Disease Screening for Endangered Whitebark Pine Ecosystem Recovery in Canada

Extension Note

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Abstract

Whitebark pine (*Pinus albicaulis*) is a high-mountain keystone and foundation species that is declining throughout most of its range in Western Canada. An introduced pathogen (*Cronartium ribicola*) causing white pine blister rust has led to the tree being listed as a federal species at risk. A disease screening program relies on carefully selecting potentially resistant parent trees, followed by testing their respective progenies. Beginning in 2011, trees were selected for controlled inoculations and field trials of seedling families. The performance of each seedling family indicates the level of disease susceptibility, implying genetic resistance in the parents. To date, we are screening hundreds of wild-collected parents. Based on post-inoculation assessments, almost one-third of our carefully selected parents have produced seedlings showing low susceptibility to disease. Numerous stakeholders are now beginning to plant disease resistant seedlings while also supporting the establishment of seed orchards and clone banks. Due to everchanging pathosystems, long-term disease screening will remain a critical contribution to the recovery of this valuable species.

Introduction

Subalpine terrain throughout much of Western Canada supports ecosystems populated with whitebark pine (*Pinus albicaulis*). This long-lived tree helps form forest habitats in harsh mountain environments. The large nutritious seed is prized by wildlife such as grizzly bears (*Ursus arctos horribilis*), foxes (*Vulpes vulpes*), and Clark's nutcrackers (*Nucifraga columbiana*). Whitebark pine stabilizes steep and shallow soils. By shading snowbanks, whitebark pine can extend water availability into late summertime. As a keystone species, whitebark pine has a disproportionally greater role than other species for community functions (Tomback et al. 2001). As a foundation species, these pines promote stable conditions in a harsh environment, thus promoting dependent organisms (Ellison et al. 2005).

In 2012, whitebark pine was listed as an endangered species in Canada, thus becoming the only designated tree in Western Canada. The primary driver of population declines in this species is a disease first introduced to western North America in 1910—white pine blister rust. The fungal pathogen, *Cronartium ribicola*, is an airborne dispersant that infects five-needle pine species. Basidiospores are transmitted from natural assemblages of secondary hosts, primarily gooseberry (*Ribes* spp.). These spores penetrate needle surfaces as disruptive hyphae, spreading within branches toward the stem (Geils & Vogler 2011).

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Cankers erupt with rust-coloured blisters producing aeciospores capable of being carried by the jet stream for distances up to 500 km (Van Arsdel et al. 1998). Cankers encircle branches and stems killing the distal portions; thus, dead tops and branch tips are often indicative of infection. Mortality is a common outcome. White pine blister rust has been found throughout Canada's whitebark pine distribution. In southeastern British Columbia and adjoining Alberta, most trees have been infected. In contrast, the West Chilcotin region supports the lowest levels of rust incidence in Canada. Additional agents of decline include anthropogenically driven changes associated with climate, fire regimes, and mountain pine beetle (*Dendroctonus ponderosae*). Cumulative mortality appears to be highest between the Selkirk Mountains and Continental Divide proximal to the US border, where greater than 80% of standing trees are dead at most sites surveyed (Murray & Moody, in press, Shepherd et al. 2018).

Naturally occurring but rare disease resistant trees may be considered the life-link to the future of whitebark pine. Identifying resistant genotypes of whitebark pine can potentially provide a pathway for long-term relief from widespread decline (Tomback & Achuff 2010, Kinloch 2003). A range-wide restoration strategy suggests that promoting such resistant trees is the topmost priority (Keane et al. 2012). In response, a growing list of stakeholders are requesting rust-resistant seedlings for multiple land objectives. Our disease screening program is designed to support this effort.

The Canadian initiative to screen whitebark pine for disease resistance launched in 2011. The program has been coordinated by the BC Ministry of Lands and Natural Resource Operations. Most screening activities are conducted at the Ministry's Kalamalka Research Station (KAL), located in Vernon, British Columbia. Procedures are adapted from the largely successful resistance program for western white pine (*P. monticola*) and reflect methods established by the US Forest Service (NAS 2019, Sniezko et al. 2011). The screening process begins with a wild healthy parent tree selection process and continues through a series of steps for a minimum of six years per parent tree (Figure 1).

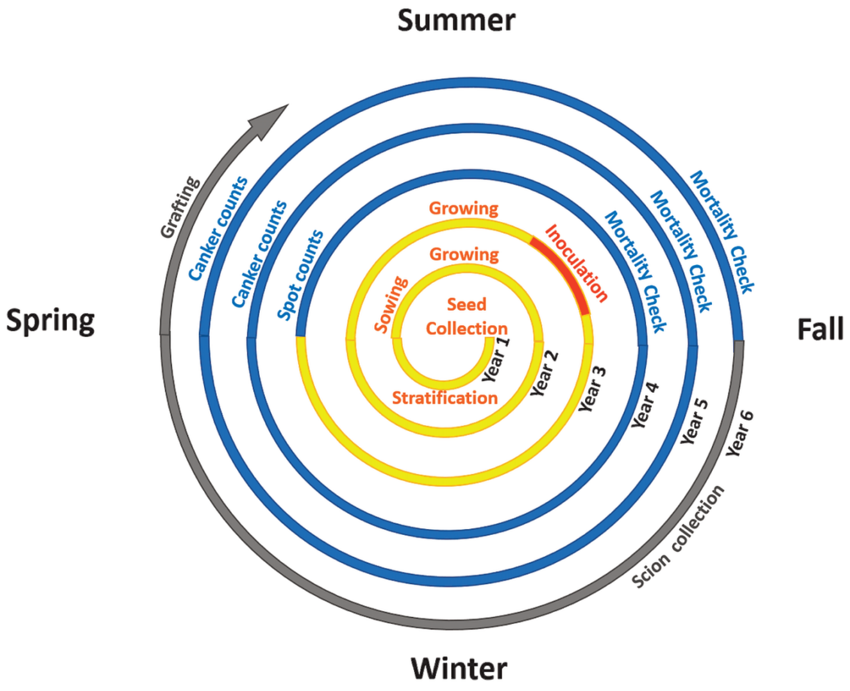

Figure 1. Starting at the center with seed collection from selected wild stand parent trees, annual activities continue for approximately six years. Yellow = seed collection and seedling growth in the greenhouse; red = inoculation; blue = canker and mortality checks in outdoor raised beds; and grey = parent tree selection, scion collection, and grafting.

Parent Tree Selection, Collection, and Propagation

Individual trees from wild stands in British Columbia and Alberta are selected for testing based on their phenotypic traits. An ideal tree has no signs of disease with a robust crown of foliage that spans most of the tree's height. Prolific cone production, freedom from defects, and proximity to roads/trails are also positive traits. Rare healthy trees within heavily impacted stands are a high priority for selection because they are more likely to be resistant (Mahalovich & Dickerson 2004). The collection of cones entails caging conelets early in the summer to prevent harvest by nutcrackers and squirrels. During September, cages are removed, and harvested cones are shipped to nurseries for extracting, stratifying, and sowing seeds. Nurseries label all seedlings according to respective parent tree. After two years of growth, seedlings are shipped to KAL for controlled inoculation and/or to field sites for natural inoculation.

Controlled Inoculation Trials

Seedlings from the selected healthy parents are exposed to the disease. The performance of seedlings indicates the level of disease susceptibility, implying genetic resistance in the parents. Seedlings from parents that are already known to be highly susceptible or resistant are also included to establish the relative effectiveness of artificial inoculation (i.e., controls).

Every August, an inoculation room is prepared with a target temperature of 16–17°C (61–62°F) and relative humidity equaling 100%, providing optimal transmission conditions. Seedlings are randomly arranged in trays before being placed on shelving units that are cloaked with saturated sheets to reduce air movement and promote uniform spore dispersal. A cultivated garden of gooseberry shrubs (*Ribes nigrum*) provides infected leaves as an inoculum source. Freshly harvested leaves are placed on wire mesh trays above the seedlings (1.3 leaves per tree seedling). Basidiospores drop from infected gooseberry leaves onto seedling foliage. Spore loading is monitored by periodically examining slides placed at tree level using a microscope. Our target spore load is 3,000–5,000/cm^2. We also install petri dishes with agar to catch spores for our estimation of the percent of spores that germinate. Once spore load target is reached (usually within 5–12 hours), the gooseberry leaves are removed from the frame. Temperature is adjusted to 20°C (68°F) and humidity is maintained at 100% for 48 hours to promote spore germination necessary to infect seedling foliage. After 48 hours, seedlings are placed in a greenhouse for overwintering.

Figure 2. Post-inoculation seedlings are assessed for cankers and vigor every spring for a minimum of four years.

Post-Inoculation Surveys and Data Collection

Needle lesions (spots) are the first post-inoculation trait observed, usually emerging in May or June of the year following artificial inoculation. After this inspection, seedlings are planted into raised beds. The first survey of stem and branch cankers occurs in October of that year. In subsequent years, canker surveys are conducted each May, when the rust-coloured blisters form on cankers (blister sacs filled with aecial spores). Mortality and tree health surveys are conducted each October. All seedlings derived from a particular parent tree (through cone collection) represent a single "family." Timing of mortality following inoculation can vary by trial and seedling family. Significant mortality typically occurs two to four years after inoculation. Thus, conclusions on the relative resistance of families are derived four years post-inoculation.

Parent Tree Ranking

Data collected from seedlings are used to compile means and summaries in order to rate each parent tree for levels of resistance. For each seedling, surveyors record the number of cankers, tree vigor, and canker severity class. Severity reflects the percent stem encirclement and vertical extent (mm) of cankers. Data are calculated separately according to each family with the following outputs: average number of cankers, average canker severity, percent seedlings with cankers, and percent seedlings dead with blister rust. We perform a linear stretch transformation to scale the values for average number of cankers and average canker severity index. This equation incorporates the range of observed data values across all families for each response variable.

$$\text{Linear Stretch Value (LS)} = [x - \min(x)]/[\max(x) - \min(x)]$$

The percent of seedlings dead from blister rust is emphasized in our calculation by applying a higher weight to this variable. We use a weight of 2/3 for the other three variables and a weight of 2 (three times as much) for the percentage killed by blister rust (these weights sum to four).

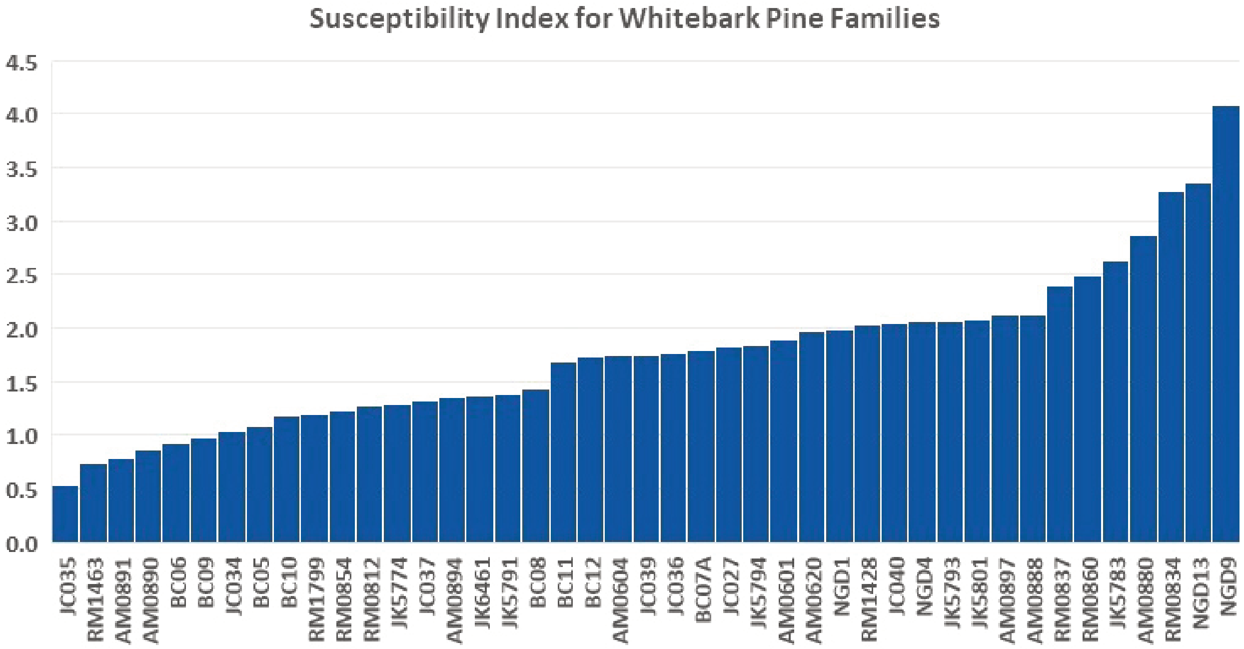

Figure 3. Susceptibility of 43 whitebark pine families to blister rust. Trees were inoculated in 2016. Lower values indicate lower susceptibility (higher resistance).

The final step entails summing all four values per family, thus providing an index of disease susceptibility. Lower index values indicate comparatively less susceptibility.

$$\text{INDEX (weighted)} = \text{LS Avg. No Cnkrs} + \text{LS Avg Sev} + \%\text{ with Cnkrs} + \%\text{ Dead Rust}$$

The resulting index values provide the basis for ranking families (Figure 3). This ranking is used to compare parents, prioritize specific parents for further seed collection in restoration efforts, target scion collection for subsequent grafting, and determine the contribution to resistance of the known maternal parent. The data also provide information on the possible inheritance of the resistance types and their geographic distribution.

Field Inoculation Trials

As a supplement to controlled inoculations of seedlings, field trials are useful for exposing families to additional natural strains of *Cronartium ribicola* and gauging long-term durability of resistance in natural high-mountain environments (Sniezko et al. 2019). By sharing the same parent tree families (between KAL and field trials), a useful means of comparing results is gained (Telford et al. 2015). To date, over 200 families are being examined at seven field sites in British Columbia. Field trial locations were chosen based on good accessibility and high blister rust hazard. Beginning in 2014, seedlings were planted along transects and monitored on an annual basis.

Preliminary Findings are Contributing to Recovery Actions

Since the first controlled inoculations were conducted at KAL in 2013, 172 families have been inoculated. Results vary widely between families. The proportion of seedlings that have died from blister rust varies from 4–79% per family. Our indexed ranking further reflects a wide span in variable responses to inoculations (Figure 3). Of the seedlings of sufficient post-inoculation age, we estimate about one-third of families as having low susceptibility to disease-caused mortality. This is a major milestone in forming a foundation for recovery in Canada. Field screening sites have not received sufficient levels of infection to draw preliminary conclusions yet. This is expected as field testing relies on uncontrolled natural transmission of spores.

The identification of rust resistant parent trees is contributing to the launch of several important initiatives. Seedlings from resistant parents, and those in the process of being screened, are being deployed for restorative planting, silvicultural obligations, and climate change mitigation. Plantings are propelled by a diverse array of stakeholders including First Nations, mining, timber, energy, BC Parks, Parks Canada, and The Nature Conservancy of Canada. Historically, annual planting of whitebark pine in British Columbia has totalled less than 11,000 seedlings per year (Figure 4). In 2021, an estimated 97,800 seedlings are being planted on land administered by BC Ministry of Lands and Natural Resource Operations.

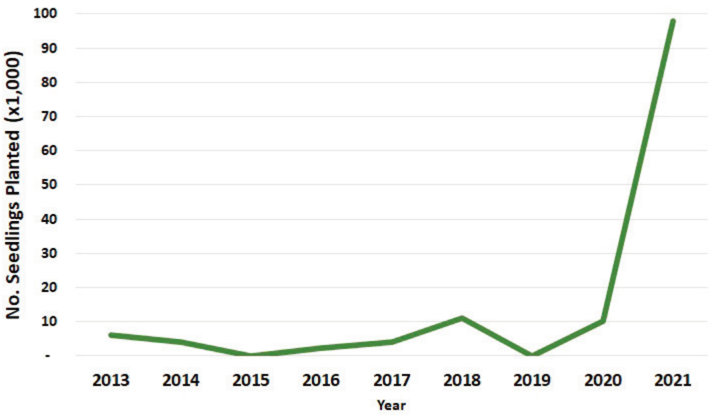

Figure 4. Annual planting of whitebark pine on land administered by BC Ministry of Lands and Natural Resource Operations.

To meet dramatic increases in demand for disease resistant seedlings, new infrastructure in the form of seed orchards is being developed. Seed orchards are long-term installations providing reliable and accessible cone production. A whitebark pine seed orchard is being established at the Ministry's Prince George Tree Improvement Station and another site is being considered near Lumby, British Columbia. Similarly, two new clone banks for genetic conservation are undergoing installation. To provide planting material for these sites, dormant shoots, known as scion, are collected from the upper (cone-bearing portion) crown of parent trees. At KAL, scion is grafted onto existing nursery rootstock (whitebark pine) for outplanting within orchards. In comparison to other conifers, whitebark pine are slow-growing. Based on seed orchard performance in Montana, we expect at least two decades to pass before Western Canada experiences substantial whitebark pine cone crops. During the interim, we are working to identify additional disease resistant sources to help bolster availability of seed. Currently, the availability of seed and seedlings is being tracked by the Whitebark Pine Ecosystem Foundation (info@whitebarkpine.ca) for the benefit of stakeholders.

Disease screening for forest improvement is not a short-term process. Plant pathogens adapt to overcome host resistance. A changing climate alters the pathogen–environment–host matrix (pathosystem). New genomic technologies will contribute to, but cannot replace, phenotypic programs for understanding and confirming durability of resistance. Thus, identifying rare resistant trees and developing an understanding of underlying mechanisms of host resistance will enhance the recovery of this valuable species.

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