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

C. Cartwright[1, *]

[1] *British Columbia Ministry of Forests, Lands and Natural Resource Operations, Cowichan Lake Research Station, Tree Improvement Branch, Mesachie Lake, British Columbia, Canada*

[*] *charlie.cartwright@gov.bc.ca*

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

INTRODUCTION

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

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

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

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

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

METHODS

Seed Sources

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

Stratification

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

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

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

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

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

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

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

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

Data Collection and Analysis

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

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

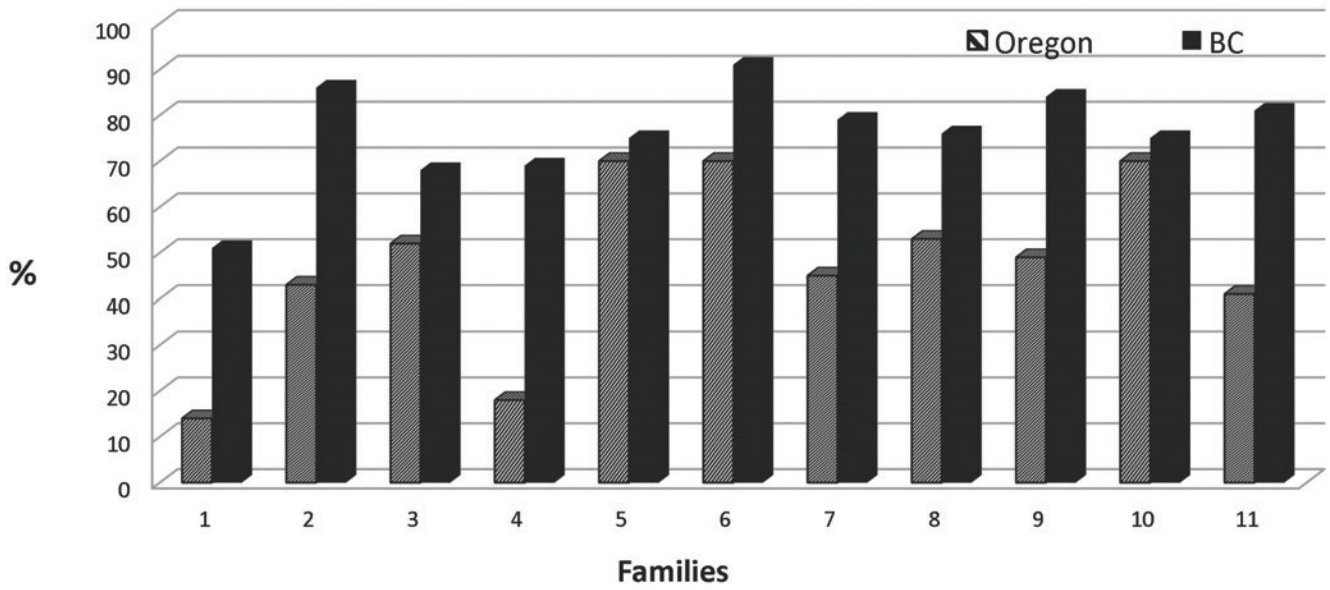

Figure 3—Percent germination of representative families (from Region 6) tested at nurseries in both Oregon and British Columbia.

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

RESULTS AND DISCUSSION

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

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

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

Variable germination rates for BC regions are difficult to explain due to effects confounded by collection in different years, by different agencies with different storage. The large regional differences suggest that

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

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

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

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

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