Preparing for Invasion: Rust Resistance in Limber, Great Basin Bristlecone, and Rocky Mountain Bristlecone Pines

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Rocky Mountain (RM) bristlecone pine (*Pinus aristata*), Great Basin (GB) bristlecone pine (*P. longaeva*), and limber pine (*P. flexilis*) are high-elevation five-needle pines threatened by the non-native pathogen Cronartium ribicola that causes the disease white pine blister rust (WPBR). The pathogen continues to spread, and the infection front is now in the southern Rocky Mountains and Great Basin. WPBR is increasing in these areas on limber pine and RM bristlecone pine as well as whitebark pine (*Pinus albicaulis*); WPBR has not yet been documented on GB bristlecone in its native range. Because many of these populations are still healthy, they offer opportunities to assess baseline frequencies of WPBR resistance traits in largely naïve populations and to make comparisons among host species (Schoettle et al. 2012). Information on genetic resistance traits and their frequencies also supports development of proactive interventions to increase the frequency of resistance in populations before pathogen invasion, to mitigate future impacts and sustain ecosystem function during pathogen naturalization (Burns et al. 2008; Schoettle and Sniezko 2007; Schoettle et al. 2019a, b).

We present results from the first extensive *C. ribicola* inoculation common garden studies to assess quantitative resistance in seedling families for limber and the two bristlecone pine species (table 1). Seedling families were grown from seed collected from stands that, at the time, had little to no WPBR infections and no WPBR-caused mortality and were therefore randomly selected from these populations. More complete analyses and interpretation of the results from these studies will be presented elsewhere. Other studies, not reported here, have been conducted to assess each of these three species for qualitative resistance to WPBR (i.e., complete resistance, major gene resistance, MGR), which was only detected in limber pine (Schoettle et al. 2014; Schoettle et al. unpublished data; Sniezko et al 2016; Vogler et al. 2006).

RM bristlecone pine, GB bristlecone pine, and limber pine are highly susceptible to WPBR in seedling screening trials though they differ in their response to inoculation (figure 1). Disease resistance was evident in some families of each species and varied among species (data not shown) resulting in differential survival after inoculation (figure 2). The RM bristlecone pine seedlings in Study B were younger at the time of inoculation than those in Study A (2 years and 3.5 years, respectively) which likely contributed to the more rapid decline in vigor and post-inoculation survival in Study B (figure 2). Variation in disease progression and rates of mortality following inoculation were observed among seedling families and source areas for each species (data not shown). These studies suggest that (1) southern Rocky Mountain sources of limber pine appear to have a very low frequency of quantitative resistance to WPBR, and (2) both bristlecone pine species have higher frequencies of quantitative resistance, which may be the highest of the North American five-needle pines. The relative susceptibility of limber pine and RM bristlecone

Figure 1. Examples of symptoms of WPBR on seedlings of RM bristlecone (left), GB bristlecone (center), and limber (right) pine observed after artificial inoculation with *C. ribicola* at DGRC (Study C).

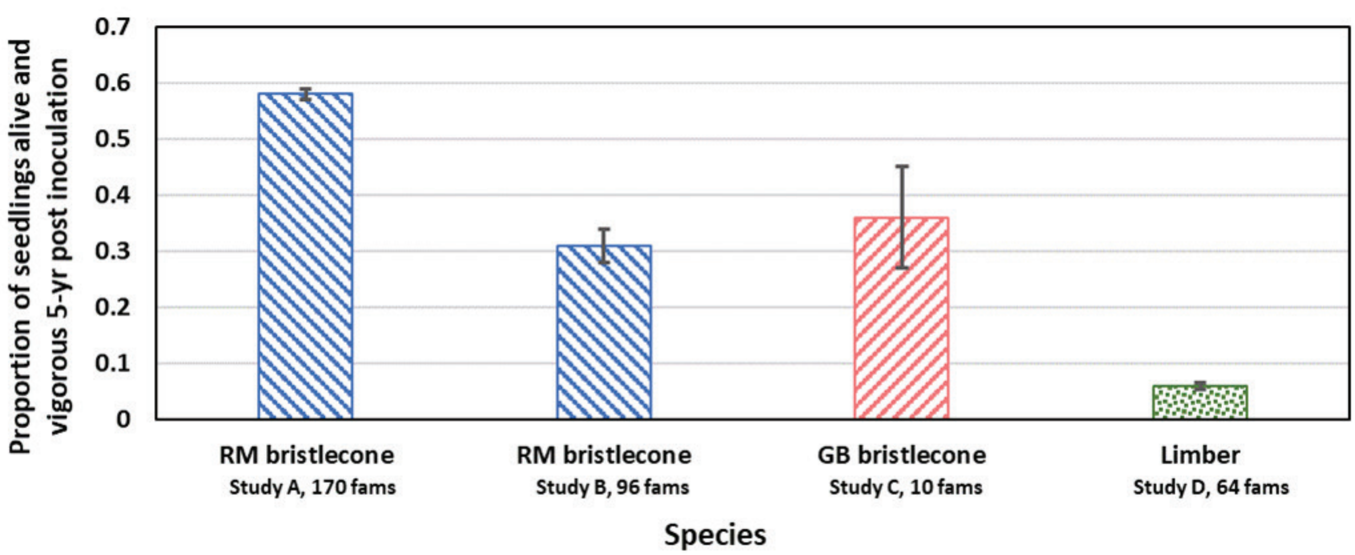

Figure 2. Preliminary results of the proportion of seedlings of each family, species and study that were alive and vigorous five years post artificial inoculation. Variation among families is shown as standard errors. MGR families of limber pine were removed from the average survival reported here. Results are from studies A, B, C (GB bristlecone only), and D (see table 1).

pine agrees with smaller, earlier trials by others (Hoff et al. 1980; Stephan 2004) though the two bristlecone species were mixed and referred to as *P. aristata* in one, and possibly both, earlier tests. As observed for quantitative resistance in other white pines, there is some evidence the resistance may be lower under higher disease pressure for RM bristlecone pine (Jacobi et al. 2018), and the future rust hazard of the environments in which these species occur under climate change may influence overall survival. These species are susceptible to WPBR suggesting that if a stand does not currently contain WPBR-diseased trees it is likely a result of ecological context, and not necessarily genetic resistance of the pines to WPBR. That is, the dry habitats and low *C. ribicola* spore availability of the Great Basin, conditions that may change in the future, likely contribute to why GB bristlecone pine is not currently diseased in the field. However, in the presence of *C. ribicola*, genetic resistance to WPBR is expected to be a significant determinant of a species' population trajectories. Field verification of WPBR resistance expression under natural conditions for RM bristlecone and limber pine families is ongoing as part of the Southern Rockies Rust Resistance Trial in southern Wyoming (Schoettle et al. 2018) and a clone bank of grafted material from the resistant limber pine seedlings has been established in Colorado.

Table 1. The first extensive artificial inoculation trials of individual-tree seed lots (families) of RM bristlecone, GB bristlecone, and limber pine for quantitative resistance to white pine blister rust. Studies are collaborations between Rocky Mountain Research Station, Rocky Mountain Region Forest Health Protection, National Park Service (Great Sand Dunes National Park & Preserve and Great Basin National Park), Pacific Northwest Region Dorena Genetic Resource Center (DGRC), and Pacific Southwest Research Station Institute of Forest Genetics (IFG). The inoculations were conducted at DGRC (Studies A, C, D, and E) and the Vogler lab at IFG (Study B) and phenotypes were assessed collaboratively. Methods are described in Schoettle et al. (2011) and Vogler et al. (2006). Study E is on-going (results not reported here).

Study	Species	# Families	Seed Sources
A	RM bristlecone	170	CO
B	RM bristlecone	96	CO
C	GB bristlecone	10	NV
C	RM bristlecone	2	CO
C	Limber	3	CO, WY
D	Limber	64	CO, WY
E	GB bristlecone	20	NV
E	Limber	20	NV

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