Virulence Gene Distribution and Dynamics of the White Pine Blister Rust Pathogen in Western North America

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ABSTRACT

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We assayed the distribution and frequency of two genes of the blister rust pathogen with specific virulence to major resistance genes in sugar pine and western white pine in inoculum from extensive parts of the hosts' ranges. The genes, *vcr1* and *vcr2*, differentially neutralize the cognate resistance alleles *Cr1* and *Cr2* of the two respective hosts and are clearly marked by their interaction phenotypes. Basidiospores from each inoculum source were cast over *Cr1* and *Cr2* host genotypes simultaneously, and interaction phenotypes scored when developed. *vcr1* was confined to sites with high concentrations of *Cr1* (mostly plantations) where

frequencies tended toward fixation. *vcr2* showed a similar tendency, except high frequencies were occasionally observed from natural and planted stands of western white pine with very low frequencies of *Cr2*. Otherwise, no pattern was evident for either allele: frequencies were very erratic from site to site within short distances (<1 to 7 km) of each other and oscillated with high amplitudes at the same sites measured in consecutive years. Intense selection for virulence by *Cr* alleles occurs locally, but spread of *vcr* alleles over the landscape is mitigated by remarkably low gene flow. Absence of heterozygotes among single telia inoculum on *Cr2* genotypes indicated cytoplasmic inheritance of *vcr2*, similar to *vcr1* (previously reported).

Additional keywords: *Cronartium ribicola*, *Pinus lambertiana*, *Pinus monticola*.

White pine blister rust, an exotic disease introduced into North America only a century ago, causes severe damage to most native white pine hosts in at least some parts of their range. Yet, evidence of highly specific interactions between sources of resistance in white pine hosts and virulence in the pathogen, *Cronartium ribicola*, implicate the existence of a simple gene-for-gene structure in this pathosystem (7). The resistance genes, *Cr1* in sugar pine (*Pinus lambertiana*) and *Cr2* in western white pine (*P. monticola*), are infrequent to rare in natural populations (3,9). Both genes condition a hypersensitive reaction (HR) in foliar tissues, but are vulnerable to virulence genes in the rust population that neutralize them differentially, such that *vcr1* overcomes *Cr1* in sugar pine but not *Cr2* in western white pine, and vice versa for *vcr2*. (A third gene, *Cr3*, exists in *P. strobiformis*, but the corresponding virulence allele in the rust has not been discovered.) We have monitored the behavior of these interacting genes for almost two decades. The distribution and frequency of *vcr1* has been reported (5), but not that of *vcr2*. This paper focuses on the distribution of *vcr2* on regional and local scales from inocula sampled from California, Oregon, Washington, and Idaho, but includes the distribution of *vcr1* from the same samples for comparison. The data permit inferences to be drawn about virulence dynamics and population structure in this pathogen, with implications for deploying major resistance genes.

MATERIALS AND METHODS

Inoculum sources. Leaves with mature telia were collected from infected bushes of alternate host *Ribes* spp. growing in for-

ests or planted stands supporting western white pine or sugar pine. Collections were made throughout much of the range of the two pine hosts between 1997 and 2000 (Table 1; Fig. 1), but were concentrated around three areas known or suspected to harbor rust populations with virulence to resistant sugar pine and western white pines. These areas included: (i) Champion Mine (CM), a high rust-hazard site on the western slope of the Cascade Range in Oregon, from which resistant selections for *Cr2* were first made and demonstrated (8) and where the first indications of defeat of this gene by a virulent race of blister rust occurred (12); (ii) Happy Camp (HC), a test plantation for sugar pine and western white pine in the Siskiyou Mountains of California, where *Ribes* spp. bushes were interplanted among the pines; and (iii) Mountain Home Demonstration State Forest (MH), California Department of Forestry, on the western slope of the southern Sierra Nevada containing natural and planted stands of sugar pine with a high frequency of resistant genotypes. In each of these three areas, collections were made in the stand or nearby plantations where resistance of known host genotypes had been observed to break down, and outward a few kilometers from collection centers. Collections were also made over consecutive years at several sites within these main areas. A single site in the Rocky Mountains of Idaho (Merry Creek) where planted western white pines selected for resistance was thought to be breaking down under intense epidemic pressure (11), was also sampled. At all sites, an attempt was made to sample as many as 20 bushes, with only one leaf per bush.

Virulence assay. Virulence in the rust population was assayed by exposing seedlings known to carry *Cr1* (sugar pine) or *Cr2* (western white pine) to each inoculum source. Test seedlings were from open-pollinated seed parents previously identified as homozygous for the respective resistance alleles, so that all seedlings were resistant genotypes and thus expressed the interaction phenotype unambiguously. Approximately 24 recently germinated test seedlings of each species, including a few susceptible

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controls (cr1cr1 or cr2cr2), were incubated with infected *Ribes* spp. leaves bearing mature telia suspended over them in a dew chamber for 48 h at 16°C, allowing basidiospores to cast naturally (5,6). Basidiospore density was monitored on glass slide cover slips. This process was reiterated for each source. The two

interaction phenotypes that developed after several weeks were yellow spots, representing high level infection type (HIT, susceptibility/virulence) and HR, representing low level infection type (LIT, resistance/avirulence) (6; Fig. 1). Because basidiospores are haploid, the phenotype denotes the genotype. LIT is

TABLE 1. Spore cast, needle symptoms (spots), and frequency of virulence phenotypes *vcr2* and *vcr1* on resistant seedling genotypes of western white pine (WWP; *Cr2*–) and sugar pine (SP; *Cr1*–), respectively, in inocula of *Cronartium ribicola* from natural and planted stands

Inoculum source	Latitude N	Longitude W	Stand type	Spore cast mm ⁻²	WWP		SP	
					Spots (no.)	% <i>vcr2</i>	Spots (no.)	% <i>vcr1</i>
1 Eightmile Creek	48.62	120.20	WWP	155	315	0.0	295	0.0
2 Darrington	48.36	121.47	WWP	7	28	0.0	8	0.0
3 Mt. Baker	47.73	121.41	WWP	11	120	0.0	160	0.0
4 Dennie Ahl	47.39	123.25	WWP	19	106	0.0	55	0.0
5 Merry Creek Plantation	46.60	116.60	WWP	116	895	0.0	460	0.0
6 Cat Creek	46.35	121.62	WWP	5	22	0.0	9	0.0
7 Government Camp	45.27	121.83	WWP	46	21	19.0	0	0.0
8 Little Catherine Creek	45.16	117.69	WWP	64	105	0.0	76	0.0
9 Bourne	44.83	118.20	WWP	3	65	0.0	9	0.0
10 Detroit Rd	44.77	122.38	WWP	52	42	0.0	102	0.0
11 Lemon Cabin	44.69	118.61	WWP	107	266	0.0	129	0.0
12 Mary's Peak Plantation	44.54	123.53	WWP	24	32	100.0	48	0.0
13 Kelly Creek	44.52	120.33	WWP	120	357	0.0	260	0.0
14 Cascadia	44.48	122.22	WWP	84	110	2.7	5	0.0
15 Near Bear Pass	44.40	122.10	WWP	60	181	0.0	174	0.0
16 Fields Creek	44.39	119.31	WWP	87	421	0.0	194	0.0
17 Bear Pass Plantation (<i>Cr2</i>) ^a	44.38	122.14	WWP	41	251	11.2	114	0.0
18 Thombstone/Bear Pass	44.37	122.06	WWP	73	87	3.4	12	0.0
19 East Strawberry	44.34	118.58	WWP	39	129	0.0	89	0.0
20 Hamner Creek/Augusta Creek	43.95	122.18	WWP	41	36	30.6	180	0.0
21 Roaring River	43.93	122.07	WWP	52	173	17.3	146	0.0
22 Lone Wolf Plantation (<i>Cr2</i>)	43.76	122.60	WWP	112	83	14.5	290	0.0
23 Prarie Camp Plantation (<i>Cr2</i>)	43.75	122.60	WWP	121	98	85.7	320	0.0
24 Ribes Garden	43.71	122.74	WWP	57	32	43.8	316	0.0
25 Harvey Creek	43.70	122.65	WWP	16	25	0.0	25	0.0
26 Blodgett Creek Plant. (<i>Cr2</i>)	43.68	122.72	WWP	38	222	100.0	220	0.0
27 Bluey Plantation (<i>Cr2</i>)	43.66	122.65	WWP	8	12	0.0	56	0.0
28 Horse Plantation (<i>Cr2</i>)	43.66	122.61	WWP	66	86	75.6	175	0.0
29 Brice Creek	43.64	122.60	WWP	61	53	0.0	14	0.0
30 Brice/Champion Jct	43.63	122.65	WWP	169	127	57.5	233	0.0
31 Brice/Grass Creek	43.62	122.58	WWP	12	36	41.7	175	0.0
32a Grass Cr. Plant. (<i>Cr2</i>), 1997	43.59	122.58	WWP	26	47	91.5	...	...
32b Grass Cr. Plant. (<i>Cr2</i>), 1998	43.59	122.58	WWP	37	36	97.2	5	0.0
32c Grass Cr. Plant. (<i>Cr2</i>), 2000	43.59	122.58	WWP	32	216	92.1	457	0.0
33a Champion Mine, 1998	43.58	122.63	WWP	128	227	2.2	...	...
33b Champion Mine, 2000	43.58	122.63	WWP	113	349	35.2	542	0.0
34 GC Ridgetop	43.59	122.59	WWP	39	126	39.7	285	0.0
35 Champion Creek	43.61	122.64	WWP	187	275	22.5	500	0.0
36 Martin/Sharp	43.58	122.75	WWP	12	30	6.7	120	0.0
37 Big River	43.53	122.90	WWP	68	64	48.4	15	0.0
38 Silica	43.51	122.82	WWP	72	72	68.1	30	0.0
39 Pass Creek	43.50	122.78	WWP	93	100	35.0	27	0.0
40 Clearwater River	43.24	122.45	WWP	103	71	83.1	12	0.0
41 Black Creek	43.18	122.80	WWP	37	74	0.0	4	0.0
42 Red Butte Plantation (<i>Cr2</i>)	43.16	122.84	WWP	16	12	33.3	2	0.0
43 Bridge Creek	42.99	121.26	WWP	49	305	0.0	450	0.0
44 East Drew	42.95	122.81	WWP	...	42	0.0	37	0.0
45 West Drew	42.81	123.02	WWP	14	19	0.0	19	0.0
46 Galice Rd	42.54	123.77	SP/WWP	35	144	0.0	261	0.0
47 Jackson Peak Plantation (<i>Cr1</i>)	41.94	123.37	SP	17	6	0.0	207	12.6
48 Thompson Ridge #5042 ^c	41.93	123.39	SP	19	56	0.0	216	82.4
49 Thompson Ridge #5059 ^c	41.93	123.39	SP	22	50	0.0	132	13.6
50 Thompson Ridge #5010 ^c	41.93	123.39	SP	29	97	0.0	97	0.0
51 Poker Flat Plantation (<i>Cr1</i>) ^c	41.93	123.52	SP	18	31	0.0	383	43.9
52 Happy Camp Plant. #1 (<i>Cr1</i>) ^c	41.83	123.40	SP	46	212	1.9	377	97.6
53 H. C. Plant. #2 (<i>Cr1</i> , <i>Cr2</i>)	41.83	123.40	SP	56	269	20.1	490	90.6
54 IFG Wild Type	38.80	120.50	SP	128	1,255	0.0	22	0.0
55 Mtn. Home, HQ Plant. (<i>Cr1</i>) ^d	36.21	118.68	SP	23	65	0.0	43	86.0
56 Mtn. Home, Balch Plant. (<i>Cr1</i>) ^d	36.22	118.68	SP	8	31	0.0	473	80.5
57 Mtn. Home, Summit Pl (<i>Cr1</i>) ^d	36.24	118.67	SP	6	18	0.0	117	9.4
58 Mtn. Home, Shake Camp ^d	36.25	118.67	SP	18	...	...	168	0.0

^a Sources followed by *Cr2* or *Cr1* or both indicate plantations containing these genotypes.

^b ... = No data.

^c Data in 2000; see Figure 4 for other years.

^d Data in 2000; see Figure 5 for other years.

the interaction phenotype of *Cr1* and *Cr2* with wild type inoculum, avirulent to these alleles by definition. We used a wild type standard inoculum (source 54, Table 1) derived from aeciospores from a large number of blister rust infections on sugar pines near the Institute of Forest Genetics in El Dorado County, California. Spots were counted on both pine host species from

each source, and the proportion of HIT spots to total spots were taken as the relative frequency of virulence genotypes in the ambient spore cloud of the inoculum source in question.

Single telia (ST), used to investigate inheritance of virulence, were sampled from infected *Ribes* spp. at HC in the plantation containing western white pines and sugar pines (source 53, Table

Fig. 1. Distribution of virulence (*vcr*) alleles of *Cronartium ribicola* corresponding to major resistance alleles in sugar pine (*Cr1*) and western white pine (*Cr2*) in sampled populations of *Ribes* spp. in the western United States.

Inheritance of virulence. Although controlled matings between virulent and avirulent thalli of the pathogen would be the most direct way to determine inheritance, they are difficult to make artificially in this kind of perennial, cauliculus rust. Con-

Chi-squared analysis was used first, to test whether frequencies of *vcr1* and *vcr2* in the ST subsample were the same as the larger sample of bulked inocula from which they derived. Then, observed ratios of telial genotypes (inferred from their basidiospore progeny) were compared with ratios expected from a nuclear gene with two alleles segregating in Hardy-Weinberg equilibrium. Basidiospore progenies of telia heterozygous for virulence should segregate in a 1:1 ratio (HIT:LIT) if a single nuclear gene conditions virulence. Furthermore, heterozygotes in the population should be at a frequency of $2pq$ (in which p and q represent the frequencies of alternate alleles for virulence and avirulence).

Inoculating *Cr2*- and *Cr1*- host genotypes with spores from the same telium enabled a simultaneous analysis of the inheritance and independent assortment of the putative *vcr1* and *vcr2* loci.

RESULTS

Virulence distribution and frequency. The first awareness of wider virulence on western white pine came in the late 1960s at the CM site when selected resistant seed parents that had survived earlier waves of the disease started becoming infected. Dating of cankers on these trees indicated that *vcr2*, as this expression of virulence came to be called (8), may have been present as early as the mid-1950s (Fig. 2), although it did not become conspicuous until over a decade later. By the mid-1970s, numbers of new infections began to decline as the parent population started dying from the disease. All originally resistant parents were dead by 1994, and virtually no white pine regeneration remained. By this time, *vcr2* was evident at neighboring sites where progenies of *Cr2* seed parents had been planted.

The inoculum surveys show very different, nearly non-overlapping distributions of *vcr2* and *vcr1* (Table 1; Figs. 1 and 3). We found *vcr2* concentrated in the central Cascade Range of Oregon and southern Washington between 43° and 45°N, with an isolated southern outlier at 42°N in the Siskiyou Mountains of California at HC, the only place where *vcr2* overlaps with *vcr1*. Within this area, frequencies were highly variable and erratic, ranging from 0.0 to 1.0. Although *vcr2* frequencies tended to be highest in stands planted with high concentrations of *Cr2*-genotypes (e.g., source 26, 1.0; source 32, 0.921, Table 1), high frequencies were also observed in some neighboring stands and plantations (sources 20 to 42, Table 1), and even a few more distant stands with no known concentration of *Cr2* (e.g., source 12, 1.0; source 40, 0.831, Table 1). However, the converse was also true: very low frequencies often existed on sites immediately adjacent to those with highest frequencies (e.g., sources 25, 27, 29, and 41, Table 1; Fig. 3). Interestingly, inoculum sampled at CM itself was intermediate in frequency in 2000 (0.352) but as low as 0.022 in 1998 (sources 33b and 33a, Table 1).

We did not detect *vcr2* in the northern Cascades, Rocky Mountains, or Sierra Nevada (Figs. 1 and 3; Table 1).

In strong contrast, we found *vcr1* only in sugar pine stands in the immediate vicinity of HC in the Siskiyou Mountains (sources 47 to 53, Table 1), and MH (sources 55 to 58, Table 1) in the southern Sierra Nevada (see also Figs. 1 and 3). But within these two areas, frequencies were also erratic with abrupt changes among sites over very short distances, as well as from year to

year at the same sites where multiple estimates were made. Figure 4 shows this for the group of inocula in the immediate neighborhood of HC (sources 48 to 51, Table 1), and Figure 5 for the group at MH (sources 55 to 58, Table 1). Thompson Ridge is approximately 2 km east and 300 m higher than is the test site at HC, and the three sites sampled there are within 1 km of each other. Poker Flat is on a line of sight 12 km northwest and 630 m higher than is HC. All sites at MH are within 4 km of each other.

As with *vcr2* and its general association with *Cr2*, frequencies of *vcr1* tend to be highest in stands planted with *Cr1*-genotypes. These included: (i) Jackson Peak (source 47, Table 1), an operational sugar pine plantation grown from bulked seed from resistant parents; (ii) Poker Flat (source 51, Table 1), a high rust-hazard site where a small planting of resistant genotypes (*Cr1*-) and susceptible (*cr1cr1*) controls were established in 1983 to monitor the possible spread or spontaneous emergence of *vcr1*; (iii) the two contiguous sources at HC (sources 52 and 53, Table 1); and (iv) three of four sources at MH (sources 55 to 58, Table 1), approximately 700 km south of the HC area. Two of the three sources on Thompson Ridge (sources 48 and 49, Table 1) were centered on demonstrated *Cr1cr1* genotypes, themselves surrounded by natural, old growth stands containing sugar pines heavily damaged from blister rust and with a very low frequency (~0.005) of *Cr1* (3).

The only inoculum sources where both *vcr1* and *vcr2* were present were in the two plantations at HC within 100 m of each other. In the main sugar pine test (source 52, Table 1), *vcr1* and *vcr2* frequencies were 0.98 and 0.02, respectively, and in the smaller test immediately adjacent (source 53, Table 1), which contained known resistant selections of sugar pine and western white pine, many of which were infected, frequencies were 0.91 and 0.20 (Table 1).

Blister rust from source 5 (Table 1), the cause of heavy infection to known resistant (but not from *Cr2*) planted western white pines (11), showed no virulence to *Cr2* or *Cr1*.

Inheritance of *vcr2*. The frequency of *vcr2* in the ST sample inocula was 0.176, and was statistically no different from that in the bulked inocula sample from which it came (0.153, $P = 0.64$, Table 2). Both frequency estimates were close to that (0.21) of the prior, independent sample of the same source (source 53, Table 1). Thus, the ST sample was representative of the larger deme.

Of the 50 ST inoculations on western white pine, 22 failed to produce any spots, five produced only one spot, and 23 produced more than one spot (range of two to nine). The last class was the only one useful in classifying telial genotypes. Genotype frequencies, estimated from basidiospore progeny of the ST inoculations, were significantly different from those expected of a nuclear gene in Hardy-Weinberg equilibrium because there were no heterozygotes, though 29% were expected on the basis of allelic frequencies (Table 3). In other words, basidiospore progeny from

Fig. 4. Frequency of *vcr1* virulence phenotypes on resistant sugar pine seedling genotypes (*Cr1*) from inoculum of *Cronartium ribicola* from five sites near Happy Camp, California from 1990 to 2000. (HC = Happy Camp Plantation, TR = Thompson Ridge, and PF = Poker Flat. (sources 48 to 52, Table 1).

Fig. 5. Frequency of *vcr1* virulence phenotypes on resistant sugar pine seedling genotypes (*Cr1*) from inoculum of *Cronartium ribicola* from four sites on Mountain Home Demonstration State Forest, California from 1998 to 2000 (sources 55 to 58, Table 1). Data from 2001 and 2002 courtesy of D. Vogler.

these ST produced either all avirulent or all virulent interaction phenotypes; there was no segregation of phenotypes from any of the ST that would have indicated a heterozygous condition.

Fifteen of the 50 ST produced infection on both species. Five of these showed virulent interaction phenotypes to both *Cr1* and *Cr2*, indicating that *vcr1* and *vcr2* can and do exist in single haploid basidiospores.

The proportion of *vcr1* in ST was 1.0, only marginally different from that in the bulked sample (0.94, Table 2). Consequently, no test for heterozygosity of this locus was possible with this data.

DISCUSSION

The distribution and frequency of *vcr* genes cognate with *Cr* genes in this extensive and wild pathosystem illustrate classical elements of virulence dynamics and epidemiology: mutation rates, selection pressure, gene flow, and chance.

Mutation and selection. Although mutation rates are not known, *vcr* alleles occur rarely in western North American populations. The first instances of *vcr1* and *vcr2* were observed in plantations (HC) or stands (CM) in areas of chronically high disease hazard and with high concentrations of *Cr1* or *Cr2* (5,8). *Cr* host alleles exert absolute selectivity on *vcr* alleles in haploid basidiospores. A perennial reservoir of these alleles thus becomes available in established stem infections, enabling exponential increase in *vcr* frequency through annual production of aeciospores.

Frequencies of *vcr1* and *vcr2* alleles are strongly affected by selection, although not necessarily to the same degree. Early epidemic waves eliminated susceptible (wild type) western white pines at CM by the mid-1950s, leaving a small residual group of resistant trees protected by *Cr2* (8). Subsequent breakdown of these naturally selected genotypes by *vcr2* progressed rapidly (Fig. 2), ending with complete extirpation of western white pine in this stand between 1970 and 1994. Thereafter, we generally found the highest concentration and frequencies of *vcr2* in neighboring stands and especially in nearby monitor plantations containing known high proportions of *Cr2* genotypes (Table 1). But there were notable exceptions. The most conspicuous of these was the Mary's Peak plantation (source 12, Table 1), about 160 km distant from CM across the Willamette Valley, but with a *vcr2* frequency of 1.0, the highest we recorded. We have been unable to trace the specific origin and genetic composition of the planting stock at this site, but there were only approximately 100 seedlings planted in 1988 for a root-rot trial. By this time, much of the western white pine seed sown in southern Oregon nurseries came from resistant seed orchard parents and would have comprised a substantial proportion of *Cr2* genotypes. Other relatively distant sites, north and south of CM, also had moderate to high frequencies of *vcr2* (Fig. 3; Table 1). A few of these were known to be near monitor plantations containing *Cr2* genotypes; others could have had small operational plantations nearby that we were unaware of. Therefore, mutation followed by selection remains a

possible explanation in these cases. Otherwise, selection is difficult to invoke as a major cause of *vcr2* presence because the frequency of *Cr2* in natural stands is only about 0.001 (9).

Another possibility is that virulent inoculum was unwittingly transported to the field. In the early days of resistance screening, inoculum was often obtained from CM because of reliable and abundant production of aeciospores at this site. Up until the early 1980s, inoculum from CM was included in a mix of diverse sources in the region to culture on *Ribes* spp. grown at the screening facility at Dorena to facilitate inoculation of candidate white pines. Further spread to remote sites on clothing or equipment of field crews in frequent contact with this inoculum could have occurred. There are even unrecorded narratives from some of the older members that spores were sometimes taken to field sites having lush stands of *Ribes* spp. and favorable environments for blister rust to conduct artificial inoculations. A specific case in point is Still Creek (G. D. Barnes, *personal communication*), a site near Government Camp (source 7, Table 1), which had a *vcr2* frequency of 0.19 in our survey. Nevertheless, all of these rationalizations carry the burden of how relatively high frequencies of *vcr2* were maintained in the apparent absence of strong selection.

The effect of selection on *vcr1* is clearer. Data in this study reinforce an earlier finding (5) that this allele occurs only at or near high concentrations of *Cr1*. In the current study, we found it only at HC and immediate environs and at MH (700 km south). At HC, which for the past two decades has been planted exclusively with *Cr1* genotypes, *vcr1* has been maintained at frequencies in excess of 0.8 (source 52, Table 1; Fig. 4). At Poker Flat (source 51, Table 1), site of a small monitor planting of *Cr1* genotypes only 12 km distant from HC, *vcr1* was absent in samples from 1991 through 1996 but then rose steadily to 0.44 in 2000 (Fig. 4) and to 0.83 in 2002 (data not shown; D. Vogler, *personal communication*). In contrast, the three sites on Thompson Ridge (sources 48 to 50, Table 1) showed highly fluctuating frequencies across the years (Fig. 4). Thompson Ridge is an old growth mixed conifer stand

TABLE 3. Observed and expected genotype frequencies at virulence loci *vcr2* and *vcr1* of *Cronartium ribicola* in single telia inoculum from Happy Camp, California^a

Genotype	Observed		Expected		<i>P</i>
	No. ^b	Frequency	No.	Frequency	
<i>vcr2/vcr2</i>	4	0.174	0.716	0.031	0.0000
<i>vcr2/AVCr2</i>	0	0.000	6.685	0.291	
<i>AVCr2/AVCr2</i>	19	0.826	15.599	0.678	
Total	23	1	23	1	
<i>vcr1/vcr1</i>	24	1.000	24.000	1.000	1.0000
<i>vcr1/AVCr1</i>	0	0.000	0.000	0.000	
<i>AVCr1/AVCr1</i>	0	0.000	0.000	0.000	
Total	24	1	24	1	

^a An independent sample in 2000 (source 53, Table 1).
^b Only single telia inoculum producing more than one spot were considered.

TABLE 2. Observed and expected frequencies of virulence phenotypes (alleles) *vcr2* and *vcr1* of *Cronartium ribicola* in bulked inoculum and single telium inoculum from Happy Camp, California^a

Allele	Bulked inoculum		Single telia				<i>P</i>
			Observed		Expected		
	No.	Frequency	No.	Frequency	No.	Frequency	
<i>vcr2</i> :	13	0.153	9	0.176	7.8	0.153	0.6406
<i>AVCr2</i> :	72	0.847	42	0.824	43.2	0.847	
Total	85	1.000	51	1.000	51.0	1.000	
<i>vcr1</i> :	71	0.934	54	1.000	50.4	0.934	0.0512
<i>AVCr1</i> :	5	0.066	0	0.000	3.6	0.066	
Total	76	1.000	54	1.000	54.0	1.000	

^a An independent sample in 2000 (source 53, Table 1).

with a very low frequency of *Cr1* in the sugar pine component (<0.01) (3), and thus, presents little selection pressure for *vcr1*. The sugar pine component in the mixed conifer forest at MH is also old growth, like Thompson Ridge, but in this case with a relatively high frequency of *Cr1* (>0.07) (3). Here, *vcr1* frequencies were variable among samples from the four small plantations distributed over approximately 1,800 ha but increased steadily over time at all of them (Fig. 5).

Gene flow. The distributions and frequencies of both virulence alleles show that gene flow in this pathogen is surprisingly limited. For years, we were unable to detect *vcr1* outside the immediate perimeter of the test plantations at HC even though its frequency within was constantly high. Just as surprising, however, was the relatively low migration of wild type from the surrounding forest back into the plantation on planted *Ribes* spp. bushes, which are presumably selectively neutral for virulence alleles on pines. Sampling points close to each other within the general areas of CM, HC, MH exhibited a wide range of allele frequencies, and patterns are lacking among more distant locations. *vcr1* frequencies at the same sites on Thompson Ridge, all within 2 km of each other, not only fluctuated widely but were also out of synchrony with each other (Fig. 4). Taken together, these facts strongly indicate that inoculum production and infection between alternate hosts is occurring very locally.

The restricted movement observed is paradoxical in view of migrational jumps of over 500 km this pathogen is known to have made in western North America (13,14). But it is consistent with data from molecular marker frequencies, which showed a very similar heterogeneity and lack of pattern over the landscape (10). Evidently, long distance migration is episodic, from few migrant spores, resulting in founder effects with low effective population sizes (10). For these reasons, we hypothesize that the conspicuous and sudden eruptions of *vcr* alleles (e.g., *vcr1* at MH, HC, and Poker Flat; *vcr2* at CM and HC) are attributable in most cases to independent mutations followed by strong selection occurring on site rather than migration from distant colonies. (Initial establishment of *vcr1* on Thompson Ridge may be an exception, since this site is only approximately 2-km distant and downwind of HC). The outbreaks of *vcr1* at HC and MH were associated with epidemic wave years in 1976 and 1989 that affected white pines in large parts of California, when inoculum potential would have been unusually high. Absolute numbers of mutant spores obviously increase with greater spore production. Chronic high hazard for disease spread and intensification at CM (8) would also promote high inoculum potential and selection of *vcr2*.

The data are ambiguous as to whether there are differences in the distributional dynamics of the two virulence loci. Unlike *vcr1*, *vcr2* occurs in places where its cognate allele for resistance, *Cr2*, is absent or at very low frequency. We have already discussed the possibilities of selection pressures from undetected plantings of *Cr2* stock and inadvertent human transport of virulent inoculum to the field. Other more basic reasons might include less cost of fitness to the pathogen for carrying *vcr2* unnecessarily than for *vcr1* (i.e., less dependence of *vcr2* on selection by *Cr2*), higher mutation rate for *vcr2* than for *vcr1*, or higher inoculum production in the generally cooler, moister environments of the Pacific Northwest, where wave years are more frequent and intense than in the Sierra Nevada, or both.

Chance. There are many places where virulence would be expected but has not yet been found, in particular, several monitor plantations with *Cr1* genotypes in California and Oregon that have been exposed to multiple wave years (5; Snieszko, unpublished). Similarly, *vcr2* was not detected on *Cr2* genotypes of western white pine in a 12-year-old plantation in British Columbia, even though all susceptible cohorts were rapidly killed over several wave years (2). As with many aspects of epidemiology, stochastic factors are also of major importance in the distribution of virulence alleles.

Virulence inheritance. The lack of segregation of *vcr2/AVCr2* in single telia from a population with intermediate frequencies of both alleles follows the identical pattern of *vcr1*, previously reported (6), and uniquely marks these virulence factors as plasmagenes. Thus, the two loci cannot recombine through sexual recombination, but can come together from sequential mutation, as our observation that 30% of ST tested produced virulent interaction phenotypes on both *Cr1* and *Cr2* genotypes shows. This may not assume any practical significance until isolation and cloning of the corresponding resistance genes becomes feasible, so that either of the host species can be transformed with the allele that it lacks and have both. Epidemiologically, the effect of uniparental inheritance through cytoplasm is a further reduction in gene flow, although probably not of major importance.

We earlier characterized the population structure of *C. ribicola*, on the basis of molecular markers, as a genetically fragmented metapopulation with low gene diversity, low gene flow, high population differentiation, and driven largely by genetic drift (10). Data from virulence gene frequencies in this study are entirely consistent with these conclusions, on local and regional scales. Implications for deployment of the corresponding major resistance genes in synthetic populations of white pines are that defeat of these genes will not occur rapidly over large areas, but that once established in individual stands, virulence alleles will be selected and increase exponentially, with devastating effects. This is most likely to occur following wave years when inoculum potential and the absolute frequency of spontaneous mutations generated are much greater than normal. The effect can be dampened silviculturally by locally reducing inoculum potential (e.g., removing alternate host *Ribes* spp.) in and around white pine regeneration, and also genetically by buffering major genes with genes controlling partial or rate-reducing resistance traits, currently being developed (4,15) that are not specifically vulnerable to matching virulence genes in the pathogen.

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