

# Past and Current Investigations of the Genetic Resistance to *Cronartium ribicola* in High-elevation Five-needle Pines

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**Abstract**—All nine species of white pines native to the U.S. or Canada are susceptible to the introduced pathogen *Cronartium ribicola*. Of the six high elevation white pine species, the severe infection and mortality levels of *Pinus albicaulis* have been the most documented, but blister rust also impacts *P. aristata*, *P. balfouriana*, *P. flexilis* and *P. strobiformis*; only *P. longaeva* has not been documented to be infected in its natural range. Early evaluations of resistance included relatively few seedlots and demonstrated that these species have some genetic resistance to blister rust but generally less than their Eurasian relatives. Recently, more extensive evaluations of these six species have begun. These recent rust tests capitalize on the methods developed from decades of prior experience by the USDA Forest Service in testing *P. monticola* and *P. lambertiana*. Following artificial inoculation, seedlings are evaluated for up to five years for an array of putative resistant responses including reduced number of needle spots, needle spot color, hypersensitive reaction in the needles, shedding of infected needles, presence or absence of stem infections, number of stem infections, latency of infection, severity of infection, bark reactions, and survival with stem infections. *P. albicaulis* has undergone the most extensive testing, with 650 families inoculated as of 2010. Seedling families of *P. albicaulis* have been tested under several inoculum densities and with different geographic sources of blister rust. Extensive work is also underway for *P. aristata* and *P. flexilis* and significant efforts have begun with *P. balfouriana*, *P. longaeva* and *P. strobiformis*. Initial results indicate that common resistance responses appear to be present in most species, that geographic variation in some types of resistance are present, and that some types of resistance may not be present in some species. Many more field selections are needed to build sufficient genetic diversity among resistant selections for each species within a breeding zone. A few field trials have been established for *P. albicaulis*, but additional trials for long-term verification of rust resistance from seedling screenings should be established to examine durability of rust resistance, correlated response with other adaptive traits, and response to climate change. Further examination of the many *P. monticola* field trials, some more than 30 years old, will help provide data on durability and stability of resistance in this species and give possible insights for examining the six high elevation white pine species. Regional programs can use resistance information to designate additional parent trees for seed collection, establish seed orchards to produce greater levels of rust resistance, and provide *ex situ* conservation of resistant individuals. The USDA Forest Service's Inland West program for *P. albicaulis* was the first to begin, but most regions are now active to varying extents. Based on results of seedling tests, the first resistant seedlings have been planted in several regions. Continued research on underlying resistance mechanisms and their inheritance, potential for greater

virulence/aggressiveness in the pathogen, and molecular tools to facilitate more efficient resistant selections are needed. Recent discussions of high levels of infection in the native ranges of some Asian species in China and South Korea suggest that a more virulent/aggressive race of *C. ribicola* may be present, and that a second species may exist; if so, then added precautions are needed to prevent importation of these pathogenic variants or other species.

## Introduction

The nine white pine species native to the United States or Canada are highly susceptible to white pine blister rust, caused by the invasive pathogen *Cronartium ribicola* J.C. Fisch. in Rabh. *C. ribicola* is currently present in the native ranges of eight of these white pine species, and it has already produced severe mortality and ecosystem disruption in some (Tomback and Achuff 2010). The focus in this paper is on continuing efforts to evaluate the genetic resistance of the six high elevation species present in the U.S. and Canada: *P. albicaulis* Engelm. (whitebark pine), *P. aristata* Engelm. (Rocky Mountain bristlecone pine), *P. balfouriana* Grev. & Balf. (foxtail pine), *P. flexilis* James (limber pine), *P. longaeva* D.K. Bailey (Great Basin bristlecone pine), and, *P. strobiformis* Engelm. (southwestern white pine). Eradication of *C. ribicola* is not feasible, and it is now a permanent resident of most of our white pine ecosystems. Genetic resistance to white pine blister rust will be a key to retaining or restoring populations of these species in areas that have been heavily impacted by the rust. Proactive measures to alleviate future impacts of rust will also require knowledge of the frequency of genetic resistance (Schoettle and Snieszko 2007).

Increasing concerns about the impact of the blister rust on natural populations of the high elevation white pine species has given impetus to make extensive seed collections for gene conservation (Snieszko and others, *Ex situ* gene conservation in high elevation white pine species in the United States—a beginning, this proceedings) and to undertake blister rust resistance testing of these species. The early work examining blister rust resistance with these white pine species used relatively few seedlots but indicated that some degree of genetic resistance was present in at least some of them (Delatour and Birot 1982; Hoff and others 1980; Stephan 1986). Additional

work is needed to elucidate the frequency and types of resistance present in each species in order to provide land managers with options to utilize this resistance.

In this paper, we summarize efforts underway to evaluate genetic resistance to white pine blister rust for these six species and prospects for the future. For most of the six species, resistance screening trials have only recently begun, and many of the specific results await summary and publication in the next few years. Key questions include: What types of resistance, if any, are present? What is the frequency and geographic distribution of the various resistance mechanisms for each species? What is the mode of inheritance for these mechanisms? How is resistance screening done and what are its limitations? What is the capacity to screen more progenies of more parent trees? How do we utilize the resistance? Will the resistance be 'durable'? How will climate change affect resistance? How soon can we utilize resistance for restoration?

## Past and Current Resistance Screening

The USDA Forest Service has over 65 years of experience in screening trees for resistance to blister rust, most of it involving *P. monticola* Douglas ex D. Don and *P. lambertiana* Douglas (McDonald and others 2004). A series of international trials involving inoculation of seedlings, proposed by Bingham and Gremmen (1971), and implemented in Idaho,

France, Germany and Japan, provides most of the pre-2000 data on resistance for some of the high elevation white pines and racial variation in virulence in the pathogen (see Hoff and McDonald 1993; Stephan 1986 for summaries).

The USDA Forest Service is the principal organization investigating blister rust resistance in these six species. Screening facilities at three Forest Service locations (see below) are currently utilized among five groups examining genetic resistance to blister rust. Testing of the six high elevation species uses protocols similar to those in the long-standing Pacific Northwest and Interior West programs for *P. monticola* and *P. lambertiana*. Forest Service programs in the Interior West and the Pacific Northwest regions have begun screening the progenies of hundreds of parent trees of *P. albicaulis* for resistance; in addition, the Rocky Mountain Research Station has led the effort to collect seed and evaluate large numbers of seedlots of *P. aristata* and *P. flexilis* for resistance to blister rust (Table 1) (Schoettle and others, Preliminary overview of the first extensive rust resistance screening tests of *Pinus flexilis* and *Pinus aristata*, this proceedings). The Institute of Forest Genetics (IFG) at the Pacific Southwest Research Station is evaluating resistance in all six species (Vogler and others 2006), and the Southwestern Region (Region 3) has expanded the testing of *P. strobiformis* at Dorena Genetic Resource Center (DRGC; Table 1). Cooperators from several other organizations in the United States and Canada have provided some seedlots and/or funding to assist with efforts.

**Table 1.** Number of seedlots of six high elevation white pine species in white pine blister rust resistance testing (inoculated with rust as of fall 2010) by test location<sup>a</sup>.

| Species                | Test location | # Individual Tree seedlots      |                                  |       | # Bulk seedlots    |                     |       |
|------------------------|---------------|---------------------------------|----------------------------------|-------|--------------------|---------------------|-------|
|                        |               | Long duration test <sup>b</sup> | Short duration test <sup>c</sup> | Total | Long duration test | Short duration test | Total |
| <i>P. albicaulis</i>   | CDA           | 200                             | 0                                | 200   | 3                  | 0                   | 3     |
|                        | DGRC          | 380                             | 0                                | 380   | 1                  | 0                   | 1     |
|                        | IFG           | 0                               | 70                               | 70    | 0                  | 0                   | 0     |
| <i>P. aristata</i>     | DGRC          | 189                             | 0                                | 189   | 11                 | 11                  | 11    |
|                        | IFG           | 0                               | 108                              | 108   | 0                  | 0                   | 0     |
| <i>P. balfouriana</i>  | IFG           | 0                               | 14                               | 14    | 0                  | 0                   | 0     |
| <i>P. flexilis</i>     | DGRC          | 70                              | 271                              | 341   | 0                  | 32                  | 32    |
|                        | IFG           | 0                               | 33                               | 33    | 0                  | 2                   | 0     |
| <i>P. longaeva</i>     | IFG           | 0                               | 84                               | 84    | 0                  | 0                   | 0     |
| <i>P. strobiformis</i> | CDA           | 0                               | 0                                | 0     | 3                  | 0                   | 3     |
|                        | DGRC          | 51                              | 1                                | 52    | 0                  | 0                   | 0     |
|                        | IFG           | 0                               | 76                               | 76    | 0                  | 0                   | 0     |

<sup>a</sup> Number of unique lots within a test location; if a seedlot was tested for both short- and long-duration testing the seedlot was listed only in the long-duration test. Some seedlots have been tested across test facilities (see text for details). See paper for estimate of number of additional seedlots for inoculation in 2011 and 2012.

<sup>b</sup> Long-duration testing is focused on identifying multiple resistance mechanisms. In general the seedlings are 2 or more years old at the time of rust inoculation, the seedlings are planted outdoors and disease symptom development and mortality are followed for up to 5 years. The outdoor planting environment allows vigorous tree growth.

<sup>c</sup> Short-duration testing is focused on identifying complete resistance mechanisms including the HR needle reaction; some other resistance types may also be identified. Seedlings for these tests are in small containers which allow for limited seedling growth. Disease symptoms and mortality are generally followed for 2 years or more, depending on the facility and species.

## Blister Rust Resistance Screening

Blister rust resistance screening, using artificial inoculation, for one or more of the six white pine species discussed here is currently underway at three regional locations: Coeur d'Alene Nursery (CDA) in Idaho and Dorena Genetic Resource Center in Oregon for *P. albicaulis*, *P. aristata*, *P. flexilis*, and *P. strobiformis*; and Institute of Forest Genetics (IFG) in California for all six species (Table 1). The CDA and DGRC programs use protocols that evolved from their long-existing operational resistance screening programs of *P. monticola* (western white pine) and *P. lambertiana* (sugar pine) in which seedlings are evaluated for 3 to 5 years after inoculation for a range of resistance types (Mahalovich 2010; Mahalovich submitted; McDonald and others 2004; Kegley and Snieszko 2004). For the high elevation white pine species, the IFG program had previously focused on short-term testing of young seedlings for one type of resistance, a hypersensitive response (HR) in the needles (Kinloch and Dupper 2002); however, IFG has recently expanded its protocols to evaluate other types of resistance in these species (Vogler and others 2006). A fourth rust resistance program in the western United States at Placerville, California, has focused on *P. lambertiana* (McDonald and others 2004), and more recently, *P. monticola*.

The basic procedure for screening parent trees for genetic resistance to white pine blister rust is to obtain wind-pollinated seed from field selections in natural stands, grow seedlings for one to three years, and then inoculate them with blister rust by suspending infected (with telial stage of *C. ribicola*) *Ribes* leaves over them under conditions of high humidity. Bulk seedlots from populations of trees are used on occasion to examine some broad trends in resistance among populations. After inoculation, resistance responses are observed for one to five years or longer to categorize these seedling families (and their parents) in a gradient from highly susceptible to varying degrees and types of resistance.

Based on resistance screening results, frequencies of various resistance types and their geographic distribution can be discerned for each species. This information can then be used to designate parent trees from which to collect seed for restoration, to select trees to put into seed orchards for future seed production, or to perform repeat inoculations to verify resistance phenotypes and to begin to ascertain their inheritance and robustness.

Artificial inoculation of seedlings under controlled environmental conditions (figures 1 and 2) helps ensure uniform infection of seedlings of the various families in trials. Subsequent resistance rating of parent trees can be done in a relatively short time period (1 to 5 years or more after inoculation, depending on the type of information desired). Each facility fine tunes its operation to help ensure that 100 percent of the seedlings are exposed to sufficient density of rust spores to infect seedlings and minimize escapes. The use of known highly susceptible and resistant checklots is essential to establish the relative effectiveness of artificial inoculation. Susceptible checklots usually show 100 percent needle and stem infection. The source of rust inoculum used by each of the three facilities generally represents that found in the regional areas in which they are located, and may come from *Ribes* gardens and/or field collections of infected *Ribes* (the main alternate host for the blister rust fungus). Little information is available on any differences in pathogenicity of these rust sources on various white pine species. The 'Champion Mine' race (Kinloch and others 2004; McDonald and others 1984), which is more virulent (to HR resistance in *P. monticola*) and possibly more aggressive than some other races of blister rust in western North America, is one of the geographic sources of rust used in inoculation trials at DGRC (Snieszko and others 2007).

Details of artificial inoculation using infected *Ribes* leaves are summarized elsewhere (Kegley and Snieszko 2004, Mahalovich and others 2006, Snieszko and others 2007, Vogler and others 2006). Very high levels of seedling

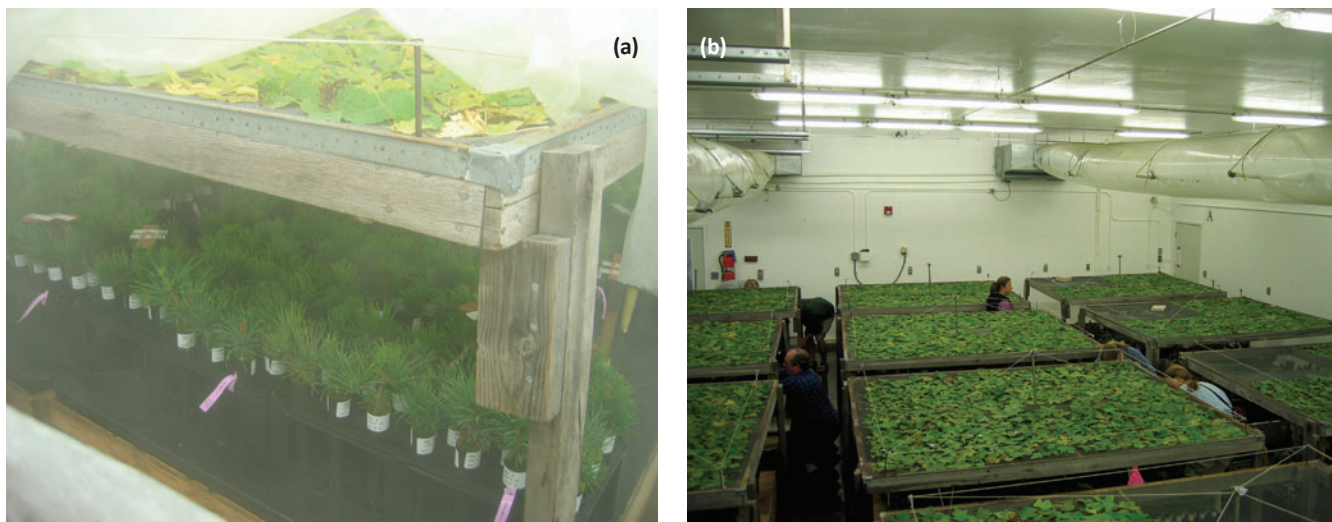


**Figure 1.** Blister rust inoculation setup at Dorena Genetic Resource Center: (a) *P. albicaulis* seedlings in 'fog' chamber for blister rust inoculation; (b) infected *Ribes* leaves over white pine seedlings in inoculation chamber.

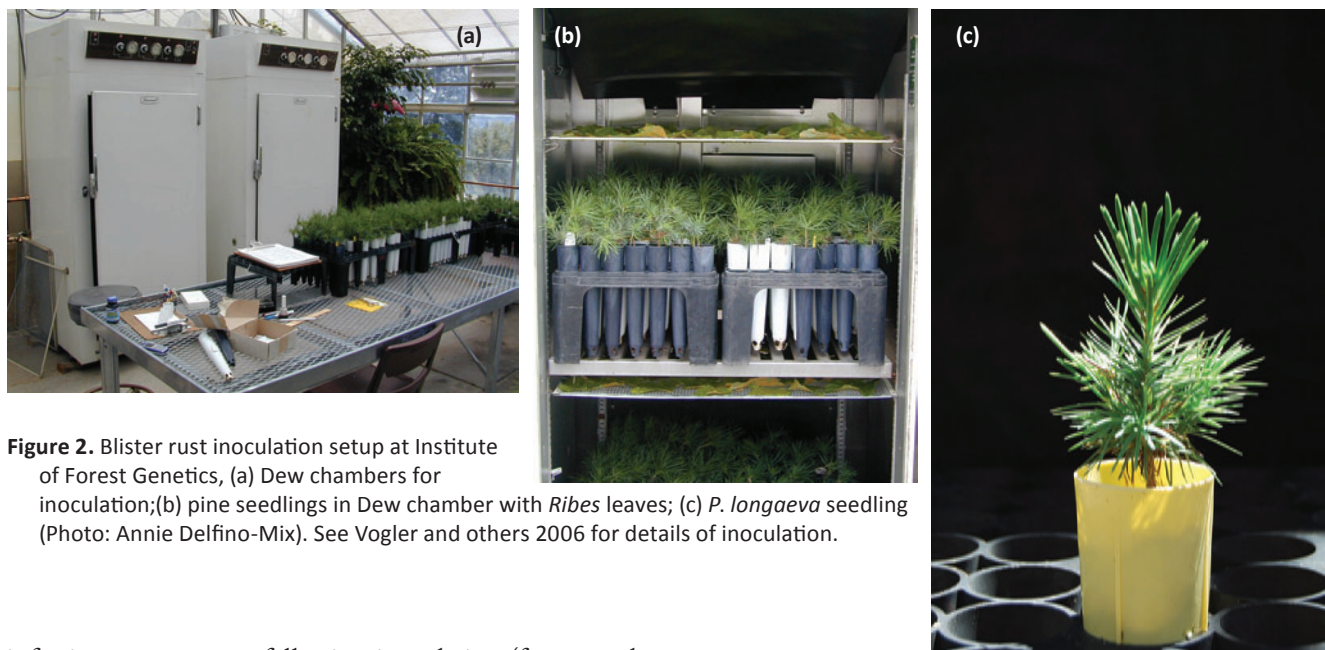


**Figure 2.** Blister rust inoculation setup at Institute of Forest Genetics, (a) Dew chambers for inoculation; (b) pine seedlings in Dew chamber with *Ribes* leaves; (c) *P. longaeva* seedling (Photo: Annie Delfino-Mix). See Vogler and others 2006 for details of inoculation.

infection are common following inoculation (for example, in trials at DGRC, >99 percent of seedlings of *P. albicaulis* and *P. flexilis* typically show needle infections). IFG strives for 100 percent “challenge”, identifies “escapes”, and removes them from the test-population (but they continue to be observed for latent infection or cryptic responses that may be signs of resistance). However, in some species, such as *P. monticola*, artificial inoculation under lower inoculum density can result in some families with less than 100 percent of seedlings with needle spots (Sniezko and Kegley, unpublished data). Families with only this type of resistance

(a higher percentage of seedlings with no needle spots and no cankers after artificial inoculation) may be useful in the field under moderate rust hazards. However, field validation is needed to confirm that these “no-needle-spot” individuals or families are not escapes.

After inoculation, test seedlings are placed in a greenhouse (IFG and DGRC) or outside (CDA and DGRC) for assessments (figure 3). The greenhouse tests are usually short duration (1 to 2 years after inoculation), while the logistics

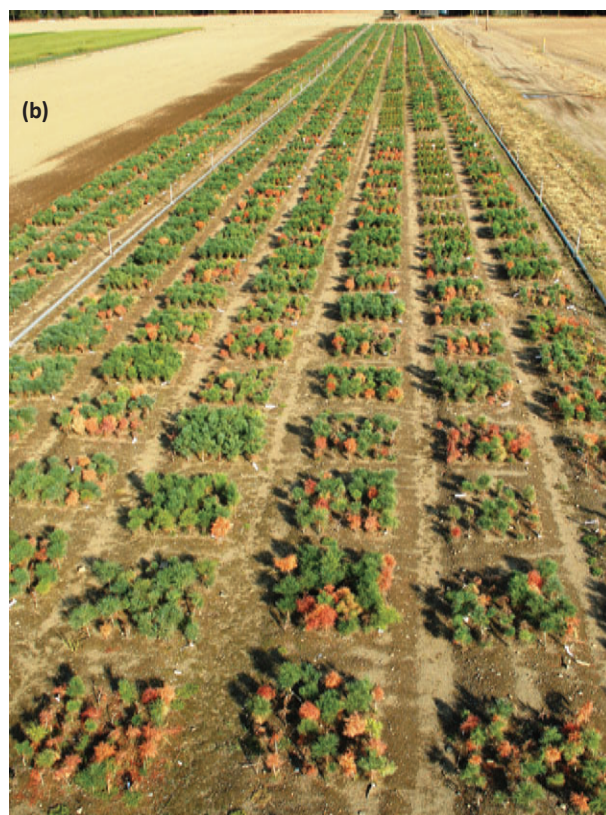


**Figure 3.** Field layout of seedlings following blister rust inoculation at (a) Dorena Genetic Resource Center; the seedlings of the five species shown here (*P. albicaulis*, *P. flexilis*, *P. lambertiana*, *P. monticola* and *P. strobiformis*) will be examined for five years post-inoculation for resistance responses; (b) Coeur d'Alene Nursery; Cycle 20 *P. monticola* and *P. albicaulis* trials (Photo: D. Foushee).

permit the outdoor tests to proceed for 3 to 5 years and longer. At various periods after artificial inoculation (at a minimum, once annually), individual seedlings are assessed for phenotypic responses. Needle lesions (or 'spots') are the first trait assessed, usually within 6 to 12 months following artificial inoculation (figure 4a). At IFG, the younger seedlings are scored for needle infection within 3 to 4 weeks or sooner, depending upon the onset and frequency of needle spots. At IFG or in the DGRC greenhouse tests, seedlings are examined early to classify spots as either susceptible or resistant hypersensitive reactions. However, in these tests, all seedlings are assessed for disease symptoms for 2 years post-inoculation to confirm and characterize associate traits for the complete resistance phenotype and gain insights into other possible resistance mechanisms. Depending on the trial and test facility, presence or absence of needle spots, number of needle spots, and color of needle spots may be noted.

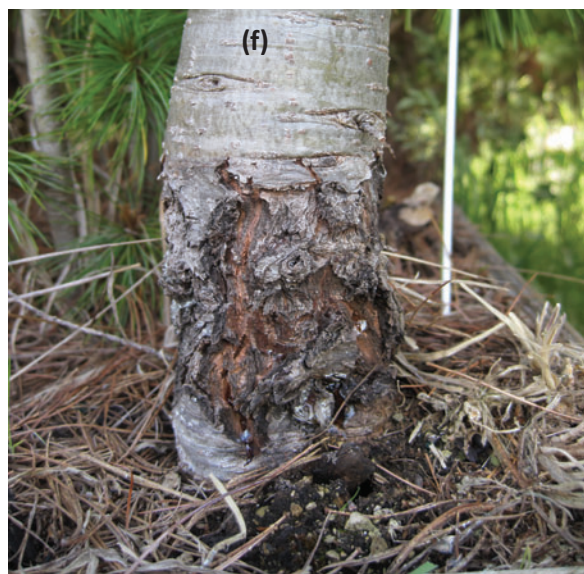
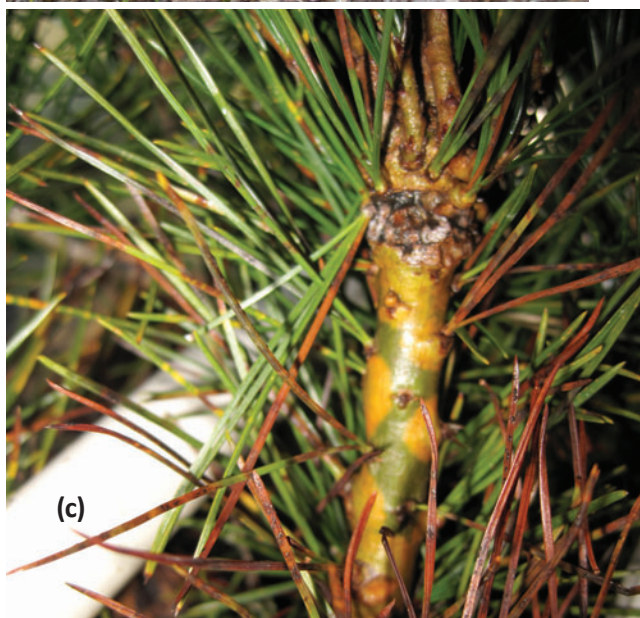
Stem infections usually appear on some seedlings within 3 to 9 months and are abundant on many seedlings within 12 to 20 months (figures 4b, 4c). The timing of the appearance of needle spots and stem infections may vary by testing location or seedling, with later appearance on a seedling being a potential expression of resistance. Recently, two new traits for assessment have been added at DGRC: the number of stem infections and their overall severity. The number of stem infections on individual seedlings can vary from 0 to 50 or more. The severity code (0 to 9) denotes the extent of damage from all stem infections (cankers and bark reactions) on the seedling, from none (0) to very extensive (6, 7, 8) to dead from rust (9). This rating changes over time as the stem infection progresses or is inactivated. Timing of mortality following inoculation can vary by trial, by species and by seedling family (note: a family is defined as the seedling progeny of a seed tree (maternal parent) that has been wind-pollinated by several pollen parents). In some cases, the most significant mortality occurs two to four years after inoculation, and even earlier on the smaller, younger seedlings at IFG and in the DGRC greenhouse trials. Terminating a trial too early can lead to over-estimating the level of survival from the rust inoculation.

Data collected are used to compile family means and summaries in order to rate each parent tree for types and levels of resistance. These data provide baseline information on the degree of rust resistance in different populations and different families for each species. This information can be used to designate specific parent trees for further seed collection in restoration efforts, for scion collection and grafting of the top-ranking trees or progeny into orchards or clone banks, and to determine the contribution to that resistance of the known maternal parent. The data also provide information on the possible inheritance of the resistance types and their geographic distribution. Information on specific protocols and summaries to date from the first *P. albicaulis* trials at CDA and DGRC is available (Mahalovich submitted; Mahalovich and others 2006; Sniezko and others 2007, 2008).

The general goal of the National Forest System genetics programs is to maintain genetic variation and adaptability of populations of trees while increasing genetic resistance to a level higher than is present in current populations. For the Forest Service research groups (IFG and RMRS), more focus is on describing the resistance/tolerance mechanisms in white pines, and on uncovering their modes of inheritance. Resistance in trees needs to be long-lasting, that is, 'durable', to be of most utility in restoration. The resistant trees need to survive to sexual maturity and produce cones. At least some of the resistances (or combinations thereof) need to last over generations to help ensure continued ecological functioning of these white pines. The resistance screening effort is complicated, because it aims to uncover an array of resistance types available in each species. Some resistance types are known to be controlled by a single gene, while others are thought to be controlled by several to many genes (Kinloch 1982). Some resistance types may be 'complete' and prevent needle and/or stem infections, while 'incomplete' or partial resistance may mean fewer infections or slower growth of the pathogen in the host tissues. Frequencies of the various resistances are low in natural populations for at least some of these species (since the North America white pines did not co-evolve with the recently introduced blister rust fungus); therefore, testing many seedling families will be necessary to maintain genetic diversity and adaptability. Fortunately, the CDA and DGRC screening facilities have capacity to undertake the screening of large numbers of seedlings as funding becomes available.

Knowledge of the types of resistance mechanisms to blister rust infection and information about their inheritance in North American white pines has been studied mostly in other white pines such as *P. monticola* and *P. lambertiana* (Hoff 1986; Hoff and others 1980; Kegley and Sniezko 2004; Kinloch 1982; Kinloch and Davis 1996; Kinloch and others 2003; Sniezko and Kegley 2003a,b), but similar resistance phenotypes appear to be present in the six high elevation white pines (Hoff 1994; Hoff and others 1980; Kinloch and Dupper 2002; Mahalovich submitted; Mahalovich and others 2006; Sniezko and others 2007, 2008; Sniezko and Kegley, unpublished data). Following artificial inoculation, seedlings are evaluated for an array of resistant types including presence and number of needle spots, needle spot color, hypersensitive reaction in the needles, shedding of infected

**Figure 4.** Seedlings after inoculation at Dorena Genetic Resource Center: (a) 100's of needle lesions ('spots') on a *P. albicaulis* seedling nine months after inoculation; (b) within-family variation in cankering in 2 *P. flexilis* seedlings, 13 cankers on seedling on left, no cankers on seedling on right, 21 months after inoculation; (c) many incipient stem infections on a *P. albicaulis* seedling, 13 months post-inoculation; (d) putative 'needle shed' mechanism on a *P. albicaulis* seedling, nine months after inoculation, seedling had 50 needle spots at 1<sup>st</sup> assessment in 2009, but is still canker-free in winter 2010; (e) bark reaction on main stem of a *P. albicaulis* pine seedling, 23 months after inoculation; (f) large bark reaction or 'inactive' canker on *P. strobiformis* seedling, 7 years after inoculation.



needles (NS), presence or absence of stem infections, number of stem infections, latency of stem infection, severity of infection, bark reactions (BR), and survival with stem infections. The array of resistant types evaluated varies somewhat by facility (for example, CDA does not evaluate for HR at this point in time). Some of these resistance types, such as HR and NS (figure 4d), prevent the rust fungus from progressing to a stem infection (McDonald and Hoff 1970; Kinloch and others 2003), while others such as BR curtail or greatly slow the progress of the fungus in the stem (figures 4e and 4f) (Hoff 1986). Needle shed can occur over different time periods and can be complete (all needles with spots are shed), or partial (Sniezko and Kegley, unpublished data). Some trees with needle shed exhibit stem cankers. However, operationally, seedlings are rated as NS only if they drop all needles with spots and do not develop stem infections. More work is needed to confirm the underlying resistance mechanisms of canker-free seedlings (other than HR) and how much they are impacted by different environmental conditions and spore densities.

Because of extensive work in *P. lambertiana* and *P. monticola*, HR is the most well documented resistance mechanism in white pines (Kinloch and Dupper 2002; Kinloch and others 1999, 2003). Vogler and others (2006) provide a useful overview of HR in white pines. HR has also been found in *P. strobiformis* and *P. flexilis*. The inheritance of HR has been found to be conditioned by different single dominant genes in three of these species (Kinloch and Dupper 2002). Work on the inheritance in a fourth species, *P. flexilis*, is underway (Schoettle and others, Preliminary overview of the first extensive rust resistance screening tests of *Pinus flexilis* and *Pinus aristata*, this proceedings). HR is very effective initially, but in at least some cases, it may not be durable over the life of the tree if virulence arises in the rust population. Unfortunately, in *P. monticola* and *P. lambertiana*, virulent strains of the rust are now known to exist in some parts of the range of these species, limiting effectiveness of HR resistance (Kinloch and others 2004). Where possible, HR resistance should be used in conjunction with other resistances, as is being done for *P. monticola* in Oregon and British Columbia, and *P. lambertiana* resistance programs in California and Oregon.

Progeny of resistant parent trees will generally be a mixture of resistant and susceptible seedlings, and 100 percent survival from natural regeneration or restoration plantings is not expected in the presence of blister rust. The level of survival in progeny of resistant parents will depend upon an array of factors, including frequency of resistance genes, type(s) of resistance and their modes of inheritance, resistance of parent trees contributing pollen, site rust hazard, virulence/aggressiveness of the rust, the age and physiological condition of hosts at time of exposure to infection in the field, and the overall environmental conditions on the site. All of this should be taken into account in planning restoration efforts. Young seedlings exposed to high levels of rust will show the fastest and highest levels of mortality. Some outstanding parents may yield progeny that show 25 to 50 percent survival or higher following inoculation testing, but

survival of plantings using seedling progeny of many parents will be reduced due to lower overall selection intensity (many resistant selections will be included, not only the very top ones). In general, extreme caution should be practiced in extrapolating the level of resistance in seedling tests to predicting field performance. Multiple tests of some seedlots are needed to confirm potential levels of resistance. Seed from seed orchards would be expected to yield the highest levels of survival, since all pollen contributions would be from presumably resistant parent trees, but large quantities of seed from orchards may not be available for a decade or more.

## Blister Rust Resistance

### Species

*Pinus albicaulis*: The small, early artificial inoculation trials in Idaho and France indicated that genetic resistance to blister rust was present in *P. albicaulis* (Delatour and Birot 1982; Hoff and others 1980). A small trial in 1992 showed large differences among stands and among families in the percentage of trees canker-free (Hoff 1994; Hoff and others 2001). The resistance mechanisms observed included: 'needle shed' (NS), 'short-shoot' (SS) and 'bark reaction' (BR) (Hoff 1994; Hoff and others 1980, 2001). Two of these, NS and SS, prevent stem infection, while BR essentially walls off the rust fungus in the stem (Hoff 1986). Families from stands experiencing high prior rust mortality had a much higher percentage of moderate to high levels of canker-free seedlings than did families from stands with low or moderate levels of rust mortality, indicating that some natural selection for resistance had occurred (Hoff 1994). Recent work at CDA and DGRC, using hundreds of families of *P. albicaulis*, has confirmed the presence of resistance in whitebark pine (Mahalovich submitted; Mahalovich and others 2006; Sniezko and others 2007, 2009; Sniezko and Kegley, unpublished data).

Of the six high elevation species, whitebark pine has the most parent trees currently in rust resistance testing (table 1; figure 5a). Although larger-scale testing of whitebark pine began nearly a decade ago (Mahalovich and others 2006; Sniezko and others 2007), the number of seedlots in testing has greatly increased since 2007 (figure 5a). Most of the seedlots currently in testing are from Pacific Northwest and Interior West U.S. (table 1), but 15 families from Alberta are included in 2010 testing at CDA, and 5 families from British Columbia and three from California were included in the 2008 inoculation trials at DGRC (figure 5a). Testing of *P. albicaulis* families from California is now underway at IFG. There have been only a few common seedlots tested at more than one facility: two seedlots tested previously at CDA were inoculated in 2008 at DGRC (DGRC trial is still underway), one additional family tested at CDA was inoculated at DGRC in 2004, and one bulk seedlot from WY has also been tested at both facilities.

Seed from over 2,400 parent trees, covering much of the range of the species, are potentially available as of 2009

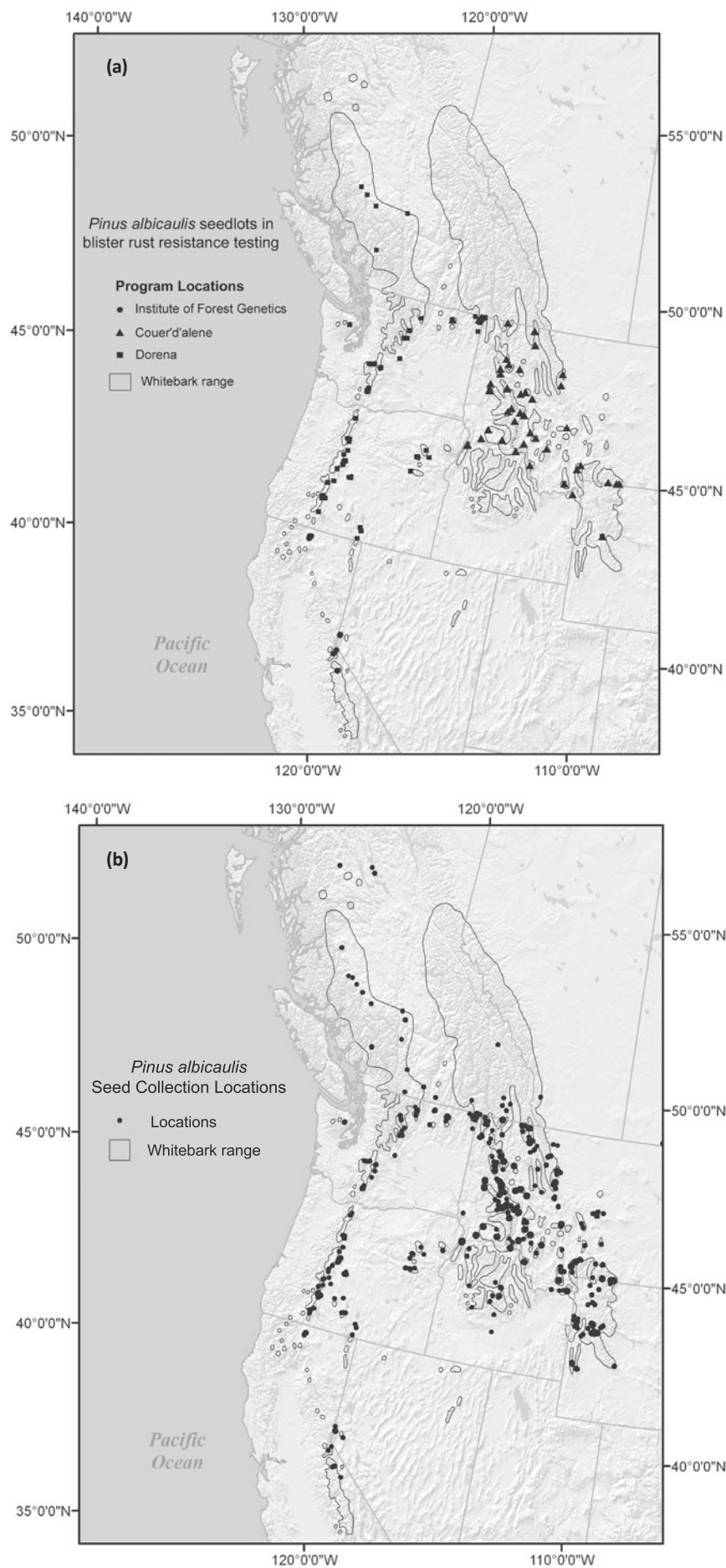


**Figure 5. (a)** Geographic distribution of parent trees of *P. albicaulis* families in blister rust testing (inoculated as of 2010); **(b)** Geographic sources of *P. albicaulis* with seed collected as of 2009 (*P. albicaulis* range from Little 1971).

for rust testing and gene conservation (figure 5b; Sniezko and others, *Ex situ* gene conservation in high elevation white pine species in the United States—a beginning, this proceedings), with additional collections underway in many areas. Through fall 2010, trials have been completed or started for 650 individual tree seed collections (table 1; figure 5a). Assessments of most of these trials are still underway. At DGRC, sowing of an additional 180 seedlots from throughout Oregon & Washington is planned for March 2011; inoculation will be in September 2012. CDA sowed 248 seedlots in 2010 for fall 2011 inoculation, and 195 seedlots in 2011 for fall 2012 inoculation. IFG sowed 70 families of California whitebark in 2007, and inoculated one set of all 70 families in September 2009 and a second set of the same families in December 2010. At IFG, replicate inoculations at different seedling ages are designed to compare host responses (for example, needle symptoms, stem reactions, pathogen establishment, and host mortality) to refine differentiation and characterization of prospective resistance mechanisms. Thirty-six families of whitebark from the Lake Tahoe region of California were sown for resistance testing in 2010, and another 100 families will be sown in 2011 for phenology assessments and for characterization for future inoculation tests.

Results from the first large-scale tests are encouraging, indicating that whitebark pine has several types of resistance and that there may be geographic trends in resistance (Mahalovich submitted; Mahalovich and others 2006; Sniezko and others 2007; Sniezko and Kegley, unpublished data). For many *P. albicaulis* families, over 90 percent of the seedlings develop stem infections, but some families in these trials and the earlier Hoff (1994) trial show much lower levels of stem infection (figures 6a, 6b, 6c; Sniezko and others 2007). Seed can now be collected from a limited number of confirmed rust-resistant parent trees for restoration, and the first re-collections have been made. Many additional seedlots need to be evaluated for each breeding zone to develop a list of parent trees that will supply rust resistance while maintaining the genetic diversity of the species. Three common families (Sources 78, 252 and 289) that ranked high in the CDA 110 seed source study (Mahalovich and others 2006) have also been tested at DGRC. At CDA, families #78 and #252 had 39 and 64 percent of seedlings with no stem infections (Mahalovich, unpublished data), while early results from the 2008 inoculation trials at DGRC showed 100 percent of the seedlings for both families had stem infections (and show >75 percent mortality two years after inoculation, additional mortality is expected). These two families ranked near the bottom for all geographic sources for rust resistance in the DGRC trial (Sniezko and Kegley, unpublished data). The third family, #289, in the CDA trial had 53 percent canker-free seedlings; in four 2004 inoculation trials at DGRC this family had 3.3 to 38.9 percent canker-free seedlings, depending upon inoculum density. Even with the higher stem infection levels at DGRC, this family showed good survival levels (30 to 68.5 percent versus the overall trial means at DGRC of 7.7 to 17.1 percent) four years after inoculation. These differences are intriguing

and could be due to several factors, including higher effective inoculation levels at DGRC, differences in geographic sources of rust, or physiological differences in seedlings from the Interior West when grown in Oregon. Further investigations are needed.

No evidence of HR resistance has been found in the seedlots of *P. albicaulis* tested so far at IFG and DGRC (Kinloch and Dupper 2002; Sniezko and others 2008; Sniezko and Kegley, unpublished data; Vogler and others 2006). Results from the current screening of hundreds of additional families of whitebark pine will greatly increase our knowledge of the frequencies of resistance types in the Pacific Northwest and Interior West portions of this species' geographic range. More extensive inoculation trials are needed for western Canada and the Pacific Southwest portions of the whitebark pine range to develop a fuller understanding of resistance mechanisms and frequency of resistance in this species and to maximize its utility to land managers.

*Pinus aristata*: The earlier international trials indicated that *P. aristata* had a surprising level of resistance and probably the highest level of rust resistance of any North American white pine species (Delatour and Birot 1982; Hoff and others 1980; Stephan 1986, 2004). However, these early trials tested few seedlots, so additional work is needed and some is underway.

The Rocky Mountain Research Station (RMRS) has been the principal coordinator of recent seed collections and rust resistance testing of *P. aristata* (Schoettle and others, Preliminary overview of the first extensive rust resistance screening tests of *Pinus flexilis* and *Pinus aristata*, this proceedings; Sniezko and others, *Ex situ* gene conservation in high elevation white pine species in the United States—a beginning, this proceedings). RMRS currently has seedlots in resistance testing at both IFG and DGRC. In the first trials at both facilities, many of the individual parent seedlots are in common, with the 109 individual tree seedlots at IFG being a subset of the 189 in testing at DGRC.

Originally, the shorter term test at IFG was designed to find HR, a hypersensitive type response in needles, which in sugar pine, western white pine, and southwestern white pine is indicative of a simply-inherited genetic mechanism for resistance in needles. However, in the test at IFG no HR was found for *P. aristata*, and many of the seedlings are now being evaluated for other resistance responses (Vogler and others 2006). The subset of families in common between IFG and DGRC will provide a comparison of resistance among families under different nursery/greenhouse cultural regimes and using different geographic sources of rust.

In the initial *P. aristata* trial at DGRC a small number of *P. monticola* and *P. lambertiana* seedlings were included as controls with the 189 *P. aristata* families. Due to the suspected higher level of resistance in *P. aristata*, the inoculum density used for inoculation in this trial was four times that typically used for *P. monticola* at DGRC. This first set of trials at IFG and DGRC are nearly complete and the data analysis is underway. Initial examination of the data indicates large differences among families in resistance (Schoettle and others, Preliminary overview of the first extensive rust

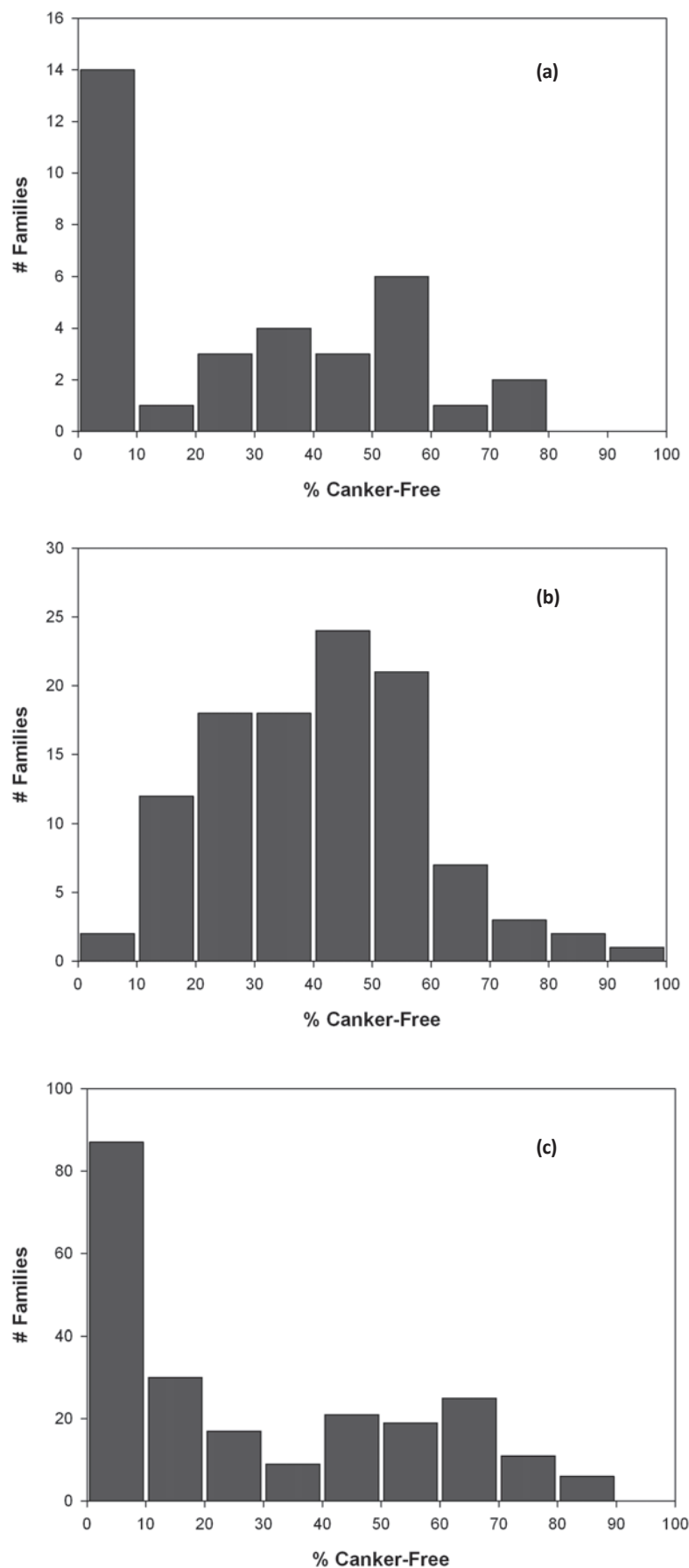


**Figure 6.** Histograms of family mean variation in percentage of canker-free seedlings for 3 trials **(a)** RMRS test of 34 Interior West families (adapted from Hoff 1994); **(b)** 108 families from Interior West 110 seed source trial at Coeur d'Alene Nursery (Mahalovich and others 2006); **(c)** early data (13 months after inoculation) for 225 families for Run 3 at Dorena Genetic Resource Center (inoculated in 2008 using AVCr2 source of rust). Note: histograms display family variation in separate trials at different facilities with generally different families and different inoculation levels (e.g., percent of seedlings with needle spots) which generally precludes direct comparisons across trials and regions. See paper for summary of three families in common between two of the testing facilities.

resistance screening tests of *Pinus flexilis* and *Pinus aristata*, this proceedings; Vogler and others 2006).

Additional testing of *P. aristata* at DGRC is underway and another 36 families will be sown in 2011. Overall, families from across the full geographic distribution of *P. aristata* are now in testing; however, more intensive and comprehensive testing throughout the range is still needed. More research on the association of resistance with other adaptive traits is also ongoing (Schoettle, unpublished data). Hundreds of additional seedlots collected throughout the range of *P. aristata* are available for testing, but funding is currently not available. Four lots from Rocky Mountain Region are being screened at CDA.

*Pinus balfouriana*: Previous tests in Idaho and Germany included very few seedlots; in these tests, *P. balfouriana* showed relatively little rust resistance (Hoff and others 1980; Stephan 1986, 2004) and no evidence of HR resistance was found in a test of six seedlots (Kinloch and Dupper 2002). IFG has recently begun testing of 14 seedlots of this species; results are pending. Many additional seedlots are potentially available for testing (Snieszko and others, *Ex situ* gene conservation in high elevation white pine species in the United States – a beginning, this proceedings).

*Pinus flexilis*: In the international trial series, a small number of seedlots of *P. flexilis* were tested for rust resistance in Idaho, France and Germany, and the level of resistance appeared to be relatively low, although at least one seedlot in the French trial showed a high level of resistance (Delatour and Birot 1982; Hoff and others 1980; Stephan 2004). In a separate test of five seedlots, a bulk seedlot of *P. flexilis* showed 19% incidence of HR phenotypes (Kinloch and Dupper 2002).

Beginning in 2006, RMRS began a series of resistance trials at DGRC, using seedlots from the Southern Rockies. In some of these trials, HR resistance, primarily, is being examined in individual seedling families, while in other trials families are being examined for additional resistance types over a more extended period of time. In another trial, 30 bulked seedlots are being evaluated for differences in the frequency of HR among populations. As of 2010, RMRS had 341 seedlots in testing at DGRC (table 1); additional lots will be sown in 2011. At IFG, 33 seedlots were sown in 2010; these are currently undergoing evaluation for symptom development and potential resistance mechanisms, including both HR and stem reactions.

*P. flexilis* has a very large geographic range, and seed collections have recently become available for some other portions of the range in the U.S. and Canada. However, no individual seedlots from Canada have been tested yet, but twelve families from Alberta sown in January 2011 will be inoculated fall 2012 at CDA. Four bulked lots from Utah will be inoculated in fall 2011 at CDA and 30 families from Montana will be inoculated in 2011 at DGRC.

Early results from the RMRS tests show that some families have moderate to high levels of resistance, but most trials are still ongoing and interpretation of the types of resistances is still underway (Schoettle, unpublished data). These trials should help delineate resistance types and their frequency in

some portions of the species' range. Many additional seedlots are available for rust testing (Snieszko and others, *Ex situ* gene conservation in high elevation white pine species in the United States—a beginning, this proceedings). Collections are still needed, particularly for Arizona, Idaho, Utah, Nevada, New Mexico, Montana, and northern Wyoming.

*Pinus longaeva*: When the earlier testing of many of the North American species was done for the international trial, *P. aristata* and *P. longaeva* were both still considered as one species, *P. aristata*, so no separate summary of rust resistance is readily available (see section above for early results from *P. aristata* testing). In a more recent trial, two seedlots tested at IFG showed no evidence of the HR resistance (Kinloch and Dupper 2002). Twenty-three California families were inoculated at IFG in 2005. Early results (4 months post-inoculation) showed that 30 percent of the seedlings were canker-free (Vogler and others 2006); a summary of final assessments is underway. IFG has recently inoculated 16 additional seedlots, and results are pending. Seventy-five seedlots from three locations in west-central, east-central, and southern Nevada are currently in stratification at IFG for inoculation in 2 or 3 years, depending upon seedling development. Hundreds of additional seedlots are potentially available for testing (Snieszko and others, *Ex situ* gene conservation in high elevation white pine species in the United States – a beginning, this proceedings), but lack of funding may limit the amount of testing for the present.

*Pinus strobiformis*: Early work with a small number of seedlots indicated that genetic resistance to the blister rust fungus is present in *P. strobiformis* (Delatour and Birot 1982; Hoff and McDonald 1980; Hoff and others 1993; Stephan 2004), and one of the resistance types is due to a simply-inherited hypersensitive response (HR) in needles (Kinloch and Dupper 2002; Vogler and others 2006). Since 1996, progeny of many more parent trees (91) have been tested or are currently under evaluation for rust resistance, with 78 and 53 families being evaluated at IFG and DGRC, respectively. Data from the 49 of the 78 parents with IFG evaluations for the HR resistance, finalized as of 2007, indicate 6 of these parents are heterozygotes for *Cr3*, the putative single dominant gene conditioning the hypersensitive response in the needles (HR) of this species. The six confirmed *Cr3* heterozygotes are from the Bradford Canyon area on the Lincoln National Forest in New Mexico, an area with a high incidence (>80%) of rust infection. These six selections were all from canker-free parent trees, and the parent trees have remained canker-free through the last field assessment in 2008. Unlike the *Cr1* and *Cr2* genes for HR in *P. lambertiana* and *P. monticola*, no strain of the rust virulent to *Cr3* in *P. strobiformis* has been documented to date. A number of the other seedlots from the other 43 parents from the Bradford Canyon area showed a low frequency of HR in the IFG testing, indicating that they were likely pollen receptors for this resistance.

Testing of progeny of 10 of the Bradford Canyon parents has been completed at DGRC (a subset of the same seedlots tested at IFG for HR). DGRC results confirmed two *Cr3* heterozygotes. In another test that included 7 of the

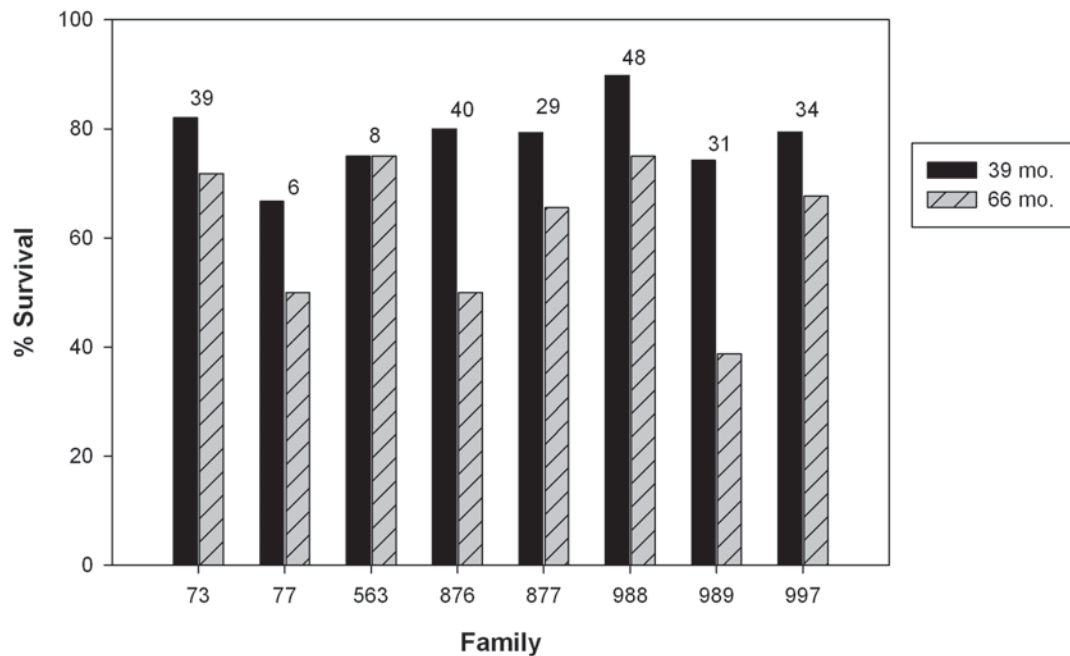


**Figure 7.** Percent survival at 39 and 66 months after inoculation in 8 *P. strobiformis* families inoculated at Dorena Genetic Resource Center (see Snieszko and others 2008 for trial background). Number of trees in family is noted above each bar.

non-HR parents, progeny of those 7 parents demonstrated other types of resistance, including bark reaction and very high survival (figure 7) (Snieszko and others 2008). Similar observations were made in Fall 2006 on the same families of *P. strobiformis*, inoculated at IFG in Fall 2005 (Delfino-Mix and Vogler, unpublished). For the DGRC trial at both 39 and 66 months after inoculation, all 8 of these families showed much higher survival than *P. lambertiana* and *P. monticola* controls included in the trial (Snieszko and others 2008; Snieszko and Kegley, unpublished data). The increase in mortality from 39 months to 66 months after inoculation in some families emphasizes the potential need to follow seedling trials over more than a few years. All 10 of these trees are from canker-free parent trees in a heavily cankered stand, and all were still canker-free in 2008. The relatively high level of resistance in the 7 families that are not from HR parents is encouraging. It is notable that, for this species, both HR resistance and other strong resistances are documented among progeny of parents from the same stand, and thus it is likely that some seedlings will have HR plus other types of resistance, effectively pyramiding the resistances. This may be the first documentation of this combination of resistances in a stand for any white pine species. The large bark reaction type response in some of the *P. strobiformis* families (figure 4f) appears similar to that in a *P. peuce* seedlot tested at DGRC (R. Snieszko, personal observation in rust resistant screening trials at DGRC, May 2010). This is also one of the first reports in a white pine species of non-HR type resistance in common seedlots from two screening facilities using different protocols and different geographic sources of the rust. Further discussion of the specific results

at DGRC and IFG of these common seedlots (and additional ones now in testing) is planned.

Forty-three additional seedlots, encompassing three National Forests in New Mexico, were inoculated at DGRC in September 2010 and will be evaluated for a range of resistant types. Some of these have been previously tested at IFG and been confirmed as non-*Cr3* parents (non-HR). Progenies of 30 parent trees, including some also in testing at DGRC, are still in rust testing at IFG. The subset of seedlots represented at both DGRC and IFG will help facilitate some comparisons of resistance under different nursery/greenhouse conditions and geographic sources of rust. One lot from the Coronado NF (AZ) and one from the Lincoln NF (NM) were inoculated in fall 2009 at CDA; percent needle spotting for both lots (58.2) was below the  $F_2$  rust-resistant *P. monticola* orchard lot (67.4). A bulk seedlot from the Nebraska Forest Service was sown at CDA in January 2011 and will be inoculated in fall 2012.

### Rust Resistance Under Field Conditions

Short-term seedling tests for rust resistance are useful if they are representative of results that occur in the field. It is possible that the high spore densities used in inoculation trials on young seedlings may make it more difficult to discern some types of partial resistance that may be more effective on many field sites, where exposures to lower spore densities are prevalent. Trees in the field will often be exposed to multiple infection events over a longer time period, compared with the usually one-time inoculation of seedlings in nursery or greenhouse trials. Field trials of the six species

of focus here are lacking or only recently established. For *P. albicaulis*, the first long-term field trial was established in 2006 at Lone Mountain in northern Idaho (using survivors from the 110-seed source study); data 10-years from seed are being evaluated for stability of rust resistance along with key climatic variables. Field trials of *P. albicaulis* using families with a range of rust resistance have recently been established at Crater Lake and Mount Rainier National Parks and on the Deschutes NF, and these seedling families will be monitored for seedling survival and rust resistance. The inclusion of families that are highly susceptible to blister rust, as controls, will provide an important monitor to gauge the first exposure and extent of rust challenge under field conditions. Additional trials with whitebark pine are planned in the Pacific Northwest. Some small plantings of *P. albicaulis* and other white pine species have been planted in northern CA and southern OR. Six long-term field trials of *P. albicaulis* will be established in Idaho and Montana following completion of the artificial inoculation trials at CDA. At least a subset of the trials needs to be followed for decades to examine what types of resistance are durable.

Until more data on field rust resistance in these six species are available, some insights can be drawn from much older and more extensive trials involving *P. monticola* and *P. lambertiana* that were established by regional resistance programs in the U.S. and Canada. The general results from the oldest trials are encouraging regarding durability of resistance (Kinloch and others 2008; Lupo 2004; Snieszko and others 2004b), but more information is needed such as the types of resistance that are effective and the level of survival to expect on higher hazard sites. For *P. monticola*, a large number of field trials have been established in the Pacific Northwest since 1996 specifically to examine family variation in field resistance. Early results are encouraging (Kolpak and others 2008; Snieszko and others 2000, 2004a, 2010), but more time is needed to follow the impacts of exposure to rust in the field on specific families and resistance types. One series of trials, replicated at six sites in Washington, includes *P. monticola* resistant seedlots from the three regional resistance programs based in Idaho, Oregon and British Columbia. In the future, these *P. monticola* trials should provide much more information on the effectiveness and durability of different resistances as well as the correspondence between short-term seedling tests and field performance for rust resistance. Although the data on *P. monticola* will be useful in making initial inferences about stability and durability of resistance in this species, data are also needed for the six high elevation species discussed here.

A second method that may be useful to examine durability of resistance is to monitor parent trees in the field. In areas where rust is present and susceptible parents succumb, what happens over time to the trees designated as resistant based upon their progeny in the seedling screening trials? The good news is that in some areas, such as Mount Rainier National Park, the surviving trees have endured the onslaught of the blister rust pathogen for decades. Early results from progeny of the first trees tested from parents from this location rated very high for resistance (Snieszko and Kegley,

unpublished data). Continued monitoring of these trees for resistance is warranted and would provide much needed data on its durability.

### **Operational Programs—Utilizing Resistance**

Some resistant parent trees have been identified from rust screening trials using their progeny over the last decade. With the levels of resistance found to date in *P. albicaulis*, *P. flexilis* and *P. strobiformis*, immediate utilization of resistance can be made by collecting seed from resistant parent trees and using it for reforestation or restoration, especially in areas where blister rust is a damaging agent. To minimize the chance of losing the high-resistant parent trees to other causes (fire, bark beetles, climate change, etc.) scion collection, grafting, and archiving of grafts in clone banks or orchards is of paramount importance (DeSpain, personal communication). These grafts should be preserved in areas that are likely to ensure their long-term survival. Some grafting has been done in both the Interior West and Pacific Northwest programs. Guiding documents for management of these species in several regions are now available and managers can now utilize rust resistance in some of the species (Aubry and others 2008; Burns and others 2008; Conklin and others 2009; Mahalovich submitted; Mahalovich and Hipkins, this proceedings; Mahalovich and Dickerson 2004; Mahalovich and others 2006). These documents can be updated as new data necessitate. Substantial additional rust screening is needed to increase the number of resistant parents available in all seed zones and to help provide greater genetic diversity in the resistant seedlots.

One example of an active program is in the Interior West for *P. albicaulis* (Mahalovich and Dickerson 2004). This program has targeted 650 selections, 100 in each breeding zone within ID, MT, and WY, and 50 for Nevada. This goal has already been exceeded with 983 selections made to date. Three short-term rust resistance screening tests have been started, and two additional tests are scheduled. The first restoration plantings began in 1996. Approximately 1,989 acres of rust-resistant seedlings have been planted to-date (1996-2010) in Idaho, Montana and Wyoming (Mahalovich, personal communication). One field test has been established (in 2006), and seven more are planned. Two seed orchards (each with 30 parents) have been established, and two additional orchards will be planted in fall 2011 (figure 8).

### **Pathogen Evolution and Durability of Blister Rust Resistance**

The rust pathogen is genetically variable and has shown the capacity to evolve more virulent strains, at least to HR type resistance (Kinloch and others 2004). Less is known for most pathogens, including *C. ribicola*, about the evolution of aggressiveness, the quantitative component of pathogenicity (Pariaud and others 2009). Long-term success in restoring areas with moderate and higher rust hazards will likely depend upon developing populations of white pines with several types of resistance. In addition, silvicultural tools

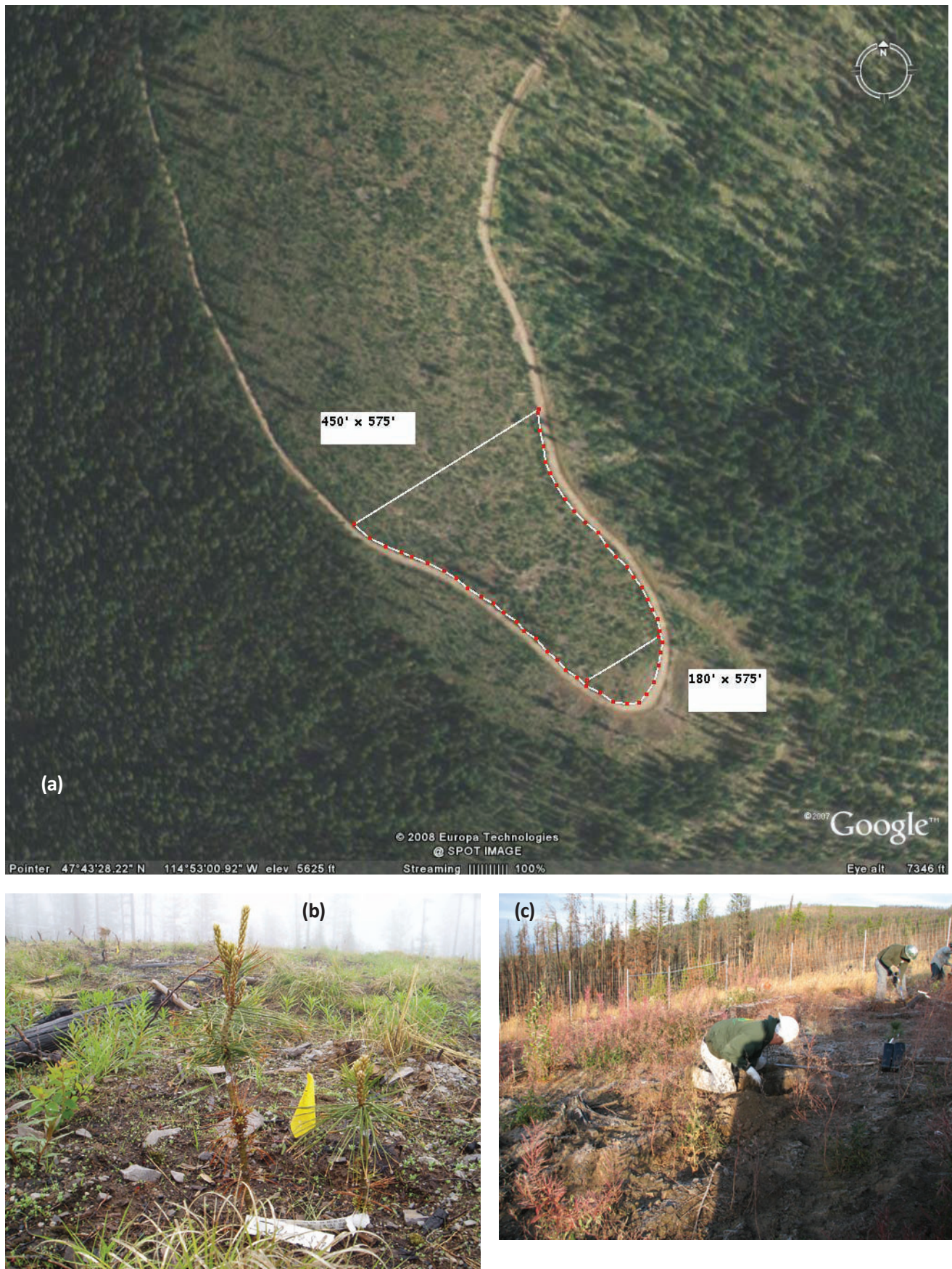


**Figure 8.** *P. albicaulis* orchard planted on Lolo National Forest, Montana, at 5650ft (1722 m): (a) aerial view of orchard; (b) close-up view of grafted tree; (c) planting grafts. First grafts planted Oct 2009. Grafted from parent trees in March 2008.

such as pruning, and measures to reduce rust hazard, such as *Ribes* removal, may aid the efforts in some areas. Avoidance of sites with extreme rust hazards may be necessary, except for small field tests to determine the effectiveness of genetic resistance under those conditions.

In resistance screening to evaluate host parents for restoration, heterogeneous mixtures of rust genotypes from *Ribes* plants in one or more geographic areas of a region are used to inoculate the seedlings in the trials. Even in the paired trials at DGRC with different geographic sources of the rust, including the 'Champion Mine' strain, it is encouraging to see some seedling families of *P. albicaulis* and *P. flexilis* consistently showing resistance (Snieszko and others 2007; Snieszko, unpublished data; Schoettle, unpublished data). The testing of common seedlots of *P. aristata* and *P. strobiformis* underway at DGRC and IFG will provide information on the effectiveness of resistance in this species to different sources of rust and host condition.

Fortunately, the white pine species of the United States and Canada appear to have one or more types of resistance to white pine blister rust. In some species such as *P. strobiformis*, there appears to be both HR resistance as well as other types of resistance. In other species such as *P. albicaulis*, no HR resistance has been found to date. From experience with the *P. monticola* and *P. lambertiana* programs, HR resistance, conditioned by a single major gene, is assumed to be the least durable, but even this will depend on the dynamics of the pathosystem (Kinloch and others 2004). Although there have been reports of some geographic sources of rust in the western United States being somewhat more virulent or aggressive than others on pines with non-HR types of resistance (Hoff and McDonald 1993; McDonald and others 1984), multiple tests at DGRC with *P. monticola*, *P. albicaulis* and *P. flexilis* (Schoettle unpublished data; Snieszko and others 2007; Snieszko and Kegley, unpublished data) have indicated that families resistant to one source of the rust are generally resistant to other sources of the rust, including the 'Champion Mine' source reported on by McDonald and others (1984). However, more work is needed on this topic.

In several previous seedling screening trials, the Eurasian species of white pines have generally shown higher levels of resistance than their North American relatives (DelaTour and Birot 1982; Hoff and others 1980; Hoff and McDonald 1993; Snieszko and others 2008; Stephan 2004). This higher level of resistance to blister rust may be due to co-evolution of these pines and the rust. However, recent reviews of the status of some white pine species in China and South Korea indicate that rust levels in plantations of *P. koraiensis* Siebold & Zucc. and *P. armandii* Franch. can reach relatively high levels (La 2009; Zhang and others 2010). The reasons for the higher level of infections in these pathosystems are unknown. It may be due to increased rust hazard from major changes in forest management and/or evolution of a more virulent race or aggressive genotypes of the rust pathogen. In the case of *P. armandii*, it may also be due to the presence of a second species of white pine blister rust (Zhang and others 2010). In general, the impact of different Eurasian races or putative species of blister rust on resistant white pine genotypes of

North American species is unknown. Further examination of the rust-pine pathosystem in Asia could be helpful in understanding possible future outcomes in our own forests.

The virulence of *vc1* and *vc2* genotypes of *C. ribicola* to HR resistance in *P. lambertiana* and *P. monticola*, respectively, is well documented (Kinloch and others 2004). Some evidence for additional racial variation and increased pathogenicity in the blister rust pathogen comes from an international trial series. In France, Germany and Japan, some resistant seedlots of *P. monticola* and *P. lambertiana* from the U.S. programs showed a much higher percentage of seedlings with cankering than in the Idaho trial (Hoff and McDonald 1993; Stephan 1986). Bulk seedlots of the high elevation white pines from the United States generally showed low to moderate levels of resistance in these trials. In the trial in Japan with resistant seedlots of *P. monticola* (from the Idaho program), little or no resistance was found in this species, while the Asian and European white pines showed high resistance (Yokota 1983). In general, the Eurasian species of pines in these trials displayed much higher levels of resistance (lower percentage of cankering) at all trial locations. In the German trial, a few F<sub>1</sub> and F<sub>2</sub> seedlots of *P. monticola* showed some resistance three years after inoculation, but much less after six years (Stephan 1986). These trials suggest evidence of virulence or increased aggressiveness of the rust in Europe and Asia to at least some sources of resistance in U.S. species, but further investigations are needed to confirm possible differences in the pathogen in Europe, Asia and North America. Hoff and McDonald (1993) suggested the need for strong quarantines on hosts of blister rust to prevent importation of spores from outside North America.

### Impacts of Climate Change

Changing climatic conditions may potentially alter dynamics of the blister rust-white pine pathosystem by influencing the geographic occurrence and intensity as well as the annual timing and duration of spread of the rust fungus. Differential changes in aggressiveness with different temperatures have been noted in various pathogens (Pariaud and others 2009). The spread of the rust to new locations may impact additional forest ecosystems where susceptible trees are present, but the spread of current local genotypes of rust is less likely to impact resistant trees. A significant increase in spore density in an area could conceivably impact some types of resistance, leading to higher frequency of stem infections, more cankers per tree, or mortality. However, the levels of rust spores used in the seedling resistance screening trials probably represent a high to extremely high level, and trees found to be resistant in these tests would likely be resistant in the field.

The timing and duration of rust infection events in the field could also be affected by changing climate, and trees (as well as their alternate hosts, such as *Ribes* spp.) could be infected at earlier or later times in the season than currently occurs. The physiology of the trees would be different during these different months, and conceivably some resistances might become less or more effective during different stages of a tree's seasonal growth or under different temperatures. Current rust

resistance screening occurs at locations with generally much milder temperatures than the natural habitat of these six high elevation species, so the trees selected as resistant in these tests should have a good chance of being resistant under the relatively more modest climatic changes expected to occur in their natural habitat.

Monitoring genetically-identified parent trees in the field for changes in resistance will help confirm changes in resistance due to changing climate or evolution of increased virulence of the rust. Changes in resistance over time in the field trials will also serve a similar function.

Movement of seed sources to different environments may influence the efficacy of resistance. The most notable example is the apparent reduced rust resistance of *P. monticola* from interior sites in Idaho planted in low elevation coastal British Columbia sites (Hunt 2004). This may indicate that moving seedlings from 'harsher' to 'milder' climates may change the efficacy of response. If so, seedling testing in a milder climate may be advantageous. Two *P. albicaulis* seedlots (from ID and MT) from the 110 seed source study showed moderately high resistance in that test at CDA, but are highly susceptible in a test at DGRC. More work is needed to examine the stability of resistance in various seed sources over a range of environments.

The presumed impact of change in environmental conditions may be seen in some inoculation trials. Among other things, the year to year fluctuation in climate may alter the physiological state of the seedling pre- or post-inoculation as well as the activity of the rust fungus post-inoculation. This may be responsible for some substantial variation observed in whitebark pine trials inoculated in different years. In inoculation trials of whitebark pine at DGRC using similar spore densities, 99.9 percent of the seedlings have needle spots; yet, the number of needle spots per seedling averaged 14.9 & 18.9 in two trials inoculated in 2004, 6.3 for the trial inoculated in 2005, and 71.3 and 126.4 in the two trials inoculated in 2008. This represents more than a ten-fold difference of needle spots among the trials. Even with this large difference in the quantity of needle spots, the preliminary results indicate that resistant families tested are consistent across trials, but ongoing study will reveal if this consistency holds for different types of resistance and whether overall survival is affected. A few families are common in these trials, so these will be examined for differences in subsequent levels of resistance.

## Summary and Information Needs

For all of these high-elevation species, we are in the relatively early stages of examining rust resistance. A compilation of screening results over the next five years should provide significant increases in knowledge about the level and geographic pattern of rust resistance in several of these white pine species. This information will be of immediate use to land managers wishing to restore populations in the face of blister rust.

The procedures to screen for rust resistance are relatively well established, and if funding permits, thousands of

additional seedlots could easily be evaluated over the next decade. Current rust resistance screening technology could be easily extended to evaluating *P. ayacahuite* Ehrenb. ex. Schltdl. and *P. chiapensis* (Martinez) Andresen from Mexico.

With only a few exceptions, no common set of seedlots have been tested among all the programs. To enable comparisons over different resistance screening programs and different test years, a set of checklots for each species would be very helpful. These checklots should span the range from highly susceptible to highly resistant, and should have large amounts of seed available. A common trial among screening facilities would allow for comparisons of results under different screening conditions and geographic sources of rust and would aid in the development of common terminology. Such a trial was proposed by all groups more than a decade ago (at a 'Rustbusters' meeting) for *P. monticola*, but did not occur due to some personnel changing careers or retiring.

Basic research is still needed to complement the more applied development of resistant populations. Relatively little is known about the impressive resistance of the Eurasian white pine species and how it may differ from that of North American species. In general, these species showed good to excellent resistance in the international trial series, while the North American species showed dramatically less resistance. The high level of resistance in these pines to races of rust present in Idaho, Oregon, France, Germany and Japan is intriguing and worth further study. What resistance mechanisms are involved and how many genes? Understanding more about the underlying nature of the resistances in these species would give us more clues on our path to augmenting resistance in North American species. Also needing study is the reported breakdown in resistance in some Asian species in China and South Korea. Was this due to an increase in virulence or aggressiveness, an increase in rust hazard, changing climate, or other factors?

More information on variability in virulence and aggressiveness in blister rust races from throughout the world would also be of interest. Now that we have identified resistance in most of the white pine species, these resistant seedlots could be tested against races of rust from the eastern U.S., Europe, and Asia. More locally, a greater understanding of the differences in virulence and aggressiveness of the geographic sources of rust currently in western North America would be useful.

A greater understanding of resistance mechanisms, their underlying inheritance, and their efficacy under different environmental conditions would also be useful. Most of the work with blister rust resistance has been conducted using bulk collections of blister rust. Although this may serve the operational programs, research using single spore isolates of the rust fungus might help increase our understanding of resistance, as would testing of more uniform seedling materials (selfs and full-sib crosses). Also needed is more information on the association of resistance traits with other adaptive traits. The development of molecular markers for resistance could potentially expedite many aspects of the screening and research resistance programs.

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## References

- Aubry, C.; Goheen, D.; Shoal, R.; Ohlson, T.; Lorenz, T.; Bower, A.; Mehmehl, C.; Snieszko, R. 2008. Whitebark pine restoration strategy for the Pacific Northwest Region 2009-2013. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Region. 212 p.
- Bingham, R. T.; Gremmen, J. 1971. A proposed international program for testing white pine blister rust resistance. *European Journal of Forest Pathology*. 1: 93-100.
- Burns, K. S.; Schoettle, A. W.; Jacobi, W. R.; Mahalovich, M. F. 2008. Options for the management of white pine blister rust in the Rocky Mountain Region. Gen. Tech. Rep. RMRS-GTR-206. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 26 p.
- Conklin, D. A.; Fairweather, M. L.; Ryerson, D. E.; Geils, B. W.; Vogler, D. R. 2009. White pines, blister rust, and management in the Southwest. R3-FH-09-01. [Place of publication unknown]: USDA Forest Service, Southwestern Region. 16 p.
- Delfino-Mix, A.; Vogler, D. R. 2010. White pine blister rust research at the Institute of Forest Genetics after major-gene resistance. Unpublished electronic presentation dated November 16, 2010, on file at: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station, Institute of Forest Genetics, Placerville, CA. 23 p.
- Delatour, C.; Birot, Y. 1982. The international IUFRO experiment on resistance of white pines to blister rust (*Cronartium ribicola*): the French trial. In: Heybroek, H. M.; Stephan, B. R.; Von Weissenberg, K., eds. Resistance to diseases and pests in forest trees: 3rd International Workshop on Genetics of Host-Parasite Interactions in Forestry; 1980 September 14-21; Wageningen, the Netherlands. Wageningen, the Netherlands: Centre for Agricultural Publishing and Documentation: 412-414.
- DeSpain, Tom. 2011. [Email to Richard Snieszko]. January 31, 2011. U.S. Department of Agriculture, Forest Service, Dorena Genetic Resource Center, Cottage Grove, OR.
- Hoff, R.; Bingham, R. T.; McDonald, G. I. 1980. Relative blister rust resistance of white pines. *European Journal of Forest Pathology*. 10: 307-316.
- Hoff, R. J. 1986. Inheritance of the bark reaction resistance mechanism in *Pinus monticola* infected by *Cronartium ribicola*. Research Note INT-361. Ogden, UT: U.S. Department of Agriculture, Forest Service, Intermountain Research Station. 8 p.
- Hoff, R. J. 1994. Artificial rust inoculation of whitebark pine seedlings—rust resistance across several populations. *Nutcracker Notes*. [Online]. Issue 4. Available: <http://www.whitebarkfound.org/Nutcracker-Notes/number4.htm> [10 January 2011].
- Hoff, R. J.; Ferguson, D. E.; McDonald, G. I.; Keane, R. E. 2001. Strategies for managing whitebark pine in the presence of white pine blister rust. In: Tomback, D. F.; Arno, S. F.; Keane, R. E., eds. Whitebark pine communities: ecology and restoration. Washington, DC: Island Press: 346-366.
- Hoff, R. J.; McDonald, G. I. 1993. Variation of virulence of white pine blister rust. *European Journal of Forest Pathology*. 23: 103-109.
- Hunt, R. S. 2004. Environmental and inoculum-source effects on resistance of Idaho F<sub>2</sub> western white pine in British Columbia. *Canadian Journal of Plant Pathology*. 26: 351-357.
- Kegley, A. J.; Snieszko, R. A. 2004. Variation in blister rust resistance among 226 *Pinus monticola* and 217 *P. lambertiana* seedling families in the Pacific Northwest. In: Snieszko, R. A.; Samman, S.; Schlarbaum, S. E.; Kriebel, H. B., eds. Breeding and genetic resources of five-needle pines: Genetics, breeding, and adaptability, Proceedings of the IUFRO 2.02.15 Working Party Conference; 2001 July 23-27; Medford, OR. Proceedings RMRS-P-32. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station: 209-225.
- Kinloch, B. B., Jr. 1982. Mechanisms and inheritance of rust resistance in conifers. In: Heybroek, H. M.; Stephan, B. R.; Von Weissenberg, K., eds. Resistance to diseases and pests in forest trees: 3rd International Workshop on Genetics of Host-Parasite Interactions in Forestry; 1980 September 14-21; Wageningen, the Netherlands. Wageningen, the Netherlands: Centre for Agricultural Publishing and Documentation: 119-129.
- Kinloch, B. B., Jr.; Davis, D. 1996. Mechanisms and inheritance of resistance to blister rust in sugar pine. In: Kinloch, B. B., Jr.; Marosy, M.; Huddleston, M. E., eds. Sugar pine: Status, values, and roles in ecosystems: Proceedings of a symposium presented by the California sugar pine management committee; 1992 March 30-April 1; University of California, Davis. Publication 3362. Oakland, CA: University of California Division of Agriculture and Natural Resources: 125-132.
- Kinloch, B. B., Jr.; Davis, D. A.; Burton, D. C. 2008. Resistance and virulence interactions between two white pine species and blister rust in a 30-year field trial. *Tree Genetics and Genomes*. 4: 65-74.
- Kinloch, B. B., Jr.; Dupper, G. E. 2002. Genetic specificity in the white pine-blister rust pathosystem. *Phytopathology*. 92(3): 278-280.
- Kinloch, B. B.; Snieszko, R. A.; Barnes, G. D.; Greathouse, T. E. 1999. A major gene for resistance to white pine blister rust in western white pine from the western Cascade Range. *Phytopathology*. 89(10): 861-867.
- Kinloch, B. B., Jr.; Snieszko, R. A.; Dupper, G. E. 2003. Origin and distribution of Cr2, a gene for resistance to white pine blister rust in natural populations of western white pine. *Phytopathology*. 93(6): 691-694.
- Kinloch, B. B.; Snieszko, R. A.; Dupper, G. E. 2004. Virulence gene distribution and dynamics of the white pine blister rust pathogen in western North America. *Phytopathology*. 94(7): 751-758.
- Kolpak, S. E.; Snieszko, R. A.; Kegley, A. J. 2008. Rust infection and survival of 49 *Pinus monticola* families at a field site six years after planting. *Annals of Forest Research*. 51: 67-80.

- La, Y. J. 2009. Korean successes in controlling blister rust of Korean pine. In: Noshad, D.; Noh, E.; King, J.; Sniezko, R., eds. Proceedings: Breeding and genetic resources of five-needle pines: Ecophysiology, disease resistance, and developmental biology; 2008 September 22-26; Yangyang, Republic of Korea. Seoul: Korea Forest Research Institute: 1-9.
- Little, E. L., Jr. 1971. Atlas of United States Trees, Volume 1, Conifers and Important hardwoods: U.S. Department of Agriculture Miscellaneous Publication 1146, 9 p., 200 maps.
- Lupo, P. 2004. Stability and expectations of blister rust resistance traits in  $F_2$  western white pine. Moscow, ID: University of Idaho. 131p. Thesis.
- Mahalovich, M. F. [Submitted May 2009]. Genetic differentiation of *Pinus albicaulis* Engelm. populations from the Northern Rocky Mountains. The Americas Journal of Plant Science and Biotechnology.
- Mahalovich, M. F. 2010. U.S.A. Inland Northwest western white pine breeding and restoration program: history, current and future directions. In: Cleary, M. R., ed. Proceedings of the 3rd Western White Pine Management Conference; 2008 June 17-18; Vernon, BC. Kamloops, BC: BC Ministry of Forests and Range, Southern Interior Forest Region: 50-74.
- Mahalovich, M. F.; Burr, K. E.; Foushee, D. L. 2006. Whitebark pine germination, rust resistance, and cold hardiness among seed sources in the Inland Northwest: planting strategies for restoration. In: Riley, L. E.; Dumroese, R. K.; Landis, T. D., tech. coords. National Proceedings: Forest and Conservation Nursery Associations—2005. Proceedings RMRS-P-43. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station: 91-101.
- Mahalovich, M. F.; Dickerson, G. A. 2004. Whitebark pine genetic restoration program for the intermountain west (United States). In: Sniezko, R. A.; Samman, S.; Schlarbaum, S. E.; Kriebel, H. B., eds. Breeding and genetic resources of five-needle pines: Genetics, breeding, and adaptability, Proceedings of the IUFRO 2.02.15 Working Party Conference; 2001 July 23-27; Medford, OR. Proceedings RMRS-P-32. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station: 181-187.
- McDonald, G. I.; Hansen, E. M.; Osterhaus, C. A.; Samman, S. 1984. Initial characterization of a new strain of *Cronartium ribicola* from the Cascade Mountains of Oregon. Plant Disease. 68: 800-804.
- McDonald, G. I.; Hoff, R. J. 1970. Resistance to *Cronartium ribicola* in *Pinus monticola*: early shedding of infected needles. Research Note INT-124. Ogden, UT: U.S. Department of Agriculture, Forest Service, Intermountain Forest and Range Experiment Station. 8 p.
- McDonald, G. I.; Zambino, P. J.; Sniezko, R. A. 2004. Breeding rust-resistant five-needle pines in the western United States: lessons from the past and a look to the future. In: Sniezko, R. A.; Samman, S.; Schlarbaum, S. E.; Kriebel, H. B., eds. Breeding and genetic resources of five-needle pines: Genetics, breeding, and adaptability, Proceedings of the IUFRO 2.02.15 Working Party Conference; 2001 July 23-27; Medford, OR. Proceedings RMRS-P-32. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station: 28-50.
- Pariaud, B.; Ravigne, V.; Halkett, F.; Goyeau, H.; Carlier, J.; Lannou, C. 2009. Aggressiveness and its role in the adaptation of plant pathogens. Plant Pathology. 58: 409-424.
- Schoettle, A.; Sniezko, R. A. 2007. Proactive intervention to sustain high-elevation pine ecosystems threatened by white pine blister rust. Journal of Forest Research. 12: 327-336.
- Schoettle, A. 2010. Rust resistance screening of *P. aristata* and *P. flexilis*—trials in progress. Unpublished data on file at: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.
- Sniezko, R. A.; Bower, A.; Danielson, J. 2000. A comparison of early field results of white pine blister rust resistance in sugar pine and western white pine. HortTechnology. 10: 519-522.
- Sniezko, R. A.; Bower, A. D.; Kegley, A. J. 2004a. Variation in *Cronartium ribicola* field resistance among 13 *Pinus monticola* and 12 *P. lambertiana* families: early results from Happy Camp. In: Sniezko, R. A.; Samman, S.; Schlarbaum, S. E.; Kriebel, H. B., eds. Breeding and genetic resources of five-needle pines: Genetics, breeding, and adaptability, Proceedings of the IUFRO 2.02.15 Working Party Conference; 2001 July 23-27; Medford, OR. Proceedings RMRS-P-32. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station: 203-208.
- Sniezko, R. A.; Hill, J.; Danchok, R. S.; Kegley, A. J.; Long, S.; Mayo, J. B.; Smith, A. J. 2010. White pine blister rust resistance in a seven year old field trial of 28 western white pine (*Pinus monticola*) families in the Coast Range of Oregon. Phytopathology 100:S120. Abstract.
- Sniezko, R. A.; Kegley A. 2003a. Blister rust resistance experiences in Oregon and Washington: evolving perspectives. In: Stone, J.; Maffei, H., eds. Proceedings of the 50<sup>th</sup> Western International Forest Disease Work Conference; 2000 October 7-11; Powell River, BC, Canada. Bend, OR: USDA Forest Service, Central Oregon Forest Insect and Disease Service Center: 111-117.
- Sniezko, R. A.; Kegley, A. 2003b. Blister rust resistance of five-needle pines in Oregon and Washington. In: Proceedings of the Second IUFRO Rusts of Forest Trees Working Party Conference; 2002 August 19-23; Yangling, China. Forest Research. 16 (Suppl.): 101-112.
- Sniezko, R. A.; Kegley A. 2010. Rust resistance screening of high elevation pines—trials in progress. Unpublished data on file at: U.S. Department of Agriculture, Forest Service, Dorena Genetic Resource Center.
- Sniezko, R. A.; Kegley, A. J.; Danchok, R. 2008. White pine blister rust resistance in North American, Asian and European species—results from artificial inoculation trials in Oregon. Annals of Forest Research. 51: 53-66.
- Sniezko, R. A.; Kegley, A. J.; Danchok, R. S.; Long, S. 2007. Variation in resistance to white pine blister rust among 43 whitebark pine families from Oregon and Washington—early results and implications for conservation. In: Goheen, E. M.; Sniezko, R. A., tech. coords. Whitebark pine: a Pacific Coast perspective; 2006 August 27-31; Ashland, OR. R6-NR-FHP-2007-01. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Region: 82-97.
- Sniezko, R. A.; Kegley, A.; Danchok, R.; Hamlin, J.; Long, S. 2009. Resistance to *Cronartium ribicola* in whitebark pine—family variation and effect of inoculum density. Phytopathology. 99: S122. Abstract.
- Sniezko, R. A.; Kinloch, B. B., Jr.; Bower, A. D.; Danchok, R. S.; Linn, J. M.; Kegley, A. J. 2004b. Field resistance to *Cronartium ribicola* in full-sib families of *Pinus monticola* in Oregon. In: Sniezko, R. A.; Samman, S.; Schlarbaum, S. E.; Kriebel, H. B., eds. Breeding and genetic resources of five-needle pines: Genetics, breeding, and adaptability, Proceedings of the IUFRO 2.02.15 Working Party Conference; 2001 July 23-27; Medford, OR. Proceedings RMRS-P-32. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station: 243-249.

- Stephan, B.R. 1986. The IUFRO experiment on resistance of white pines to blister rust (*Cronartium ribicola*) in northern Germany. In: Proceedings 18<sup>th</sup> IUFRO World Congress; 1986 September 7-21; Ljubljana, Yugoslavia. Division II, Vol. I: 80-89.
- Stephan, B. R. 2004. Studies of genetic variation with five-needle pines in Germany. In: Snieszko, R. A.; Samman, S.; Schlarbaum, S. E.; Kriebel, H. B., eds. Breeding and genetic resources of five-needle pines: Genetics, breeding, and adaptability, Proceedings of the IUFRO 2.02.15 Working Party Conference; 2001 July 23-27; Medford, OR. Proceedings RMRS-P-32. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station: 98-102.
- Tomback, D. F.; Achuff, P. 2010. Blister rust and western forest biodiversity: ecology, values and outlook for white pines. *Forest Pathology*. 40(3-4): 186-225.
- Vogler, D. R.; Delfino-Mix, A.; Schoettle, A. W. 2006. White pine blister rust in high-elevation white pines: screening for simply-inherited, hypersensitive resistance. In: Guyon, J. C., comp. Proceedings of the 53<sup>rd</sup> Western International Forest Disease Work Conference; 2005 September 26-30; Jackson, WY. Ogden, UT: U.S. Department of Agriculture, Forest Service, Intermountain Region: 73-82.
- Yokota, S. 1983. Resistance of improved *Pinus monticola* and some other white pines to the blister rust fungus, *Cronartium ribicola* of Hokkaido, Japan. *European Journal of Forest Pathology*. 13: 389-402.
- Zhang, X. Y.; Lu, Q.; Snieszko, R. A.; Song, R. Q.; Man, G. 2010. Blister rusts in China: hosts, pathogens, and management. *Forest Pathology*. 40(3-4): 369-381.

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