Genetic Specificity in the White Pine–Blister Rust Pathosystem

Bohun B. Kinloch, Jr. and Gayle E. Dupper

Institute of Forest Genetics, U.S. Department of Agriculture Forest Service, Pacific Southwest Research Station, Berkeley, CA 94701 and Placerville, CA 95667.

Accepted for publication 6 November 2001.

ABSTRACT

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

Four of eight white pine species native to western North America surveyed for resistance to white pine blister rust by artificial inoculation showed classical hypersensitive reactions (HR) at frequencies ranging from very low to moderate. Mendelian segregation, indicating a single dominant allele for resistance (Cr3), was observed in southwestern white pine (*Pinus strobiformis*), as it was previously in sugar pine (*P. lambertiana*, Cr1) and western white pine (*P. monticola*, Cr2). HR was present at a relatively high frequency (19%) in one of five bulk seed lot sources of limber pine (*P. flexilis*), and was also presumed to be conditioned by a single gene locus, by analogy with the other three species. HR was not

found in whitebark pine (*P. albaulis*), Mexican white pine (*P. ayacahuite*), foxtail pine (*P. balfouriana*), or Great Basin bristlecone pine (*P. longaeva*), but population and sample sizes in these species may have been below the level of detection of alleles in low frequency. When challenged by (haploid) inocula from specific locations known to harbor virulence to Cr1 or Cr2, genotypes carrying these alleles and Cr3 reacted differentially, such that inoculum virulent to Cr1 was avirulent to Cr2, and inoculum virulent to Cr2 was avirulent to Cr1. Neither of these two inocula was capable of neutralizing Cr3. Although blister rust traditionally is considered an exotic disease in North America, these results, typical of classic gene-for-gene interactions, suggest that genetic memory of similar encounters in past epochs has been retained in this pathosystem.

White pine blister rust, caused by *Cronartium ribicola*, was introduced into both eastern and western North America a century ago and has caused extensive damage to many native white pine populations (13,15). Yet, major genes for resistance have been found at low frequencies in both sugar pine (*Pinus lambertiana*) and western white pine (*P. monticola*). The resistance alleles, designated Cr1 and Cr2, respectively (11), show similar phenotypes, in that they condition a classical hypersensitive reaction (HR) in foliar tissues, similar to resistance (R) genes in other plant taxa. Both Cr1 and Cr2 also have been neutralized by blister rust inocula from certain locations, suggesting that these inocula harbor alleles with specific virulence to Cr1 and Cr2 (8,11). Demonstration of differential interactions between the two host genes and rust inocula would provide the critical evidence for this hypothesis.

Observations of resistant phenotypes in other white pine species in natural stands in western North America that have been heavily impacted by blister rust suggest that the same or similar R genes also may exist in these species. Here, we explore this possibility in a survey of HR in available seedling populations of six previously untested species. We then characterize the interactions between all identified host R genes and inocula from rust populations having known or suspected wider virulence.

MATERIALS AND METHODS

To assay for major gene resistance (MGR), seed collections of different species and populations of white pines (sub. gen. *Strobus*, sect. *Strobus*) (1) from the western United States were obtained

from inventories at the U.S. Forest Service (USFS) Institute of Forest Genetics (IFG), Placerville, CA, and from cooperators at other USFS facilities in California and Oregon (Table 1). Population samples were represented by open-pollinated seed from single trees or bulk collections from several trees in a stand.

Seedlings were inoculated in the cotyledon and early primary needle stage, following standard procedures (7). Briefly, seedlings were incubated in a dew chamber with artificially inoculated European black currant (*Ribes nigrum*) leaves bearing mature telia suspended over them for 48 h at 15°C. Inocula for the potted currant plants were aeciospores bulked from infected sugar pines near the IFG in El Dorado County (ED), known to be avirulent to both Cr1 and Cr2 (7,11).

To test for interactions among putatively different R genes and inoculum sources, seedlings of sugar and western white pine parents homozygous for Cr1 or Cr2, respectively, and 1-year-old southwestern white pine seedlings (*P. strobiformis*) that had previously shown HR were inoculated as above with inocula from sources known to be able to neutralize at least one of these genes. These sources included Happy Camp (HC) and Mountain Home State Demonstration Forest (MH) in California, virulent to Cr1, (8,9), and Grass Creek (GC) in Oregon. GC is within 2 km of Champion Mine, an area long known to have rust populations virulent to Cr2 (11,12). These inocula were from bushes of *Ribes* spp. with mature telia of blister rust collected in the wild.

Host–rust interaction phenotypes were scored 4 to 6 weeks after inoculation on cotyledon leaves. The two phenotypes were either typical yellow spots, indicating compatible (i.e., susceptible, or virulent) interactions, or necrotic spots, indicating incompatible (resistant) interactions. When both phenotypes occurred on the same MGR seedling genotype, up to 100 spots were counted and assigned to the appropriate phenotypic class. Basidiospores are haploid; therefore, the percentage of virulent phenotypes determined was interpreted as the relative proportion of virulent rust genotypes in the ambient basidiospore cloud of the inoculum source in question.

Corresponding author: B. Kinloch; E-mail address: bkinloch@fs.fed.us

Publication no. P-2002-0117-02R

This article is in the public domain and not copyrightable. It may be freely reprinted with customary crediting of the source. The American Phytopathological Society, 2002.

RESULTS

HR was observed in four of the eight species screened (Table 1). Two of these, sugar pine and western white pine, carry the dominant R alleles, Cr1 and Cr2, respectively, that segregate in typical Mendelian ratios (10,11). Southwestern white pine showed HR reactions in seedlings of several families from the Sacramento Mountains of New Mexico. Overall frequency of HR in this stand was 5%, mostly from MGR pollen receptors (seedlings of susceptible seed parents that derive MGR resistance from wind-pollination) (9). A family from one phenotypically resistant seed parent, however, segregated 24:18 HR:susceptible, the near 1:1 ratio ($\chi^2 = 0.86$, $P = 0.35$) expected of a seed parent heterozygous for a dominant R allele that is fertilized from an ambient pollen cloud with a low frequency of the same allele. This is strong evidence of an R gene in this species. Limber pine had a high frequency (19%) of seedlings with HR phenotypes in one source (from the Front Range of the Rocky Mountains in Colorado), but, because this was a bulk seed lot, Mendelian ratios could not be tested or the true frequency of the putative allele calculated.

Except for ED, which was completely avirulent to MGR genotypes in all species, no other inoculum source was purely virulent or avirulent (Fig. 1). Virulence among and within the four inocula ranged from 0.0 to 92%, but affected the three hosts with R genes differentially: MH was highly virulent to sugar pine, but avirulent to western white pine; GC was virulent to western white pine, but not to sugar pine; HC was virulent to both sugar pine and western white pine; and no inoculum source was virulent to southwestern white pine. Thus, the allele in southwestern white pine is different from the other two, and we designate it Cr3. So far, limber pine has only been challenged with ED.

DISCUSSION

The differential interactions expressed in this pathosystem are typical of a classic gene-for-gene relationship. For example, resistance to GC inoculum in sugar pine with Cr1 is complete, but lacking in western white pine with Cr2 (Fig. 1). The reverse is true for MH inoculum. Both Cr1 and Cr2 are neutralized by HC inoculum. The qualitative nature of these interactions imply corresponding gene pairs in the rust for avirulence/virulence, which we provisionally designate AVCr1/vcr1 and AVCr2/vcr2 (following the convention for dominance of AVR, even though not relevant for haploids). Frequencies of these genes vary in different populations (Fig. 1) (*unpublished data*). Extension of this gene-for-gene relationship to Cr3 in southwestern white pine seems likely. We have not found virulence to Cr3 in southwestern white pine in inocula reported here, or in extensive tests of sources from the Sierra Nevada and Cascade Range (data not shown). We surmise it will most likely arise by mutation and natural selection in rust populations recently established in New Mexico (4,17), where Cr3 is currently under intense pressure.

Formal proof of a Cr gene in limber pine is lacking, but seems likely, by analogy with the three other species that express similar

HR. With the exception of the unusually high frequency ($\approx 9\%$) in one limber pine population, all Cr genes are at low frequencies; and may exist in some of the other four species surveyed with frequencies that were below our level to detect in the limited number and size of our samples. Whitebark pine is a case in point. This species is reputedly the most susceptible of the white pines, but we and others (5) have observed individual trees free of rust infection in stands that have been heavily and chronically impacted by blister rust over several decades, where probabilities of escape were low. Our samples came from only a few populations in California, but remarkably high levels (up to 44%) of rust-free seedlings were reported by Hoff et al. (5) in inoculated progenies of phenotypically resistant seed parents naturally selected by heavy general infection in northern Idaho stands. Qualitative resistance at this level is consistent with a major gene, but it was not clear from their data whether test material was from individual seed parents or bulk seed lots, or whether HR was expressed on any of the resistant seedlings.

The white pine-blister rust gene-for-gene system is unusual, in that its structure appears to be relatively simple, with few R genes dispersed among different species with apparently a single locus each. This compares with other relatively well studied gene-for-gene systems in phylogenetically younger pathosystems, which can have large numbers of alleles at complex loci (2,16). Most intriguing, though, is the presence of a gene-for-gene relationship to a pathogen presumed to be an exotic species of recent introduction. This begs the question of how these genes could arise and be maintained in North American host populations, apparently in the absence of selection pressure, at frequencies that, although low, are yet much higher than the mutation rate. For example, the average frequency of Cr1 in sugar pine is $\approx 2 \times 10^{-2}$ (7) and Cr2 in western white pine between 10^{-3} and 10^{-4} (*unpublished data*). Mutation rates, by contrast, are often assumed to be $\approx 10^{-6}$.

Recently, it has become clear that R genes are ubiquitous and abundant in plants, making up as much as 1% of the genome (2).

Fig. 1. Percentage of virulence phenotypes induced by haploid basidiospore inoculum from the source indicated. (ED, wild type; HC, Happy Camp; MH, Mountain Home; GC, Grass Creek. Hosts: SWWP, southwestern white pine; SP, sugar pine; WWP, western white pine).

TABLE 1. Presence (P) or absence (A) of a hypersensitive reaction (HR) in white pine species screened by artificial inoculation with white pine blister rust

White pine host	Number		HR	Segregation (ref.) ^a
	Populations	Seedlings		
Whitebark pine (<i>P. albicaulis</i>)	6	367	A	No
Mexican white pine (<i>P. ayacahuite</i>)	4	506	A	No
Foxtail pine (<i>P. balfouriana</i>)	6	487	A	No
Limber pine (<i>P. flexilis</i>)	5	386	P	No
Sugar pine (<i>P. lambertiana</i>)	41	>69,000	P	Yes (10)
Great Basin white pine (<i>P. longaeva</i>)	2	120	A	No
Western white pine (<i>P. monticola</i>)	6	>10,000	P	Yes (11)
Southwestern white pine (<i>P. strobiformis</i>)	10	830	P	Yes (this article)

^a Mendelian segregation for HR observed.

Large clusters of these genes originate as tandem duplications, on which mutation, deletion and insertion events, and unequal crossing over act to generate variation. Structural elements common to the most prevalent type of R gene are the nuclear binding site (NBS)/leucine rich repeat (LRR) motifs. These motifs have recently been found in sugar pine (14). Functionality of resistance is conserved (as in HR), but specificity is conferred largely by the hyper-variable LRR domain, thought to be responsible for recognition of pathogen elicitors produced by AVR genes. Thus, although there is a mechanism and raw material for the generation of R genes, maintenance of specific alleles at frequencies biologically significant for defense in host populations still depend on selection by the cognate AVR alleles.

The gene center for *C. ribicola* is in Asia, within the ranges of some of the known native hosts that include *P. armandii*, *P. koraiensis*, *P. pumila*, *P. siberica*, and *P. wallichiana* (1). Somehow, the disease reached the Baltic provinces of Russia in the middle of the nineteenth century, possibly through serial transmission from botanical garden specimens (15). In less than four decades it had swept across Europe, wherever the silviculturally popular eastern white pine (*P. strobus*) had been planted as an exotic. The highly susceptible and widely cultivated European black currant (*R. nigrum*) provided abundant inoculum to ignite the epidemic. From Europe, blister rust was introduced to both the east and west coasts of North America around 1900 on infected planting stock of eastern white pine seedlings from European nurseries (13,15).

The coevolved Asian white pines are relatively resistant to blister rust and at least one (*P. armandii*) is polymorphic for HR when challenged with North American inocula (6), suggesting that R genes in contemporary populations may have derived from ancestors common to both Asian and North American white pines. But this hypothesis is burdened by the duration of time that it would take to maintain these genes in North American host populations in the absence of the disease as a selective agent. Although it is not possible to fix the time of separation of Asian and North American species even approximately, it would have to be measured in geological epochs, at least hundreds and perhaps even thousands of tree generations. Assuming, for the sake of argument, ≈ 1 century per generation on average, over 100 have occurred since the end of the Pleistocene, when the last land bridge existed between the two continents across the Bering Strait. Many such bridges existed during glacial periods of the Pleistocene (≈ 2 mega annum [Ma], millions of years before present), but during these episodes no plausible habitat for pine was available in far northern latitudes, and most populations were likely in retreat to the south on both continents.

Neutral or nearly neutral alleles are expected to go to fixation or loss over time, through genetic drift. Cr alleles in white pines are typical of R alleles in many plant taxa in that they show HR and specificity in interaction with different inocula, and have no obvious pleiotropic effects affecting their selection and maintenance in host populations (7). The probability of ultimate fixation of a neutral allele is its frequency (3); therefore, wild-type alleles at Cr loci in North American white pines should have been fixed long ago in the absence of positive selection for mutant alternative alleles conferring resistance. It is thus paradoxical that Cr alleles exist in contemporary populations of North American white pines at frequencies at least 1 or 2 orders of magnitude greater than likely mutation rates.

An alternative hypothesis, that Cr alleles may have been under selection by a related endemic pathogen in the more recent past, has been proposed (7) but not yet established. According to this idea, some of the white pines of western North America in subsections *Strobi* (*P. flexilis*, *P. lambertiana*, *P. monticola*, and *P. strobiformis*) and *Cembrae* (*P. albicaulis*) that are presently under the greatest jeopardy from *C. ribicola* had distributions close to or overlapping two of the pinyon pines (*P. monophylla* and *P. edulis*,

subsection *Cembroides*) of the western high desert during the Pleistocene (and perhaps earlier epochs). Both of these pinyon pines are parasitized by *C. occidentale*, the pinyon blister rust (PBR), and have wide distributions even in contemporary populations that, in places, impinge closely upon those of the white pines we have identified with Cr alleles. Symptoms and life cycle of PBR are similar to white pine blister rust, and the two share alternate hosts in native *Ribes* spp. Although PBR is not known to attack any white pines outside of *Cembroides* naturally, we have been able to force parasitism by artificial inoculation (*unpublished data*). Stahl et al. (16) described evidence for R gene polymorphisms in *Arabidopsis* spp. that have existed almost 10 Ma. They used a "trench warfare" metaphor to rationalize the persistence of these genes in populations, where the ceaseless advance and retreat of host resistance and pathogen virulence is mediated by frequency-dependent selection of R genes and matching AVR genes. A more intimate association of species in *Strobi* with the pinyon pines and their rust in the past might have provided the challenge and pressure necessary to select and maintain Cr alleles. The strongest evidence for this hypothesis would be demonstration that Cr alleles in *Strobi* can also protect against PBR.

ACKNOWLEDGMENTS

We thank C. I. Millar, R. M. Lanner, and P. T. Spieth for critical review of an earlier draft.

LITERATURE CITED

1. Critchfield, W. B., and Little, E. L. 1966. Geographic distribution of the pines of the world. U.S. Dep. Agric. For. Serv. Misc. Publ. 991.
2. Ellis, J., Dodds, P., and Pryor, T. 2000. Structure, function, and evolution of plant disease resistance genes. *Curr. Opin. Plant Biol.* 3:278-284.
3. Fristrom, J. W., and Spieth, P. T. 1980. Principles of Genetics. Chiron Press, New York.
4. Hawksworth, F. G. 1990. White pine blister rust in New Mexico. *Plant Dis.* 74:938.
5. Hoff, R. J., Ferguson, D. E., McDonald, G. I., and Keane, R. E. 2001. Strategies for managing whitebark pine in the presence of white pine blister rust. Pages 346-366 in: *Whitebark Pine Communities. Ecology and Restoration*. D. F. Tomback, S. F. Arno, and R. F. Keane, eds. Island Press, Washington, D.C.
6. Hoff, R. J., and McDonald, G. I. 1972. Resistance of *Pinus armandii* to *Cronartium ribicola*. *Can. J. For. Res.* 2:303-307.
7. 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.
8. Kinloch, B. B., Jr., and Comstock, M. 1981. Race of *Cronartium ribicola* virulent to major gene resistance in sugar pine. *Plant Dis.* 65:604-605.
9. Kinloch, B. B., Jr., and Dupper, G. E. 1999. Evidence of cytoplasmic inheritance of virulence in *Cronartium ribicola* to major gene resistance in sugar pine. *Phytopathology* 89:192-196.
10. Kinloch, B. B., Jr., and Littlefield, J. L. 1977. White pine blister rust: Hypersensitive resistance in sugar pine. *Can. J. Bot.* 55:1148-1155.
11. 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.
12. McDonald, G. I., Hansen, E. M., Osterhaus, C. A., and Samman, S. 1984. Initial characterization of a new strain of *Cronartium ribicola* from the Cascade Mountains of Oregon. *Plant Dis.* 68:800-804.
13. Mielke, J. L. 1943. White pine blister rust in western North America. *Yale Univ. School For. Bull.* No. 52.
14. Sheppard, L. A., James, S. G., Delfino-Mix, A., Bassoni, D., Neale, D. B., and Kinloch, B. B., Jr. 2000. Resistance gene analogs in sugar pine. In: *W 115, Plant Animal Genome VIII Conf.*, San Diego, CA.
15. Spaulding, P. 1929. White pine blister rust: A comparison of European with North American conditions. U.S. Dep. Agric. Tech. Bull. No. 87.
16. Stahl, E. A., Dwyer, G., Mauricio, R., Kreitman, M., and Bergelson, J. 1999. Dynamics of disease resistance polymorphism at the Rpm1 locus of *Arabidopsis*. *Nature* 400:667-671.
17. Van Arsdel, E. P., Conklin, D. A., Popp, J. B., and Geils, B. W. 1998. The distribution of white pine blister rust in the Sacramento Mountains of New Mexico. Pages 275-284 in: *Proc. First IUFRO Rusts For. Trees Work. Party Conf.* Saariselka, Finland. R. Jalkanen, P. E. Crane, J. A. Walla, and T. Aalto, eds. *Finn. For. Res. Inst. Res. Pap.* 712.