beaker using a gauze funnel, which allowed the passage of spores but retained leaf fragments.

The initial volume of the suspension was recorded, and an aliquot of the mixture was collected and placed in a Neubauer chamber or hemocytometer for observation under a microscope and to count spores in order to obtain the initial concentration. The concentration was adjusted to 105 spores/mL, and the mixture was applied with a micropipette on the top of the branch, in the first pair of leaves of *Eucalyptus* and *S. jambos* seedlings. For more efficient anatomical analysis, adhesive tape with a hole in its centre was used to delimit the spore deposition site.

Following inoculation, seedlings were kept in a dark chamber for 48 hours. They were then transported to the inoculation room in the Department of Plant Protection, School of Agriculture, UNESP, where temperature, photoperiod, and air relative humidity were controlled, which allowed better fungal growth.

2.3.2 Post-infection anatomical studies After 15 and 30 days of inoculation, the marked leaves were collected, and anatomical analysis was conducted to detect the presence of the fungus and changes it caused to the leaf structures.

The technique used for post-infection anatomical analysis, considering leaf fixation and infiltration processes, was the same as that used for healthy plants, except that tissue measurements and multivariate statistical tests were not employed.

The data were considered sufficient to discriminate between leaf stages and to establish a differentiation grid and a visual scale based on photographs of leaf maturity stages of eucalyptus plants.

3.1 Analysis of Quantitative Traits

The quantitative traits analyzed during this study are represented in Table 1, showing the rate of growth over time.

TABLE 1   *Mean values of morphological characteristics of eucalyptus leaves in five maturity stages*

Stage	Blade length (cm)	Blade width (cm)	Leaf area (cm^2)	Petiole diameter (mm)
1	2.04	0.56	0.47	0.58
2	3.79	1.33	2.92	0.86
3	5.72	2.31	8.00	0.96
4	9.04	4.16	23.50	1.31
5	10.20	5.09	31.75	1.49

A cluster analysis of the data on the quantitative traits of leaves was conducted using the mean Euclidean distance among the five leaf maturity stages. Leaf maturity stages formed three clusters at Euclidean distance 0.20: the first group included stages 1 and 2; the second group included stage 3; and the third group included stages 4 and 5 (Figure 1).
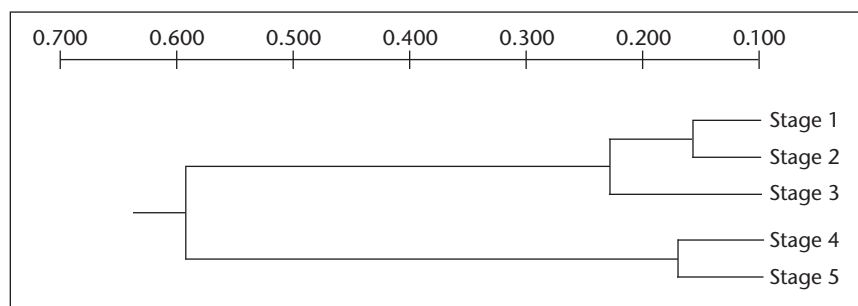
FIGURE 1   *Cluster analysis of quantitative traits for the five maturity stages of eucalyptus leaves, based on Euclidean distance.*

Correlations among the four quantitative traits of the five maturity stages of eucalyptus leaves and the two first principal components (Y1 and Y2) are shown in Table 2. Satisfactory values, close to 1, were obtained for blade length, blade width, leaf area, and petiole diameter. These values indicate that the analyzed characteristics had high discriminatory potential regarding the leaf maturity stages; that is, the analyzed quantitative traits are sufficient to separate the three clusters formed among leaf stages. The high contribution of such quantitative characteristics to the differentiation among leaf maturity stages is related to the higher absolute values of Y1, which was responsible for 99.03% of variability accounted for by the traits, and shows its high efficiency as a dissimilarity indicator.

In the principal components analysis of the five maturity stages of eucalyptus leaves (Figure 2), the clustering of stages was identical to that formed by the

TABLE 2   *Correlations among the quantitative traits of the five maturity stages of eucalyptus leaves for the first two principal components (Y1 and Y2)*

Quantitative traits	Y1	Y2
Blade length	0.9947	−0.0952
Blade width	0.9994	−0.0254
Leaf area	0.9937	0.1119
Petiole diameter	0.9903	−0.1060
Retained information (%)	99.03	0.83
Accumulated information (%)	99.03	99.86
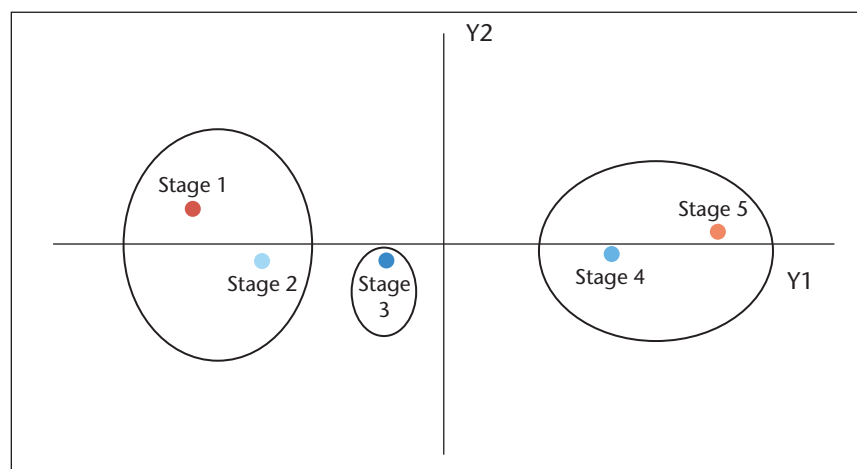
FIGURE 2   *Dispersion of the five maturity stages for the quantitative traits of eucalyptus leaves.*

cluster analysis (Figure 1). Stages 4 and 5 showed the highest values for blade length, blade width, leaf area, and petiole diameter, while stages 1 and 2 had the lowest values, and stage 3 showed intermediate results (Table 2; Figure 2).

3.2 Analysis of Qualitative Traits

Blade shape did not show discriminatory potential because it was oval in all stages; the same was found for blade apex shape, which was always acute (Table 3). Blade base shape was important for differentiating the first stage, which was the only stage that had an acute blade base. Blade colour allowed for differentiation of the first and fifth stages but was not useful for the other stages, among which the characteristic was equal.

TABLE 3   *Qualitative leaf traits*

Stage	Blade shape[a]	Blade apex shape[a]	Blade base shape[a]	Blade colour (colour no.)[b]
1	Oval	Acute	Acute	Chocolate (7)
2	Oval	Acute	Obtuse	Olive green (84)
3	Oval	Acute	Obtuse	Olive green (84)
4	Oval	Acute	Obtuse	Olive green (84)
5	Oval	Acute	Obtuse	Ivy green (70)

a   Classification of leaves (Hickey 1973).
b   Exotica Horticultural Color Guide (Graf 1970).

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3.3 Identification Grid for the Five Maturation Stages of Eucalyptus Leaves, and Visual Scale (photographic)

Quantitative and qualitative analyses were grouped to develop a differentiation grid for the maturity stages of eucalyptus leaves (Table 4) and a photographic visual scale (Figure 3), both of which allow feasible and practical diagnosis of the maturity stage of a leaf of *Eucalyptus urophylla* either in the field or in the laboratory. Learning the maturity stage of a eucalyptus leaf, infected or not by the studied pathogen, is easy with the proposed differentiation grid or photographic scale since such information can be obtained by simply measuring the blade length, as well as the other proposed traits: blade width, leaf area, petiole diameter, blade base shape, and blade colour. If the leaf maturity stage needs to be detected in the field, use of the photographic scale is the best option since no other tool is required, although accuracy may be reduced.

TABLE 4   *Differentiation grid for the five maturity stages of eucalyptus leaves based on quantitative and descriptive traits*

Stage	Blade length[a] (cm)	Blade width[a] (cm)	Leaf area[a] (cm^2)	Petiole diameter[a] (cm)	Blade base shape[b]	Blade colour[b] (colour no.)
1	2.04	0.56	0.47	0.58	Acute	Chocolate (7)
	(1.66–2.38)	(0.45–0.65)	(0.31–0.58)	(0.51–0.65)	73%	71%
2	3.79	1.33	2.92	0.86	Obtuse	Olive green (84)
	(3.45–4.31)	(1.18–1.45)	(2.27–3.75)	(0.79–0.91)	73%	53%
3	5.72	2.31	8.00	0.96	Obtuse	Olive green (84)
	(5.02–6.17)	(2.19–2.46)	(7.19–8.98)	(0.88–1.03)	73%	51%
4	9.04	4.16	23.50	1.31	Obtuse	Olive green (84)
	(8.34–9.51)	(3.75–4.67)	(19.22–27.57)	(1.20–1.38)	60%	84%
5	10.20	5.09	31.75	1.49	Obtuse	Ivy green (70)
	(10.11–10.28)	(4.78–5.69)	(30.43–33.23)	(1.41–1.62)	73%	98%

a Minimum and maximum mean values of quantitative traits.
b Frequency of descriptive traits.
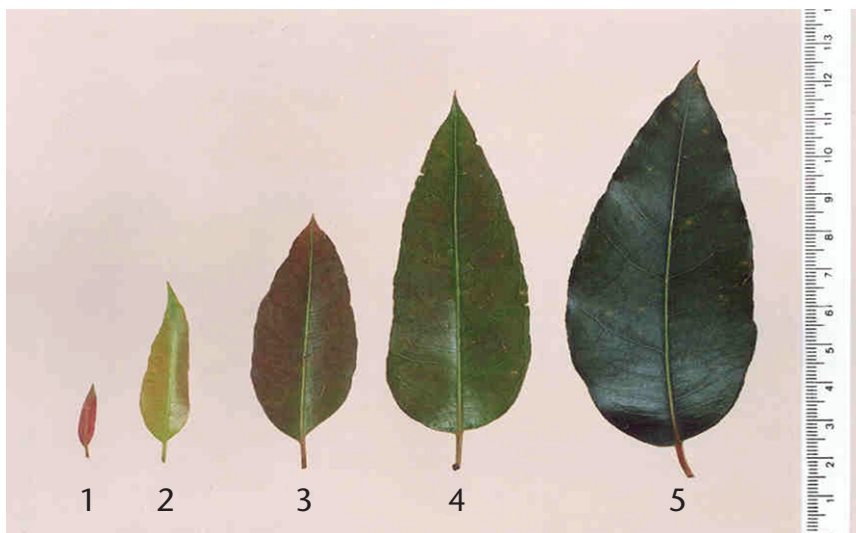
FIGURE 3   *Photographic visual scale of eucalyptus leaves in five maturity stages.*

3.4 Anatomical Studies of Healthy Leaves

The first contact between the pathogen and the host is through the cuticle, a lipid layer that covers the cell wall of epidermal cells. The presence of wax and an insoluble polymer gives a hydrophobic character to this structure, which prevents water film formation and consequent fungal germination. Thus, cuticle thickness is considered a pre-formed structural resistance factor. Structural barriers formed after penetration of the fungus and its progress through leaf tissues also provides plant resistance to a pathogen attack.

The mean values of 13 quantitative structural traits for *E. urophylla* and *S. jambos*, in three leaf maturity stages, are shown in Tables 5 and 6. The values in Table 7 correspond to the coefficient of correlation among the 13 quantitative structural traits and the two first principal components (Y1 and Y2). These two components were responsible for 68.82% of information accounted for by the evaluated traits, and show them as indicators of dissimilarity between the studied species and their corresponding leaf stages. The high contribution of these quantitative traits to species differentiation is related to the higher absolute values of Y1 and Y2, which are characterized as having greater discriminatory potential.

TABLE 5   *Mean values of six quantitative structural traits of the midvein region of the leaves of* Eucalyptus urophylla *and* Syzygium jambos *in three maturity stages*

Species	Leaf stage	Midvein regions[a]					
		UE (%)	LE (%)	VB (%)	COL (%)	SCL (%)	PAR (%)
Eucalyptus urophylla	1	3.27	2.85	27.42	15.04	13.11	38.31
	3	3.19	3.56	30.16	11.89	8.66	42.53
	5	2.55	3.11	30.26	9.71	9.53	44.83
Syzygium jambos	1	3.96	4.35	27.56	9.32	9.02	45.79
	3	3.67	3.50	32.95	9.01	9.06	41.81
	5	3.25	3.78	37.87	8.86	5.35	40.88

a   UE: upper epidermis (adaxial), LE: lower epidermis (abaxial), VB: vascular bundle, COL: collenchyma, SCL: sclerenchyma, PAR: parenchyma.

TABLE 6   *Mean values of seven quantitative structural traits of the interveinal region of the leaves of* Eucalyptus urophylla *and* Syzygium jambos *in three maturity stages*

Species	Leaf stage	Interveinal regions[a]						
		UE (%)	LE (%)	UPP (%)	LPP (%)	LP (%)	LT (μm)	OCA ($\times 10^{-4}$ mm^2)
Eucalyptus urophylla	1	9.57	5.47	26.65	33.39	24.93	171.26	17.87
	3	8.54	6.93	23.38	0	61.15	161.56	48.29
	5	7.84	6.95	24.64	0	60.58	187.22	48.68
Syzygium jambos	1	8.67	5.27	12.20	19.15	54.72	229.41	45.07
	3	7.09	4.36	22.30	0	66.26	305.72	55.42
	5	5.75	4.01	20.37	0	69.87	337.53	50.55

a   UE: upper epidermis (adaxial), LE: lower epidermis (abaxial), UPP: upper palisade parenchyma, LPP: lower palisade parenchyma, LP: lacunar parenchyma, LT: leaf thickness, OCA: oil cavity area.

Coefficients of correlation among the 13 quantitative structural traits of the leaf blade of Eucalyptus urophylla *and* Syzygium jambos *and the two first principal components (Y1 and Y2)*

Traits	Y1	Y2
% upper epidermis (midvein)	−0.4985	0.4891
% lower epidermis (midvein)	−0.2754	0.4467
% vascular bundle (midvein)	0.7538	0.1030
% collenchyma (midvein)	0.8116	0.1911
% sclerenchyma (midvein)	0.6640	0.4612
% parenchyma (midvein)	−0.8832	−0.3422
% upper epidermis (interveinal region)	−0.6810	0.2248
% lower epidermis (interveinal region)	−0.2743	0.5036
% upper palisade parenchyma (interveinal region)	0.0048	0.8124
% lower palisade parenchyma (interveinal region)	−0.9360	0.0706
% lacunar parenchyma (interveinal region)	0.8168	−0.4755
Leaf thickness (interveinal region)	0.5827	−0.5930
Oil cavity area	0.7136	−0.3971
% variability accounted for	52.37	16.45
% cumulative variability accounted for	52.37	68.82

According to Table 7, for the principal component Y1, the most relevant traits for discriminating between the studied species were vascular bundle, collenchyma, and parenchyma, belonging to the midvein; and lower palisade parenchyma, lacunar parenchyma, and oil cavity, corresponding to the interveinal region of the leaf. This first principal component (Y1) accounted for 52.37% of the variation in the data set, which is considered satisfactory for explaining the contribution of the analyzed structural traits to the clustering of the two studied species for the three leaf maturity stages 1, 3, and 5.

The second principal component (Y2) accounted for 16.45% of the variation in the data set (Table 3), which is considered low; consequently, only the upper palisade parenchyma of the interveinal region had satisfactory discrimination potential; the remaining traits did not contribute to differentiation.

According to Figures 5 and 11, corresponding to the interveinal region of the leaf, in the first maturity stage, there is isobilateral parenchyma; that is, the parenchyma has palisades on both faces. In the third maturity stage (Figures 7 and 13), some differentiation is noted with the formation of lacunar parenchyma on the lower surface, which is more evident in the fifth stage (Figures 9 and 15). The formation of lacunar parenchyma was also reported for other species of Myrtaceae and can be considered an anatomical characteristic of the family. This trait can be related to the pathology of the rust, since in the first stage cells are more closely grouped, which allows better internal fungal growth. However, in more advanced stages, such as the third and fifth stages, larger intercellular spaces are formed, which may impair the action of the pathogen.
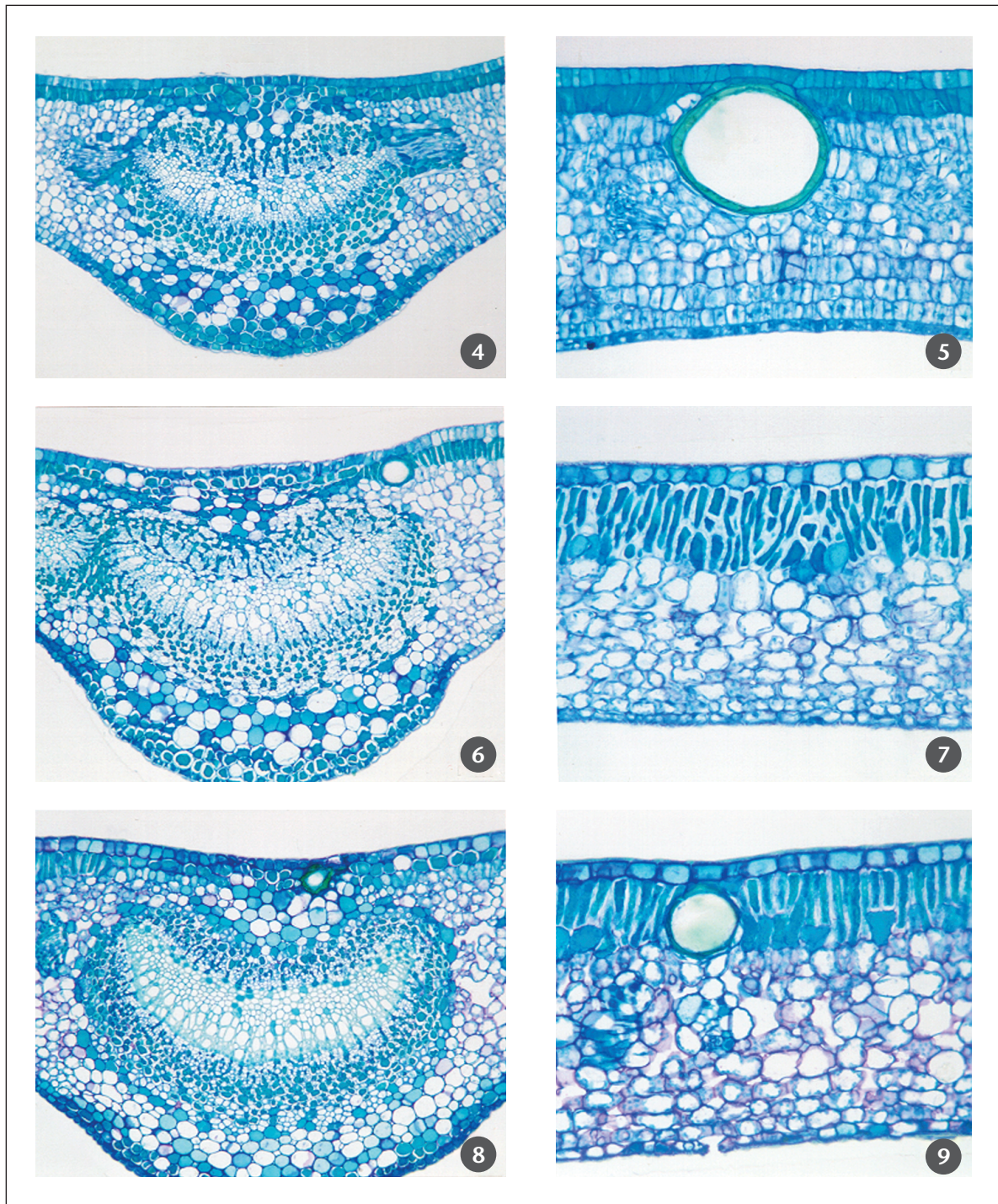
FIGURES 4–9   *Leaf blade structure of* Syzygium jambos *at three maturity stages. Figures 4, 5: stage 1; Figures 6, 7: stage 3; Figures 8, 9: stage 5; Figures 4, 6, 8: transverse sections of the midvein; Figures 5, 7, 9: transverse sections of the interveinal region. Bar = 92 µm (Figures 4, 6, 8); 46 µm (Figures 5, 7, 9).*

FIGURES 10–15 *Leaf blade structure of* Eucalyptus urophylla *at three maturity stages. Figures 10, 11: stage 1; Figures 12, 13: Stage 3; Figures 14, 15: stage 5; Figures 10, 12, 14: transverse sections of the midvein; Figures 11, 13, 15: transverse sections of the interveinal regions. Bar = 92 μm (Figures 10, 12, 14), 46 μm (Figures 11, 13, 15).*

3.5 Anatomical Analysis of Infected Plants

Figures 16–19 show the formation of rust sori on the epidermis of the leaf abaxial and adaxial faces as well as uredospore release. Sori are developed by sporulation from the upper and lower palisade parenchymas after fungal colonization of leaf tissues. This was recorded for both *S. jambos* and *E. urophylla*, which shows their susceptibility to *A. psidii*.
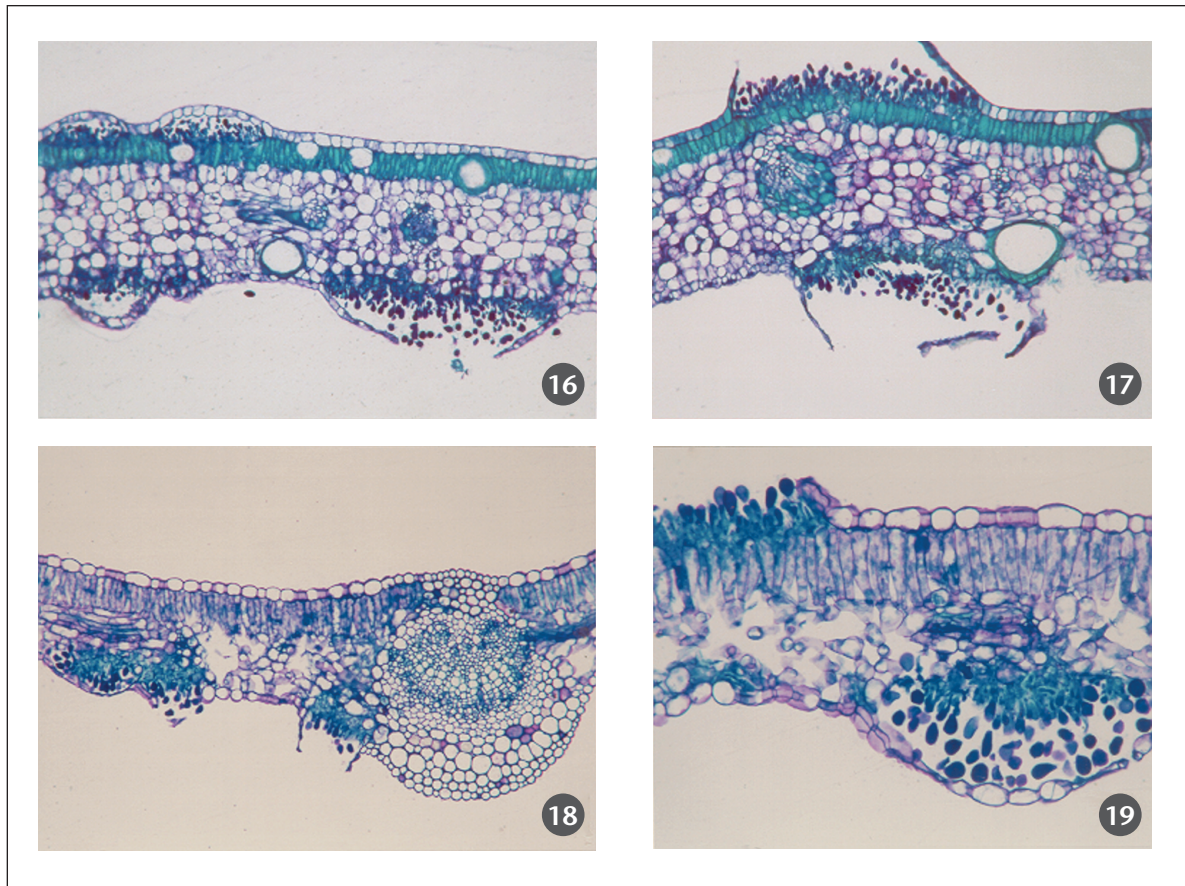
FIGURES 16–19 *Transverse sections of the leaf blade structure of* Syzygium jambos *and* Eucalyptus urophylla *at 15 days after inoculation with* Austropuccinia psidii. *Figures 16, 17:* S. jambos; *Figures 18, 19:* E. urophylla. *Bar = 92 μm (Figures 16, 17, 18); 46 μm (Figure 19).*

Absence of contents retained in the oil glands after pathogen attack makes this leaf structure worthy of note, and the oil can be considered a probable nutrient source that favours fungal growth.

4   CONCLUSIONS

- The parameters vascular bundle, collenchyma, and parenchyma of the midvein and the interveinal region, as well as lower palisade parenchyma, lacunar parenchyma, leaf thickness, and oil cavity area showed discriminatory potential for the three leaf maturity stages: 1, 3, and 5.
- The evaluated parameters allowed for differentiation between the genera *Eucalyptus* and *Syzygium* at leaf maturity stages 1 and 5.
- Leaf maturity stages 2 and 3 were correlated with the susceptibility of *S. jambos* and *E. urophylla* to the fungus *A. psidii*.
- Inoculation tests demonstrated the susceptibility of *S. jambos* and *E. urophylla* to the fungus *A. psidii* based on their similarity in stages 2 and 3.

- *S. jambos* and *E. urophylla* in the first maturity stage have isobilateral parenchyma; i.e., their parenchyma is palisade on both leaf faces.
- *S. jambos* and *E. urophylla* in the third maturity stage show differentiation due to lacunar parenchyma formation on the lower face, which is more evident in the fifth stage.
- Absence of content retained in the oil glands after the pathogen attack makes this leaf structure worthy of note, and the oil can be considered a probable nutritional source that favours fungal growth.

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21. Alien Rust Fungi in Switzerland

Ludwig Beenken[1][**] and Valentin Queloz[1][*]

1 Swiss Federal Research Institute WSL, Forest Health and Biotic Interactions
*Presenting author
**Corresponding author: ludwig.beenken@wsl.ch

ABSTRACT

Rust fungi (Pucciniales) are obligatory biotrophic plant parasites that form up to five spore states on up to two host plant species. This complex life cycle and the fact that most rust fungus species have high host specificity make it difficult for a fungus species to establish in a new area. The corresponding plant host species must be present for the fungus to complete its life cycle. Nevertheless, 26 rust fungi were detected among 283 neomycetes in Switzerland. These included species that can complete their entire life cycle on one host species. For example, *Puccinia lagenophorae* was introduced from Australia and grows on the European *Senecio vulgaris*, or both the rust fungus and its host were introduced, as with *Cumminsiella mirabilissima* on *Mahonia aquifolium* from North America and *Puccinia komarovii* on *Impatiens parviflora* from Central Asia. In contrast, some alien rust fungi need two different hosts. One or both of the original hosts can be replaced by native plants; for example, *Melampsoridium hiratsukanum* on the native *Larix decidua* and *Alnus incana*, and *Cronartium ribicola* on the native *Pinus cembra* or the alien *Pinus strobus* as aecial host and native *Ribes* species as telial hosts. Rust species in which both hosts are alien are correspondingly rare: for example, *Puccinia sorghi* with *Oxalis stricta* and *Zea mays* as alternating hosts. None of the introduced rust fungi causes serious damage to native or introduced plants. No dangerous invasive rust fungi have been detected in Switzerland so far.

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22. The Effect of the Alien Rust Fungus *Melampsoridium hiratsukanum* on Riparian Alder Formations in the Eastern Italian Alps

Salvatore Moricca,[1*] Claudia Maria Oliveira Longa,[2] Alessandra Benigno,[1] and Giorgio Maresi[3]

1 Department of Agricultural, Food, Environmental and Forestry Science and Technology (DAGRI), Plant Pathology and Entomology Division, University of Florence, Florence, Italy
2 Department of Sustainable Agroecosystems and Bioresources, Research and Innovation Centre, Fondazione Edmund Mach., San Michele all'Adige, Trento, Italy
3 Centre for Technology Transfer, Fondazione Edmund Mach, San Michele all'Adige, Trento, Italy
*Presenting and corresponding author: salvatore.moricca@unifi.it

ABSTRACT

In approximately 20 years, the full-cyclic, alder rust pathogen *Melampsoridium hiratsukanum* has spread from the Baltics, where it was introduced from eastern Asia, through most of the European continent down to the Mediterranean countries. This exotic leaf parasite has now pervasively dispersed through several valleys of the eastern Italian Alps, where it causes yearly outbreaks on grey alder (*Alnus incana*). Owing to its high reproduction and dispersal capacity, conferred by its repeating uredinial stage, this rust has spawned repeated infection waves in valleys in the Alps for at least 10 years. The disease causes serious defoliation of grey alder during the summer, and has an infection rate that varies in relation to temperature and moisture trends but always remains quite high. *M. hiratsukanum* is damaging to grey alder stands, but the main concern is its possible effect on riparian alder formations due to the ecological processes it could trigger. Because of the high incidence and severity of the rust on grey alder, the tree species seems to be gradually declining in occurrence along mountain waterways and is being replaced by various non-native herbaceous and shrub species. The replacement of grey alder, a dominant riparian species in the Alps, by non-native invasive plants is causing a loss of stability in banks along mountain streams, which is leading to an increase in landslides. It seems, therefore, that we are witnessing a classic and all-too-common theme of invasion ecology: the arrival of a non-native organism into a new habitat, with negative effects on the native flora. *M. hiratsukanum* is altering the structure and dynamics of riparian ecosystems in the Alps by weakening grey alder and causing it to be driven out by invading competitors.

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23. *Pinus cembra*'s Last Stands? Small and Isolated Populations of Stone Pine in the Eastern Alps in Past and Current Climate Change

Berthold Heinze,[1*] Martin Gollobich,[2] Hannah Hochsattel,[3] and Lara Eigner[3]

1 Research Scientist
2 Former Master's student
3 Current Master's students, Unit of Genome Research, Department of Forest Genetics, Austrian Federal Research Centre for Forests (bfw), Vienna, Austria
*Presenting and corresponding author: berthold.heinze@bfw.gv.at

SUMMARY

This presentation focusses on small and isolated populations of stone pine (*Pinus cembra*) in the Eastern Alps during past and current climate change. We want to determine how the species survived past climate fluctuations, how those changes determined the species' current distribution range margins, and which conclusions this might provide for current climate-change scenarios.

P. cembra is the only five-needle pine in the Alps, and is a close relative of *P. sibirica* and *P. albicaulis*. It occurs in the subalpine belt in the drier inner Alps, together with *Larix decidua, Picea abies,* and *Pinus mugo*. It seems to be at an advantage in these more continental zones, which are characterized by less precipitation than the outer chains, and by higher temperature amplitudes (Tschermak 1950). *P. cembra* exhibits a close ecological "partnership" with the Eurasian Nutcracker (*Nucifraga caryocatactes*), which distributes the pine's wingless, heavy seeds by creating caches intended for winter supplies. *P. cembra* was an early pioneer after glaciation, when it formed the highest forest belt, which moved up the slopes, generation after generation.

Presently, *P. cembra* seems to be threatened by climate change, as in many instances there does not seem to be enough room to move further upward in elevation if other species advance from below—at least this seems to be the case in the margins of *P. cembra*'s range, where mountain chains and peaks are not as high. Can isolated outposts act as models for climate-change effects?

Compared with *P. cembra* stands in the Eastern Alps more than 100 years ago (Nevole 1914), the species' current distribution range seems to have contracted, and the stands appear to be smaller and more fragmented, at least at the margins of the species' range. For example, the Koralpe chain, which occurs at the southeast edge of the Alps in Styria and Carinthia (Austria), where its highest elevation is approximately 2000 m above sea level, was a grazing pasture, with *P. cembra* present in the uppermost forest belt. However, *P. cembra* has completely disappeared from that area, aside from recent reforestation trials. Nevole (1914) also described declining populations at neighbouring Zirbitzkogel, just to the west across a wide valley (approximately 2300 m elevation), but today, these are vital forests, with only few old trees remaining. Additionally, a small stand of apparently fewer than 100 trees exists south of Koralpe at Mount Petzen on the

border with Slovenia. Why did stands such as those on Zirbitzkogel recover but not others such as those on Koralpe? How did stands such as those at Mount Petzen become isolated, and will they be able to recover? We want to determine whether there are any genetic reasons for these distributions, and whether other marginal populations go through similar phases.

The stands of *P. cembra* often appear to be "trapped" on mountaintops. For example, in Gesäuse National Park (northern Styria), a main population occurs around the central mountaintops, but on Tamischbachturm, just across the narrow valley of the Enns River, there are fewer than 10 trees. We speculate that the behaviour of the Eurasian Nutcracker may be responsible for these differences because the birds usually cache seeds in the vicinity (often upward in elevation) of existing stands but may not cross deep valleys when carrying seeds.

In a genetic comparison among stands (Gollobich 2016), six stands on west–east and north–south transects in the Eastern Alps were sampled, and 24 adults from each stand were analyzed with simple sequence repeat (also called microsatellite) DNA markers. Although within-stand relatedness was somewhat low in the extremely marginal Petzen population (compared to theoretical expectations), some other stands had similar values (Figure 1).
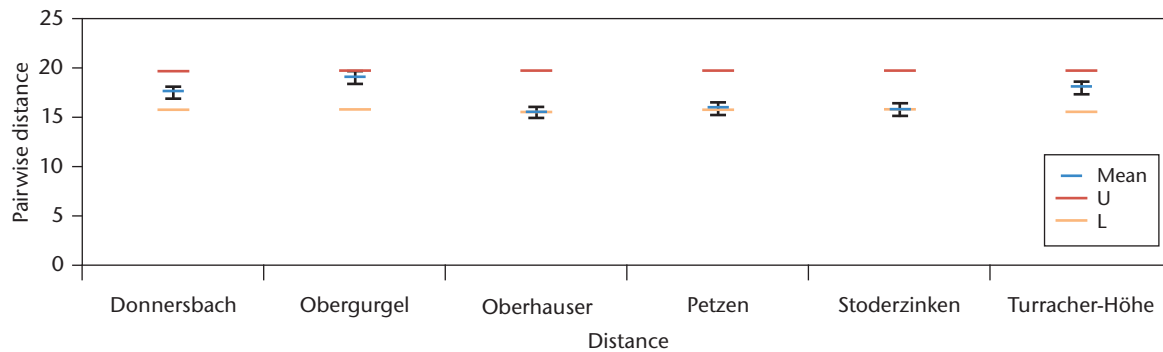
FIGURE 1   *Mean within-population pairwise distance values, as calculated by the GenAlEx software (http://biology-assets.anu.edu.au/GenAlEx/Welcome.html, accessed Dec. 31, 2019 [Peakall and Smouse 2012]), for six* Pinus cembra *stands in the Austrian Alps.*

A scatterplot showed that the very small Petzen stand still retains much genetic variation, and that geographic distance does not offer a good explanation for the observed genetic distances among the sites (Mantel test, $R^2 = 0.0167$) (Gollobich 2016). In a model-based clustering approach implemented in the software Structure (Hubisz et al. 2009), only slight differentiation among the sites was detectable. We conclude that under the very long generation times of these pines, genetic diversity still reflects the past distribution of *P. cembra*, but the genetic data are compatible with a contracting range. There is no significant inbreeding yet. The contracting range is a recent phenomenon in terms of tree generations.

Can genetic data from current recruitment explain the slow-to-absent genetic erosion? In an ongoing study in the Berchtesgaden National Park in southern Germany (H. Hochsattel, unpubl. data), where *P. cembra* is present in the subalpine belt (1400–1914 m elevation), we are examining whether there is an eventual depletion of genetic variation from the parents to the next generation by estimating gene flow among three close subpopulations. We want to determine whether conservation of the small stands is feasible under these circumstances. In the three subpopulations—Funtensee, Sittersbachtal (Blaueistal/Klausbachtal), and

Reiteralp—five plots with a total of 288 trees were established; adults and young trees were sampled and analyzed with nuclear and chloroplast microsatellites. Young trees in these subpopulations seem to "collect" more diversity than adults locally offer—an indication of intact gene flow mediated by pollen. There was greater (chloroplast genetic; i.e., pollen-mediated) diversity in young trees than in adult trees in three of four cases. The three sites are differentiated (based on nuclear microsatellites), but not the adult versus the young trees at individual sites. In a sibship and parentage reconstruction exercise, we found local parents for the young trees. We are currently collecting more data about genetic recruitment patterns in a different national park (Gesäuse, Styria; L. Eigner, unpubl. data), where another extremely small, detached fragmented stand of fewer than 10 trees was discovered.

Our preliminary conclusion is that recruitment seems to be episodic—there are plots with higher and lower genetic variation. In some cases, more trees may contribute to the next generation than in other cases, depending on each specific plot. This resembles a metapopulation structure: small, marginal populations contract and expand over time. Only in their entirety do they conserve the standing genetic variation. It may be better to conserve "several (or all) small" rather than "one large" population (if this means disregarding the small ones) at the margins of the species' range.

In a model of *P. cembra*'s historical range and elevation shifts (Mayer 1974; Heinze and Holzer 2013), warmer climate periods in the past have led to the disappearance of the species in lower mountain ranges (with less Massenerhebung effect) because other competitors, such as *Picea abies* and *Larix decidua*, were advancing upward in elevation. During the cooler last few centuries, *P. cembra* could thus not regain ground on the lower mountain ranges and remained "trapped" on the higher ones.

Building a future for *P. cembra* on the basis of the effects of past climate change and on current differentiation and recruitment data thus requires a consideration of geography—the overall size of a mountain range (large ones may buffer in times of climate change). Small, marginal ranges may retain *P. cembra* or not, in a somewhat stochastic process. We need to study generation times in the past in more detail, and their relation to climate-change events. Currently, restoration is also hampered by tourism, grazing, and increased game pressure, and the fact that most land is privately owned.

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24. Evolution and Speciation of the Chinese White Pines

Xiao-Xin Wei,[1*] Yan-Yan Liu,[1,2] Wei-Tao Jin,[1] and Xiao-Quan Wang[1]

1 State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences, Beijing, China
2 College of Plant Protection, Henan Agricultural University, Zhengzhou, China
*Presenting and corresponding author: weixx@ibcas.ac.cn

ABSTRACT

East Asia, one of the two species diversity centres of five-needle pines, harbours 11 species and some varieties. Despite a long history of systematic study, there are still many taxonomic uncertainties in these species, especially those in subtropical China, due to their similar morphological traits, broad introgression and recent divergence, and restricted or discontinuous distributions. We investigated the evolutionary relationships of the seven species of five-needle pines in subtropical China by using phylotranscriptomic analysis. Combined with population genetic analysis and ecological niche modelling, we also explored the process of speciation in *Pinus armandii*, an endemic five-needle pine in China that has the broadest geographic distribution of the group. Our study generated a well-resolved phylogeny of these species, and found that all of them form their own monophyletic groups. Two main clades, one including *P. armandii, P. dabeshanensis,* and *P. fenzeliana*, and the other comprising *P. bhutanica, P. kwangtungensis,* and *P. wangii*, were recognized, which largely correspond to their geographic distribution and seed characteristics. Molecular dating suggests that the initial uplift of the Hengduan Mountains probably served as a major force in the separation of the ancestor of the *P. armandii* group from the Himalayan white pine (*P. wallichiana*). We also found that cryptic speciation has occurred between the northern and southern *P. armandii* subdivisions. During the process of speciation, geologically induced formation of Mount Gongga and other massive peaks might have triggered the initial vicariance isolation of the two lineages, and ecologically based selection then reinforced their differentiation and local adaptation. These findings imply that geography and ecology both contributed significantly to the high conifer species diversity in southwest China.

25. Whitebark Pine Restoration Strategy for the U.S. Forest Service, Pacific Northwest Region

Andrew Bower[1*]

1 U.S. Department of Agriculture Forest Service, Olympic National Forest, Olympia, Wash.

*Presenting and corresponding author: abower@fs.fed.us

ABSTRACT

There are more than 1.1 million acres (0.4 million hectares) of whitebark pine (*Pinus albicaulis*) habitat in Oregon and Washington, 96% of which is on U.S. Forest Service land. The U.S. Forest Service Pacific Northwest Region (Oregon and Washington) has allocated substantial financial and human resources for the conservation and restoration of whitebark pine. These efforts have followed a plan outlined in the *Whitebark Pine Restoration Strategy for the Pacific Northwest 2009–2013* (Aubry et al. 2008), which presents a comprehensive plan for reaching the goal of "a network of viable populations of whitebark pine throughout the Pacific Northwest." The key actions prescribed include:

- collecting seed for gene conservation, rust resistance screening, and reforestation;
- assessing stand conditions in priority management units;
- planting seedlings in areas where natural regeneration is lacking;
- thinning or removing competing vegetation to release whitebark pine;
- treating for mountain pine beetle in high-risk management units; and
- continuing a rust screening program.

Highlights of some of the activities that have been undertaken as prescribed in this strategy will be discussed.

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26. Progress and Challenge in Tree Breeding of Korean Pine (*Pinus koraiensis*) in South Korea

Kyu-Suk Kang,[1*] Yang-Gil Kim[1]

1 Department of Forest Sciences, Seoul National University, Seoul, Republic of Korea
*Presenting and corresponding author: kangks84@snu.ac.kr

ABSTRACT

Pinus koraiensis is a coniferous white pine that is native to Korea, Japan, and the Ussuri River basin of China and Russia. In the north of its range, it grows at moderate altitudes, typically 600–900 m, whereas further south, it grows at 2000–2600 m altitude. In subalpine forests, where *P. koraiensis* grows at 1300–2500 m, it can reach heights up to 30 m. The Korean pine is a softwood that is harvested for both its timber, used for furniture and construction, and for its edible seeds, which are a food staple in northern Asia. The wood is versatile and light, with a straight grain, and is easy to work with. It can easily be broken down into chips, or pulp for paper, or it can be used to create particle board. The seeds are edible and are sold commercially (Figure 1A). The seed oil contains the uncommon fatty acid pinolenic acid, which is used to make lubricants and soap. The Korean pine is also used as an ornamental tree. It is tolerant of several soil types (Figure 1B) and thrives in urban settings. It is adapted to climates with very cold winters. In South Korea, *P. koraiensis* is one of the major target species in tree breeding programs due to its economic, social, and ecological values. We will present the major achievements and status of tree breeding and gene conservation in five-needle pines, including *P. koraiensis, P. strobus,* and *P. pumila*, in South Korea (Figure 1C).

FIGURE 1   *(A) Cone and pine seeds of the Korean pine, (B)* Pinus strobus *plantation, (C) Korean pine seed orchard.*

27. Clonal Variation of Strobilus and Seed Production in a 1.5-Generation Seed Orchard of *Pinus koraiensis*

Yang-Gil Kim,[1*] Da-Bin Yeom,[1] Ji-Min Park,[1] Ki-Won Kim,[1] Hae-In Kang,[1,2] Sung-Joon Na,[2] Soon-Ho Kwon,[1] and Kyu-Suk Kang[1**]

1 Department of Forest Sciences, Seoul National University, Seoul, Republic of Korea
2 Department of Forest Bioresources, National Institute of Forest Science, Suwon, Republic of Korea
*Presenting author: rladidrlf@snu.ac.kr
**Corresponding author: kangks84@snu.ac.kr

ABSTRACT

Pinus koraiensis Siebold & Zucc., commonly known as the Korean pine, is a native five-needle pine species of Korea, northeastern China, eastern Russia, and Japan. Since its seed is edible and has high commercial value, studying its strobilus and seed production is important. For 5 consecutive years (2014–2018), we measured strobilus and conelet production among 53 clones in a 1.5-generation seed orchard of Korean pine in Gangwon province, Republic of Korea. In 2015, we collected the cones from each clone to analyze their morphological traits and seed productivity. First, the data showed a large difference in strobilus production among clones. Variation in male strobilus production was much larger than that for females. From 2014 to 2015, only two clones contributed half of all male strobilus production. Second, there was great annual variation in seed-producing strobilus. Top pruning was applied in December 2015; it reduced female strobilus and conelet production but increased male strobilus production. Lastly, cone analysis in 2015 showed high degrees of variation in morphological characteristics and viable seed numbers. From a commercial standpoint, clonal variation in seed production could be used for breeding Korean pines that have high seed productivity. However, the imbalance among the gamete contributions of each clone would reduce the genetic variation in seeds. Therefore, consistent monitoring and proper management should be used to maintain genetic diversity in seeds produced from the 1.5-generation seed orchard of *P. koraiensis*.

28. Field Testing of Range-Wide Populations of Whitebark Pine in British Columbia

Charlie Cartwright[1*]

1 B.C. Ministry of Forests, Lands, Natural Resource Operations and Rural Development, Cowichan Lake Research Station, Mesachie Lake, B.C.
*Presenting and corresponding author: Charlie.Cartwright@gov.bc.ca

ABSTRACT

Provenance tests with whitebark pine (*Pinus albicaulis*) were established at a dozen sites in British Columbia using range-wide seed sources. Almost 500 open-pollinated families from more than 60 populations were deployed. The primary purpose of the trials is to identify white pine blister rust (*Cronartium ribicola*)–resistant populations and parent trees for use in restoration. Test sites were selected to represent all biogeoclimatic zones in which the species grows in British Columbia, based on the expectation that the diverse conditions would allow for development of sound Climate Based Seed Transfer guidelines. Additionally, having multiple installations allows for the quantification of genotype-by-environment interaction as it pertains to rust resistance and plant vigour. Long-term field tests also permit the assessment of trait durability through different life stages. Early growth performance across a subset of sites is reported, in addition to survival data.

29. Pines (Including White Pine) versus Spruce: Comparative Fitness in an Elevated CO_2 × Soil Moisture Stress Factorial

John E. Major[1*] and Alex Mosseler[1]

1 Natural Resources Canada, Canadian Forest Service–Atlantic Forestry Centre, Fredericton, N.B.
*Presenting and corresponding author: john.major@canada.ca

ABSTRACT

Genetic variation in total biomass, biomass allocation, and physiological traits of four species of pines (*Pinus strobus, P. resinosa, P. banksiana,* and *P. rigida*) and spruce (*Picea glauca, P. rubens, P. mariana,* and *P. abies*) were quantified under a factorial of ambient and elevated CO_2 and soil moisture stress. A comparison of total biomass under elevated CO_2 (eCO_2) in relation to ambient CO_2 (aCO_2) by species showed that overall, the greater the species mass, the greater the mass gain under eCO_2 and the greater the mass loss under drought conditions. In addition, our results for spruces lend strong support to the theory that late-successional species have greater growth responses under eCO_2 than early to mid-successional species. A diverse portfolio of tree species for artificial reforestation would help forest management adapt to the many uncertainties about future environments and markets, but our results on responses in spruces and pines to eCO_2 and soil moisture stress support a shift toward increased use of pines in forest management and artificial reforestation.

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30. Identification of *Phytophthora* Species Associated with Western White Pine Mortality in British Columbia Tree Seed Orchards

Nicolas Feau,[1*] Berni van der Meer,[1] Wendy van der Linden,[1] Geoff Bradley,[2] Nicholas Ukrainetz,[2] Harry Kope,[3] and Richard C. Hamelin[1,4]

1 Department of Forest and Conservation Sciences, University of British Columbia, Vancouver, B.C.

2 B.C. Ministry of Forests, Lands, Natural Resource Operations and Rural Development, Vernon, B.C.

3 B.C. Ministry of Forests, Lands, Natural Resource Operations and Rural Development, Victoria, B.C.

4 Faculté de foresterie et géomatique, Institut de biologie intégrative et des systèmes (IBIS), Université Laval, Québec City, Que.

*Presenting and corresponding author: feaunico@mail.ubc.ca

ABSTRACT

British Columbia is a world leader in breeding trees that are well-suited to current and future ecological conditions. For example, western white pine (*Pinus monticola*) has been selected for local adaptation to the environment and for tolerance to the white pine blister rust caused by *Cronartium ribicola*. Over several years in seed orchards on Vancouver Island (Saanich) and in the Okanagan (Skimikin, Bailey Road, and Kalamalka), sudden mortality of western white pine that was resistant to white pine blister rust was observed. Diseased trees were light green to yellowish, with needles sharply bent downward at a point just below their attachment to the branch; mortality followed quickly. On dead trees, needles were brown and roots had necrotic tips typical of *Phytophthora* root rots. In total, 280 mycelial cultures obtained from the soil, root-baiting, and direct plating of symptomatic root tissues on CMA-PARPBH selective media were identified by DNA barcoding. Two *Phytophthora* species were detected: *Phytophthora cryptogea* on 32% of the trees from Saanich, and *Phytophthora cactorum* on 9% and 33% of the trees at Saanich and Kalamalka, respectively. In the two other orchards, only *Pythium* was found at high frequency. In artificial inoculation experiments, two isolates of *Phytophthora cryptogea* caused symptoms on western white pines and were readily re-isolated from necrotic roots, which suggested that this pathogen could be responsible for the mortality in the British Columbia seed orchards. Additional pathogenicity tests showed high susceptibility of whitebark pine (*Pinus albicaulis*) and Douglas-fir (*Pseudotsuga menziesii*) to *Phytophthora cryptogea*, which suggests that these conifers could become severely affected in contaminated seed orchards and nurseries.

31. Chemical and Anatomical Defences of Lodgepole Pine Relate to Infection by Western Gall Rust, *Endocronartium harknessii*

Jennifer G. Klutsch,[1*] Chen. X. Kee[1], and Nadir Erbilgin[1]

1 University of Alberta, Department of Renewable Resources, Edmonton, Alta.
*Presenting and corresponding author: klutsch@ualberta.ca

ABSTRACT

The distribution, composition, and concentration of chemical and physical defence characteristics determine tree resistance to attack by insects and pathogens. Our research investigates the association of western gall rust infection (causal agent *Endocronartium harknessii*, syn. *Peridermium harknessii*) of lodgepole pine (*Pinus contorta* var. *latifolia*) with chemical and anatomical defences. The mature lodgepole pine trees we examined were from four progeny trials in northern Alberta that were made up of 40 families. At each progeny site, more than half the trees were infected at a range of severities. By measuring monoterpene compounds and resin duct characteristics, we showed that there is an effect of infection on defences and that it can vary with family and site. Furthermore, we assess rust infection–induced changes in growth and defence relationships. Understanding the relationship between western gall rust infection and pine defence characteristics can contribute to our understanding of biological and co-evolutionary relationships, and can help inform tree breeding efforts to sustain healthy forests in Alberta.

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Poster 1 Whitebark Pine (*Pinus albicaulis*) Recovery: Testing Assisted Migration and Direct Seeding as Possible Restoration Methods

Iain R. Reid,[1][*] Sierra C. McLane,[2] and Sally N. Aitken[3]

1 MSc student, Department of Forest and Conservation Sciences, University of British Columbia, Vancouver, B.C.
2 National Park Service Director, Murie Science and Learning Center, Denali, Alaska
3 Professor and Associate Dean, Department of Forest and Conservation Sciences, University of British Columbia, Vancouver, B.C.
*Presenting and corresponding author: iain.reid@ubc.ca

ABSTRACT

Species distribution models predict that climatically suitable habitat for whitebark pine (*Pinus albicaulis*) will change substantially in the future, with losses in the southern part of the species' range and new potential habitat in northern and western British Columbia. However, due to the migration lag caused by delayed reproduction and slow dispersal, whitebark pine has not migrated fast enough since the last glacial maximum to fill the geographic distribution of its modelled climatic niche. We re-assessed an assisted migration trial of whitebark pine planted in 2007 and 2008 at eight sites ranging from 600 km southeast to 800 km northwest of the northern range margin. Seed was planted in areas predicted to be climatically suitable for whitebark pine both currently and in projected climates of the 2050s. Half the seeds were stratified and had seed coats clipped to facilitate faster germination (treated); the other half were left untreated. The Smithers common garden had the highest survival between 2010 and 2017 (76.3%); Whistler had the lowest (13.2%). Site climate variables that influenced survival included relative humidity ($r^2 = 0.32$) and spring precipitation ($r^2 = 0.30$), which were ranked based on r^2 values of linear quadratic models. Survival of seedlings originating from untreated seed was better in the long term compared to treated seed. Additionally, site climate was a much stronger predictor of treated seed survival compared to untreated seed. Growth was slow: seedlings at one of the northernmost sites near Haines Junction were the tallest (mean = 20.2 cm), whereas seedlings at the nearby but colder, drier Atlin site were shortest (mean = 5.1 cm). Site climate variables that influenced height included summer climatic moisture deficit ($r^2 = 0.29$) and degree-days above 18°C ($r^2 = 0.28$). Provenance differences were weak for both survival and growth. This study demonstrates that whitebark pine can survive in a model-predicted range, and direct seeding could be an effective restoration method in remote areas.

McLane, S.C. and S.N. Aitken. 2012. Whitebark pine (*Pinus albicaulis*) assisted migration potential: testing establishment north of the species range. Ecol. Appl. 22(1):142–153.

Poster 2 Development and Application of Marker-Assisted Selection Tools for Breeding of Western White Pine (*Pinus monticola* Douglas ex D. Don) Resistance to White Pine Blister Rust (*Cronartium ribicola* J.C. Fisch.) in British Columbia

Jun-Jun Liu,[1]* Holly Williams,[1] Arezoo Zamany,[1] Xiao-Rui Li,[1] Savannah Gellner,[1] and Richard A. Sniezko[2]

1 Pacific Forestry Centre, Canadian Forest Service, Victoria, B.C.
2 U.S. Department of Agriculture Forest Service, Dorena Genetic Resource Center, Cottage Grove, Oreg.
*Presenting and corresponding author: jun-jun.liu@canada.ca

ABSTRACT

Cronartium ribicola is a pathogenic fungus that causes white pine blister rust on five-needle pines worldwide. After accidental introduction into North America approximately 100 years ago, this invasive fungus has caused heavy mortality in western white pine (*Pinus monticola*) and other related white pine species across North America. Breeding by selection of rare resistant trees is time-consuming; therefore, the use of genomics-based tools is highly desirable to speed up the breeding process. Our laboratory previously constructed genetic maps for the *P. monticola Cr2* locus that confers major gene resistance to white pine blister rust and identified serval nucleotide-binding site leucine-rich repeat genes as *Cr2* positional candidates. In the present study, single nucleotide polymorphism (SNP) loci within *Cr2* candidate genes (conitg58688 and contig41490) were selected to develop qPCR-based genotyping arrays using two SNP genotyping technologies (TaqMan and Kompetitive Allele Specific PCR-KASP). To evaluate the utility and efficiency of these marker-assisted selection tools in the British Columbia breeding program, we analyzed 46 open-pollinated seedlots that were randomly selected across the British Columbia landscape. Single nucleotide polymorphism genotyping results were highly consistent, with matching rates of more than 99% between TaqMan and KASP arrays for each specific SNP locus. The *Cr2*-linked alleles were absent in all or approximately 90% of genotyped samples for the snp58688-82Y and snp41490-1778M loci, respectively. These results demonstrate that both TaqMan and KASP SNP genotyping arrays developed here are powerful marker-assisted selection tools for prediction of *Cr2* resistance in wild parental trees and for *Cr2* selection among progeny following cross-pollination between trees with partial resistance and major gene resistance across the British Columbia landscape.

Poster 3 Monitoring of *Cronartium ribicola* in Québec Forest Tree Nurseries Using Genome-Enhanced DNA Detection Assays

Philippe Tanguay,[1*] Marie-Josée Bergeron,[1] Nicolas Feau,[2] and Richard C. Hamelin[2,3]

1 Laurentian Forestry Centre, Canadian Forest Service, Natural Resources Canada, Québec City, Que.

2 Forest Sciences Centre, Department of Forest and Conservation Sciences, University of British Columbia, Vancouver, B.C.

3 Institut de biologie intégrative des systèmes (IBIS), Université Laval, Québec City, Que.

*Presenting and corresponding author: Philippe.Tanguay@canada.ca

ABSTRACT

Early investigations of the white pine blister rust (*Cronartium ribicola*) symptomology revealed that discriminant symptom development typically takes 2 years following initial infection, which results in an incipient canker, and 1 extra year for characteristic signs of *C. ribicola* to show as bright orange aecia. In Québec, 2 million 2-year-old white pine seedlings are planted annually. Given the large occurrence of white pine blister rust in young plantations, and the inadequacy of visual inspection to detect white pine blister rust–infected seedlings when shipping from nurseries, concerns have been raised about the level of white pine blister rust infection occurring in nurseries. Considering that identification of *C. ribicola* through isolation is almost impossible because of its obligate biotrophic life cycle, a culture-independent detection strategy was required. Whole-genome comparison was used to identify genome regions that are unique to the white pine blister rust fungus. These unique regions were then translated into three species- and two genus-specific real-time polymerase chain reaction assays. The developed assays were tested over a wide range of samples, including different spore types, different infected plant parts on the pycnio-aecial or uredinio-telial host, and captured insect vectors. One hundred percent detection accuracy was achieved for the targeted genus and species. The *C. ribicola* molecular assays were used in a 5-year trial to assess the level of white pine blister rust infection on stocks of seedlings that were ready to be shipped from nurseries for planting. Despite a few positive samples, most of the white pine seedlings were rust-free when leaving the nurseries.

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Poster 4 Whitebark Pine and Limber Pine Conservation in the Canadian Mountain National Parks

Natalie Stafl,[1*] Brenda Shepherd,[2] Robert Sissons,[3] Gregg Walker,[4] and Jane Park[5]

1 Ecologist Team Leader, Parks Canada Agency, Mount Revelstoke and Glacier National Parks
2 Conservation Biologist, Parks Canada Agency, Jasper National Park
3 Ecologist Team Leader, Parks Canada Agency, Waterton Lakes National Park
4 Acting Fire and Vegetation Specialist, Parks Canada Agency, Kootenay, Yoho and Lake Louise Field Unit
5 Fire and Vegetation Specialist, Parks Canada Agency, Banff National Park
*Presenting and corresponding author: Natalie.stafl@canada.ca

BACKGROUND

Whitebark pine (*Pinus albicaulis*) (Endangered [*Species at Risk Act*]) and limber pine (*Pinus flexilis*) (Endangered [Committee on the Status of Endangered Wildlife in Canada]) are key components of forested and subalpine ecosystems in the Rocky Mountains and Columbia Mountains. Both species are experiencing population declines and are at risk due to the combined effects of non-native white pine blister rust (*Cronartium ribicola*), fire and fire exclusion, mountain pine beetle, and climate change.

Five-needle pine restoration is not a new concern, and work is ongoing across the species' ranges in western North America. Within Parks Canada areas, a monitoring program for five-needle pine was established in 2003, and the first steps toward active restoration began approximately 10 years ago. In 2014–2015, the mountain parks were awarded a joint pool of Conservation and Restoration funding to continue and expand this work. Substantive progress has been made, but restoration efforts for these species require long-term commitments, with the involvement of partners, to effectively implement recovery actions.

The seven Canadian mountain national parks (Banff, Jasper, Waterton, Mount Revelstoke, Glacier, Yoho, and Kootenay) recently completed 5 years of conservation and restoration work for whitebark pine and limber pine in 2018. The outcomes of restoration treatments to promote blister rust resistance and conserve genetic diversity from 2014 to 2018 are presented, along with proposed restoration actions for the next 5 years.

VISION

To establish self-sustaining, rust-resistant metapopulations of whitebark pine and limber pine throughout their Canadian ranges.

A series of workshops that followed the methods established by the Open Standards for the Practice of Conservation was held in January 2018 and April 2019 with participants from not-for-profit, provincial, and federal agencies with the aim of developing a conservation strategy for whitebark pine and limber pine in order to implement the federal recovery strategy. During the meetings, it was determined that it is most appropriate for the mountain parks to work within a greater landscape area, along with not-for-profit, provincial, and federal partners. As a result, the scope of the project encompasses the entire range of limber pine and whitebark pine in Canada.

Through the Open Standards process, the group developed results chains that identify actions needed to improve the status of the species:

1. Increase the frequency of occurrence of trees that have resistance to white pine blister rust.
   1.1 Identify high-infection stands.
   1.2 Identify rust-resistant plus trees within high-infection stands.
   1.3 Conduct needs and options analyses for rust screening capacity.
   1.4 Increase rust screening capacity to match restoration needs.
   1.5 Collect scions and cones from plus trees and confirm rust resistance through testing.
   1.6 Establish seed orchards using scions to increase seed production capacity.
   1.7 Plant seedlings and establish a consistent monitoring protocol.

2. Minimize losses of trees and genetic diversity to mountain pine beetle.
   2.1 Identify high-value and high-risk stands, and conduct mountain pine beetle infestation and susceptibility mapping.
   2.2 Remove infected trees to reduce the spread, where effective.
   2.3 Apply pheromones (green leaf volatiles and verbenone) to protect plus trees.
   2.4 Mitigate stand susceptibility through fire and forest management.

3. Implement prescribed fire on the landscape to restore five-needle pines.
   3.1 Develop and implement a communication strategy to build agency and public support for fire on the landscape.
   3.2 Increase fire expertise and capacity within and among agencies via inter-agency knowledge sharing.
   3.3 Include the ecological role of fire in agency mandate.
   3.4 Develop a prescribed burn plan for five-needle pine.

4. Reduce mortality of five-needle pine stands from wildfire.
   4.1 Consolidate and communicate fire-effects research for five-needle pine restoration.
   4.2 Identify high-value five-needle pine stands and plus trees as values at risk.

5. Improve mapping and inventory data for whitebark pine and limber pine.

WHAT HAS BEEN ACCOMPLISHED (2014–2018)

In the first 5 years, the seven mountain parks collectively:

- identified approximately 740 whitebark pine and 120 limber pine plus trees;
- collected seeds from 280 of those trees;
- sent seeds from 159 parent trees for rust resistance testing;
- used pheromones to protect more than 250 plus trees from mountain pine beetle attacks;
- used prescribed fire across more than 1100 ha of whitebark pine and 7 ha of limber pine habitat;
- thinned approximately 30 ha of pine habitat to reduce competition and wildfire risk;
- planted roughly 20 800 whitebark pine and 1500 limber pine seedlings, with first-year survival rates of more than 90%;
- developed interpretive programs—*Indiana Cones and the Quest for the Keystone* and *Gnarly Pines*—both of which won awards from Interpretation Canada; and
- implemented five-needle pine–themed guided hikes, geocaching, and interpretive signs within the mountain parks.

SCALING UP/FUTURE FORWARD (2019–2024)

Dedicated Conservation and Restoration funding in the amount of $4.1 million has been secured for the next 5 years (2019–2024) to support five-needle pine conservation. Intended outcomes over the next 5 years include:

- restoring 28 stands in whitebark pine and limber pine habitat (mechanical removal of competing species and/or creating Clark's Nutcracker openings);
- contributing seeds from 150 new parent trees to blister rust resistance testing;
- protecting an average of 400 high-value trees per year with pheromones to prevent mountain pine beetle attack;
- planting 43 500 putatively resistant whitebark pine and limber pine seedlings;
- hosting and/or facilitating the establishment of joint whitebark pine and limber pine seed orchards;
- supporting blister rust resistance testing facilities;
- supporting research into whitebark pine and mycorrhizal associations; and
- creating and enhancing outreach opportunities for five-needle pine (interpretive programs, displays, multi-media).

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Poster 5 Investigating the Antagonistic Activity of Fungal and Bacterial Endophytes of Western White Pine against the Pathogen *Cronartium ribicola*

Emily Martin,[1*] Beau Larkin,[2] and Lorinda Bullington[1]

1 Molecular Ecology and Bioinformatics, mpg Ranch, Missoula, Mont.
2 Forestry and Conservation, mpg Ranch, Missoula, Mont.
*Presenting and corresponding author: EMartin@mpgranch.com

ABSTRACT

North American white pine populations have experienced increased mortality due to the invasive fungal pathogen *Cronartium ribicola*. White pines may defend against *C. ribicola* infection by hosting diverse fungal endophyte communities. Endophytes can be vertically transmitted, through seeds, from parent to offspring, as well as horizontally by wind and rain. We are currently screening previously isolated vertically and horizontally transmitted endophytes collected from western white pines (*Pinus monticola*) throughout the Pacific Northwest for the production of organic compounds that can inhibit *C. ribicola* growth in vitro. To do this, we are developing a method using microspectrophotometry, which allows us to monitor pathogen metabolism and the inhibitory effects of toxic antimicrobial compounds produced by isolated endophytes with a high degree of sensitivity. With these methods, we can screen many fungal and bacterial endophytes at a time, and quantify and compare their inhibitory effects. Bacterial and fungal endophytes that exhibit the most effective antagonistic activity will be selected and used in a western white pine seedling inoculation trial this summer.

Poster 6 Host Jump Drives Diversification of *Chrysomyxa* Species

Chongjuan You[1,2*] and Chengming Tian[1]

1 Beijing Forestry University, Beijing, China
2 University of British Columbia, Vancouver, B.C.
*Presenting and corresponding author: chongjuanyou@gmail.com

ABSTRACT

Chrysomyxa Unger can cause serious spruce rust diseases, which have become important fungal diseases in northeast and northwest China. Most *Chrysomyxa* species are heteroecious and complete their life cycle on two different plant hosts belonging to two unrelated genera: *Picea* and *Rhododendron*. However, there have been no systematic studies on the *Chrysomyxa* in China, especially the phylogenetic relationships among *Chrysomyxa* species, and co-evolution of *Chrysomyxa* and its host plants are still undetermined. In this study, based on concordant results from Generalized Mixed Yule-Coalescent and Poisson tree processes analyses, 11 *Chrysomyxa* species in China were recognized. To determine the evolutionary history of *Chrysomyxa* species, *Coleosporium* was selected as a reliable calibration point to estimate the divergence time of *Chrysomyxa*. The mean age of the node marking the split of *Chrysomyxa* is the Miocene period in the Cenozoic era, approximately 12.99 million years ago. The evolutionary relationships of *Chrysomyxa* and their hosts were investigated by comparing the cophylogenetic analysis of *Chrysomyxa* and *Picea* and *Rhododendron* species, based on three chloroplast genes for *Rhododendron* (trnH-psbA, matK, and rbcL) and three mitochondrial genes for *Picea* (trnS-trnG, trnL-trnF, and ndhK/C). The results showed that high discordance between topologies of the parasites and hosts, and host jump, rather than cospeciation, were the main speciation events that drove the diversification of *Chrysomyxa*.

Poster 7 Returning Sugar Pine and Western White Pine to the Pacific Northwest Forests—A Collective Effort

Richard A. Sniezko,[1] Robyn Darbyshire,[2] Holly Kearns,[3] Ellen Goheen,[3] Brennan Ferguson,[3] Brent Oblinger,[3] Betsy Goodrich,[3] Blakey Lockman,[3] Josh Bronson,[3] Michael McWilliams,[3] Mike Crawford,[4] George McFadden,[4] Jeff DeBell,[5] Dan Omdal,[5] Amy Ramsey,[5] Scott Kolpak,[6] Jim Hargrove,[7] Doug Manion,[8] Timothy Larkoski,[9] Gerald Barnes,[10] Rick Kern,[11] Dan Cress,[12] Bill Marshall,[13] Donald J. Kaczmarek,[14] Jun-Jun Liu,[15] Martin Nicholson,[16] Shawn Barnes,[17] Nicholas Ukrainetz,[18] Lorinda Bullington,[19] Lisa Winn,[1] Robert Danchok,[1] Emily Boes,[1] and Angelia Kegley[1*]

1 U.S. Department of Agriculture Forest Service, Dorena Genetic Resource Center, Cottage Grove, Oreg.

2 U.S. Department of Agriculture Forest Service, Pacific Northwest Region, Beaverton, Oreg.

3 U.S. Department of Agriculture Forest Service, Pacific Northwest Region, Forest Health Protection, Wash. and Oreg.

4 U.S. Department of Interior Bureau of Land Management, Lorane, Oreg.

5 Washington Department of Natural Resources, Olympia, Wash.

6 U.S. Department of Agriculture Forest Service, Genetic Resource Program, Corvallis, Oreg.

7 Quinault Indian Nation, Aberdeen, Wash.

8 Confederated Tribes of Warm Springs, Warm Springs, Oreg.

9 Kalispel Natural Resources, Usk, Wash.

10 G. Barnes Enterprises, Cottage Grove, Oreg.

11 Josephine County Forestry, Grants Pass, Oreg.

12 Regenetics Forest Genetics Consulting, Seattle, Wash.

13 Cascade Timber Consulting, Inc., Sweet Home, Oreg.

14 Oregon Department of Forestry, Saint Paul, Oreg.

15 Canadian Forest Service, Pacific Forestry Centre, Victoria, B.C.

16 Hoyt Arboretum, Portland, Oreg.

17 Geneseed Northwest, Cottage Grove, Oreg.

18 B.C. Ministry of Forests, Lands, Natural Resource Operations and Rural Development, Surrey, B.C.

19 MPG Ranch, Missoula, Mont.

*Presenting and corresponding author: angelia.kegley@usda.gov

SUMMARY

Sugar pine (*Pinus lambertiana*) and western white pine (*P. monticola*) are important species in western North American forests (Figures 1 and 2); they provide wood products and ecosystem services, including enhancing biodiversity of our forests. However, both species are very susceptible to the non-native fungal pathogen *Cronartium ribicola*, causal agent of white pine blister rust disease (Goheen and Goheen 2014; Koester et al. 2018; Sniezko et al. 2019). Mortality from white pine blister rust has caused these species to be extirpated in some areas, and the frequency of their occurrence has diminished over time in many parts of their
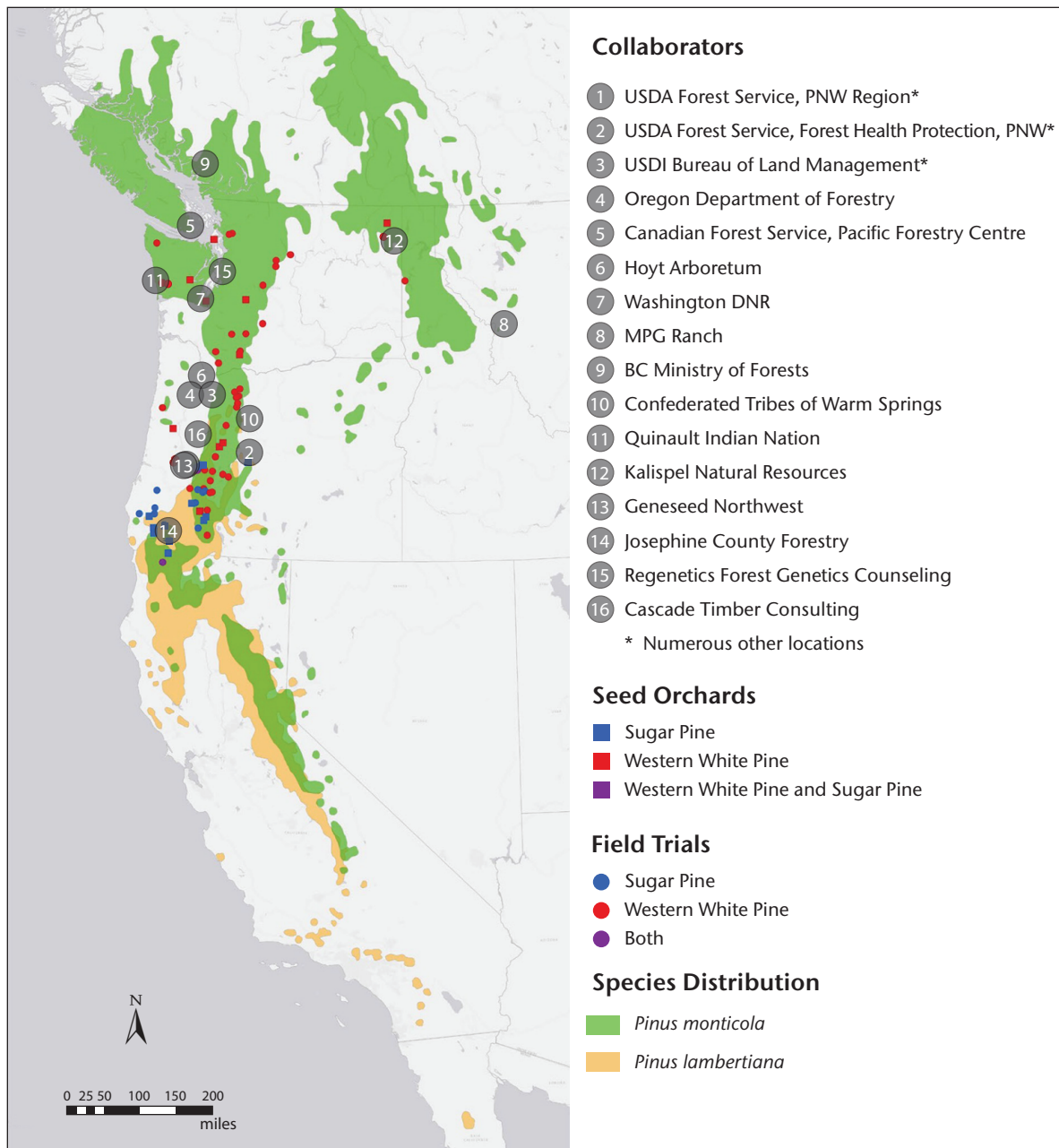
FIGURE 1 *Geographic range of western white pine* (Pinus monticola) *and sugar pine* (P. lambertiana), *locations of seed orchards, and partners and collaborators who are assisting with developing genetic resistance to white pine blister rust (USDA: U.S. Department of Agriculture; PNW: Pacific Northwest; USDI: U.S. Department of the Interior; DNR: Department of Natural Resources; B.C.: British Columbia).*

ranges (Farjon 2013; Goheen and Goheen 2014; Lintz et al. 2016). In addition, land managers have been reluctant in the past to use these species for reforestation and restoration because of potential losses due to rust and to the lack of white pine blister rust–resistant planting stock. Breeding programs to create genetic resistance to white pine blister rust in both species have been underway for more than 50 years at the U.S. Department of Agriculture Forest Service (USFS) Dorena

Genetic Resource Center, and seed orchards are now producing seed that is suitable for many areas in the Pacific Northwest that are within the species' ranges. A wide range of cooperators and partners has assisted with varying components of these resistance breeding programs (Figures 1, 2, and 3). With the combination of available seed from resistance programs, rust hazard rating of sites, and early pruning of trees, these species are again becoming part of the silvicultural toolbox needed to maintain healthy forests.

Using selections from the white pine blister rust resistance program, seed orchards for sugar pine and western white pine have been established throughout their ranges in the Pacific Northwest on U.S. Forest Service, Department of the Interior Bureau of Land Management, tribal, Washington Department of Natural Resources, Josephine County, and private lands (Figures 1 and 2), which has made improved seed available in many areas. Field trials established to examine the durability and stability of resistance are also distributed throughout much of
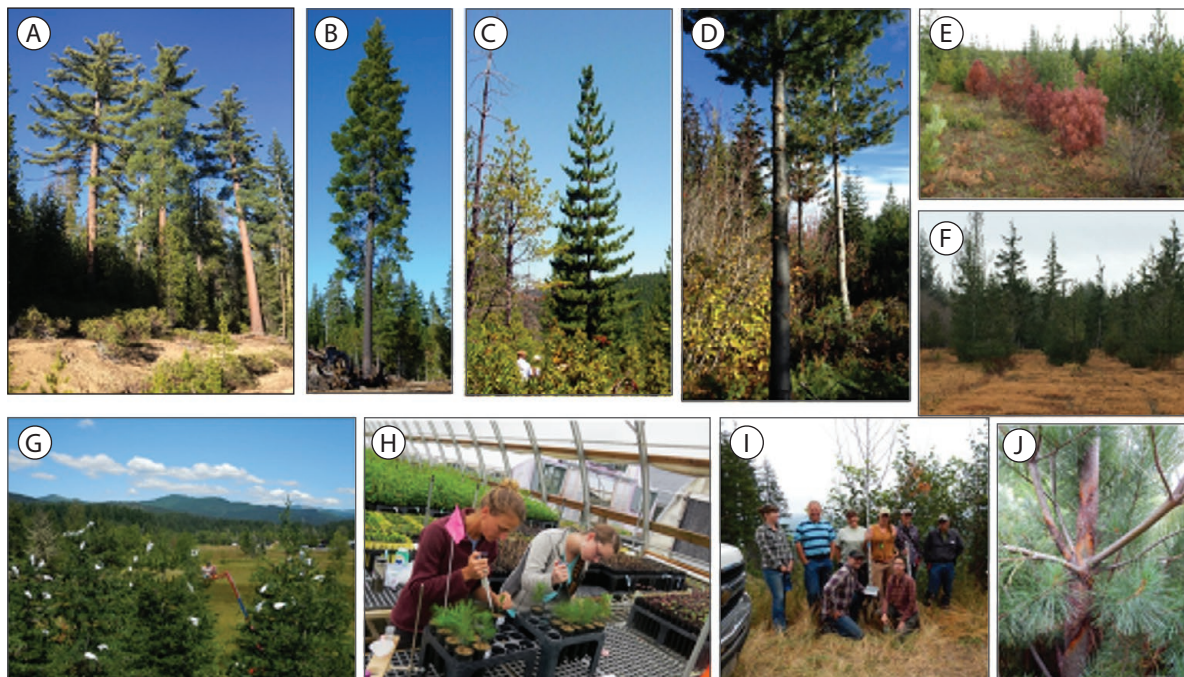
FIGURE 2   *(A, B) Healthy sugar pines; (C) healthy western white pine in area with very high incidence of white pine blister rust, Grass Creek trial in Cottage Grove Ranger District, Umpqua National Forest; (D) pruning of western white pine in a Confederated Tribes of Warm Springs Reservation planting; (E) effect of a virulent race of white pine blister rust on western white pine with major gene resistance (dead) in an Oregon field trial on Bureau of Land Management land: note healthy western white pine with quantitative resistance (see RV7 in Sniezko et al. [2019] for details); (F) Quinault Indian Nation's western white pine seed orchard provides seed for annual planting needs; (G) controlled pollination on western white pine in clone bank at Dorena Genetic Resource Center; (H) MPG Ranch and Dorena Genetic Resource Center personnel setting up a western white pine–fungal endophyte treatment prior to inoculation with white pine blister rust; (I) Washington Department of Natural Resources and U.S. Forest Service (Forest Health Program and Dorena Genetic Resource Center) employees at a western white pine field trial on Washington Department of Natural Resources land; (J) western white pine with numerous white pine blister rust cankers at a field trial on Washington Department of Natural Resources land (photo credits: Brent Oblinger [A, D], Bob Danchok [B], Richard Sniezko [C, E, I, J], Jim Hargrove [F], Emily Boes [G], Lorinda Bullington [H]).*

TREE SELECTION AND TESTING
- Breeding for WPBR resistant parent trees
- Trees are selected throughout Oregon and Washington for:
  - WPBR resistance testing on seedling progeny
  - Field trials to confirm adaptability

Collaborators: 1, 3, 4, 7, 9, 10, 11, 12, 13, 14, 16

REFORESTATION AND RESTORATION
- Planting genetically diverse seedlings with an adequate level of WPBR resistance will be key for forest health and species diversity
- Conduct rust surveys and pruning treatments to monitor disease and reduce incidence of cankers
- Silviculturists, pathologists, entomologists, geneticists and foresters provide key inputs on management, conservation education and choice of seed sources to maximize the success

Collaborators: 1, 2, 3, 6, 7, 9, 10, 11, 14, 16

SEED ORCHARDS AND SEED BANKS
- Provide high-quality, genetically diverse seedlots for each breeding zone in the PNW
- Essential for land owners who want to reforest or restore an area

Collaborators: 1, 3, 4, 7, 9, 10, 11, 13, 14, 15

SUCCESS

RESEARCH
- New technologies such as the use of genomic resources offers the potential to develop resistance more efficiently
- Investigation of fungal foliar and mycorrhizal endophytes may provide a complementary path to strengthen resiliency against WPBR
- Investigation of climate-change adaptation strategies including assisted migration and gene conservation

Collaborators: 1, 5, 8, 9

FIGURE 3 *Past and present collaborative activities underway to help retain the use of western white pine and sugar pine for reforestation and restoration in the presence of white pine blister rust. See Figure 1 for collaborator numbers (WPBR: white pine blister rust; PNW: Pacific Northwest).*

the species' ranges (Figures 1 and 2) (Omdal et al. 2015; Sniezko et al. 2019), and two new trials are slated for establishment in 2020 on private industry lands in Oregon. The Dorena Genetic Resource Center continues breeding for increased resistance in these species, which offers even more promise for the future (Sniezko et al. 2014). The development of genomic resources for these species (Liu et al. 2014, 2019; Stevens et al. 2016) has the potential to help increase breeding efficiencies in the future.

The USFS Forest Health Protection specialists and their counterparts from the Washington Department of Natural Resources and Oregon Department of Forestry provide managers with information on best site choices, rust pressure, and considerations for pruning treatments (Singleton and Oblinger 2017). In

collaboration with forest health specialists and others, each organization's silviculturists will direct the management of sugar pine and western white pine on their lands.

The interest in sugar pine and western white pine has brought together a wide array of federal, state, provincial, tribal, county, and private organizations throughout the Pacific Northwest. They all work together to increase white pine blister rust resistance and provide a variety of improvements to forest health through management activities. Seed from thousands of candidate tree selections has been screened for white pine blister rust resistance at the Dorena Genetic Resource Center. The parents rated as resistant, or selections from their progeny, have been grafted into seed orchards or clone banks, and advanced-generation breeding to increase resistance continues. These concerted efforts to conserve sugar pine and western white pine from seed to tree to orchard will help maintain healthy, diverse forests in the Pacific Northwest.

ACKNOWLEDGEMENTS

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Poster 8 Ecology and Evolution of a Tree Species Challenged by Dual Threats

Kristen Waring,[1] Sam Cushman,[2] Andrew Eckert,[3] Lluvia Flores-Renteria,[4] Richard Sniezko,[5] Christopher Still,[6] Christian Wehenkel,[7] Amy Whipple,[1] Michael Wing,[6] Justin Bagley,[3] Ethan Bucholz,[1] Socorro Gonzalez-Elizondo,[8] Jessica Hartsell,[1] Cory Garms,[6] Evan Heck,[5] Jeremy Johnson,[1,5]* Erin Landguth,[9] Alejandro Leal Sáenz,[7] Mitra Menon,[3] Ehren Moler,[1] Gerald Page,[6] John Shaw,[2] Andrew Shirk,[10] and Jared Swenson[1]

1 School of Forestry, Northern Arizona University, Flagstaff, Ariz.

2 U.S. Department of Agriculture Forest Service, Rocky Mountain Research Station, Fort Collins, Colo.

3 College of Humanities and Sciences, Department of Biology, Virginia Commonwealth University, Richmond, Va.

4 San Diego State University, San Diego, Calif.

5 U.S. Department of Agriculture Forest Service, Dorena Genetic Resource Center, Cottage Grove, Oreg.

6 Department of Forest Ecosystems & Society, College of Forestry, Oregon State University, Corvallis, Oreg.

7 Universidad Juárez del Estado de Durango, Durango, Mexico

8 Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional (CIIDIR), Unidad Durango, Instituto Politecnico Nacional, Durango, Mexico

9 School of Public and Community Health Sciences, University of Montana, Missoula, Mont.

10 College of the Environment, University of Washington, Seattle, Wash.

*Presenting and corresponding author: jeremy.johnson@nau.edu

ABSTRACT

We are studying the ecological and evolutionary processes that affect the geographical distribution of southwestern white pine (*Pinus strobiformis*), an important tree species of the southwestern United States and Mexico. Southwestern white pine viability is threatened by changing climate and an invasive tree disease, white pine blister rust (caused by the fungal pathogen *Cronartium ribicola*). Rapid climate change is negatively affecting southwestern white pine by causing increased heat and drought stress, and thus challenging its ability to adapt. White pine blister rust causes extensive tree decline and mortality, including in southwestern white pine. The dual threats of climate change and invasive species make forecasting future tree distributions at continental scales an urgent challenge. The goal of our project is to determine how gene movement among populations, adaptation to disease and drought, heritable changes beyond DNA mutations, and a changing environment interact to govern the success of southwestern white pine. We are using and developing tools to help forecast and manage the future of the species, including those from genomics, common gardens, tree disease resistance testing, engineering, and technology innovation to measure drought tolerance and physiological response. Results will be synthesized and included in cutting-edge landscape genomics models to meet our overarching

goal. We will present our framework and current project status, including results. Completed products include development of landscape genomic models, a species distribution model that incorporates climate change, and adaptive trait, phenotype plasticity, and genomic variation analyses. Continuing work incorporates these aspects in addition to transgenerational plasticity research and a genome-wide association study.

Poster 9 Finding and Using Genetic Resistance to White Pine Blister Rust in Alberta Populations of Limber Pine (*Pinus flexilis*)

Richard A. Sniezko,[1*] Jodie Krakowski,[2] Robert Sissons,[3] Genoa Alger,[3] Jun-Jun Liu,[4] Megan Lewien,[1] Robert Danchok,[1] Rachel Zitomer,[5] and Angelia Kegley[1]

1 U.S. Department of Agriculture Forest Service, Dorena Genetic Resource Center, Cottage Grove, Oreg.
2 Alberta Agriculture and Forestry, Edmonton, Alta.
3 Parks Canada, Waterton Lakes National Park, Alta.
4 Canadian Forest Service, Canadian Forestry Centre, Victoria, B.C.
5 Oregon State University, Department of Forest Ecosystems and Society, Corvallis, Oreg.
*Presenting and corresponding author: richard.sniezko@usda.gov

SUMMARY

Limber pine (*Pinus flexilis* James) is a long-lived conifer with a very wide geographic range in western North America (Figure 1). In the northernmost part of its range, 90% of which occurs in Alberta and 10% of which occurs in British Columbia, limber pine has been greatly affected by the disease white pine blister rust (Figure 2), which is caused by the non-native fungal pathogen *Cronartium ribicola* J.C. Fisch. Many populations have more than 70% infection; some have more than 95% infection and high mortality (Figure 2). Limber pine is considered Endangered under Alberta's *Wildlife Act*, and in 2014, it was recommended for Endangered status under the federal *Species at Risk Act*. Alberta has made a concerted effort to promote recovery of limber pine in the province, including partnering with the U.S. Department of Agriculture Forest Service to identify disease-resistant parent trees to provide seed for restoration. Genetic resistance to white pine blister rust is a key component of limber pine recovery in Alberta. Resistance testing is still in the early phases for Alberta candidate parent trees. Work to restore limber pine is underway, and phenotypically selected plus trees, as well as plus trees still being tested, are used as seed sources. Agencies in Alberta are aiming to build a diverse base of genetically resistant parent trees for each seed zone. Long-term seed collections for gene conservation are established and are being augmented.

 All nine North American white pine species, including limber pine, are very susceptible to white pine blister rust (Hoff et al. 1980; Stephan 2004; Sniezko et al. 2008). Genetic resistance programs for commercial white pine species began more than 50 years ago. Findings indicate that both the level and type of resistance in white pines varies by species (Sniezko et al. 2011, 2019). In 2014, in the first small trial to include seedling families from Alberta, along with some seedlots from British Columbia and Oregon, only one of 13 families tested showed notable resistance; this Alberta family was later confirmed to have major gene resistance (Sniezko et al. 2016). Major gene resistance can confer high levels of survival (more than 50%) in the presence of non-virulent white pine blister rust races. Virulence to major gene resistance in two other white pine species, western white pine (*P. monticola* Dougl. ex D. Don) and sugar pine (*P. lambertiana* Dougl.)
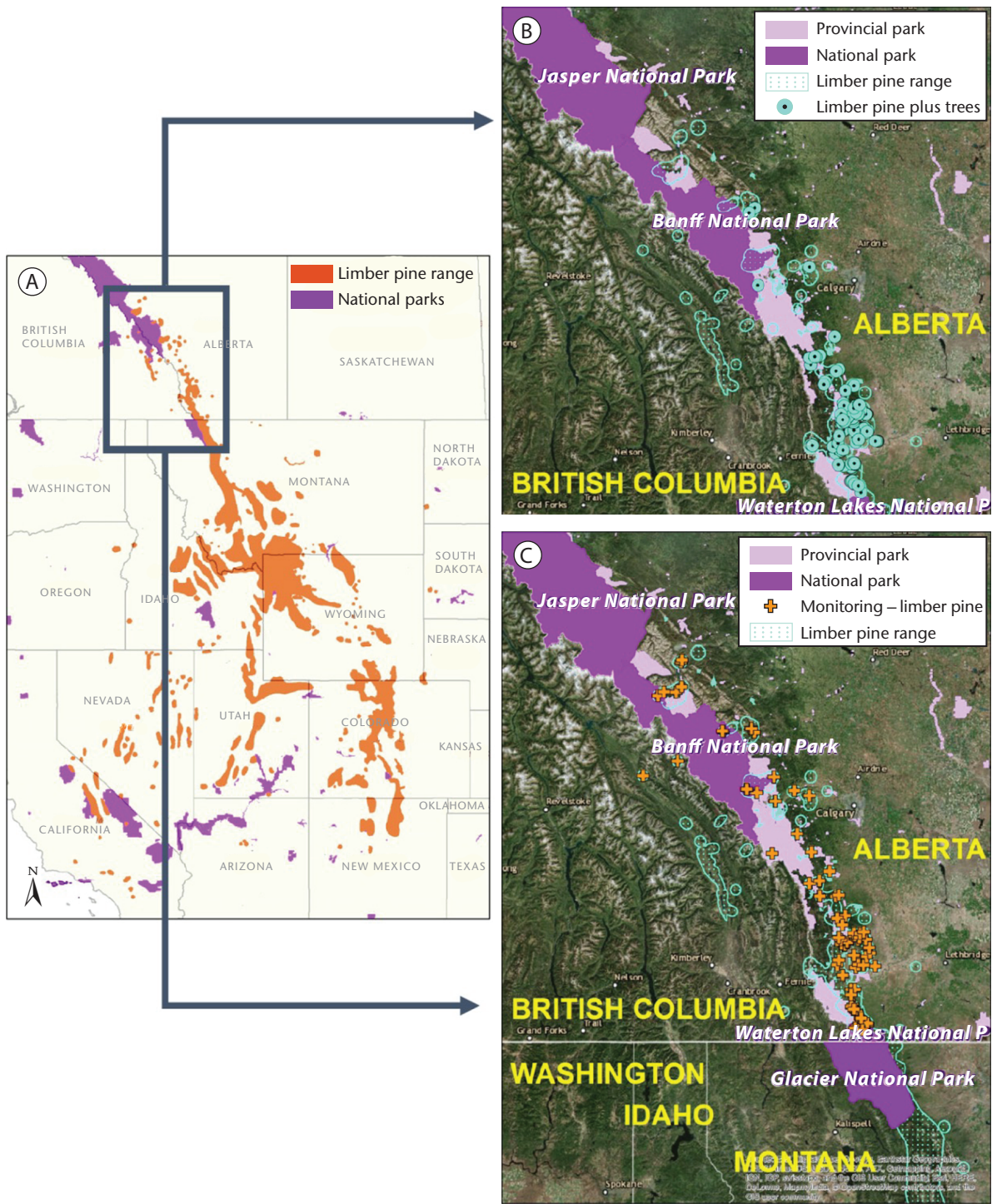
FIGURE 1   *(A) Range of limber pine; (B) distribution of Alberta limber pine plus trees; (C) distribution of limber pine monitoring plots in Alberta.*
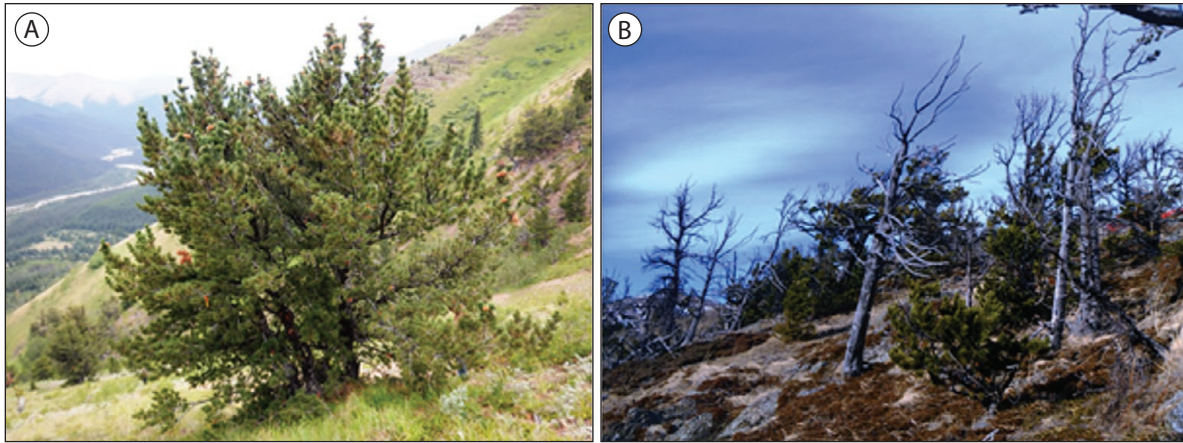
FIGURE 2   *(A) A mature limber pine in Alberta; (B) high mortality in a limber pine forest in southern Alberta caused by white pine blister rust.*

has been documented (Kinloch et al. 2004; Sniezko et al. 2019), but whether a virulent white pine blister rust race to major gene resistance in limber pine exists is currently unknown. Presence of virulence limits the effectiveness of major gene resistance, resulting in very high mortality of major gene resistance trees if they have no other type of resistance (Sniezko et al. 2019). Quantitative disease resistance to white pine blister rust has also been documented in several white pine species (Sniezko et al. 2019) and is considered more durable in regard to a potentially evolving pathogen such as *C. ribicola* (Sniezko et al. 2019). However, little or no quantitative disease resistance was found in the 2014 trial or has been reported in the few previous trials of limber pine conducted to date.

A concerted effort is underway to expand the search for both major gene resistance and quantitative disease resistance in limber pine from Alberta. Seedling families of 183 Alberta candidate parent trees were sown for resistance testing from 2016 to 2019 at Dorena Genetic Resource Center, Cottage Grove, Oregon. Most of the parent tree candidates selected were canker-free in stands with more than 75% infection. These trials will provide information about the frequency and types of resistance present in Alberta populations. These trials will also provide some information on the percentage of susceptible trees still uninfected in native stands, as progeny of such trees are expected to show a high rate of infection symptoms.

Two types of trials are used at Dorena Genetic Resource Center to characterize resistance. Seedlings 3–6 months old are inoculated and assessed for approximately 1–2 years to test for major gene resistance (see Sniezko et al. 2016); 2-year-old seedlings are inoculated and monitored for up to 5 years post inoculation to document quantitative disease resistance. In 2016, 10 previously untested Alberta families from Waterton Lakes National Park were placed in a major gene resistance test; 60 Alberta families (including the 10 in the major gene resistance test) were established in the quantitative disease resistance test. In the major gene resistance trial, four of the 10 families showed canker-free ratios, which suggests that the maternal parents have major gene resistance (Sniezko, unpubl.). Single nucleotide polymorphism genotyping results indicated that most of the major gene resistance seed families from Alberta and those previously identified in U.S. populations may carry the same gene (*Cr4*), alleles, or closely linked genes for white pine blister rust resistance (Liu, unpubl. data). Some evidence from the

parent tree status, the seedling phenotypes, and preliminary single nucleotide polymorphism genotyping investigations suggests that one limber pine parent may have a different major gene resistance gene and/or that virulence to major gene resistance may have already arisen in at least one Alberta population (Sniezko and Liu, unpubl. data); further investigation of this result is underway.

Surviving canker-free test seedlings from the 2016 major gene resistance test were planted over nearly 10 ha in Alberta as part of two larger restoration projects; they will provide data on the durability of major gene resistance in high rust hazard areas. Ongoing monitoring of these seedlings and their parent trees will identify changes in the rust fungus or in environmental conditions that may affect the durability or stability of the resistance.

The inoculation trial of the 60 seedling families sown in 2016 and inoculated in 2017 was very effective, with 80.2% of seedlings showing stem symptoms 1 year after inoculation and stem infection ranging from 28 to 100% among families (Figure 3A). Some seedlings displayed many cankers (Figure 3B). The wide range in infection among families may suggest that both major gene resistance and quantitative disease resistance may be present among at least some of the 60 families. Further assessments and resistance determinations are scheduled in late 2019 and beyond.
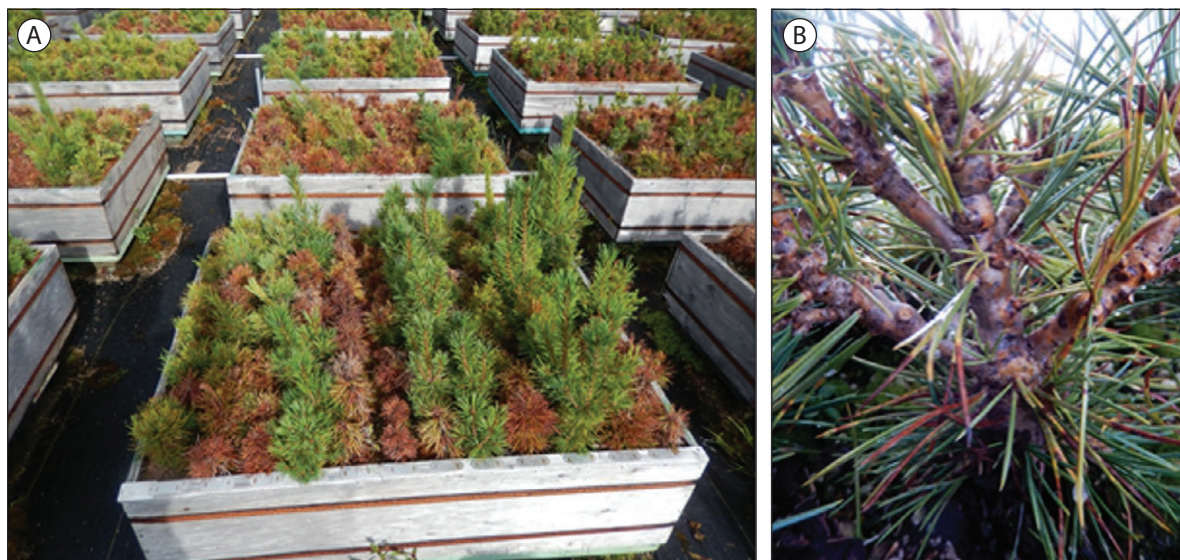
FIGURE 3 *(A) Genetic variation in white pine blister rust resistance: 60 seedling families were sown in 2016 and inoculated with rust spores in September 2017. In June 2019, large differences in resistance (survival) were evident among families (each family was planted in a 10-tree row plot); (B) limber pine seedling with many cankers in the 2016 seedling inoculation trial at Dorena Genetic Resource Center.*

The information from resistance testing will be integrated into future management and restoration plans by Alberta agencies, including planned development of seed orchards. Within 5 years we expect to have:

- increased knowledge of the frequency, type, and level of genetic resistance in limber pine;
- more habitat restored by planting seedlings from plus trees and tested resistant parent trees; and
- continuing field validation of seedling resistance ratings and of the durability of genetic resistance.

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Poster 10 The Search for White Pine Blister Rust Resistance in Foxtail Pine (*Pinus balfouriana*)

Richard A. Sniezko,[1] John Gleason,[2] Brianna McTeague,[3] Robert Danchok,[1] Megan Lewien,[1] and Angelia Kegley[1*]

1 U.S. Department of Agriculture Forest Service, Dorena Genetic Resource Center, Cottage Grove, Oreg.
2 U.S. Department of Agriculture Forest Service, El Dorado National Forest, Placerville Nursery, Camino, Calif.
3 Weyerhaeuser Company, Olympia, Wash.
*Presenting and corresponding author: angelia.kegley@usda.gov

SUMMARY

Foxtail pine (*Pinus balfouriana*) is a long-lived, high-elevation white pine species that is endemic to California and has two disjunct populations (Figures 1 and 2). Due to foxtail pine's small and disjunct range and to concerns about the potential effects of a changing climate and air pollution, the IUCN Red List has listed this species as "Near Threatened" (Farjon 2013). In addition to these threats, foxtail pine, like the other eight white pine species that are native to the United States, appears to be very susceptible to the non-native fungal pathogen *Cronartium ribicola*, causal agent of white pine blister rust disease (Hoff et al. 1980; Stephan 2004). However, for foxtail pine, this rating is based on only a few seedlots. More information is needed to further characterize susceptibility/resistance in this species, including the frequency, geographic distribution, and level of any genetic resistance to white pine blister rust. Ex situ gene conservation efforts, principally storage of seed from individual tree collections, and some small plantings (Figure 2D), have recently been undertaken for this species, and current data suggest that the seed can be stored for at least several decades and retain viability (Sniezko et al. 2017).

To examine white pine blister rust resistance in foxtail pine, 21 seedling families from four national forests were sown in a trial conducted in 2014 at the U.S. Department of Agriculture Forest Service's Dorena Genetic Resource Center. The seedlings were inoculated with white pine blister rust basidiospores in 2015 after their second growing season. In this trial, all foxtail pine seedlings showed heavy needle spotting (first symptoms of infection) within 4 months of inoculation, and 100% were cankered within 14 months of inoculation. The appearance of needle spots and cankers occurred much more rapidly relative to the other four white pine species inoculated in 2015 (McTeague et al. 2017; Sniezko, unpubl. data). Only two of 705 foxtail pine seedlings in this trial survived 3 years after inoculation (Figure 2F), and both were cankered. The high early infection and mortality in this trial suggests that foxtail pine may be the most susceptible of all native U.S. white pine species.

Subsequent trials sown in 2018 and 2019 at two U.S. Department of Agriculture Forest Service facilities—Dorena Genetic Resource Center (Oregon) and Placerville Nursery (California)—have greatly expanded the number of families that will be tested for white pine blister rust resistance. Between the two facilities, nearly 200 seedlots have been sown, and many of the families will be evaluated at both.
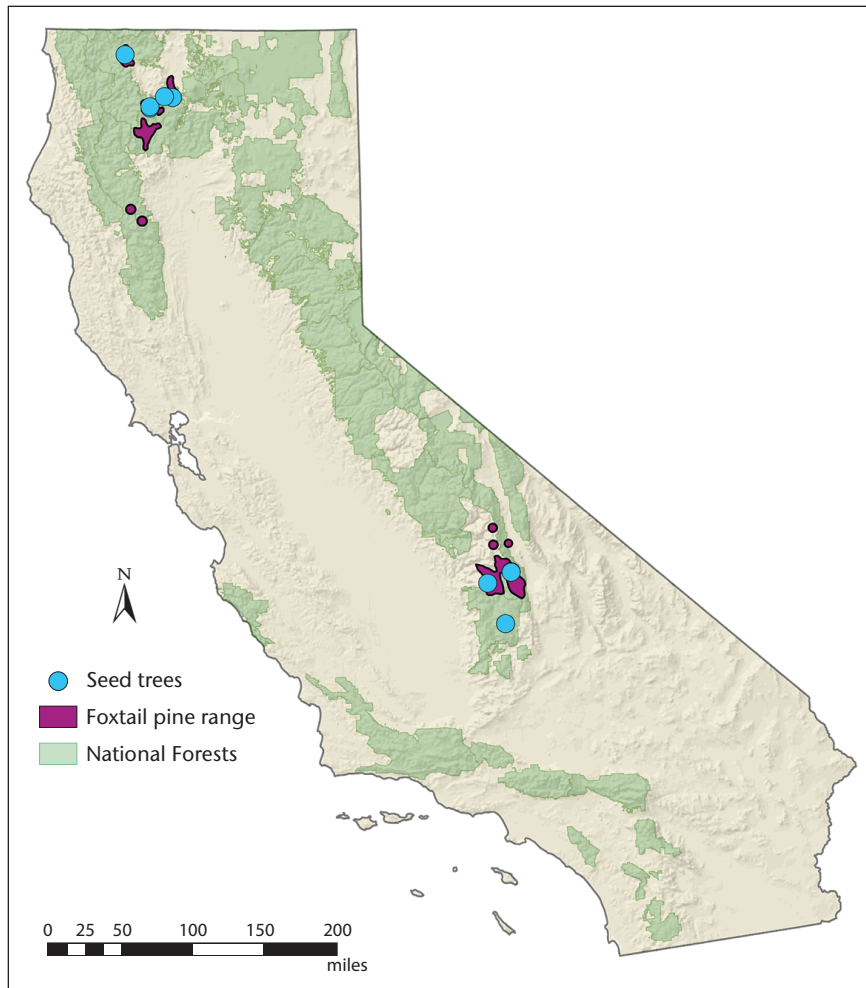
FIGURE 1 *Geographic range of foxtail pine and location of the 21 parent (seed) trees represented in the 2014 white pine blister rust inoculation trial.*

Germination of stored foxtail pine seed was generally very good but varied widely by seedlot (Figure 3). Further examination of the data will be needed to more fully understand the variation. Family variation in seedling colour was evident in young seedlings, and chlorophyll-deficient seedlings were present in some families, possibly indicating some inbreeding (Figure 2G).

The trials sown in 2018 and 2019 include seedling families of parent trees from throughout most of the species' range. The 2018 trials were inoculated with white pine blister rust in September 2019 (Oregon) and are tentatively planned for December 2019 (California); the 2019 trials will be inoculated in 2020. Results for the level of genetic resistance to white pine blister rust will be forthcoming in the next few years. In addition to the main resistance tests with a standard inoculum level, subsets of the seedling families will be (1) tested at differing inoculation densities to evaluate whether a lower inoculum level is needed in this species to detect resistance and to characterize resistances that may be expressed at different (low to high) inoculum densities, and (2) included in a field trial at low elevation to examine resistance, validate the seedling inoculation results, and examine the durability of resistance. The field trial will also serve as a sentinel
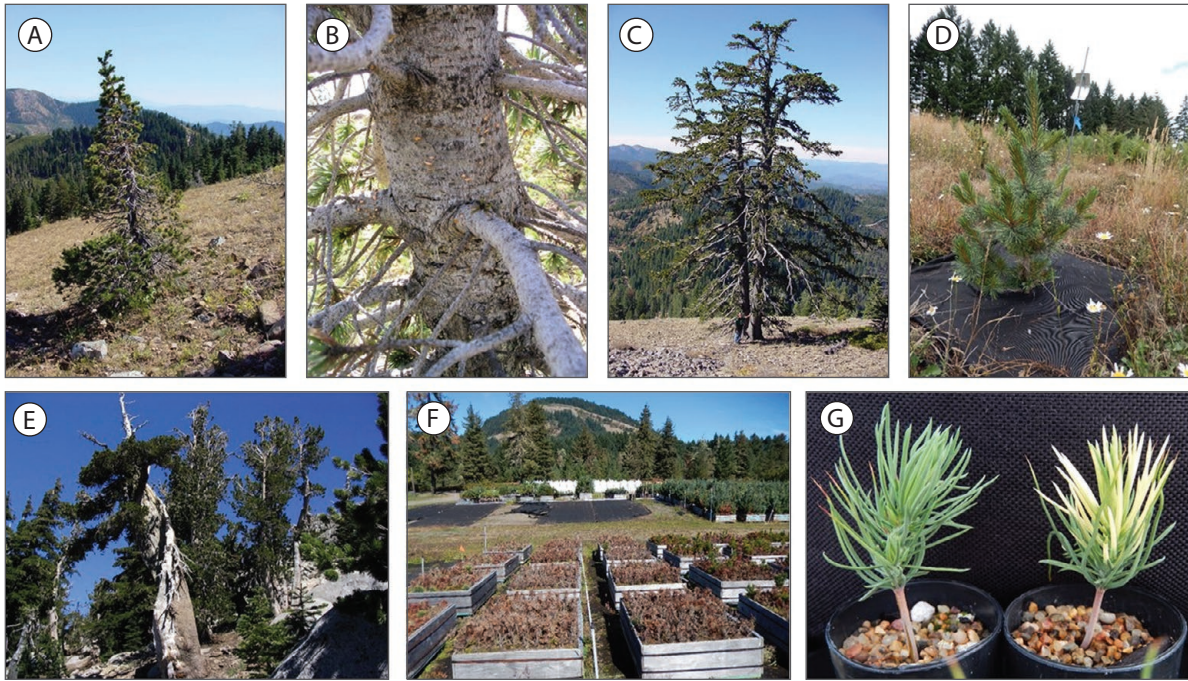
FIGURE 2 *(A, B) Foxtail pine infected with white pine blister rust at Lake Mountain; a canker with aecia present on main bole; (C) large foxtail pine at Lake Mountain; (D) young seedling at low-elevation ex situ conservation planting in western Oregon at Bureau of Land Management Tyrrell Seed Orchard; (E) foxtail pines in Trinity Alps; (F) extremely high mortality in the 2014 white pine blister rust trial (eight boxes) at Dorena Genetic Resource Center; (G) two seedlings, one with normal foliage (left) and one with chlorophyll-deficient foliage (right) from Sow# 255 in 2019 sowing at Dorena Genetic Resource Center (photo credits: Deems Burton [A, B, C, E], Richard Sniezko [D, F, G]).*

FIGURE 3 *Relationship between filled seed and germination for foxtail pine seedlots sown in 2019, by geographic source (National Forest). Percentage filled seed is based on X-ray images of 100 seeds. Each seedlot is from a single parent tree.*

planting for effects by other biotic and abiotic agents. By conducting a more comprehensive resistance assessment for the species and potentially identifying the first resistant parent trees, these trials will provide critical information to land managers for foxtail pine conservation 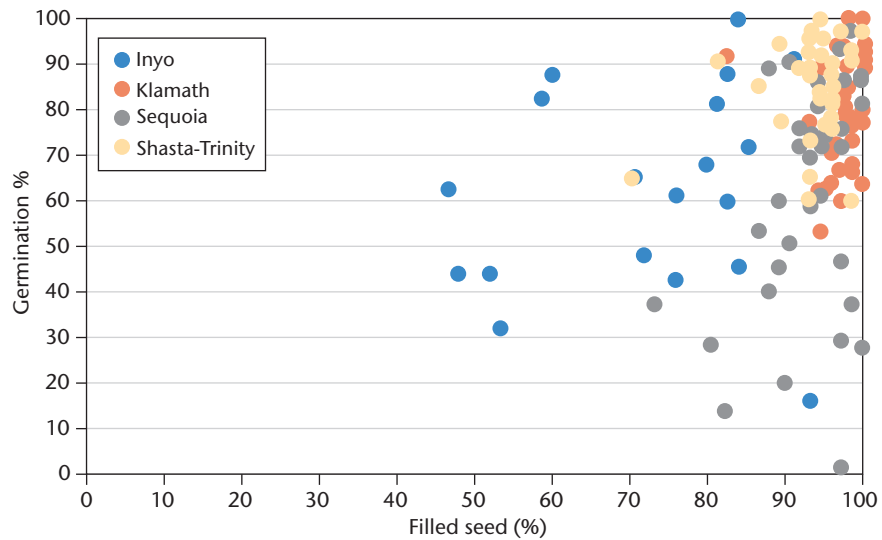efforts and for maintaining the species and its ecological function. Furthermore, germination data for this range-wide collection will provide an update on the potential for ex situ conservation using seed storage.

ACKNOWLEDGEMENTS

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Poster 11 Toward Evolutionary and Functional Characterization of an Avirulence Gene in the Poplar Rust Fungus *Melampsora larici-populina*

Pascal Frey,[1*] Clémentine Louet,[1] Agathe Maupetit,[1] Méline Saubin,[1] Antoine Persoons,[1] Stéphane De Mita,[1] Sébastien Duplessis,[1] and Fabien Halkett[1]

1 Institut national de la recherche agronomique (INRAE), Lorraine University, (UMR) Unité Mixte de Recherche, (IAM) Interactions Arbres-Microorganismes, Nancy, France
*Presenting and corresponding author's email: pascal.frey@inra.fr

ABSTRACT

Poplar rust, caused by the pathogenic fungus *Melampsora larici-populina* (Basidiomycota, Pucciniales), is the main phytosanitary constraint for commercial poplar cultivation in Europe and other parts of the world. In the last 50 years, many rust-resistant cultivars have been bred and released, but all the qualitative resistance genes (i.e., major resistance genes) released were overcome by pathogen evolution within a short period. Qualitative resistance is particularly subject to breakdown by pathogen evolution for perennial hosts, such as poplar trees, because of the wide inequality between the pathogen's rapid generation time and the time needed to deploy new host varieties. For instance, resistance R7 was released in poplar plantations in the 1980s, and some R7 cultivars were widely planted in northwestern Europe. In 1994, breakdown of the R7 resistance was detected in Belgium and northern France, and virulent 7 *M. larici-populina* individuals spread all over western Europe in less than 5 years, causing very destructive epidemics, and leading to a complete replacement of the pathogen's populations, on both cultivated and wild poplars. Through a genome-wide association study, we recently identified a locus in the genome of *M. larici-populina* that likely corresponds to the Avr7 avirulence locus, whose mutation is responsible for R7 resistance breakdown in poplar. The candidate avirulence gene possesses some characteristics of canonical effectors (coding a small cysteine-rich protein, specific to *M. larici-populina*, early expressed and of unknown function) but lacks a secretion signal. Our aim is to functionally validate this avirulence locus and study its evolution over time in natural populations of the pathogen. This would be the first avirulence gene identified in this biotrophic fungal species. A better understanding of the evolution of the pathogen's genome and the genetic architecture of virulence would help in defining breeding strategies for durable resistance, especially for perennial crops, such as poplars.

Poster 12 Partnerships in the Pacific Northwest to Help Save an Endangered Species, Whitebark Pine (*Pinus albicaulis*)—Research, Rust Resistance, and Restoration

Richard A. Sniezko,[1][*] Kristen Chadwick,[2] Robyn Darbyshire,[3] Jennifer Hooke,[4] Chris Jensen,[3] Dan Omdal,[5] Amy Ramsey,[5] Betsy Goodrich,[2] Michael P. Murray,[6] Charlie Cartwright,[6] George McFadden,[7] Michael Crawford,[7] Doug Manion,[8] Whitney Matthes-Finley,[9] Jun-Jun Liu,[10] Lorinda Bullington,[11] Anna Conrad,[12] Enrico Bonello,[12] Iain Reid,[13] Ehren Moler,[14] Jennifer Gruhn,[15] Martin Nicholson,[16] Eric Sprague,[17] Spus Wilder,[18] Tyler Hale,[19] Rob Mangold,[20] Diana Tomback,[20,21] Robert Danchok,[1] Kristin Silva,[1] Emily Boes,[1] and Angelia Kegley[1]

1 U.S. Department of Agriculture Forest Service, Dorena Genetic Resource Center, Cottage Grove, Oreg.

2 U.S. Department of Agriculture Forest Service, Forest Health Protection, Pacific Northwest Region, Sandy, Oreg.

3 U.S. Department of Agriculture Forest Service, Pacific Northwest Region, Beaverton, Oreg.

4 Crater Lake National Park, Oreg.

5 Washington Department of Natural Resources, Olympia, Wash.

6 B.C. Ministry of Forests, Lands, Natural Resource Operations and Rural Development, Nelson, B.C.

7 U.S. Department of the Interior Bureau of Land Management, Lorane, Oreg.

8 Confederated Tribes of Warm Springs, Warm Springs, Oreg.

9 Yakama Nation Wildlife, Range, and Vegetation Resource Management Program, Yakima, Wash.

10 Canadian Forest Service, Pacific Forestry Centre, Victoria, B.C.

11 MPG Ranch, Missoula, Mont.

12 College of Food, Agricultural, and Environmental Sciences, The Ohio State University, Columbus, Ohio

13 Department of Forest and Conservation Sciences, University of British Columbia, Vancouver, B.C.

14 School of Forestry, Northern Arizona University, Flagstaff, Ariz.

15 Washington University, St. Louis, Mo.

16 Hoyt Arboretum, Portland, Oreg.

17 American Forests, Washington D.C.

18 Confederated Tribes of the Colville Reservation—Bureau of Indian Affairs, Colville, Wash.

19 American Public Gardens Association, Maine

20 Whitebark Pine Ecosystem Foundation, Missoula, Mont.

21 Department of Integrative Biology, University of Colorado, Denver, Colo.

*Presenting and corresponding author: richard.sniezko@usda.gov

SUMMARY

Whitebark pine (*Pinus albicaulis*) is a keystone species that is distributed widely at high elevations across western North America (Tomback and Achuff 2010). Whitebark pine faces many threats, including white pine blister rust caused by the

non-native pathogen *Cronartium ribicola*, mountain pine beetle (*Dendroctonus ponderosae*) predation, climate change, and altered fire regimes (Loehman et al. 2011; Leirfallom et al. 2015). Whitebark pine is listed as Endangered on the IUCN Red List (Mahalovich and Stritch 2013). It is also a federally listed endangered species in Canada under the *Species at Risk Act* and has been proposed for listing under the *Endangered Species Act* in the United States. A concerted effort is needed to reverse the decline of this keystone species and enhance opportunities for its retention as a viable component of high-elevation forest ecosystems. The National Whitebark Pine Restoration Plan[1] is a collaborative partnership among American Forests, the Whitebark Pine Ecosystem Foundation, and the Washington office of the U.S. Department of Agriculture Forest Service that is guiding an inter-agency, range-wide strategic approach to restoring whitebark pine. However, a strategic plan depends on appropriate tools and techniques to build resilient whitebark pine populations (e.g., Keane et al. 2012). In the Pacific Northwest portion of the whitebark pine range, partnerships among organizations and land managers are essential to understand the current status of the species, undertake research to understand its biology (including patterns of genetic variation and resistance to white pine blister rust and mountain pine beetle), develop populations of parent trees with durable genetic resistance to white pine blister rust for each seed zone to use for seed collection to produce seedlings for restoration, provide conservation education, and implement restoration. Integrating these efforts will maximize the potential for successful retention of whitebark pine ecosystems into the future.

A U.S. Department of Agriculture Forest Service regional information survey on the status of whitebark pine in Oregon and Washington that was conducted in the early 1990s highlighted concerns about the future viability of populations of whitebark pine in the Pacific Northwest (Sniezko et al. 1994). Screening seedling families for genetic resistance to white pine blister rust began in 2002. Subsequently, an initial regional restoration plan was developed for U.S. Department of Agriculture Forest Service lands (Aubry et al. 2008). Information needs and recommendations were further delineated in a report commissioned by the Western Wildland Environmental Threat Assessment Center (Tomback and Sniezko 2017). Concerns about the effects of the biotic and abiotic threats to whitebark pine in the Pacific Northwest have led to ongoing cooperative efforts between federal, tribal, state, provincial, university, non-profit, and private groups, notably in studying genetic variation, screening for white pine blister rust resistance, and gene conservation activities (see below for some activities by various groups) (Figures 1 and 2). These efforts have helped with a broad range of initiatives: gathering on-the-ground information on the status of whitebark pine populations, beginning parent tree seed collections to use for testing more than 1300 seedling families for genetic resistance to white pine blister rust (Sniezko et al. 2007, 2011, 2018; Murray and Berger 2018; Savin et al. 2018), and initiating investigations into genetic variation and disease resistance (Hamlin et al. 2012; Gruhn 2016; Syring et al. 2016; Liu et al. 2016, 2017; Bair 2017; Beck and Sniezko 2018; Bennett et al. 2018; Cartwright 2018; Lea et al. 2018). Other applications include examining the possibilities and considerations for the use of biotechnology (National Academies of Sciences, Engineering, and Medicine 2019), gathering seed and information to aid ex situ conservation (Sniezko et al. 2017), and undertaking studies regarding endophytic microbial communities associated with whitebark

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1 https://whitebarkfound.org/our-work/national-whitebark-pine-restoration-plan/.

pine (Bullington et al. 2018; Moler and Aho 2018). Partners have begun grafting to develop seed orchards of resistant parents, establish provenance trials and white pine blister rust resistance field trials and conservation plantings (Cartwright 2018; Omdal et al. 2018), and initiate the first restoration plantings (Figure 1). With the cooperation of this diverse group of land managers, scientists, forest health specialists, resource professionals, and concerned non-profit organizations, in conjunction with regional and national restoration plans, the groundwork to begin successful restoration of whitebark pine has been laid (Figure 3).
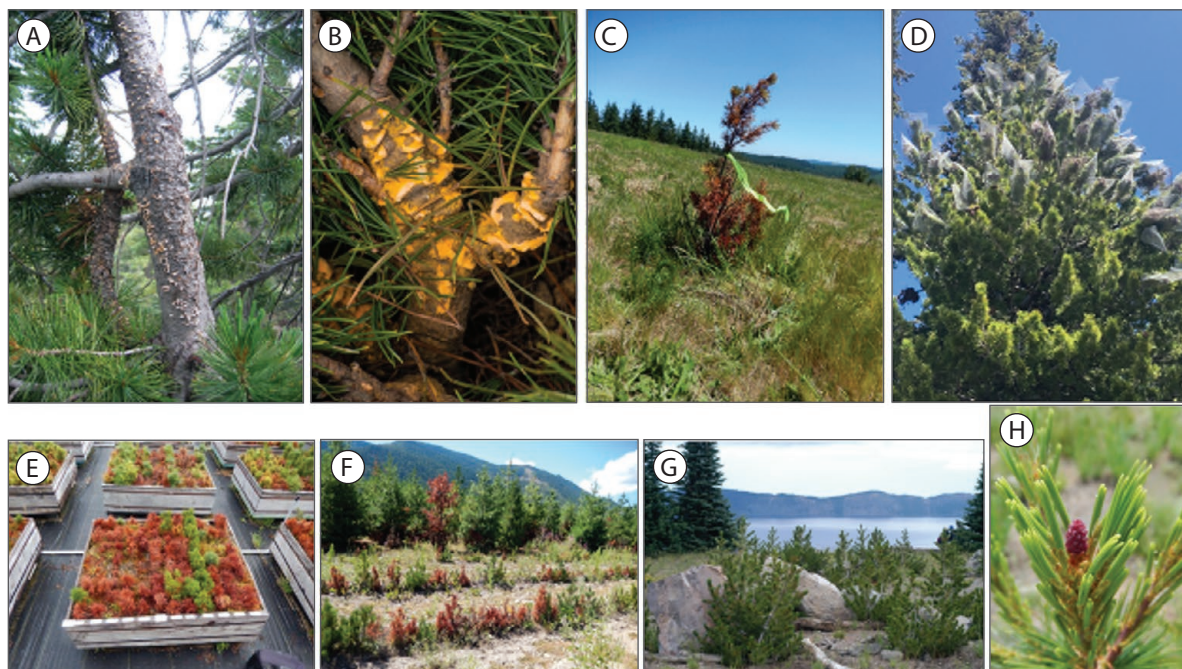
FIGURE 1   *(A) Whitebark pine at Crater Lake National Park infected with white pine blister rust; aecia present on main bole; (B) whitebark pine infected with white pine blister rust (WPBR), showing aecia present; (C) seedling progeny of Crater Lake National Park parent tree CL28, with white pine blister rust canker at the low-elevation field trial at Bureau of Land Management Tyrrell orchard, Oregon; (D) elite tree selection on Tyee Mountain (Entiat Ranger District, Okanogan–Wenatchee National Forest in Washington) from the 2018 cone collection, showing 128 cages with three to four cones per cluster; (E) white pine blister rust SY2016 resistance inoculation trial showing seedling family variation (by row) in resistance to white pine blister rust, with seedlings from the Deschutes National Forest parent tree 011192 showing very high survival; (F) whitebark pine provenance disease resistance trial at Skimikin Seed Orchard site, British Columbia, where whitebark pine was planted at a low-elevation site with an adjacent western white pine trial and infected* Ribes *spp. plants (the alternate host for white pine blister rust fungus) nearby; (G) whitebark pine restoration planting at Rim Village, Crater Lake National Park; (H) the first seed cone from one of the seedlings planted for restoration at Crater Lake National Park (photo credits: Richard Sniezko [A, C, E, F, G, H], Iain Reid [B], Eireann Pederson [D]).*

FIGURE 2   (A) Whitebark pine cone collection on the McKenzie Pass lava fields; (B) measuring seedlings in Crater Lake National Park Horse Trail endophyte trial; (C) parent tree 011192, Deschutes National Forest of sow #54 in SY2016 whitebark pine trial, which shows one of the highest survival rates of its progeny of any of the 1300 families tested in rust screening trials at Dorena Genetic Resource Center; (D) Michael Murray demonstrating placing protection bags over conelets at Crater Lake National Park; (E) planting a seedling for restoration in 2016 at Crater Lake National Park; (F) Washington Department of Natural Resources staff planting whitebark pine on Darland Mountain in Washington; (G) U.S. Forest Service Deschutes National Forest, in conjunction with Mount Bachelor Ski Area, planting approximately 100 seedling progeny of resistant parent trees in 2019 (photo credits: Chris Jensen [A, C], Jennifer Hooke [E], Dan Omdal [F], Matt Horning [G], Richard Sniezko [B, D]).

FIGURE 3   *Components of whitebark pine (WBP) biology, management, and restoration that will help maximize the potential for success in retaining whitebark pine in Pacific Northwest ecosystems (WPBR: white pine blister rust).*

GROUPS WORKING WITH WHITEBARK PINE IN THE PACIFIC NORTHWEST (NOT A COMPREHENSIVE LIST):

U.S. Department of Agriculture Forest Service helps coordinate and support efforts among geneticists, plant pathologists, entomologists, botanists, foresters, and silviculturists to develop populations of white pine blister rust–resistant whitebark pine for restoration. Efforts involve field surveys to monitor the current status of whitebark pine; seed collection for genetic conservation across 15 national forests, as well as national parks, and tribal lands; and cone collection from more than 1300 parent trees for white pine blister rust resistance testing at Dorena Genetic Resource Center (Figure 1E). Seeds from parent trees that are rated

resistant to white pine blister rust are collected for gene conservation and future restoration (Figures 1 and 2). Many of these white pine blister rust–resistant parents or their progeny are also being grafted to place into orchards or clone banks for gene conservation and future seed production. Restoration plantings using white pine blister rust–resistant seedlots have begun.

U.S. Department of the Interior National Park Service: Six restoration plantings have been established in Crater Lake National Park since 2009 using seed from white pine blister rust–resistant trees (Figure 1G), and seed from 126 parent trees has been collected for rust resistance testing and genetic conservation. The seedlings in the restoration are identified by parent tree origin to also serve as a genetic trial. Rust-resistant trees are monitored annually and are protected from mountain pine beetle. In Mount Rainier National Park, trees have been selected and cones have been collected for white pine blister rust resistance testing, the first restoration plantings with seedling families of white pine blister rust–resistant trees have been established, and measurements are made of permanent whitebark pine health transects across the park (Rochefort et al. 2018). In North Cascades National Park, trees have been selected and cones have been collected for white pine blister rust resistance testing, and permanent whitebark pine health transects have been established across the park (Rochefort et al. 2018).

Canadian Forest Service is using genetics research tools to study genetic variation in whitebark pine and to further understand resistance to white pine blister rust (Liu et al. 2016, 2017), and is leveraging transcriptomics to identify genes associated with blister rust resistance.

British Columbia Ministry of Forests, Lands, Natural Resource Operations and Rural Development has established field trials at eight sites, three nursery bed locations, and four small tests to assess white pine blister rust resistance and other traits. Together, these trials include more than 600 parent trees and families from populations that represent much of the whitebark pine's range.

Bureau of Land Management has provided a site for two whitebark pine trials, which allows for the study of the species in a low-elevation environment and the evaluation of the seedling families for field resistance to white pine blister rust, and provides opportunities for conservation education.

Hoyt Arboretum has joined efforts in ex situ genetic conservation, and offers opportunities for conservation education, research, and restoration.

American Public Gardens Association supports the dispersal of whitebark pine seedlings from the U.S. Department of Agriculture Forest Service Dorena Genetic Resource Center to public gardens and arboreta around the country. The Association produces and distributes outreach and education materials, including a series of youth education materials on white pine blister rust and its effects on *Pinus albicaulis* as part of the Plant Heroes program.

American Forests is a long-time partner in the whitebark pine restoration effort. Since 1990, American Forests has partnered with the U.S. Department of Agriculture Forest Service to plant 500 000 whitebark pine trees across more than

809 ha (2000 acres) in the United States and Canada—this is 40% of all whitebark pine restoration since 2006. The organization also provided significant funding for the 2018 whitebark pine cone collections in Oregon and Washington. The agreement between the U.S. Department of Agriculture Forest Service and American Forests, and resulting funding in 2018, were essential in making it a successful year for cone collection in this relatively rare bumper cone crop and providing seed for the ensuing restoration efforts over the next few years. American Forests is a key partner in the implementation of the National Whitebark Pine Restoration Plan.

Whitebark Pine Ecosystem Foundation is a leader in championing whitebark pine restoration and partnering with federal agencies on related projects. Outreach, education, and information sharing has been accomplished through newsletters, community activities, annual workshops, and major conferences that bring together the scientific and management community. The Foundation is the science lead in the National Whitebark Pine Restoration Plan.

University of British Columbia is testing the potential of assisted migration and direct seeding of whitebark pine by re-assessing eight common gardens planted up to 800 km north of the current whitebark pine northern range limit. The University is also phenotyping seedlings (217 families, 46 populations) for white pine blister rust to examine variation in rust resistance and climate variables related to growth and rust resistance at parent tree locations.

Ohio State University is developing a tool for predicting white pine blister rust resistance based on spectroscopy analysis of whitebark pine needles.

Idaho State University has conducted research on the fungal ecology of whitebark pine phyllospheres in the southern Cascades, including changes in the composition of whitebark pine fungal phyllospheres following a controlled inoculation of whitebark pine with a putative fungal antagonist of *Cronartium ribicola* (Moler 2015; Moler and Aho 2018).

Washington University has conducted research on genetic variation in whitebark pine, such as the PhD thesis Ecological and Genetic Consequences of Climate Change Impacting Species Distributions, with Specific Cases in Whitebark Pine (*Pinus albicaulis* Engelm.) (Gruhn 2016).

MPG Ranch has collaborated with U.S. Department of Agriculture Forest Service to investigate the influence of genetics, defensive chemistry, and the fungal microbiome on disease outcome in whitebark pine trees (Bullington et al. 2018).

Yakama Nation is collaborating with the U.S. Department of Agriculture Forest Service Forest Health Protection group to study whitebark pine population status and collect seed for resistance testing at Dorena Genetic Resource Center. Restoration and gene conservation are proceeding via matching funds provided by the Yakama Nation and a Forest Health Protection grant received in 2016.

Colville Confederated Tribes are currently testing seedlots from tribal land for white pine blister rust resistance; testing is being conducted at Dorena Genetic Resource Center. Plans for whitebark pine restoration in burned areas are being developed. A white pine blister rust resistance and gene conservation planting

was established in 2018 in conjunction with the Washington Department of Natural Resources and Dorena Genetic Resource Center (Omdal et al. 2018).

Confederated Tribes of Warm Springs has documented white pine blister rust resistance in some of their parent trees based on testing at Dorena Genetic Resource Center, but 90% of the first selected trees were destroyed by wildfire; this emphasizes the importance of ex situ in addition to in situ conservation. Cones from additional parent trees have also been collected, and testing for white pine blister rust is underway.

Washington Department of Natural Resources and Dorena Genetic Resource Center have coordinated the establishment of four field trials for genetic conservation, white pine blister rust resistance testing, and seed production (Omdal et al. 2018).

ON THE ROAD TO SUCCESS

Tremendous progress has been made for in situ and ex situ genetic conservation efforts across the Pacific Northwest portions of the whitebark pine range. The joint efforts of the many land managers and stewards across organizational boundaries in this part of the species' range have provided opportunities to identify white pine blister rust–resistant parent trees, collect seed for long-term genetic conservation, initiate provenance trials, plant the first restoration trials, and study genomic resources. These efforts will lead to a better understanding of whitebark pine's genetic variation, including how the species may fare under challenges presented by climate change. Due to extensive white pine blister rust testing, many resistant parent trees have been documented, and the large seed collection effort in 2018 (a relatively rare excellent cone crop year) bodes well for restoration efforts and provides further candidates for white pine blister rust testing. There is already evidence of success—in 2015, the U.S. Fish and Wildlife Service downgraded the endangered species listing priority number for whitebark pine, but a final decision is slated for 2020. This was due, in part, to the increased survival and propagation of genetically resistant trees in the Pacific Northwest portion of whitebark pine's range. Continued cooperative effort across organizational boundaries will provide the best avenue for dynamic genetic conservation and retention of whitebark pine as a keystone species in forest ecosystems (Figure 3).

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Poster 13 Reach for the Top: Implementing Whitebark Pine and Limber Pine Recovery in Alberta

Jodie Krakowski,[1]* Robin Gutsell,[2] and Brad Jones[3]

1 Forest Stewardship and Trade Branch, Alberta Agriculture and Forestry, Edmonton Alta.
2 Resource Stewardship Division, Fish and Wildlife Policy Branch, Alberta Environment and Parks, Edmonton Alta.
3 Resource Stewardship Division, South Saskatchewan Region, Alberta Environment and Parks, Calgary Alta.

*Presenting and corresponding author: jodie.krakowski@gov.ab.ca

SUMMARY

Alberta has made significant progress toward recovery of whitebark pine (*Pinus albicaulis* Engelm.) and limber pine (*P. flexilis* James). In 2003, the Province started assembling a team of stakeholders and subject matter experts, which led to a multi-agency working group. In 2008, both species were listed as Endangered under the provincial *Wildlife Act*. A smaller recovery team, formed out of the working group, finalized Minister-approved recovery plans in 2013 (whitebark) and 2014 (limber) (Alberta Whitebark and Limber Pine Recovery Team 2013, 2014). An updated plan combining the status and recovery actions for both species was developed using the Open Standards for the Practice of Conservation (CMP 2013) and has been completed for both species, based on their shared geography, threats, and recovery goals and actions (in review; the plan will be posted at https://www.alberta.ca/plant-species-at-risk.aspx).

After approval of the recovery plans, the working group was reconvened as a recovery implementation team. Stakeholder commitment to formal and informal interagency collaboration has enabled the use of consistent protocols, the sharing of resources, information, material, and expertise, and broader geographic access to implement recovery actions. Agencies that have engaged in and supported whitebark pine and limber pine recovery to date in Alberta include provincial government departments in Alberta and British Columbia; federal government agencies in Canada (notably Parks Canada) and the United States, particularly the U.S. Department of Agriculture Forest Service; academic institutions throughout the Intermountain and Pacific Northwest regions; and non-governmental organizations with mandates that support conservation and species at risk (Table 1).

TABLE 1 *Partners in whitebark pine and limber pine recovery in Alberta*

Type		Agency/Department
Provincial government	Alberta	Alberta Agriculture and Forestry • Forest Stewardship and Trade Branch • Alberta Wildfire Alberta Environment and Parks • Alberta Parks • Resource Stewardship Division: Fish and Wildlife Policy; Resource Management, South Saskatchewan Region
	British Columbia	B.C. Ministry of Forests, Lands, Natural Resource Operations and Rural Development B.C. Ministry of Environment BC Parks
Federal government	Canada	Parks Canada Agency • Rocky Mountain national parks Natural Resources Canada-Canadian Forest Service • Pacific Forestry Centre • Laurentian Forestry Centre • Atlantic Forestry Centre
	United States	U.S. Department of Agriculture Forest Service • Coeur D'Alene Forest Nursery • Dorena Genetic Resource Center • Rocky Mountain Research Station • Intermountain Research Station
Academia		Kings University College Montana State University University of Alberta University of British Columbia University of Calgary University of Northern British Columbia University of Victoria
Non-governmental organizations		Whitebark Pine Ecosystem Foundation Crown Managers Partnership High-5 Working Group Nature Conservancy of Canada Oldman River Watershed Council
General public		Private landowners

Successes to date reflect the strong cross-agency collaborations, science-based prioritization, and sharing of data and material needed to recover these slow-growing pine species throughout their wide-ranging, difficult-to-access ecosystems. Key achievements are detailed on the provincial whitebark and limber pine recovery website, and include:

- establishing and monitoring a network of more than 250 transects across the Rocky Mountains of Canada to assess status and trends in health and stand dynamics;
- establishing a diverse provincial seed archive with hundreds of single-tree and population accessions representing the species' ranges;
- building a library of putatively disease-resistant/tolerant trees ("plus trees") in the field (in situ) and seed bank (ex situ) that contains more than 210 limber pine and 50 whitebark pine genotypes (so far) selected from highly infected stands using standardized protocols;
- screening more than 200 limber pine and 20 whitebark pine plus trees for heritable disease resistance (to date);
- establishing seed zones, seed transfer guidelines, and associated provenance trials for each species (Krakowski 2018);
- installing restoration plantings of nearly 10 000 seedlings and 20 ha in priority areas (so far);
- establishing replicated controlled silviculture trials to evaluate effectiveness of different treatments for whitebark pine release;
- improving operational protocols and practices in whitebark pine and limber pine habitat, which has resulted in the development of best practices guidelines;
- addressing and minimizing effects on whitebark pine and limber pine in tenure holder forest management plans and operational practices;
- creating a completely revised and updated recovery plan for both species;
- producing habitat suitability mapping that is freely available online (Krakowski et al. 2017);
- standardizing and sharing data to improve outcomes during planning, management, and conservation;
- supporting research and sharing material with collaborators to fill knowledge gaps;
- sharing knowledge with many end users via diverse extension venues; and
- conducting cross-training among agencies to ensure consistent, high-quality data collection.

Successful recovery of these extremely slow-growing mountain pine species will require a century or more because a seed planted now will take at least 50–80 years to mature and produce enough cones and pollen to reproduce itself. While incremental measures are key to funding, tracking and monitoring, sustained commitment, and an adaptive approach are essential to ensuring that recovery progresses. Next steps include supporting the establishment of seed orchards comprising disease-resistant and/or disease-tolerant parent trees to produce seed from each seed zone for restoration purposes, evaluating silviculture options for whitebark pine competition release, expanding restoration planting in priority areas, and monitoring the effectiveness of different recovery actions.

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Last name	First name	Affiliation	Country	Email
Allan	Kiah	Univ. British Columbia	Canada	kiahallan@hotmail.com
Bower	Andrew	U.S. Dep. Agric. For. Serv.	United States	andrew.bower@usda.gov
Bullington	Lorinda	MPG Ops.	United States	lbullington@mpgranch.com
Cartwright	Charlie	B.C. Min. FLNRORD[a]	Canada	charlie.cartwright@gov.bc.ca
Cooke	Janice	Univ. Alberta	Canada	janice.cooke@ualberta.ca
Cross	Patrick	Yellowstone Ecol. Res. Cent.	United States	cross@yellowstoneresearch.org
Erbilgin	Nadir	Univ. Alberta	Canada	erbilgin@ualberta.ca
Fan	Lijun	Inst. Bot., Chinese Academy Sci.	China	weixx@ibcas.ac.cn
Feau	Nicolas	Univ. BritishColumbia	Canada	Fnicolas.feau@ubc.ca
Frey	Pascal	INRAE, l'institut national de recherche pour l'agriculture, l'alimentation et l'environnement	France	pascal.frey@inra.fr
Furtado	Edson Luiz	Fundunesp, Fundação para o Desenvolvimento da Unesp	Brazil	administracao@fundunesp.unesp.br
Gass	Barb	Agric. Can.	Canada	barb.gass@agr.gc.ca
Haeussler	Sybille	Skeena Forestry Consultants/Univ. Northern British Columbia	Canada	sybille.haeussler@unbc.ca
Hamelin	Richard	Univ. British Columbia	Canada	richard.hamelin@ubc.ca
Heinze	Berthold	Austrian Fed. Res. Cent. For. (BFW)	Austria	berthold.heinze@bfw.gv.at
Isik	Fikret	N.C. State Univ.	United States	fisik@ncsu.edu
Johnson	Jeremy	North. Ariz. Univ.	Canada	jeremy.johnson@prescott.edu
Kang	Kyu-Suk	Dep. For. Sci., Seoul Natl. Univ.	Korea	kangks84@snu.ac.kr
Kegley	Angelia	Dorena Resource Cent.	United States	angelia.kegley@usda.gov
Kim	Yang-Gil	Dep. For. Sci., Seoul Natl. Univ.	Korea	rladidrlf@snu.ac.kr
King	Scott	Louisiana-Pacific Canada Ltd.	Canada	scott.king@lpcorp.com
Klutsch	Jennifer	Univ. Alberta	Canada	klutsch@ualberta.ca
Kolpak	Scott	Umpqua Natl. For.	United States	scott.kolpak@usda.gov
Lickl	Eleonore	HBLVA	Austria	eleonore@lickl.net
Liu	Jun-Jun	Can. For. Serv.	Canada	jun-jun.liu@canada.ca
Major	John	Nat. Resources Can., Can. For. Serv.	Canada	john.major@canada.ca
Marino	Celso Luis	Fundunesp	Brazil	cl.marino@unesp.br
Martin	Emily	MPG Ops.	United States	emartin@mpgranch.com
McLaughlin	Marie	U.S. Dep. Agric. For. Serv., Sandpoint Ranger Distr.	United States	marie.mclaughlin@usda.gov
McPeak	Eden	Univ. Alberta	Canada	emcpeak@ualberta.ca
McRae	Nick	Canfor	Canada	nick.mcrae@canfor.com
Moltzan	Bruce	U.S. Dep. Agric. For. Serv.	United States	bruce.moltzan@usda.gov
Moody	Randy	Moody Tree	Canada	whitebarkrandy@gmail.com
Moricca	Salvatore	Dep. Agric., Food, En	Italy	salvatore.moricca@unifi.it
Murray	Michael	B.C. Min. FLNRORD	Canada	michael.murray@gov.bc.ca
Neale	David	Univ. Calif. Davis, retired	United States	dbneale@ucdavis.edu
Oline	David	South. Oreg. Univ.	United States	olined@sou.edu
Olson	Åke	Swedish Univ. Agric. Sci.	Sweden	ake.olson@slu.se

Last name	First name	Affiliation	Country	Email
Pigott	Don	Yellow Point Propagation Ltd.	Canada	ypprop@shaw.ca
Queloz	Valentin	Swiss Fed. Res. Inst. WSL	Switzerland	valentin.queloz@wsl.ch
Ramsfield	Tod	Nat. Resources Can.	Canada	tod.ramsfield@canada.ca
Reich	Richard	College New Caledonia	Canada	reichr1@cnc.bc.ca
Reid	Iain	Parks Canada	Canada	iain.reid@canada.ca
Sacenieks	Kristine	B.C. Min. FLNRORD	Canada	kristine.sacenieks@gov.bc.ca
Sniezko	Richard	Dorena Resource Center	United States	Richard.sniezko@usda.gov
Stafl	Natalie	Parks Canada	Canada	natalie.stafl@canada.ca
Strong	Ward	B.C. Min. FLNRORD, retired	Canada	ward.strong@gov.bc.ca
Tanguay	Philippe	Nat. Resources Can.	Canada	philippe.tanguay@canada.ca
Ukrainetz	Nicholas	B.C. Min. FLNRORD	Canada	nicholas.ukrainetz@gov.bc.ca
Vance	Marie	B.C. Min. FLNRORD	Canada	marie.vance@gov.bc.ca
Weber	Anne	U.S. Dep. Agric. For. Serv.	United States	aweber@fs.fed.us
Wei	Xiao-xin	Inst. Bot., Chinese Academy Sci.	China	weixx@ibcas.ac.cn
Woods	Alex	B.C. Min. FLNRORD	Canada	alex.woods@gov.bc.ca
You	Chongjuan	Beijing For. Univ.	China	chongjuanyou@gmail.com

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