Update on the Southern Rockies Rust Resistance Trial (SRRRT)

Biological Evaluation R2-18-01

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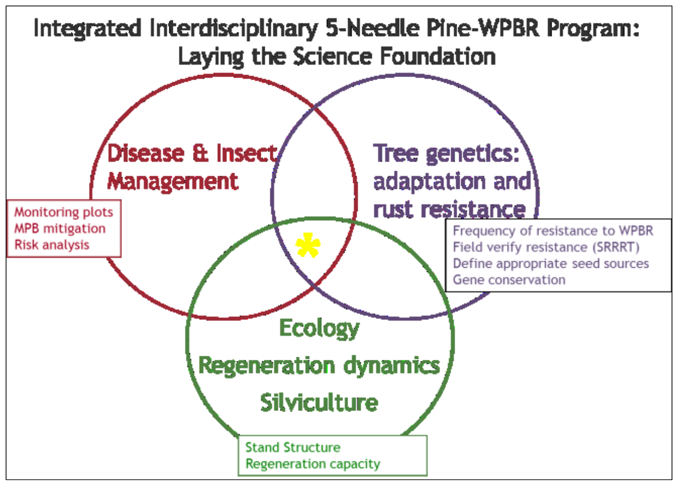
Introduction



The nonnative pathogen *Cronartium ribicola,* that causes white pine blister rust (WPBR), is spreading through limber pine (*Pinus flexilis*, PIFL) and Rocky Mountain bristlecone pine (*P. aristata*, PIAR) forests of the Southern Rocky Mountains.

An integrated regional program – the Proactive Strategy – is characterizing the infestation and gaining genetic and ecological knowledge of these formerly under-studied ecosystems to provide the science foundation for early interventions to mitigate the development of ecological impacts to the high mountain headwater ecosystems (Burns et al. 2008, Schoettle et al. 2011a). The Proactive Limber Pine Conservation Strategy recommends identifying and developing planting material with genetic resistance to WPBR as essential to sustain these forests into the future (Schoettle et al. in press).

Objectives

The Southern Rockies Rust Resistance Trial (SRRRT) was initiated in 2013 to verify the stability of genetic resistance to WPBR identified during artificial inoculation screening tests for limber and Rocky Mountain bristlecone pines. The artificial inoculation tests were conducted by RMRS in collaboration with R2-FHP and USFS Dorena Genetic Resource Center (Cottage Grove, OR) and USFS Institute of Forest Genetics (Placerville, CA) (Schoettle et al. 2011b, 2014). Growing conditions and inoculum sources can affect the expression of disease resistance, so verification that the resistance is stable under natural growth and inoculation conditions provides the scientific justification for seedling deployment for proactive plantings and increases the potential for restoration success. The objectives of the SRRRT are (1) test the effectiveness of this location at infecting seedlings in a timely manner, and (2) field verify resistance expression and durability as estimated in controlled inoculation screenings.

Installation

An existing administrative site on the Pole Mountain Unit of Laramie Ranger District, Medicine Bow National Forest (MBNF) that was used as a USDA Forest Service nursery in the past was revitalized for this project. Administrative approval and site preparation were completed in 2012 and 2013. An area less than two acres was cleared and fenced by MBNF in 2013 for the SRRRT planting. The fenced area was made large enough to host other future plantings.

Limber pine and bristlecone pine seed from previously-tested resistant and susceptible individual-tree collections (i.e. families) was sown and seedlings grown at the Colorado State Forest Service Nursery (Fort Collins, CO). Thirteen limber pine families and eleven Rocky Mountain bristlecone pine families are included in the study and represent seed sources from throughout the Southern Rocky Mountains (Fig. 1).

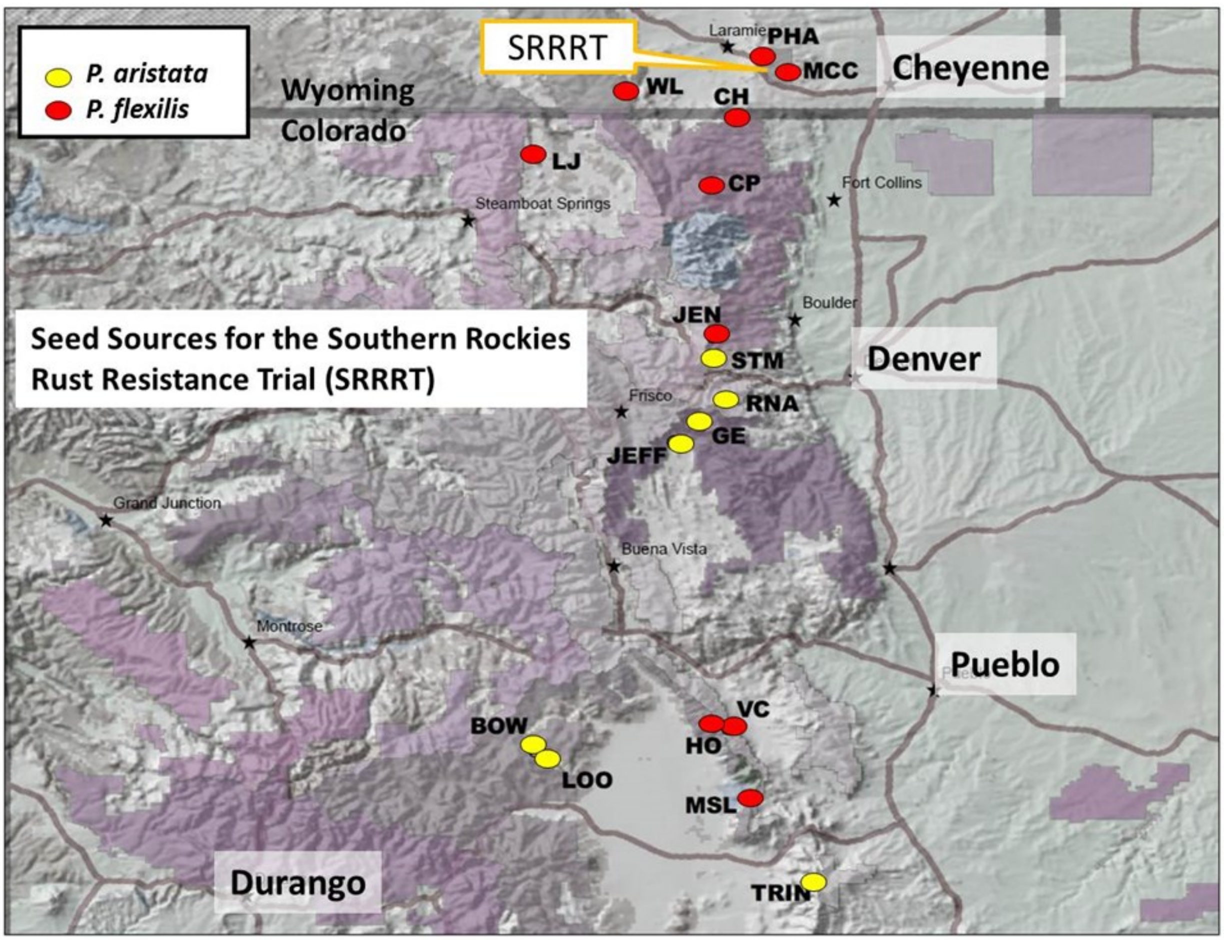


Figure 1. *Map of seed sources planted in 2013 and 2014 at the SRRRT site.*

Seven hundred and twenty seedlings were outplanted in the fall 2013 and another 720 seedlings in spring 2014; fall and spring planted seedlings were interspersed (Fig. 2). Each family is represented by 60 seedlings; the field layout included 6 blocks with 10 seedling per family in each block (see Appendix).

Weed barrier and a drip irrigation system were installed prior to planting. Peat moss was mixed in with the soil at planting to increase porosity of the very fine loess soils. WPBR is common in the forests in and around the SRRRT site providing a natural source of *Cronartium ribicola* inoculum to challenge the seedlings. Periodic assessments for seedling survival, growth, and signs and symptoms of WPBR were scheduled bi-annually over the next 10 years.

Figure 2. *Planting the site.*

Seedling Establishment and Growth

Limber pine had better seedling establishment than bristlecone pine and the fall planting had superior survival for both species. Overall, bristlecone pine survival in the first two years after planting was approximately 15% less than that of limber pine and the spring planting resulted in 24% less survival than the fall panting (Fig. 3).

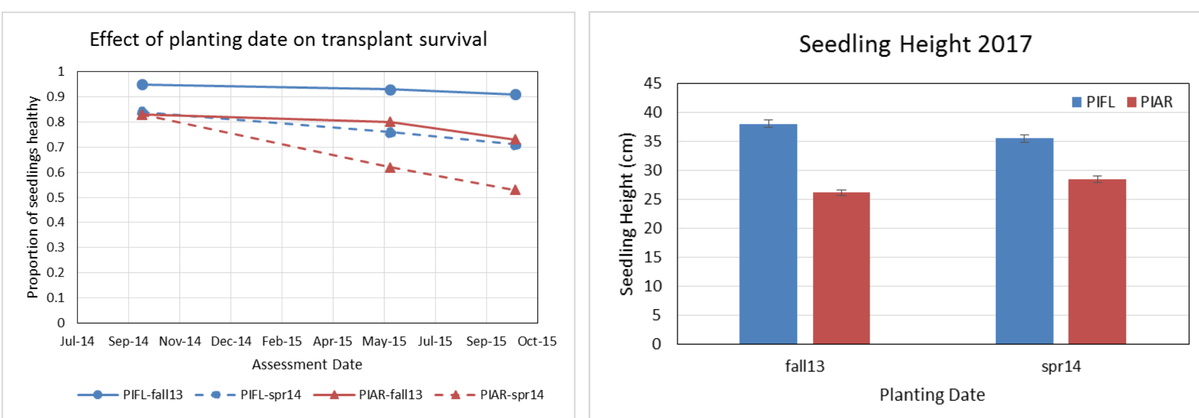


Figure 3. *Effect of planting date on seedling survival and seedling height as of the summer of 2017.*

Seedlings have grown well at the site. The limber pine seedlings are consistently taller than the bristlecone seedlings. Limber pine seedlings planted in the fall are taller than those planted the following spring while the reverse is true for bristlecone pine seedlings (Fig. 3).

White Pine Blister Rust Infection

WPBR infections were first assessed in August 2016 on limber pine. At that time 28% of the seedlings had signs and symptoms of WPBR. In August 2017, the proportion of limber pine seedlings with WPBR increased to 46%; 56% of the bristlecone pines were symptomatic for WPBR in 2017.

The study includes three known susceptible families (two limber pine and one bristlecone pine) which previously tested to have near 100% susceptibility to inoculation with *C. ribicola*. These families serve as monitor for exposure to *C. ribicola* since they should become symptomatic with WPBR if they are exposed. As of August 2017 infection levels were at 57% and 59% for the susceptible limber pine families; the known susceptible bristlecone pine family was 78% symptomatic for WPBR. These results suggest that overall, it is likely that 57% - 78% of the seedlings have been exposed to *C. ribicola* spores thus far. For comparison with the artificial inoculation studies, the goal is for all of the seedlings in this field trial to be challenged with *C. ribicola* so we can be confident that seedlings that are free of disease are likely to be expressing resistance to the pathogen rather than having escaped exposure. Depending on the weather conditions, we estimate it may take another 2-5 years for all seedlings to be exposed to the pathogen, and another few years to show definitive signs of disease.

For those seedlings that are infected and symptomatic, limber pine seedlings had on average 2.1 cankers while bristlecone seedlings had on average only 1 canker per seedling. However, the severity of the infections was more advanced on bristlecone pine than limber pine and has led to greater seedling mortality (Fig. 4). Both species showed evidence of partial bark reactions and complete bark reactions, consistent with observations from the artificial inoculation studies. Results thus far suggest that bristlecone pine seedlings are more susceptible to expressing WPBR symptoms at SRRRT than under the controlled inoculation conditions; this preliminary finding was unexpected and will be further explored as data collection continues.

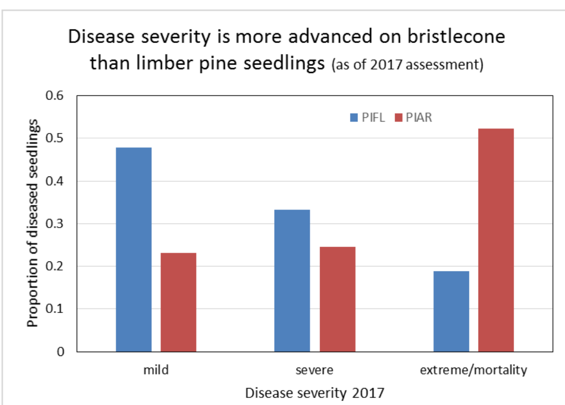


Figure 4. *WPBR severity on limber pine and bristlecone pine seedlings. The proportion of diseased seedlings by species in each severity class is shown as of summer 2017.*

Figure 5. *Symptoms of WPBR on limber pine seedlings in 2017 at SRRRT. From left to right: mature WPBR canker with orange aecia spores; early canker with* C. ribicola *spermatia (also known as pycnia) on a branch; early canker with* C. ribicola *spermatia on the main bole.*

Outcome to Date

The SRRRT site has proven to be a good location for exposing seedlings to *Cronartium ribicola* inoculum and providing the conditions for disease expression and development. To complete the comparison with the controlled inoculation trials, the study should continue for at least another 5 years to provide ample opportunity for all of the seedlings to be challenged. During that time, watering will be needed intermittently as well as weeding. The weed barrier is deteriorating and will need to be repaired, patched or replaced with time.

The site is now a model for two similar installations being installed in Region 3 by FHP and Northern Arizona University.

A Proposal for the Future

Given the effective inoculation of seedlings at the SRRRT site, we propose that this site be further utilized as a prescreening facility for proactive rust resistance testing. Because the Proactive Strategy recommends screening progeny from trees in stands that have not yet been invaded by WPBR, we do not have the advantage of selecting phenotypically resistant seed trees in the field as we would if we were sampling stands in which WPBR had already caused extensive disease or mortality. Consequently, seed trees for testing are not preselected for resistance and therefore many of them have been fully susceptible. The goal of screening for resistance is two-fold: first to estimate the frequency of resistance in the stand and second to identify trees with heritable resistance to be used as seed sources for seedling plantings. Our current seed tree sampling satisfies the first goal but is inefficient at achieving the second. Compounding the problem is the expense of testing seedling families at the remote screening facilities, especially if many of the families prove to be fully susceptible. We propose that seedling families be prescreened at the SRRRT site – this would enable the Region to identify those families that appear to be more resistant than most and then invest funds to have those select families further tested at a controlled inoculation facility. Field screening sites are being used in Canada for WPBR resistance and for programs for other diseases (Woodcock et al. 2017). If this proposal is acceptable to the MBNF, it will also require Regional support.

Challenges

Available water on site, provided by MBNF, has been essential to achieve the high level of seedling survival. Seedlings were watered as needed, generally 3-4 times per summer. The site is in a cold air drainage and we have had problems with freezing pipes in the fall. We are working on improving communication among the cooperators to avert this situation; a frost-proof hydrant would also facilitate keeping the pipes drained and preventing damage. The weed barrier helped but weeds in the growing in the planting holes has required constant attention. The planting requires weeding at least three times per summer and each weeding campaign takes two people approximately a week to complete; RMRS has been conducting this with intermittent assistance from R2-FHP.

Marmots were a problem in the years immediately following planting. Control was achieved with buried perimeter fencing, MBNF trapping, and sonic ground stakes. No traps or stakes have been deployed for in the past two years. Small gophers and voles have burrowed under the weed barrier and cut holes in the weed cloth (Fig. 6); they have caused some seedling damage in the northern part of the planting but less than 10 seedling were killed. No control measures have been attempted.

Figure 6. *Rodent damage.*

Evidence of wintertime ice abrasion and sun scald to seedlings was obvious in 2014 on the seedlings planted in fall 2013. Inspection during the following winter revealed that the snow was blown from the site (Fig. 7) and therefore the seedlings were not being protected by snowcover. Snow fences were erected in October 2015 to increase snow retention on the seedlings; winter damage to seedlings has since been nominal.

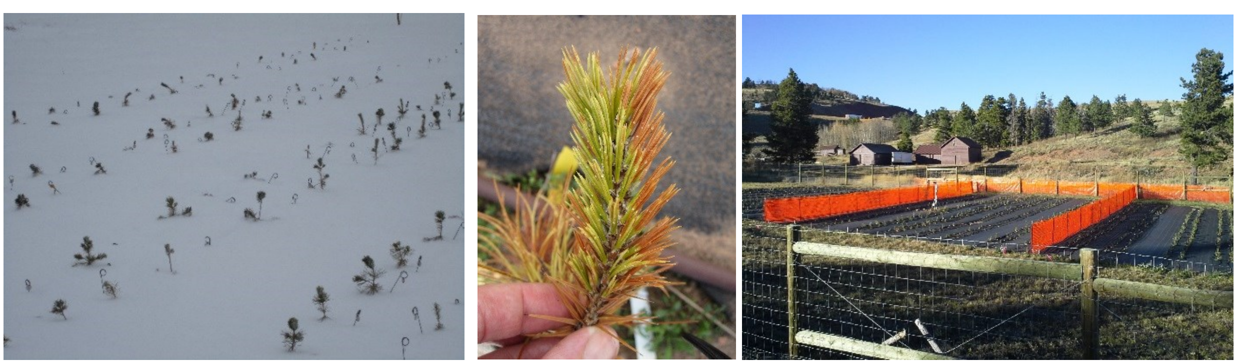


Figure 7. *Inadequate snow cover to provide protection to seedlings (Feb. 2015), resultant ice/sun scald on bristlecone, and the snow fence installation to increase snow retention (Oct 2015).*

In the future, we recommend that disease assessments begin as early as the first year post-planting. Disease symptoms were slower to develop under controlled conditions in bristlecone pine and hence we delayed assessments until 2017; this was not a good decision as it appeared that some seedlings had already died from WPBR but it was very difficult to confirm it on a dead seedling. Consequently, the bristlecone pine mortality estimates from WPBR may be unreliable.

Funding

Installation of this project was funded largely by the Rocky Mountain Region - Regional Office, with in-kind contribution from MBNF, RMRS, R2-FHP, Colorado State University, Wyoming Conservation Corps, and community volunteers. Maintenance and monitoring is funded by RMRS and MBNF with in-kind contribution from R2-FHP, CSU, and community volunteers.

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Appendix – SRRRT plot layout

Southern Rockies Rust Resistance Trial

6 blocks of 10-sdlg family row plots (5 sdlgs/fam planted in 2013, 5 planted in 2014)
The seedlings spaces 1-10 in block 1 row 1 are all one family, positions 11-20 are a second family, etc
For each species, families row plots were randomized within each block
Seedlings within a row are spaced 2 ft apart (each square is 2x2ft)
Space between pair of rows is 2ft
Space between blocks is 4 ft
13 PIFL families; 11 PIAR families
See study summary for more details