White Pine Blister Rust in High-Elevation White Pines: Screening for Simply-Inherited, Hypersensitive Resistance

Detlev R. Vogler, Annette Delfino-Mix, and Anna W. Schoettle

Introduction

Recent concern about survival and recovery of high-elevation white pine ecosystems has returned white pine blister rust (caused by *Cronartium ribicola*) to prominence as a significant threat to forest health in the western U.S. (Samman et al., 2003). This, in turn, has spurred new research into potential rust-resistance mechanisms in high-elevation white pines, including whitebark (*Pinus albicaulis*), foxtail (*P. balfouriana*), Rocky Mountain bristlecone (*P. aristata*), and Great Basin bristlecone (*P. longaeva*).

The impacts of *C. ribicola* on low- and mid-elevation western white (*P. monticola*) and sugar pine (*P. lambertiana*) are well documented. Although limber (*P. flexilis*) and whitebark pine have been infected for over 60 years in the northern United States, the consequences of the disease for these ecosystems are only just becoming recognized (see Tomback et al., 2001). The disease continues to spread into the southern species and populations, including southwestern white pine (*P. strobiformis*), foxtail, and Rocky Mountain bristlecone pine; Great Basin bristlecone pine is the only North American white pine not yet infected with the disease in the field. Blister rust is likely to impact the high elevation species' distributions, their population dynamics, and the functioning of their ecosystems (Schoettle, 2004).

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Detlev R. Vogler and Annette Delfino-Mix are Research Geneticist/Plant Pathologist and Greenhouse Manager, respectively, with USDA, Forest Service, Pacific Southwest Research Station, Institute of Forest Genetics, 2480 Carson Road Placerville, CA 95667. Anna W. Schoettle is a Research Plant Ecophysiologist with USDA Forest Service, Rocky Mountain Research Station, 240 W. Prospect Rd, Fort Collins, CO 80526.

The role major genes can play in fortifying the high-elevation white pine species against the impacts of the fungus deserves greater attention. Compared to non-specific, complexly-inherited forms of resistance*, relatively simply-inherited, specific mechanisms that prevent the pathogen from growing out of infected needles and into branches, thereby preventing sporulation, remain potent tools for reducing the impact of blister rust. We report here early work in bringing knowledge gained from the lower-elevation white pine species that have been screened intensively for major gene interactions with the blister rust fungus, to greenhouse inoculation studies of resistance in high-elevation white pines.

Before proceeding, it is important to address three terms that are fundamental to our discussion, and to substantiate them with formal definitions. These terms are *resistance*, *resistance phenotypes*, and *heritable resistance*.

Resistance

Resistance is an *active* host response. In a strict sense, it is the genetically-determined ability of a plant to actively resist inoculation, infection, growth, and sporulation by a pathogen, ranging from *complete* (pathogen may infect specific tissues, but is walled off or dies before extensive establishment and sporulation) to *partial* (pathogen survives and perhaps sporulates, but established infection does not prevent host reproduction or survival). Hypersensitive resistance is a form of complete resistance.

* Such **non-specific** mechanisms are passive defense responses against all pathotypes, are often controlled by multiple genes, lack definitive, readily-scoreable phenotypes, and merely limit physical damage to the host so that it can survive to reproductive age. By contrast, **specific** mechanisms are active defense responses by particular host genotypes that cause a hypersensitive reaction against matching pathotypes.

Resistance Phenotypes

In our research, we are looking for experimentally repeatable host/pathogen interaction *phenotypes* that represent potential mechanisms of resistance. These include a range of host reactions to pathogen inoculation and infection (including, but not limited to, needle spots, needle shed, twig blight, and bark lesions) that are robust, readily characterized, experimentally repeatable, and demonstrably heritable.

Heritable Resistance

Once resistance has been demonstrated, it must be amenable to practical *deployment* in hosts under field conditions. Whether resistance is simply-inherited (a single major gene) or complexly-inherited (multi-genic), for it to be useful in disease management it must be amenable to selection, breeding, and deployment so that future host populations comprise a measurable component of desired disease-preventing and disease-limiting traits.

Background

Since 1970, white pine blister rust resistance research has operated in the presence of a paradigm that was familiar in agriculture as the gene-for-gene hypothesis (Flor, 1956), but seldom if ever recognized in forestry. This paradigm addresses the powerful role of major gene (simply-inherited) immune resistance (MGR[†]), which was first reported in forestry in a wild, uncultivated conifer in 1970 (Kinloch et al.). The conifer was sugar pine, the host resistance gene came to be known as Cr1[‡], and the pathogen that was recognized by this gene, preventing infection beyond the needle, was *C. ribicola*.

In sugar pine, the presence of MGR was inferred by whether the host, in response to inoculation and invasion by the pathogen through stomata, developed a discrete, *necrotic needle spot*, within which the fungus was prevented from developing further (an active hypersensitive response by the host, indicating the pine had at least one copy of the dominant Cr1 allele, designated either Cr1cr1 or Cr1Cr1[§] in the diploid state), or developed diffuse, yellow, *chlorotic needle spots* from which the fungus rapidly colonized the needle, twig, and stem (essentially a non-response by the host, indicating alleles at the Cr1 locus were in the homozygous recessive state, cr1cr1, and the susceptible tree was incapable of recognizing and reacting to fungal invasion).

Simple plant-resistance genetic systems such as this are usually complemented by a similarly simple genetic system in the pathogen. A dominant resistance gene in the host (in this case, Cr1) is reciprocally complemented by an active avirulence gene in the pathogen (Avcr1)[**]. If the pathogen is inactive (vcr1) at the avirulence locus, however, it is then capable of infecting both resistant and susceptible pines (i.e., both resistant, Cr1_[††], and susceptible, cr1cr1, hosts), whereas the Avcr1 pathogen is only capable of infecting susceptible cr1cr1 hosts (Table 1).[‡‡]

Hypersensitive needle resistance has been documented also in western white pine (Cr2, *P. monticola*) (Kinloch et al., 1999) and in southwestern white pine (Cr3, *P. strobiformis*) (Kinloch and Dupper, 2002), and shown by experimental inoculation to segregate in a Mendelian manner (i.e. simply-inherited). The latter data were derived from control inoculations with *C. ribicola* at the Institute of Forest Genetics (IFG) greenhouse in Placerville, using seed collected from maternal parent trees (families) that were open-pollinated by local paternal sources. Blister rust inoculum in these tests was known by repeated experiments to be avirulent to Cr1 (i.e., lacking the vcr1 allele), and was designated

[†] The term MGR is used to describe one form of specific resistance in white pines that induces a hypersensitive reaction in the host and prevents colonization beyond a constricted infection spot in the needle.

[‡] The letters denote that this gene is active against *Cronartium ribicola* ("Cr"); the numeral "1" denotes that this is the first such gene described in the *C. ribicola* pathosystem, in this case associated with sugar pine.

[§] Convention dictates that dominant alleles are denoted with a leading capital letter (i.e., Cr1 or Avcr1) while recessive alleles are denoted by all lower case letters (i.e., cr1 or vcr1).

[**] This discussion can be confusing, since resistance is dominant in the diploid plant host, while avirulence (in which the pathogen generates a signal that is recognized by the host, and is thereby prevented from further development *in planta*) is the active allele in the haploid pathogen. Alternatively, virulence (the ability of the pathogen to circumvent host defenses by avoiding recognition and thus causing disease) is inactive in this pathosystem. The term **active** refers to the genetic capability of the pathogen to generate a signal or product that is recognized by the Cr1 host and leads to a hypersensitive response.

[††] The notation "Cr1_" indicates that, whether an individual sugar pine is heterozygous resistant (Cr1cr1) or homozygous resistant (Cr1Cr1), the phenotype of that tree against wild-type inoculum remains the same (i.e., a resistant, hypersensitive needle spot).

[‡‡] One assumption of this complementary genetic system is that the host is diploid and the pathogen, which infects pines via uninucleate basidiospores, is haploid. Thus, the pathogen is either Avcr1 (avirulent against Cr1_ hosts, but virulent against cr1cr1 hosts), or vcr1 (virulent against Cr1_ and cr1cr1 hosts).

El Dorado wild-type because it was and continues to be collected (and monitored for virulence) from selected sugar pine populations east of the Institute in El Dorado County.

If a maternal parent carries one copy of the Cr allele and is exposed to the El Dorado wild-type inoculum, then 50% of her seedling progeny will be resistant; if she carries two copies of the Cr gene, all her progeny will be resistant; if she has no copies of the Cr allele, then all progeny will usually prove susceptible. In some cases, low levels of resistance (1-15%) are found in families in which the maternal parent has no copies of the Cr allele; this resistance derives from local pollen donors that carry a copy of the Cr allele. Thus, one may infer from bulk-lot seedling experiments whether hypersensitive needle resistance is present within a population, which is indicative of a Cr phenotype, but cannot confirm the presence of the Cr allele without demonstrating that the phenotype is simply-inherited.

Table 1. General scheme for gene-for-gene interactions between a diploid host and a haploid pathogen. Resistance is dominant in the host, while avirulence is active in the pathogen. For a resistant interaction to occur, the pathogen must produce an active signal that is recognized by the resistant host. In susceptible interactions, either the pathogen (designated vr) produces no signal, or else the host (rr) is incapable of recognizing signals produced by the pathogen, and thus cannot respond to invasion with a hypersensitive reaction. This table demonstrates what H.H. Flor asserted, that "… for each gene conditioning rust reaction in the host there is a specific gene conditioning pathogenicity in the parasite" (Flor 1956).

Pathogen avirulence genes[a]	**Host resistance genes**[a]		
	RR[b]	Rr[b]	rr
Avr	–[c]	–	+
vr	+	+	+

[a] Generic labels for pathogen and host genes: Avr, active avirulence; vr, inactive virulence; RR, homozygous resistant; Rr, heterozygous resistant; rr, homozygous recessive.

[b] Note that, since resistance (R) is dominant, the RR and Rr states in hosts yield identical interactions with the pathogen.

[c] Types of host/pathogen interactions: –, no disease (resistant interaction); +, disease (susceptible interaction).

Hypersensitive needle resistance has been documented in limber pine (*Pinus flexilis*) (resistance gene tentatively denoted Cr4, pending confirmation), but inheritance has not been determined because all seed tested so far have been from bulk lots or from small, usually single-family collections that exhibited no resistance (Kinloch and Dupper, 2002). Lacking a family structure to seed collections, it is impossible to confirm inheritance without experimentally controlling the maternal contribution of half the alleles that are expressed in the progeny. Assuming that Cr resistance is rare in most white pine species[§§], bulk lot inoculations can only provide an indication of the presence of the resistant phenotype and a preliminary estimate of the presence of resistance alleles across the landscape. (e.g., limber pine, Table 2). Greenhouse inoculations at IFG and at the Placerville Nursery (J. Gleason, personal communication) have been and are being carried out on family-level whitebark and foxtail pine seed collections, but no evidence of needle hypersensitivity resistance has yet been documented in these species. Tests for simply-inherited resistance mechanisms in Rocky Mountain and Great Basin bristlecone pine are underway, and will be described below.

Virulence conferred by vcr1 has been documented in the sugar pine/*C. ribicola* pathosystem at two sites separated by several hundred miles in California (Kinloch & Comstock, 1981; Kinloch, 1996): at Happy Camp, Siskiyou Co., a sugar pine testing site where all survivors of field inoculations there carried the Cr1 allele (otherwise, they would not have initially survived the heavy inoculum load at this location); and at Mountain Home Demonstration

§§ This is a tenable assumption, since we know from several decades of resistance testing (both at IFG and at the nearby USDA, FS, Placerville Nursery, where the Region 5 Rust-Resistance Screening Program (RRSP) has been testing white pines for several decades) that gene frequency for Cr1 in sugar pine throughout California is approximately 0.02 overall, ranging from 0.001 in the southern Cascade Mtns., to 0.03-0.05 in the central Sierra Nevada, to 0.05-0.07 in the southern Sierra, and then dropping to 0.03-0.06 in Southern California and to 0.00 in northern Baja California (the southern-most distribution of *P. lambertiana*) (Kinloch 1992).

Table 2. Results of limited inoculation tests on mixed lots of limber pine (*Pinus flexilis*) seed collected from 4 western U.S. States. Seedlings were inoculated at the cotyledon stage with El Dorado County, CA wild-type *Cronartium ribicola* inoculum in dew chambers at the Institute of Forest Genetics, Placerville, CA (data from Kinloch and Dupper, 2002, and from unpublished records of recent inoculations archived at the Institute).

Seed Source (State)	No. of parents	Seedlings inoculated	Inoculation results[a]				Allele frequency
			S	R	?	D	
Montana	1	43	43	0	0	0	0.00
Colorado	Bulk lots	185	152	18[b]	13	2	0.05
Arizona	1	196	192	0	4	0	0.00
California	2	147	144	0	2	1	0.00
Total seedlings tested:		571	531	18	19	3	<0.02
Percent of all seedlings:			93.0	3.2	3.3	0.5	—

[a] S, diffuse chlorotic spots on needles, with subsequent stem infection; R, discrete hypersensitive spots on needles, with no subsequent stem infection; ?, needle and stem reactions unclear; D, test seedling died before needle symptoms or stem infection could be assessed.

[b] Assuming that each resistant individual was heterozygous for the putative Cr4 allele, the total number of resistance alleles was assumed to be one per individual, out of a total pool of 2 x 185 seedlings, or 370 total alleles at this genetic locus.

State Forest, Tulare Co., a mixed conifer-Giant Sequoia forest with large *Ribes* populations distributed throughout, and a moderately high rust resistance in sugar pine (Cr1 frequency ~0.08). Abundance of inoculum on *Ribes*, locally conducive climates, and, most prominently, moderate to high frequencies of Cr1 in sugar pine hosts have contributed to selection within the local *C. ribicola* population of rare fungal mutants that are vcr1.

Within a few miles of each of these sites, the frequency of vcr1 drops to zero, supporting the hypothesis that both locations, with frequencies of vcr1 approaching 1.00, are isolated occurrences that arose because of a particular set of rust-conducive circumstances that is exceedingly rare elsewhere (Kinloch et al. 2004). We continue to test this hypothesis by making annual telial *Ribes* leaf collections at and near both Happy Camp and Mountain Home to monitor changes in vcr1 frequency over time. Plans are also underway for regularly testing other populations of *C. ribicola* from selected locations in California where frequencies of Cr1 are known to be higher than in the landscape at large; these sites are associated with USDA, FS, Region 5 Genetic Resources Program monitor plantations, comprising characterized lots of both resistant and susceptible sugar pine. To date, however, no occurrences of vcr1 have been documented at any monitoring plantations that are not in the immediate vicinity of either Happy Camp or Mountain Home.

Virulence to major gene resistance in western white pine (Cr2) has been detected at Happy Camp and at varying frequencies throughout central and southern Oregon (Kinloch et al. 2004), and has been designated vcr2. Reciprocal inoculations of vcr1 and vcr2 onto homozygous-resistant families of sugar pine (Cr1) and western white pine (Cr2) revealed that vcr1 is avirulent against Cr2 in western white pine, and vcr2 is avirulent against Cr1 in sugar pine. (Table 3). Inoculations of vcr1 and vcr2 onto resistant southwestern white pine (Cr3) also demonstrated that these pathotypes are avirulent against Cr3. As yet, no complementary pathotype (putatively denoted vcr3) is known to occur that is virulent against Cr3 (Kinloch and Dupper, 2002; Vogler, unpublished data) (Table 3). If there were such a pathotype, it would presumably be virulent only against major gene resistance in *P. strobiformis* and not against resistance alleles in sugar pine or western white pine (Cr1 and Cr2, respectively), but this hypothesis is yet to be tested.

Table 3. Interactions between virulence genes and resistance genes in different white pine hosts, exhibiting how virulence alleles interact specifically with a complementary host resistance allele, but not with non-complementary alleles. All non-resistant pines will, however, be susceptible to all 3 pathotypes.

Host resistance genes	Pathogen virulence genes[a]		
	vcr1	vcr2	(vcr3)[b]
Sugar pine (Cr1_)	+	–	(–)
Western white pine (Cr2_)	–	+	(–)
Southwestern white pine (Cr3_)	–	–	(+)

[a] **+**, susceptible interaction (disease); **–**, resistant interaction (no disease).
[b] Pathogen alleles and interactions in brackets are hypothetical, since they have not been fully documented experimentally.

The current state of knowledge about major resistance genes in western North American white pines is summarized in Table 4. So far, major gene resistance has been documented in four white pine species. In sugar and western white pine, operational screening protocols[***] are well established and routine; for limber and southwestern white pine, protocols are being developed experimentally at IFG. For southwestern white pine, family-level inoculation tests have identified several heterozygous resistant parents; these yield 50% resistant progeny (Kinloch and Dupper, 2002; Vogler, unpublished data). For limber pine, family-level inoculation trials have not been conducted to any significant extent. For the latter two species, when possible, homozygous resistant host genotypes (progeny 100% resistant) must be identified and developed as seed sources for experimental determination of putative virulence alleles vcr3 and vcr4[†††].

Until such time as these homozygous-resistant seed sources become available, accurate detection and delineation of *C. ribicola* pathotypes that are virulent against Cr3 and Cr4 will be difficult, though not impossible[‡‡‡].

Inoculation tests of high-elevation white pines

Thus far, all inoculation tests that have demonstrated major gene, hypersensitive resistance to white pine blister rust have been with white pines within Section *Strobus*, Subsection *Strobi* (Table 5). According to the phylogeny developed by Price et al. (1998), the high-elevation pines that have exhibited no evidence of major genes for resistance, or have yet to be tested, are either within Section *Strobus*, Subsection *Cembrae* (*P. albicaulis*) or in Section *Parrya*,

[***] Beginning in the early 1970's, these protocols were developed experimentally by B.B. Kinloch, Jr. and colleagues at IFG, and later transferred to the USDA, FS, Region 5 Rust Resistance Screening Program at the Placerville Nursery in Camino, CA, where they were developed further and refined for operational use by S. Samman, P. Zambino, J. Gleason, J. Dunlap, and others.

[†††] Heterozygous resistant seed sources are not ideal for this purpose, since 50% of their progeny become infected with Avcr inoculum, and thus it is difficult to determine whether infection was initiated by wild-type or by virulent inoculum. With homozygous resistant seedlings, we count and assess the phenotype of each and every needle spot, allowing us to detect those initially rare susceptible-interaction phenotypes that indicate virulence in the pathogen.

[‡‡‡] With heterozygous resistant seed sources, individual seedlings with both resistant and susceptible needle spots indicate that test inoculum is a mixture of Avcr and vcr basidiospores, from which one may infer that virulence has arisen within the local *C. ribicola* population.

Subsection *Balfourianae* (*P. balfouriana*, *P. aristata*, and *P. longaeva*). A more recent molecular phylogenetic analysis (Gernandt et al. 2005), though supporting placement of all of the above except whitebark in Subsections *Strobi* and *Balfourianae*, nevertheless groups whitebark pine closely with pines in Subsection *Strobi*.

Inoculations performed to date with *P. albicaulis* and *P. balfouriana* have revealed no evidence of MGR, but they have been too few to be conclusive. The potential close affinity between whitebark pine and sugar and western white pine revealed by Gernandt et al. (2005)[§§§] suggests that whitebark, if family-level seed collections were surveyed from throughout its extensive range, might be a promising candidate for MGR. To date, our whitebark seed collections have been limited, nevertheless we will continue to test *P. albicaulis* for MGR as seed become available from cooperators.

For this discussion we will focus on the three high-elevation pine species in Subsection *Balfourianae*. As illustrated in Table 4, limited inoculation tests have been done with foxtail pine, but hypersensitive needle spots indicative of MGR were not observed (Delfino-Mix, unpublished data, J. Gleason, personal communication), leading to speculation that simply-inherited resistance mechanisms may not be found in this species. Foxtail pine is confined to two disjunct locations in California: high-elevation wilderness stands in the Klamath Mountains in the north of the State, and remote portions of Sequoia and Kings Canyon National Parks in the southern Sierra Nevada. We have not yet collected an extensive sampling of foxtail pine seed for testing. We have, however, collected a sizeable family-level seed collection of Rocky Mountain bristlecone pine from throughout Colorado and a smaller collection of Great Basin bristlecone pine from the White Mountains of California. Our current inoculation efforts have therefore focused on the latter two species.

Table 4. Summary of experimentally-determined major resistance genes in western U.S. white pines, and corresponding virulence genes in *Cronartium ribicola*.

White pine hosts		WPBR-related genes[a]	
Common name	Scientific name	Resistance	Virulence
Sugar pine	*Pinus lambertiana*	Y	Y
Western white pine	*P. monticola*	Y	Y
SW white pine	*P. strobiformis*	Y	N
Limber pine	*P. flexilis*	Y	?
Whitebark pine	*P. albicaulis*	(N)	?
Foxtail pine	*P. balfouriana*	(N)	?
Rocky Mtn. bristlecone	*P. aristata*	P	?
Great Basin bristlecone	*P. longaeva*	P	?

[a] Y, major gene resistance in the host or virulence in the pathogen have been documented in this species; N, resistance or virulence have not been documented by greenhouse tests; (), tests for major gene resistance have been conducted, but results are still too limited for definitive conclusions; ?, no controlled inoculation tests have been done with these species; P, inoculation tests are underway, but results are still pending.

An early trial with *P. longaeva* had shown that seedlings inoculated with blister rust within 3-6 months post-germination became infected and died rapidly, well before needle spots or stem symptoms could be scored with assurance. We therefore modified our standard protocols (which had been to inoculate seedlings in the cotyledon stage, when only primary needles had developed) so as to inoculate bristlecone in the second year after sowing. This necessitated over-wintering seedlings in the lath house, and then returning them to the greenhouse the following spring, which dramatically improved their hardiness prior to inoculation. Consequently, at inoculation, cotyledons were either moribund or shed, and seedlings comprised mostly primary needles with secondary needles just beginning to expand.

[§§§] *P. albicaulis* is intermediate between the latter two taxa in the authors' strict consensus of 55,536 trees based on *rbcL* and *matK* sequence data (Fig. 2, Taxon 54: 33-34).

Table 5. Phylogeny of western North American white pines within the genus *Pinus* (Price et al., 1998).

Genus *Pinus*
- Subgenus *Pinus* (hard pines)
- Subgenus *Strobus* (soft pines)
  - Section *Strobus*
    - Subsection *Strobi*
      - *Pinus monticola, P. lambertiana, P. flexilis, P. strobiformis*
    - Subsection *Cembrae*
      - *P. albicaulis*
  - Section *Parrya*
    - Subsection *Balfourianae*
      - *P. aristata, P. longaeva, P. balfouriana*

For inoculation of *Cronartium ribicola* onto *Pinus aristata* and *P. longaeva*, we used El Dorado wild-type inoculum amplified via urediniospores on leaves of multiple ramets from a single *Ribes nigrum* clone[****]; because of their large size and tolerance of rust infection, leaves of this clone are ideal for controlled inoculations. Formation of telia was induced by cultivating inoculated ramets in the greenhouse with temperatures not exceeding 23° C in daytime and 18° C at night, and relative humidity in the range of 50-70%.[††††] Just prior to pine inoculation, telial *R. nigrum* leaves were harvested and soaked in sterile distilled water for 1 hour, and then placed telia-down on 64 cm x 58 cm wire-mesh racks with 5 mm-square openings. *Ribes* leaves were covered with moistened cheesecloth and racks were placed in the dew chamber[‡‡‡‡] so that exposed telia were 5-10 cm above the tops of the test seedlings. Two 98-well supercell racks of pine seedlings were placed on the shelf directly below the ripe telia. *Ribes* leaves and pine seedlings were inoculated in the dark at 15° C and 100% relative humidity for 72 hours. At the end of inoculation, chambers were switched off, chamber doors opened, racks of telial leaves removed, autoclaved, and discarded, and racks of seedlings left in place for 4-6 hours until equilibrated with greenhouse temperature and humidity. Seedling racks were then placed on greenhouse benches for *C. ribicola* incubation, needle spot development, and scoring of phenotypes. For long-term observations and analysis, seedlings are over-wintered in the lath house.

[****] Ramets of a single, highly susceptible, and pathogen-compliant *R. nigrum* clone were generated in the 1970's, and have been used ever since as the preferred uredinial/telial host for all *C. ribicola* inoculations in disease-resistance research at IFG and in the Rust-Resistance Screening Program at the Placerville Nursery. Ramets are serially propagated by vegetative cuttings, and inoculated in dew chambers with either aeciospores or urediniospores of El Dorado wild-type inoculum.

[††††] Temperature and relative humidity settings were optimized to encourage telial development and forestall basidiospore production.

[‡‡‡‡] IFG employs three Percival model I-35D dew chambers.

Table 6. Infection results for Rocky Mountain bristlecone pine *(Pinus aristata*) 12 months post-inoculation. Seed were sown in spring 2002, inoculated with *Cronartium ribicola* (El Dorado wild-type) in May 2004, and scored for stem symptoms and signs in May 2005. Trees noted as dead died for reasons unrelated to rust infection.

Stem symptoms & signs[a]	No. of trees	Percent
None[b]	294	22%
Discolored or swollen[c]	152	11%
Discolored & swollen, spermatial, or aecial[d]	883	65%
Dead (non-rust)	26	2%
TOTAL	1355	100%

[a] Symptoms are evidence of host response to infection (i.e., stem discoloration, swelling, or both); signs are evidence of the pathogen (i.e., spermatia (pycnia) or aeciospores).
[b] Within this category, 132 seedlings exhibited no needle spots attributable to infection; the remainder of seedlings in this category (162) developed needle spots.
[c] Symptoms suggestive of successful infection, but remaining unresolved.
[d] Definitive symptoms and signs of infection.

Results of inoculations are shown in Tables 6 and 7 for *Pinus aristata* and *P. longaeva*, respectively. The former were rated for needle spots, symptoms of infection, and signs of fungal development three times, the last at one year post-inoculation, and thus provide the more complete picture of potential resistance mechanisms for these two species. The latter (*P. longaeva*) has been rated once at 4 months post-inoculation; one-year evaluations will be conducted in June 2006. The 1355 *P. aristata* seedlings inoculated in May 2004 represent 108 open-pollinated families from throughout Colorado. Number of seedlings per family ranged widely, from 1 to 55, based on quality of seed and percent germination. Overall, two-thirds of the trees that survived one year after inoculation became infected. Percent trees infected per family ranged from 0 to

Table 7. Infection results for Great Basin bristlecone pine (*Pinus longaeva*) 4 months post-inoculation. Seed were sown in spring 2003, inoculated with *Cronartium ribicola* (El Dorado wild-type) in June 2005, and scored for stem symptoms and signs in October 2005. Trees noted as dead died for reasons unrelated to rust infection.

Stem symptoms & signs[a]	No. of trees	Percent
None[b]	159	30%
Discolored or swollen[c]	101	10%
Discolored & swollen, spermatial, or aecial[d]	258	59%
Dead (non-rust)	3	1%
TOTAL	521	100%

[a] Symptoms are evidence of host response to infection (i.e., stem discoloration, swelling, or both); signs are evidence of the pathogen (i.e., spermatia (pycnia) or aeciospores).
[b] Within this category, 19 seedlings exhibited no needle spots attributable to infection; the remainder of seedlings in this category (140) developed needle spots.
[c] Symptoms suggestive of successful infection, but remaining unresolved.
[d] Definitive symptoms and signs of infection.

100%, with some families appearing to segregate 50:50 for susceptibility and non-susceptibility. With the latter families, however, replicate inoculations will be required to determine whether these ratios are statistically robust and therefore indicative of Mendelian segregation.

Needle spot characteristics did not correlate with whether or not an individual seedling ultimately became infected. Spots on primary and secondary needles ranged in color from yellow to brown to orange to red, and ranged in morphology from discrete to diffuse and spreading. However, there was no evidence of a correlation between any particular spot color or morphology and subsequent success or failure of *C. ribicola* to colonize the stem from needles. Thus, there was no evidence in this species for classic needle hypersensitivity (Kinloch and Littlefield, 1977), as evidenced with MGR in sugar, western white, and southwestern white pine, and suspected in limber pine. Although evaluations of *P. longaeva* inoculations are still incomplete, preliminary observations of the 23 families tested confirm that needle spot phenotypes in this species likewise do not correlate with subsequent infection or non-infection by blister rust.

One may be tempted to conclude from these results that simply-inherited resistance does not occur in either *P. aristata* or *P. longaeva*, but that would be confusing the mechanism of resistance with the mode of inheritance. Apparent absence of needle hypersensitivity as a mechanism, or phenotype, of resistance in these species does not preclude the possibility that other simply-inherited resistance mechanisms nevertheless may yet be found in *P. aristata* or in *P. longaeva*; the mechanisms and timing of resistance are not yet obvious. As noted earlier for *P. aristata*, there is suggestive, though very preliminary, evidence for simple segregation within families for resistance and susceptibility. Early results from *P. longaeva* show a similar trend. At this early stage of research, it seems possible that both species will exhibit a range of inherited resistance to *C. ribicola*, but it is not yet possible to state with certainty either the mode of inheritance or the mechanism by which it operates.

One caveat in interpreting early inoculation results is that failure of infection to develop is not evidence that resistance is occurring. Often, the simplest explanation for apparent resistance is that the fungus failed to inoculate and infect the host (a phenomenon known as "escape"). Although we closely monitor the amount and distribution of inoculum during dew chamber inoculations, occasionally individuals or small groups of seedlings are not directly challenged by basidiospores. Thus, when assessing the status of seedlings that did not become infected, we record whether or not they first developed needle spots consistent with invasion and establishment by *C. ribicola*. As shown in Table 6 for *P. aristata*, some 45% (132/294) of the seedlings that did not become infected did not exhibit pathogen-associated needle spots prior to evaluation at one year post-inoculation. To confirm whether these seedlings are actually resistant and not merely escapes, we have re-inoculated this set, and are currently evaluating them for needle spots and subsequent symptom development.

As to the remainder of the seedlings that did not develop symptoms or signs but did exhibit needle spots (Table 6: 162 *P. aristata*; Table 7: 140 *P. longaeva*), we continue to monitor them closely for

any and all phenotypes that may represent a hypersensitive or walling-off response elsewhere in the plant than in the needles, which theoretically may prevent establishment and subsequent sporulation of the rust fungus. Among the 162 asymptomatic *P. aristata*, some 20% exhibited pitchy lesions on the stem at or near the base of infected needles; these were often associated either with necrotic, occasionally purple, bark patches, or with bumpy, constricted swellings. Such lesions may be evidence of a hypersensitive reaction at the needle base, similar to the bark reaction that has been reported in sugar pine (Kinloch and Davis, 1996). At this time, and until repeat inoculations have been conducted with *P. aristata* and *P. longaeva* families selected from these initial tests, the inheritance, durability, and robustness of these putative resistance mechanisms remain to be determined.

In summary, preliminary results support several conclusions: 1) some individual seedlings (and possibly some families) of *Pinus aristata* and *P. longaeva* exhibit reduced susceptibility to *Cronartium ribicola*, possibly resistance; 2) classic, simply-inherited hypersensitive needle-spotting does not appear to be a mechanism of resistance in these white pine species; 3) more detailed observations and re-inoculations of seedlings that did not express needle spots are needed to substantiate the possibility that these individuals actively resisted fungal invasion and did not merely escape infection; 4) replicate inoculations of putatively resistant families are needed to substantiate hypothesized mechanisms of resistance and modes of inheritance; and 5) experience gained from the bristlecone pines may benefit resistance screening efforts with foxtail pine, which, though closely related to the bristlecones, has so far exhibited little evidence of resistance to blister rust.

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Literature Cited

Flor, H.H. 1956. The complementary genic system in flax and flax rust. Adv. Genet. 8: 29-54.

Gernandt, D.S., Lopez, G.G., Garcia, S.O., and Liston, A. 2005. Phylogeny and classification of *Pinus*. Taxon 54: 29-42.

Kinloch, B.B., Jr. 1992. Distribution and frequency of a gene for resistance to white pine blister rust in natural populations of sugar pine. Can. J. Bot. 70: 1319-1323.

Kinloch, B.B., Jr. 1996. Breakdown of major gene resistance to white pine blister rust in sugar pine at Mountain Home Demonstration State Forest: What are the implications? Pp. 20-26 *in* Proc. 45th Ann. Meeting of the California Forest Pest Council, November 13-14, Sacramento, CA

Kinloch, B.B., Jr., and Comstock, M. 1981. Race of *Cronartium ribicola* virulent to major gene resistance in sugar pine. Pl. Dis. Rep. 65: 604-605.

Kinloch, B.B., Jr., and Davis, D. 1996. Mechanisms and inheritance of resistance to blister rust in sugar pine. Pp. 125-132 *in* Kinloch, B.B., Jr., Marosy, M., and Huddleston, M.E., eds., Proc. of a Symposium by the California Sugar Pine Management Committee, Sugar pine: status, values, and roles in ecosystems, Publ. No. 3362, Univ. of Calif., Div. of Agriculture and Natural Resources, Davis, CA.

Kinloch, B.B, Jr., and Dupper, G.E. 2002. Genetic specificity in the white pine–blister rust pathosystem. Phytopathology 92: 278-280.

Kinloch, B.B., Jr., and Littlefield, J.L. 1977. White pine blister rust: hypersensitive resistance in sugar pine. Can. J. Bot. 55: 1148-1155.

Kinloch, B.B, Jr., Parks, G.K., and Fowler, C.W. 1970. White pine blister rust: simply inherited resistance in sugar pine. Science 167: 193-195.

Kinloch, B.B, Jr., Sniezko, R.A., Barnes, G.D., and 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: 861-867.

Kinloch, B.B, Jr., Sniezko, R.A., and Dupper, G.E. 2004. Virulence gene distribution and dynamics of the white pine blister rust pathogen in western North America. Phytopathology 94: 751-758.

Price, R.A., Liston, A., and Strauss, S.H. 2000. Phylogeny and systematics of *Pinus*. Pp. 49-68 *in* Richardson, D.M., ed., Ecology and Biogeography of *Pinus*, University Press, Cambridge.

Samman, S., Schwandt, J.W., and Wilson, J.L. 2003. Managing for healthy white pine ecosystems in the United States to reduce the impacts of white pine blister rust. USDA, Forest Service, Report no. R1-03-118. 10 p.

Schoettle, A.W. 2004. Ecological roles of five-needle pines in Colorado: Potential consequences of their loss. Pp 124-135 *in* Sniezko, R.A., Samman, S., Schlarbaum, S.E. and Kriebel, H.B., eds., Breeding and genetic resources of five-needle pines: growth, adaptability, and pest resistance, Proceedings of IUFRO Working Party 2.02.15, 23-27 July 2001, Medford, OR, USA. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fort Collins, CO. Report No. RMRS-P-32.

Tomback, D.F., Arno, S.F., and Keane, R.E., eds. 2001. Whitebark Pine Communities. Island Press, Washington, D.C. 440 p.