

Blister Rust Resistance Among 19 Families of Whitebark Pine, *Pinus albicaulis*, From Oregon and Washington – Early Results From an Artificial Inoculation Trial

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Whitebark pine is considered one of the most susceptible white pine species to white pine blister rust, the disease caused by the non-native pathogen *Cronartium ribicola*. High mortality from blister rust and other factors in much of the range in the United States and Canada have raised serious concerns about the future viability of this high-elevation forest tree species (USFWS 2011). A major effort is now underway to collect seed for gene conservation, to evaluate trees for genetic resistance to blister rust, and to establish restoration plantings (Aubry et al. 2008, Mangold 2011; Sniezko et al. 2011a, 2011b).

Seed collected from 19 whitebark pine parent trees at four locations in Oregon and Washington were sown in 2005 (fig. 1). The number of families represented in this study varied by geographic source: eight from Mt. Rainier National Park, three from the Colville National Forest, two from the Deschutes National Forest, and six from the Fremont National Forest. The number of seedlings available per family for blister rust inoculations ranged from 21 to 60.

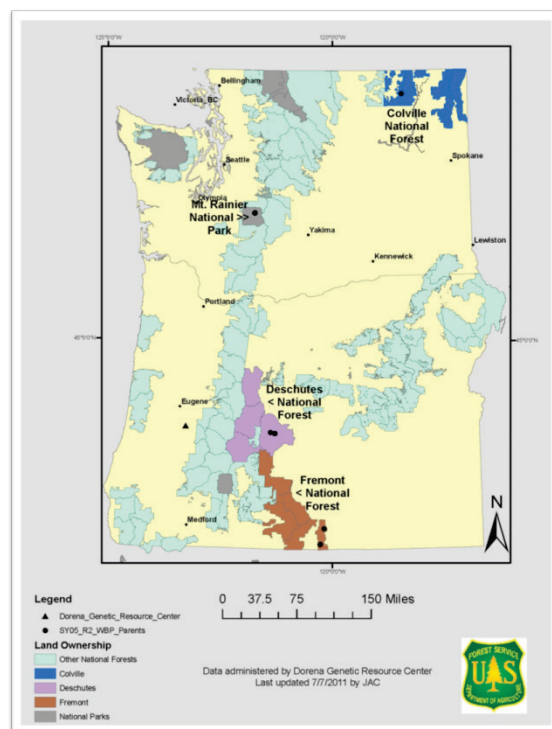


Figure 1—Parent tree locations for 19 whitebark pine families sown in 2005 for a white pine blister rust resistance screening trial.

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A randomized complete block design was used, with each of the 19 families distributed randomly in row plots in six blocks. Within each family, the number of seedlings in each row plot was made as equal as possible between the blocks, but varied between families (from 3 to 10). Seedlings were inoculated with *C. ribicola* twice, initially in 2006, and again in 2009, using the well-established protocols at Dorena Genetic Resource Center (DGRC), Cottage Grove, Oregon. The first inoculation was very effective in producing needle spots (on >99 percent of the seedlings), but, unexpectedly, less than 10 percent of the seedlings later developed cankers. The second inoculation was also very effective in producing needle spots (on >99 percent of the seedlings), and subsequently led to the expected high levels of stem infection (fig. 2). This second inoculation will be particularly interesting to follow; the trees were 5 years old and much larger than any whitebark pine previously subjected to artificial inoculation at DGRC.



Figure 2—(a) Seedlings of whitebark pine in April 2010 (following September 2009 inoculation); (b) needle lesions in June 2010 from blister rust inoculation; (c) and (d) many stem infections on seedlings in early November 2010. (Photo credits: Richard Snieszko)

As of November 2010, 76.6 percent of trees had stem symptoms (SSp). The Deschutes and Fremont locations from eastern Oregon averaged 97 percent and 96 percent SSp, respectively; the Colville location (northeastern Washington) averaged 71 percent SSp, and the Mt. Rainier location (west-central Washington) averaged 58 percent SSp. Family means ranged from 46.9 percent (a Mt. Rainier family) to 100 percent (four families, all in the Fremont population).

The number of stem symptoms per infected tree (excludes trees with needle spots, but no stem symptoms) at the November 2010 assessment averaged 16.4, with family mean number of stem symptoms per infected tree ranging from 7.3 (a Mt. Rainier family) to 28.3 (also a Mt. Rainier family). One individual seedling had 90 stem symptoms. In addition to normal cankers, some bark reactions (complete and partial) were apparent; families from the Colville and Mt. Rainier locations had the highest levels of bark reaction.

To estimate the effect of geographic source (population) and family within a source on tree resistance, analyses of variance (ANOVAs) were conducted on row plot means using SAS PROC GLIMMIX (SAS 2008). For these analyses, population and family within population were considered fixed effects, while block and associated interactions were treated as random effects.

The first response examined was the presence or absence of stem symptoms. A binomial model with logit link was applied to the data. In cases where the proportion of trees with a stem symptom was 0 or 1 in a row plot, an adjustment of +0.01 or -0.01 was applied to the row plot mean, respectively. The analysis indicates that there is strong evidence of an association between the odds of getting a stem symptom and the geographic source of the family parent ($p < 0.0001$, fig. 3). In addition, within a source there is suggestive evidence of a family difference in the odds of getting a stem symptom ($p = 0.055$). It is estimated that the odds of getting a stem symptom for seedlings with parents in the Deschutes and Fremont National Forests are 19.6 and 30.7 times higher (respectively) than for seedlings with parents in Mt. Rainier National Park; the 95 percent confidence intervals with Tukey-Kramer adjustment are from 3.9 times higher to 99.2 times higher and from 7.0 times higher to 134.3 times higher for Deschutes and Fremont, respectively.

The second response examined was the number of stem symptoms per infected tree. A negative binomial model with log link was applied to the data. There is suggestive but inconclusive evidence of an association between the number of stem symptoms on an infected tree and the geographic source of origin of the family parent ($p = 0.057$, fig. 3). However, within sources, there is convincing evidence of family differences in the mean number of stem symptoms per infected tree ($p < 0.0001$). If all trees (including those with no stem symptoms at this stage) are included in the analysis, then the evidence of an association between the number of stem symptoms and the geographic source of origin is highly significant ($p = 0.0002$), with families from the two northern sources generally having much lower means than those from the Deschutes and Fremont National Forests.

The early results for this trial show similar trends to those observed from previous inoculation trials at DGRC. Families from Mt. Rainier were highest in resistance: all families from Mt. Rainier showed fewer seedlings with stem symptoms and tended to have fewer stem symptoms than those from Deschutes and Fremont. The two northern-most sources (of the four tested here), Mt. Rainier and Colville, in this trial have the highest level of resistance. Other recent trials have suggested possible geographic trends in the percentage of seedlings developing stem symptoms (Snieszko et al. 2007; Snieszko, unpublished).

This trial included relatively few families from each of the four populations, but the trends for SSp observed in this trial are consistent with population results from other trials at DGRC (Snieszko, unpublished). However, screening of more families from each population will likely identify families with some level of resistance even amongst populations such as Deschutes and Fremont that have very high SSp in this trial.

At this stage of the trial, there are differences in both the percent of trees with stem symptoms (between populations and between families within populations) as well as in the number of stem symptoms per tree (between families). Both of these traits may have utility in selecting parents that will be most resistant to blister rust in the field. The first field trials for whitebark pine have recently been established and will serve to help validate seedling resistance screening as well as monitor the durability of rust resistance.

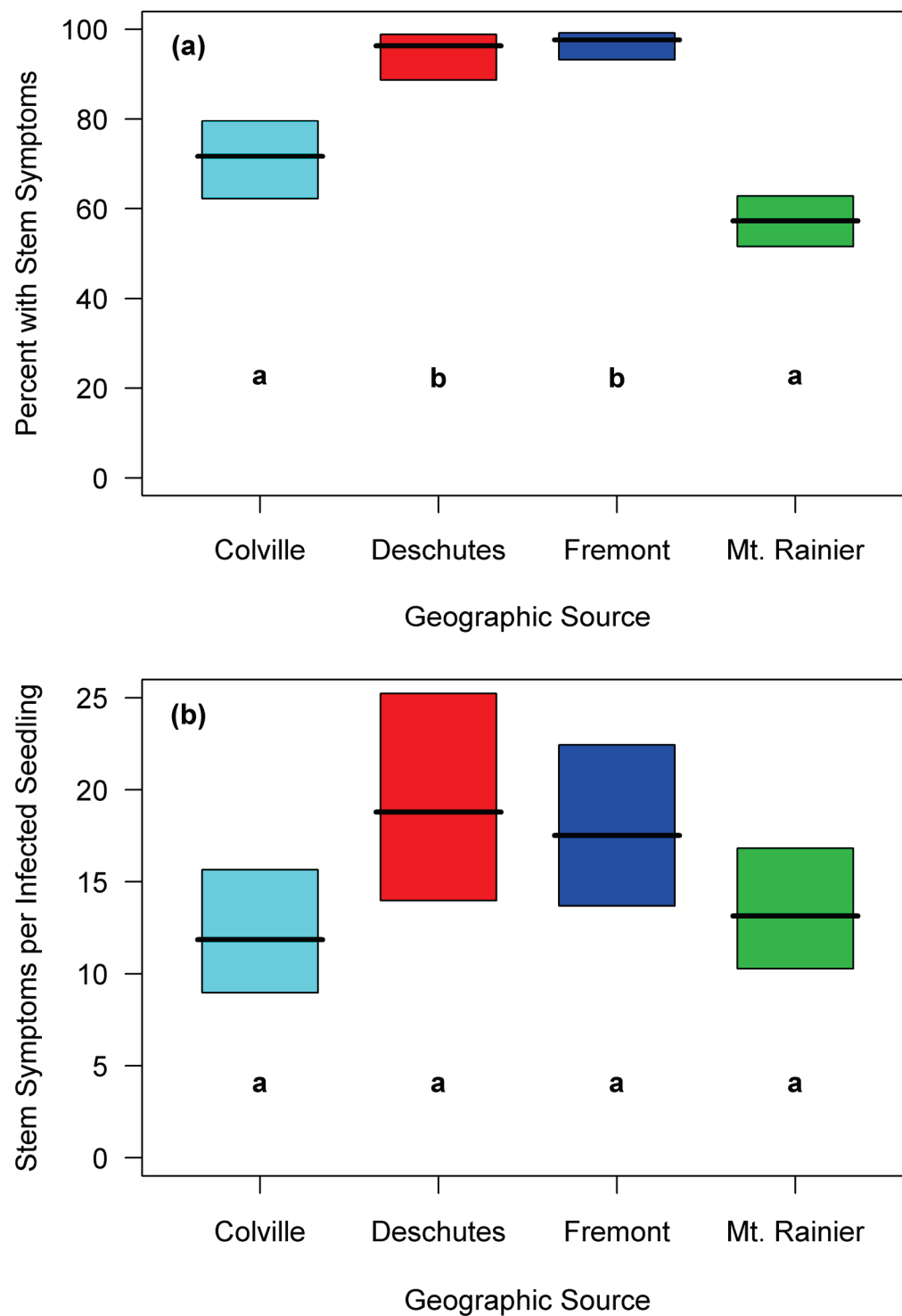


Figure 3—Least squares means and 95 percent confidence intervals for (a) the percent of seedlings with stem symptoms, and (b) the number of stem symptoms per infected seedling for each source using the final models described in the text. Locations sharing the same letter indicate no significant difference at the $\alpha=0.05$ level after Tukey-Kramer adjustment.

Canker severity is currently low (3 on a 9 point scale), but is expected to increase dramatically over the next year. Mortality from blister rust is currently low (3.7 percent), but is expected to continue, with very high mortality expected over the next 2 years. Seedlings will be assessed annually over the next several years for additional stem symptom development and mortality. The high levels of canker-free seedlings in some families at this stage are encouraging, but at least one additional year of assessments will be needed to discern latent stem symptoms. The parents identified with genetic resistance to blister rust in seedling screening trials will be a key component to successful restoration of whitebark pine. Seedlings from top families can be grafted for gene conservation, and land managers will be contacted concerning the potential to collect seed from the top parents to use for restoration.

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