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

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

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

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

INTRODUCTION

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

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

METHODS

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

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

RESULTS AND DISCUSSION

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

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

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

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

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

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

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

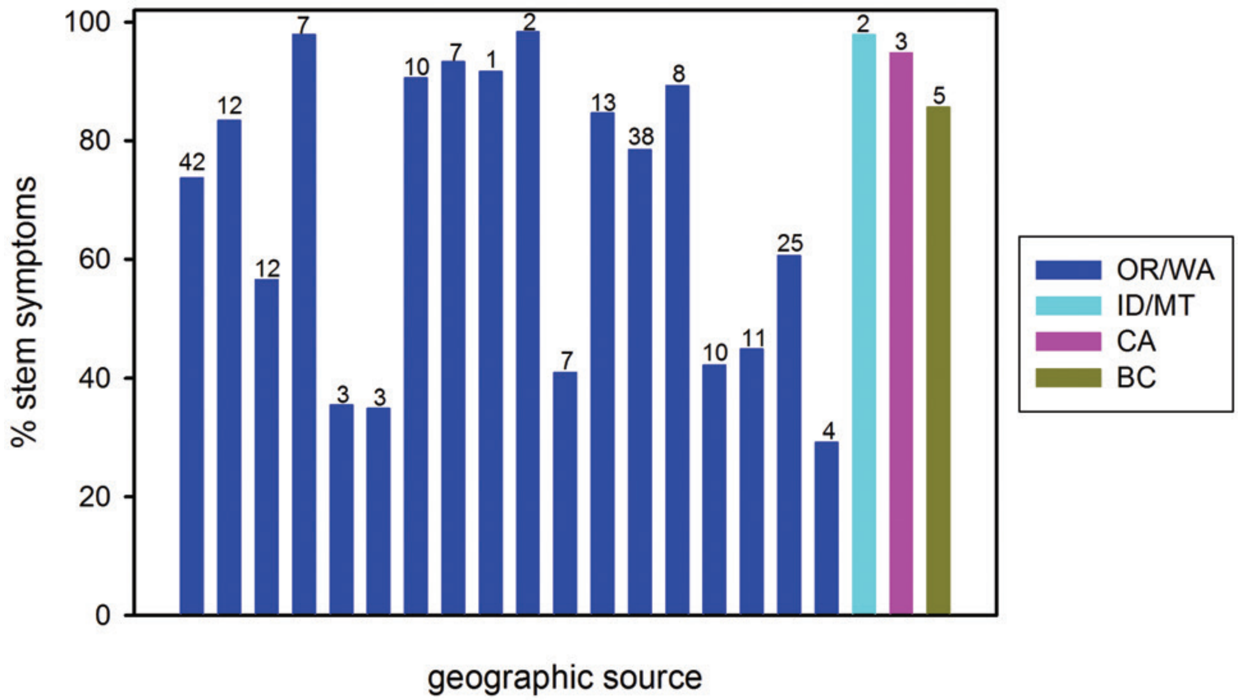

Figure 4—Genetic variation among seed sources in percentage of seedlings with early blister rust stem symptoms ~15 months post-inoculation. The number of half-sib families represented in each seed source mean is indicated above the bar (varies from 1 to 42). The 18 Oregon and Washington sources refer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

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

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

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

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

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

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

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

ACKNOWLEDGMENTS

We thank all those who contributed seed and the technicians who completed many of the assessments. Funding for this project was through the USDA Forest Service Genetic Resource and Forest Health Protection programs in Region 6 as well as Crater Lake National Park (CRLA) and Mount Rainier National Park (MORA). We thank Regina Rochefort (MORA), and Laura Hudson and Jen Beck (CRLA) for their assistance. We thank Michael Murray, Jodie Krakowski, Everett Hansen, Bohun Kinloch, Jr., Bruce Moltzan, Jun-Jun Liu, and James Jacobs for their reviews of an earlier version of this paper.

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