A Major Gene for Resistance to White Pine Blister Rust in Western White Pine from the Western Cascade Range

Bohun B. Kinloch, Jr., Richard A. Sniezko, Gerald D. Barnes, and Tom E. Greathouse

First author: USDA Forest Service, Institute of Forest Genetics, Box 245, Berkeley, CA 94701; second author: USDA Forest Service, Dorena Genetic Resource Center, Cottage Grove, OR 97424; third author: Tree Improvement Enterprises, Cottage Grove, OR 97424; and fourth author: Box 392, Newcastle, New Brunswick, Canada E1V 3M5.
Accepted for publication 9 June 1999.

ABSTRACT

Kinloch, Jr., B. B., 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.

A dominant gene for resistance to white pine blister was indicated by Mendelian segregation in full-sib families of western white pine parent trees selected for phenotypic resistance in six heavily infected stands in the Western Cascades of Oregon and Washington. Seedlings were artificially inoculated three times between 1959 and 1964 and observed for development of stem infection. Segregation at this locus (Cr2) occurred in only two of the six parent populations sampled: one a natural stand, Champion Mine (CM), and the other a plantation of unknown seed origin. At CM, reduced penetrance of this gene was expressed by altered Mendelian ratios (mostly less-than-expected resistant phenotypes) in families of specific combinations of certain parents, indicating the presence of modifier genes with effects that ranged from mild to almost complete suppres-

sion of Cr2. Between 1968 and 1994, an apparent shift in virulence at CM caused all of the resistant selections to become infected and die. Recent inoculations of many of the same or related families from these parents, made from grafted ramets in a seed orchard, showed that Cr2 conditions a classical hypersensitive reaction (HR) in needle tissues, the primary infection courts. In the latter tests, seedlings were challenged with wild-type and four other sources of inoculum at and near CM that were also suspected of having wider virulence than wild type. No seedlings segregating for HR that were inoculated with wild type subsequently developed stem symptoms, but the other inocula induced both susceptible and HR needle spots on Cr2- genotypes, and many of these seedlings did develop stem infections. This implied that spore genotypes with specific virulence to Cr2 are carried in these inocula.

Additional keywords: *Cronartium ribicola*, genetic background, hypersensitivity, *Pinus monticola*.

Western white pine (*Pinus monticola* Douglas) has historically been one of the most valuable conifers in western North America, both economically and ecologically. It has the potential to grow to great size and stand densities, producing large amounts of clear wood of unsurpassed quality. A seral species at middle and upper elevations, it ranges widely in the western cordillera of Canada and the United States (4). Its relative resistance or tolerance to several native root pathogens allows it to dominate many sites where more shade-tolerant climax species would dominate in the absence of these pathogens (5). But after the introduction of white pine blister rust (caused by *Cronartium ribicola* J.C. Fisch.) almost a century ago, many populations of this and other white pine species have been severely damaged. In the northern Rocky Mountains, for example, up to 95% of the once nearly pure stands of white pine have been destroyed (15). The disease kills seedlings and saplings directly and large trees indirectly by damaging their crowns so badly that they become stressed and predisposed to bark beetles. Most importantly, regeneration is severely limited in areas of high rust hazard, altering patterns of succession. After the abandonment of massive programs to eradicate alternate hosts (1) or to control the disease directly by antibiotic sprays (17), genetic resistance came to be considered the most feasible and effective method of control.

In the late 1950s, the U.S. Forest Service, Pacific Northwest Region, began a tree improvement program to select, breed, and test

candidate western white pine parent trees for resistance to blister rust for the Cascade and Coast Ranges of Oregon and Washington. The program was developmental, modeled on earlier research by R. T. Bingham (2) and associates at Moscow, ID, for the blister rust problem in the northern Rocky Mountains. The early results from artificial inoculations, recorded, filed, and summarized in internal office memoranda, strongly suggested that a qualitative resistance existed in a significant number of full-sib families from phenotypically resistant parents. This was surprising at the time, since resistance to blister rust was thought to be entirely quantitative (3). Other ambiguities remained: parents in only two of the six populations sampled expressed this kind of resistance; but in both of these, the frequencies were so high the data were considered suspect. Also, progenies of certain of these parent trees did not perform consistently in different crosses. Furthermore, in subsequent years, the apparent resistance evident earlier was seen to erode or become erratic in expression. New infections started to be observed even in some of the originally resistant parent selections in one of the stands around 1970, and within 2 decades, all were dead. During this interval, comparison of infection by inoculum from alternate host *Ribes* spp. from this stand, called Champion Mine, with that of presumed wild-type inoculum from elsewhere on western white pine families of known resistant parents led to the conclusion that a new strain of blister rust with wider virulence had arisen (12). Meanwhile, changes in personnel, policy, and program direction occurred. Vanderplank's (19) emphasis on the (theoretical) stability of horizontal resistance, compared with the notorious lability of vertical resistance, dominated strategic thinking, and interest in a possible single gene for resistance that had been suggested by the early data diminished. Results of the early work were never published, and institutional memory of them faded as key personnel departed from the program over the years.

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

Publication no. P-1999-0820-02R

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, 1999.

Our purpose in this paper is to document some of the more important early observations of this program, retrieved from old files as well as from the memory of two of us (G. D. Barnes and T. E. Greathouse) associated with the program in its formative stages. We will then relate these observations to recent experiments designed to test hypotheses suggested by the early data. Specifically, we wished to confirm the inheritance and dominance relationships of a putative major gene for resistance in western white pine; determine its mechanism and mode of expression; and describe its interaction with two known blister rust races of wider virulence than wild type (10,12).

MATERIALS AND METHODS

Candidate selection. Resistant phenotypes of western white pine were sought in the Cascade Range of Oregon and Washington.

TABLE 1. Rust-free seedlings of western white pine full-sib families from phenotypically selected parents after three artificial inoculations with white pine blister rust, 1959 to 1964

Source (National Forest)	Parents tested		Nonsegregating families			
	Total	Segregating	No.	Mean no. of seedlings	Rust-free (%)	
					Mean	Range
Gifford Pinchot	15	0	23	80	2.2	0–12
Mt. Hood	9	0	13	62	1.4	0–6
Olympic	7	0	5	30	0	
Snoqualamie	11	0	16	79	5.2	0–14
Umpqua (CM) ^a	26	22	4	124	11.1	3–21
Willamette (BP) ^a	27	10	32	50	1.9	0–9
Total/mean	95	32	93	70.8	3.6	0–21

^a CM = Champion Mine, and BP = Bear Pass.

TABLE 2. Observed and expected ratios (rust-free/infected) of western white pine full-sib families after three inoculations with white pine blister rust, 1959 to 1964 (Bear Pass group)

Parents		No. of seedlings	% Rust-free	Ratio tested ^a	<i>P</i>
Seed	Pollen				
3	12	28	32	1:1	0.057
4	1	40	58	1:1	0.312
4	33	41	47	1:1	0.701
4	04-01	20	40	1:1	0.371
4	04-02	67	54	1:1	0.513
9	33	77	44	1:1	0.292
9	36	67	48	1:1	0.743
9	04-02	61	54	1:1	0.532
10	04-01	21	48	1:1	0.855
10	04-02	80	59	1:1	0.107
11	35	25	64	3:1	0.204
14	36	29	45	1:1	0.590
15	35	32	41	1:1	0.309
16	2	28	36	1:1	0.138
18	35	49	47	1:1	0.674
19	32	32	59	1:1	0.309
28	12	21	38	1:1	0.271
32	15	98	51	1:1	0.843
32	18	88	42	1:1	0.133
32	33	94	48	1:1	0.698
32	36	131	44	1:1	0.170
32	04-02	95	52	1:1	0.697
35	12	25	92	3:1	0.050 ^b
35	15	22	41	1:1	0.399
37	12	31	65	3:1	0.199
37	15	35	34	1:1	0.058
37	18	37	49	1:1	0.903
39	18	31	55	1:1	0.578
Pooled ratios for classes					
3:1		81	72.8	3:1	0.681
1:1		1,324	47.7	1:1	0.114

^a Thirty-one nonsegregating families (both parents susceptible) not shown.
^b Tested ratio significantly greater than expected at *P* = 0.05.

TABLE 3. Observed and expected ratios (rust-free/infected) of western white pine full-sib families after three inoculations with white pine blister rust, 1959 to 1964 (Champion Mine group)

Parents		No. of seedlings	% Rust-free	Ratio tested	<i>P</i>
Seed	Pollen				
801	816	112	72	3:1	0.463
802	820	127	69	3:1	0.118
802	836	69	75	3:1	1.000
802	Wind	80	68	3:1	0.148
803	816	94	73	3:1	0.654
803	841	119	74	3:1	0.801
806	837	67	76	3:1	0.850
806	840	65	46	1:1	0.193
806	846	92	82	3:1	0.121
813	816	52	72	3:1	0.617
813	836	37	75	3:1	1.000
813	844	51	84	3:1	0.138
814	816	123	81	3:1	0.124
814	820	88	81	3:1	0.194
814	837	36	81	3:1	0.406
814	841	134	81	3:1	0.109
814	872	125	86	3:1	0.005†† ^a
816	836	52	67	3:1	0.183
816	837	117	65	3:1	0.012 ^{a,b}
820	816	134	58	3:1	0.000***
820	836	139	73	3:1	0.586
820	836	102	52	3:1	0.000***
820	837	136	63	3:1	0.001**
820	841	160	59	3:1	0.000***
834	816	24	92	3:1	0.054
834	816	62	55	3:1	0.000***
834	836	35	86	3:1	0.133
834	836	52	58	3:1	0.005**
835	836	155	63	1:1	0.001**
836	836	49	78	3:1	0.628
836	836	108	76	3:1	0.810
836	837	87	67	3:1	0.085
836	841	63	62	3:1	0.017*
836	846	80	74	3:1	0.836
836	847	122	63	3:1	0.002**
837	836	63	81	3:1	0.271
837	841	69	64	3:1	0.035*
837	844	78	71	3:1	0.415
839	836	34	79	3:1	0.590
839	841	52	83	3:1	0.183
840	816	72	6	1:1	0.000***
840	836	82	6	1:1	0.000***
840	837	183	39	1:1	0.003**
840	837	124	35	1:1	0.001***
840	839	90	50	1:1	1.000
840	840	139	4	?	
840	841	134	31	1:1	0.000***
840	844	94	42	1:1	0.121
840	846	93	30	1:1	0.000***
840	Wind	188	22	?	
841	816	37	84	3:1	0.206
841	836	109	57	3:1	0.000***
841	837	18	56	3:1	0.063
841	841	91	69	3:1	0.186
841	847	47	83	3:1	0.205
844	836	59	75	3:1	1.000
844	841	136	74	3:1	0.788
844	862	103	66	3:1	0.035*
845	836	83	80	3:1	0.293
845	841	87	66	3:1	0.053
845	844	69	74	3:1	0.848
851	851	140	10	?	
852	816	30	63	3:1	0.129
852	836	26	88	3:1	0.126
852	841	24	67	3:1	0.365
852	841	80	74	3:1	0.836
854	836	67	82	3:1	0.186
854	841	121	80	3:1	0.204
854	844	106	75	3:1	1.000
863	836	96	57	1:1	0.170
863	837	114	54	1:1	0.393
863	841	99	60	1:1	0.047†
871	836	62	87	3:1	0.029†
871	841	29	94	3:1	0.018†
872	816	149	57	3:1	0.000***
872	820	35	77	3:1	0.785
872	836	110	65	3:1	0.015*
872	841	173	61	3:1	0.000***
Pooled ratios for classes					
3:1		5,075	72.7	3:1	0.000***
1:1		1,401	39.9	1:1	0.000***

^a †, †† = Tested ratio significantly greater than expected at *P* = 0.05 and 0.01, respectively.
^b *, **, *** = Tested ratio significantly less than expected at *P* = 0.05, 0.01, and 0.001, respectively.
^c ? = Ratio unknown.

Criteria for selection were sexual maturity and absence of blister rust infection in stands where heavy infection was pervasive. Candidates were controlled pollinated in the wild, using highly fecund trees to test several other pollen parents in each selection unit. A total of 95 parents was selected and bred in the wild in six National Forests (NF), and 136 full-sib families were produced by 1964 (Table 1). Many crosses were later made on ramets of the same parents in a grafted seed orchard.

Two stands in the Western Cascades of Oregon that yielded families segregating for resistance were Bear Pass (BP), in the Willamette NF, and Champion Mine (CM), in the Umpqua NF. The BP stand was an old plantation, established in 1916 and at least partially reestablished in 1936 after much of the original stand failed. Stand establishment records are incomplete, and we have been unable to positively determine the seed origin of the plantation. The CM site was named after a gold mine that operated intermittently from 1896 to 1941. Situated in a narrow, steep canyon drained by Champion Creek, its topography and north-facing aspect make it an unusually cool and moist site for the area, an ideal habitat for blister rust and, therefore, for phenotypic selection. The parent trees selected on this site, starting in 1957, were second growth, dating from about 1920. The area of western white pine regeneration was only about 100 acres, between an elevation of 4,200 and 4,800 feet. A few scattered old-growth western white pines still survive on the ridge tops, but the site is otherwise isolated from other western white pine stands, and the trees at CM may have represented a relatively narrow genetic base.

Progeny testing: Early inoculations (1959 to 1964). Usually, 100 seed were sown for each full-sib family, and seedlings were

grown in metal one-gallon pots, one seedling to a pot, initially at a U.S. Forest Service nursery at Wind River, OR, and then at a work center at Layng Creek near Cottage Grove, OR. After their second growing season, seedlings were moved into an open-faced equipment storage shed in the autumn for inoculation and arranged in a quasi-random manner. The face of the shed was covered with canvas and the canvas, floor, and walls saturated with water, as well as the seedling potting soil. Whole branches of different *Ribes* spp. (mainly *R. bracteosum* Douglas and *R. sanguineum* Pursh, but occasionally including *R. roezlii* Regel var. *cruentum* Greene (Rehd.) and *R. lacustre* Pers. (Poir)) with heavily rust-infected leaves were stuck into the pots, where they remained for 3 days under highly humid conditions. The inoculum was obtained from various NF sites in the region, which almost always included CM (Umpqua NF) and Still Creek (Mt. Hood NF) because of their abundant and dependable yields. Telia were monitored for germination, and water droplets on needles were sampled and examined microscopically for presence of basidiospores, but inoculum density was not quantified. Each family was inoculated in this manner in 3 successive years.

Foliage symptoms were noted, but no attempt was made to quantify amounts of infection or to distinguish between different needle spot phenotypes. Seedlings were scored only for presence or absence of stem infection 1 year after each inoculation.

Progeny testing: 1996 to 1997 inoculations. Seed from many of the same or similar families in the early tests were cold-stratified for 120 days and sown into Leach Super cells (164 cm³; Ray Leach Nursery, Canby, OR) in racks, 98 per rack, in early March. In the 1996 test, seedlings were grown with the photoperiod ex-

Fig. 1. Morphology and histology of the hypersensitive reaction in foliage of western white pine. Cr2 genotypes attacked by **A and C**, virulent and **B and D**, avirulent genotypes of blister rust. Note the masses of mycelium (blue) in and surrounding the central vascular cylinder of the virulent phenotype, and the cellular disorganization, necrosis, and lack of viable mycelium in the avirulent phenotype. **B and D**, Fluorescent illumination.

tended to 16-h days by 400-W high-pressure sodium lamps and with weekly, dilute fertilization to promote growth and early secondary needle production. They were inoculated in September, after most had formed secondary needles but still had viable cotyledons and primary needles. Seedlings in the 1997 test were inoculated only 6 weeks after sowing, while still in the cotyledon and early primary needle stage.

Inoculum for the 1996 test included sources from CM and vicinity, including the Layng Creek *Ribes* garden (used as a source of inoculum for annual screening tests) and Bryce Creek, 2 km downslope from CM. CM and Layng Creek were sources known or thought to have factors for wider virulence than wild type (12). Inoculum was also sampled from the U.S. Forest Service test site near Happy Camp, source of the race of rust virulent to major gene resistance in sugar pine, in the Siskyou mountains of northern California (10). Pooled inoculum from several sites in El Dorado County, CA, remote from sites known to harbor wider virulence, was chosen, based on previous experience, to represent wild-type inoculum. At each site, *Ribes* leaves with mature telia were collected from many different bushes, mixed, and refrigerated in polyethylene bags until used, usually within 3 days. All sources of inoculum were kept and used separately. Thus, inoculum was homogenized for each source, but variation in virulence among individual basidiospores within a source, if any, could be detected by differences in needle spot phenotypes on test seedlings with a resistance gene. Seedlings in the 1997 test were inoculated with wild type only.

Seedlings were incubated at $15 \pm 0.6^\circ\text{C}$ for 48 h in darkness in dew chambers (model I-35D; Percival Manufacturing Co., Boone, IA) with detached *Ribes* leaves suspended over the seedlings on wire frames. Our objective was to induce heavy basidiospore production in order to minimize escapes and to enhance the unambiguous expression of any qualitative phenotypic differences in needle symptoms. Inoculum density was quantified by counts of spores cast onto microscope slides placed horizontally at seedling height, two per rack (one per square foot), sampled through an ocular grid. Fine dewdrops condensed on seedling foliage, without runoff. At the end of incubation, dew chamber doors were opened and seedlings allowed to dry gradually for the next 24 h, before being returned to greenhouse benches.

RESULTS

1959 to 1964 inoculations. Overall results of the early tests are given in Table 1. Although needle symptoms were observed, only presence or absence of stem symptoms were recorded, 1 year after each of the three inoculations. Thirty-two parents had families with very high proportions of rust-free seedlings after the third inspection, with modal points around 50 and 75%. These were interpreted as segregating for a major gene for resistance (Tables 2 and 3). Parents with this gene came from only two sources: the BP stand in the Willamette NF (10 parents), and the CM stand in the Umpqua NF (22 parents). Parents from the other four NFs had few or no rust-free offspring. The mean percentage of rust-free seedlings for 93 non-segregating, full-sib families from all forests was only 3.6%, although a few families ranged considerably higher than this, from about 10 to 21% (Table 1).

Parent genotypes were inferred from progeny performance. Most parents with the dominant gene appeared to be heterozygotes; no families with nonsegregating rust-free offspring were found. Chi-square tests for conformance to hypothesized Mendelian segregation ratios (1:1 and 3:1, representing parental genotypes $Rr \times rr$ and $Rr \times Rr$, respectively) are given in Tables 2 and 3 for BP (Willamette NF) and CM (Umpqua NF) parent groups, respectively.

We designate this locus Cr2, following suggested nomenclature for resistance gene loci (22), to distinguish it from a similar gene in sugar pine, Cr1 (formerly R) (8,11).

BP group. In this group, 10 of 26 parent trees were inferred to be heterozygous at Cr2, based on Mendelian segregation ratios of their progenies. Of 28 segregating families, 25 segregated in 1:1 and 3 in 3:1 ratios (Table 2). None of the observed ratios was significantly different from those expected, although four approached significance with probabilities ranging from 0.05 to 0.10. Pooled ratios for the two classes (1:1 and 3:1) were also nonsignificant. An anomalous progeny (16×2) appeared to segregate in a 1:1 ratio when no other families of either of these parents did.

CM group. The CM group contrasted strongly to the BP group in two ways. First, many more parents (22 of the 26) appeared to be segregating at Cr2 in almost all of their crosses (Table 3). Parent 835, appearing in only one cross with a heterozygote, was ambiguous, as was to some extent parent 863, appearing in three crosses

TABLE 4. Percentages and expected ratios of western white pine full-sib families expressing a hypersensitive reaction (HR) in cotyledons after challenge with white pine blister rust and percentage of HR seedlings developing stem symptoms from different inoculum sources, 1996

Parents		No. of seedlings	% HR	Ratio tested	P	% HR seedlings (number challenged) with stem symptoms					
Seed	Pollen					Wild type	Champion Mine	Layng Creek	Bryce Creek	Happy Camp 1	Happy Camp 2
802	845	80	68.8	3:1	0.299	0 (20)	76.5 (17)			50 (2)	80 (15)
813	813	56	80.4	3:1	0.440	0 (24)	81 (21)				
814	814	58	74.1	3:1	0.901	0 (17)	94.1 (17)			0 (2)	85.7 (7)
F1	F1	21	95.2	1:0		0 (20)					
820	820	73	75.3	3:1	0.956	0 (29)	88.9 (18)				
824	840	56	71.4	3:1	0.617	0 (23)	88.2 (17)				
824	841	60	68.3	3:1	0.338	0 (23)	95 (20)				
836	845	126	78.6	3:1	0.443	0 (28)	92.3 (26)	95.5 (22)	50 (16)	83.3 (6)	
839	845	152	75.7	3:1	0.878	0 (28)	78.3 (23)	65.2 (23)	94.4 (18)	8.3 (12)	37.5 (8)
840	840	55	52.7	3:1	0.003	0 (15)	50 (8)			0 (4)	
840	841	137	43.1	3:1	0.000	0 (15)	61.9 (21)		91.7 (12)	0 (4)	0 (6)
841	814	104	53.8	3:1	0.000	0 (13)	93.8 (16)		100 (18)	25 (4)	25 (4)
841	851	138	26.8	1:1	0.000	0 (10)	44.4 (9)		90 (10)	0 (6)	0 (2)
851	Wind	142	19.7				87.5 (8)		57.1 (7)	0 (1)	25 (4)
862	840	128	66.4	3:1	0.073	0 (19)	100 (19)	76.9 (13)	95.7 (23)	14.3 (7)	100 (1)
862	845	74	71.6	3:1	0.586	0 (17)	89.5 (19)			41.7 (12)	50 (4)
389	380	147	0	0:1							
389	383	98	2	0:1							0 (1)
389	390	72	51.4	1:1	0.814	0 (18)	68.4 (19)				
390	386	77	72.7	3:1	0.708	0 (25)	90 (20)			0 (3)	20 (10)
391	392	90	51.1	1:1	0.833	0 (15)	88.9 (9)	66.7 (9)		0 (1)	45.5 (11)
392	392	128	76.6	3:1	0.737	0 (21)	83.3 (24)	60 (20)	94.7 (19)	0 (8)	40 (5)
399	389	151	0	0:1							
399	395	125	54.4	1:1	0.325	0 (14)	83.3 (18)	90 (10)	94.7 (19)	0 (2)	33.3 (6)
Mean (total)						0 (394)	81.8 (349)	71.8 (75)	90.4 (148)	12.6 (84)	46.7 (90)

with other heterozygotes. In these early tests, only parents 840, in 11 crosses, and 851, in a single selfed cross, appeared to lack the dominant allele.

Secondly, many more progenies of heterozygous parents had skewed ratios. Of the 76 families, 26 departed significantly from ratios expected and tested by chi-square. Most (22 families) had significantly fewer numbers of rust-free seedlings than expected, while only four had greater-than-expected numbers of rust-free seedlings. Pooled ratios for classes (1:1 and 3:1) were highly significant.

Almost half of the parents produced families with skewed ratios, but three parents stood out, especially as seed parents: 820 (four out of five families), 840 (six out of nine families), and 872 (three out of four families). As pollen parents, 820 performed as expected in three crosses and 840 in one cross, while 872 had significantly higher numbers of rust-free seedlings in one cross. Parent 840 was the most conspicuous deviant; in crosses with confirmed Cr2cr2 pollen parents 816 and 836, it suppressed expression of Cr2 almost completely—6% rust-free seedlings in each (versus 50% expected). With pollen parents 837, 841, and 846, proportions of rust-free seedlings were only partially reduced. On the other hand, a few trees such as 836 and 841 had more deviant families as pollen parents.

1996 to 1997 inoculations. All seedlings in the 1996 sowing had healthy cotyledons and primary needles at the time of inoculation (autumn), and most also bore secondary needles. Inoculum density averaged 99.6 basidiospores per mm², and infection was moderate to heavy with all inocula except Happy Camp, which only cast six spores per mm² with correspondingly lighter infection. Reinoculation of escapes in July 1997 with this source, however, resulted in heavy infection.

Needle symptoms started to appear about 2 weeks after inoculation. There were two main types: typical yellow (occasionally red) spots on known susceptible control seedlings (Fig. 1A) and brown necrotic spots on seedlings with Cr2, a classic hypersensitive reaction (HR) (Fig. 1B). Yellow or red spots were also induced on Cr2- genotypes by virulent inoculum. HR symptoms were indistinguishable from susceptible symptoms in very early stages, until a thin, barely visible, necrotic band appeared at the margins of the spots. Necrosis then spread inward until the entire spot was brown. Symptoms were the same on all foliar types, but the earliest and most definitive expression was on cotyledons.

Histological examination of HR showed characteristic cellular degeneration of both host and fungal cells (Fig. 1D), similar to HR in sugar pine (6), while yellow spots (susceptible reactions) had masses of viable and vigorous mycelium (Fig. 1C).

The two phenotypic classes assigned for analysis of Mendelian segregation were presence or absence of HR. Seedlings with mixed reactions were designated HR, because HR only occurs on Cr2- genotypes. There were no differences among inocula in the segregation ratios of different full-sib families, and needle symptom data were pooled over all inoculum sources (Table 4). Segregation ratios (3:1 and 1:1) for presence/absence of HR were virtually identical to those for rust-free/rust-infected in the earlier inoculations involving many of the same parents (cf. Table 3), based on stem infection only, demonstrating that HR was the mechanism of resistance to stem infection. The single family from a cross of two F1 offspring from different heterozygous parents was 95% HR, indicating that at least one of the F1 parents is probably homozygous (Cr2Cr2). Four families, however, deviated significantly from ratios expected under the dominant gene hypothesis. These were among the same parents and families that had significant deviations in the early tests, namely 814, 820, 840, and 841 (cf. Table 3).

In contrast to the clear segregation of needle spot phenotypes, development of stem infection differed greatly among the inocula. Wild-type inoculum was the only source completely avirulent to Cr2, inducing only HR lesions on Cr2- genotypes (Table 4). No seedlings with HR subsequently got stem infections from wild-type inoculum, but most seedlings (72 to 90%) (Table 4) with HR did get stem infections from inocula from CM and the three neighboring

sites (Bryce Creek, Grass Creek, and Layng Creek). All inocula except wild type also induced some yellow (susceptible) needle symptoms on seedlings with predominantly HR symptoms, implying the presence of virulent rust genotypes in these inocula at low frequencies. None of the inocula were completely virulent. Accurate counts for comparison of relative frequencies of the two spot classes on individual seedlings was not possible, because infection was heavy and yellow spots coalesced too rapidly. Happy Camp inoculum had the lowest incidence of seedlings with stem infections in both inoculations with this source (13 and 47%) and also induced the fewest yellow spots.

The 1997 inoculation included as many families as available of parents that showed skewed ratios in either the early tests or the 1996 test. Emphasis was on families of 814, 820, 840, and 841, all in the CM group, both as seed or pollen parents. Inoculation was with wild type only. All but 2 of the 30 families performed as expected, based on previous results (Table 5). One of these, 841 × 813, segregated in a ratio of 1:1 versus the 3:1 ratio expected from other crosses involving these parents in this and the earlier tests. In the other, 840 × 846, the HR was almost completely suppressed: only a single seedling showed this trait, when 30 (out of 40) were expected to. But as pollen parent in four crosses (with seed parents 803, 813, 817, and 823, all heterozygous at Cr2), 840 also performed as a Cr2 heterozygote (as it did with seed parent 824 in the 1996 inoculation) (Table 4).

Combining data from Tables 3 to 5 shows that a quarter (32 of 120) of the segregating families in the CM group had fewer than expected offspring expressing Cr2 (compared with 0 of 28 families for BP) (Table 2). These were distributed among many of the parents, but over half derived from parents 820, 840, and 872,

TABLE 5. Percentages and expected ratios of western white pine full-sib families expressing a hypersensitive reaction (HR) in cotyledons after challenge with wild-type inoculum, 1997

Parents		No. of seedlings	% HR	Ratio tested	P
Seed	Pollen				
802	845	35	77.1	3:1	0.770
803	840	52	88.5	3:1	0.025† ^a
813	840	46	82.6	3:1	0.233
814	852	36	66.7	3:1	0.248
814	862	40	65	3:1	0.144
814	867	40	77.5	3:1	0.715
814	869	40	77.5	3:1	0.715
817	840	40	75	3:1	1.000
819	845	44	72.7	3:1	0.728
820	820	28	67.9	3:1	0.383
820	510	14	78.6	3:1	0.758
823	840	40	77.5	3:1	0.715
824	845	40	67.5	3:1	0.273
836	845	28	75	3:1	1.000
839	845	40	75	3:1	1.000
840	846	40	2.5	3:1	0.000**** ^b
841	841	32	84.4	3:1	0.221
841	813	31	48.4	3:1	0.001***
841	814	40	80	3:1	0.465
841	851	51	41.2	1:1	0.208
841	852	39	66.7	3:1	0.229
841	862	40	80	3:1	0.465
841	867	35	65.7	3:1	0.205
841	869	40	72.5	3:1	0.715
854	820	40	72.5	3:1	0.715
868	820	40	82.5	3:1	0.273
870	820	40	77.5	3:1	0.715
876	820	39	84.6	3:1	0.166
896	820	39	74.4	3:1	0.926
389	383	38	0.0	0:1	
Pooled test				3:1	0.036*
Pooled test, excluding 840 × 846				3:1	0.718

^a † = Tested ratio significantly greater than expected at $P = 0.05$.

^b *, ***, **** = Tested ratio significantly less than expected at $P = 0.05$ and 0.001, respectively.

mainly as seed parents. For example, deficient numbers of offspring expressing Cr2 occurred in four of eight families of 820 as seed parent, but in none of the 10 families of the same tree as pollen parent. The corresponding figures for 840 were 12 out of 12 as seed parent, but only three out of nine as pollen parent (only one of seven, if two selfed families are excluded). Only five families expressed greater-than-expected numbers of resistant seedlings.

Sixteen families were represented in two different inoculation years. In at least six of these comparisons, results were inconsistent between years (820×836 , 834×816 , and 834×836 [Table 3]; 840×840 [Tables 3 and 4]; 840×846 [Tables 3 and 5]; and 841×814 [Tables 4 and 5]).

DISCUSSION

Evidence from all the test inoculations leads consistently to three main conclusions: (i) a dominant gene controlling classical HR to white pine blister rust exists in some natural populations of western white pine; but (ii) penetrance of this gene can be strongly affected by the genetic background, resulting in altered Mendelian ratios; and (iii) the CM race of rust capable of neutralizing this gene exists in some blister rust populations at low but varying frequencies.

Mendelian segregation patterns for HR in foliage in the 1996 to 1997 inoculations were identical to those for rust-free stems in the 1959 to 1964 inoculations (Tables 3 to 5), and no seedlings challenged with wild-type inoculum that had HR symptoms subsequently developed stem symptoms. The HR in western white pine is similar to the HR in many other plant pathosystems and, in particular, to the HR conditioned by major gene resistance in sugar pine foliage (6). Since similar disease reaction phenotypes can be controlled by different genes, this does not necessarily imply that the two genes identified are the same in the two pine species. However, we have recent evidence (data not shown) that CM and CM-related inoculum does not neutralize Cr1 in sugar pine, suggesting that genetic specificity exists among resistance and virulence loci in this pathosystem. Nor do our data prove that Cr2 is the only major gene locus for resistance in these western white pine populations or even identical in all of the genotypes tested; formal proof would require precise genetic control of test inoculum rather than the bulk mixes we used.

Penetrance of Cr2 ranged from complete to almost nil, depending on the specific combination of parents in the cross. This was most evident in the CM group. About a third of the families showed altered ratios (mostly fewer-than-expected numbers of rust-free or HR seedlings), but some parents influenced this tendency to a much greater extent than others. Parent 840 was the most conspicuous example, particularly as a seed parent, in which all of its 12 families showed suppression of Cr2, ranging from moderate to nearly complete. As seed parent, 840 often suppressed both its own Cr2 allele as well as that of other heterozygote pollen parents (e.g., 816, 836, self, and 846) (Tables 3 to 5), although duplicate trials of the same crosses were inconsistent in two cases (selfed and with 846). As a pollen parent, however, 840 performed like a normal heterozygote in all but one case (with seed parent 806) (Table 3). Parents 820 and 872 had similar patterns, if less pronounced in effect. The significance of these apparent maternal effects on a demonstrably nuclear gene are difficult to evaluate from these data, but it is clear that expression of Cr2 is profoundly affected by modifying genes in certain genetic backgrounds.

Although the influence of genetic background on the expression of disease phenotype has long been recognized in a general way, recent emphasis has focused on suppressors and modifiers with major effects on R genes. Examples include a dominant suppressor specific to the Pm8 allele for resistance to powdery mildew in wheat (18); another locus in wheat that suppresses up to three R genes for stem rust resistance (21); three suppressors in barley that differentially affect some specific powdery mildew R genes but not

others (7); and in *Arabidopsis*, the suppression by a mutated R gene against one pathogen of R genes that function against different pathogens (20). The literature was reviewed by Jorgensen (7), who noted that differential effects of suppressors on different R genes imply that these R genes employ or involve different cascade pathways in defense. Thus, suppression or modification of expression can occur either to regulatory genes or to structural genes downstream in the cascade. This provides a heuristic rationalization of both the qualitative and quantitative effects of the R gene modification we document here.

Altered Mendelian ratios could also be explained by pollen contamination. While pollen contamination cannot be disproved in any given cross, we consider it an unlikely cause of the large numbers of discrepancies observed. First, significantly altered ratios occurred only in the CM group in the early tests, even though matings of both CM and BP groups were made by the same personnel during the same period of time. Secondly, repeat trials of some of the same CM parents (e.g., 840 and 841) in the 1996 to 1997 inoculations produced similar results as with the earlier inoculations (cf. Tables 4 and 5 with Table 3).

Virulence to Cr2 is clearly present in some rust populations, most notably at CM (where we expected to find it) (12) and neighboring sites. Virulence was indicated in these inocula by presence of susceptible (yellow)-type needle spots in low frequency on Cr2- seedlings (seedlings with mostly HR reactions) and by the subsequent development of stem symptoms and signs on a high proportion of these seedlings (Table 4). In contrast, no trace of virulent needle spot phenotypes was observed from inoculum from the El Dorado, CA, site, which we chose to represent typical wild type, and no HR phenotypes induced by this inoculum developed stem symptoms.

Although incidence of stem infection on Cr2- genotypes from CM and near-neighbor inocula was high, the actual frequency of yellow spots (the phenotypic marker of virulence) on the same seedlings was low. Precise counts of virulent and avirulent spot phenotypes were not possible, but we estimate that the frequency of virulence was less than 3%. This was still more than enough to cause a high incidence of stem infection, however, since a single compatible needle infection can cause stem infection. The stem infection that did result provides a ready explanation for the apparent erosion of resistance observed in annual screening tests after the appearance of the CM strain in the late 1960s. Virulence was also evident in inoculum from Happy Camp, about 110 miles southwest of CM, though at a lower frequency than CM and neighboring inocula. We do not know whether this represents a spontaneous separate occurrence or migration from CM.

In the initial characterization of the CM strain (12), resistant western white pines were used to compare the effects of CM and wild-type inocula. Parent genotypes and their resistance mechanisms were not described, but records at the U.S. Forest Service Dorena Tree Genetic Resource Center show that all parents used in the referenced test were from the CM population. Several symptom traits induced by CM inoculum were described that appeared to differ quantitatively from the other inocula used. Our data suggest, more simply, that a specific resistance gene in the host is neutralized by a corresponding gene for virulence in the rust, very similar to the situation in sugar pine (10) and in gene-for-gene systems in general.

The existence of rust-free seedlings at low frequency (3.6%) (Table 1) in nonsegregating families in almost all seed sources used in the early (1959 to 1964) inoculations is evidence that other strong resistance mechanisms may exist in western white pine. Because all families were inoculated three times in successive years, the challenge was severe and the probability of escape was low. Although we did not observe similar low-level resistance in the 1996 to 1997 inoculations, these involved a narrower population base composed almost entirely of CM parents.

Absence of Cr2 in any seed sources other than BP and CM is curious and might be explained by a combination of the low fre-

quency of this allele and the relatively fewer parents tested in the other sources. The frequency of Cr2 in the original, unchallenged pine populations at BP and CM cannot be estimated from these data, but must have been low even at CM, since none of the 22 parents with this allele was homozygous. The difference in the frequency of Cr2- genotypes between the CM and BP groups is more problematic and adds to the frustration of our uncertainty about the seed origin of BP. We do know that natural selection against susceptible genotypes in both populations was intense; the CM site, in particular, was known to lie in an area of severe rust hazard long before 1957, when selections for resistant phenotypes were begun there. The completely susceptible families produced by heavily challenged, resistant phenotypes at BP could be explained by ontogenetic resistance, a type of resistance that develops with age and is known in other white pines (9,16). However, there was little evidence for this mechanism at CM.

Equally curious is the apparent absence of Cr2 in northern Rocky Mountain populations, where selection and breeding for blister rust resistance has been ongoing since 1950 (2). Reactions similar to HR have been documented but attributed to recessive genes at two different loci (6,13,14). Clearly, a comprehensive screening involving all major populations of *P. monticola* is needed to accurately resolve the distribution and frequency of Cr2.

Like all HR genes, Cr2 is very strong, yet completely vulnerable to a gene in the pathogen with specific virulence to it, as the complete extirpation of the CM parent population over little more than 2 decades clearly indicates. Its value in protecting synthetic populations of western white pines being developed in breeding programs in western North America will depend somewhat on the distribution, frequency, and potential migration rate of the presumed virulence gene in the CM and like populations of rust. Standing alone, the durability of Cr2 will probably be relatively short-lived. But buffered with rate-reducing and other resistance mechanisms, it could be extremely effective.

ACKNOWLEDGMENTS

We thank A. Delfino-Mix and G. Dupper for implementing the 1996 to 1997 experiments at the Institute of Forest Genetics, and D. B. Neale, A. Yanchuck, and two anonymous reviewers for constructive suggestions of earlier drafts.

LITERATURE CITED

- Benedict, W. V. 1981. History of White Pine Blister Rust Control—A Personal Account. U.S. Dep. Agric. For. Serv. FS-355.
- Bingham, R. T. 1983. Blister rust resistant western white pine for the inland empire: The story of the first 25 years of the research and development program. U.S. Dep. Agric. For. Serv. Gen. Tech. Rep. INT-146. Intermountain Forest and Range Experiment Station, Ogden, UT.
- Bingham, R. T., Olson, R. J., Becker, W. A., and Marsden, M. A. 1969. Breeding blister rust resistant white pine. V. Estimates of heritability, combining ability, and genetic advance based on tester matings. *Silvae Genet.* 18:23-38.
- Graham, R. T. 1990. *Pinus monticola* Dougl. ex D. Don. western white pine. Pages 385-394 in: *Silvics of North America*. Vol. 1: Conifers. R. M. Burns and B. H. Honkala, technical coordinators. Agric. Handb. 654. Washington, DC.
- Harrington, T. C., and Wingfield, M. J. 1998. Diseases and the ecology of indigenous and exotic pines. Pages 381-404 in: *Ecology and Biogeography of Pinus*. D. M. Richardson, ed. Cambridge University Press, Cambridge.
- Hoff, R. J., and McDonald, G. I. 1971. Resistance of *Pinus monticola* to *Cronartium ribicola*: Short shoot fungicidal reaction. *Can. J. Bot.* 49: 1235-1239.
- Jorgensen, J. H. 1996. Effect of three suppressors on the expression of powdery mildew resistance genes in barley. *Genome* 39:492-498.
- Kinloch, Jr., B. B. 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, Jr., B. B., and Byler, J. W. 1981. Relative effectiveness and stability of different resistance mechanisms to white pine blister rust in sugar pine. *Phytopathology* 71:386-391.
- Kinloch, B. B., and Comstock, M. 1981. Race of *Cronartium ribicola* virulent to major gene resistance in sugar pine. *Plant Dis.* 65:604-605.
- Kinloch, Jr., B. B., and Littlefield, J. L. 1977. White pine blister rust: Hypersensitive resistance in sugar pine. *Can. J. Bot.* 55:1148-1155.
- 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.
- McDonald, G. I., and Hoff, R. J. 1970. Resistance of *Pinus monticola* to *Cronartium ribicola*: Early shedding of infected needles. U.S. Dep. Agric. For. Serv. Res. Note INT-124. Intermountain Forest and Range Experiment Station, Ogden, UT.
- McDonald, G. I., and Hoff, R. J. 1971. Resistance of *Pinus monticola* to *Cronartium ribicola*: Genetic control of needle-spots-only resistance factors. *Can. J. For. Res.* 1:197-202.
- Monnig, E., and Byler, J. 1992. Forest Health and Ecological Integrity in the Northern Rockies. U.S. Dep. Agric. For. Serv. FPM Rep. 92-7.
- Patton, R. F. 1961. The effect of age upon susceptibility of eastern white pine to infection by *Cronartium ribicola*. *Phytopathology* 51:429-434.
- Peterson, R. S., and Jewell, F. F. 1968. Status of North American pine stem rusts. *Annu. Rev. Phytopathol.* 6:23-40.
- Ren, S. X., McIntosh, R. A., Sharp, P. J., and The, T. T. 1996. A storage-protein marker associated with the suppressor of Pm8 for powdery mildew resistance in wheat. *Theor. Appl. Genet.* 93:1054-1060.
- Vanderplank, J. E. 1963. *Plant Diseases: Epidemics and Control*. Academic Press, New York.
- Warren, R. F., Henk, A., Mowery, P., Holub, E., and Innes, R. W. 1998. A mutation within the leucine-rich repeat domain of the *Arabidopsis* disease resistance gene RPS5 partially suppresses multiple bacterial and downy mildew resistance genes. *Plant Cell* 10:1439-1452.
- Williams, N. D., Miller, J. D., and Klindworth, D. L. 1992. Induced mutations of a genetic suppressor of resistance to wheat stem rust. *Crop Sci.* 32:612-616.
- Yoder, O. C., Valent, B., and Chumley, F. 1986. Genetic nomenclature and practice for plant pathogenic fungi. *Phytopathology* 76:383-385.