Race of *Cronartium ribicola* Virulent to Major Gene Resistance in Sugar Pine

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ABSTRACT

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Differential foliar and bark reactions to two different sources of inoculum on sugar pine seedlings of known genotype confirmed the existence of a race of the white pine blister rust pathogen virulent to major gene resistance.

Additional key words: *Pinus lambertiana*

Major gene resistance (MGR) to white pine blister rust (*Cronartium ribicola* Fisch.) in sugar pine (*Pinus lambertiana* Dougl.) is conditioned by a single dominant gene that causes a hypersensitive reaction in mesophyll tissue of infected needles (5). Small, necrotic flecks produced by this incompatible reaction are easily distinguished from the larger, bright yellow or red lesions of compatible genotypes on all kinds of leaves, including cotyledons (4). In incompatible needle reactions, the pathogen does not usually penetrate the leaf endodermis and central vascular cylinder, which is the normal path of entry into bark tissue. When the pathogen does penetrate, it triggers a hypersensitive reaction in the bark tissue and does not develop further.

Other, more virulent reactions are rare. Known resistant genotypes of sugar pine have been inoculated repeatedly with the pathogen, and virulence to MGR has seldom been detected. The inocula originated from sources throughout California and were presumed to have a broad genetic base (4,5, *unpublished data*).

A sudden rust infection of trees known to carry MGR is a recent indication that resistance has been broken by a virulent race. The infected trees had remained free of rust under conditions of chronic and intense disease hazard for up to 14 yr in field progeny tests (3). We sought to confirm the existence of this race by determining foliar and bark reactions of MGR carriers inoculated with virulent and avirulent inocula.

MATERIALS AND METHODS

Aeciospores of the race believed to be virulent (V) to MGR were collected in 1979 from sporulating rust cankers on 15 sugar pines identified as MGR carriers in a field progeny test near Happy Camp, CA (3). Pedigrees of these trees included five different parents that were homozygous or heterozygous for MGR. The progeny were established between 1963 and 1968 and had remained rustfree until 1978, long after the death of nearly all susceptible control seedlings (3). Additional progeny were established annually on the same site through 1975.

We pooled aeciospores from rust-infected trees having a common resistant parent (three collections from each of the five parents) and used them to inoculate potted bushes of *Ribes nigrum* L. When telia were well formed on the *Ribes*, we harvested leaves of the five groups, mixed them thoroughly, and inoculated test seedlings.

The avirulent race (AV) was derived from aeciospores collected from rusted sugar pines in each of 10 widely distributed areas throughout California (5). *Ribes* were inoculated with these collections, and the resulting urediospores were pooled in equal amounts for each collection area. The urediospore mixture was then propagated on *Ribes* as needed.

To reduce the chance that the *Ribes* clone most often used would select certain genotypes, we periodically collected aeciospores from inoculated seedlings in other experiments and recycled them into the urediospore mix; occasionally, we added aeciospore collections from infected trees in natural stands. Despite the heterogeneous origin, inocula from this source over the years have produced consistent reactions on control seedlings of known genotype (4,5).

Test seedlings were inoculated with basidiospores cast from telia-bearing *Ribes* leaves that were suspended over the seedlings for 72 hr at 9–22 C and close to 100% relative humidity. The V and AV inocula were cultured separately on *Ribes* and used in two different chambers.

While still in the cotyledon stage, test seedlings were identified as MGR carriers by inoculation with AV (4). Resistant segregants were taken from 31 open-pollinated families that segregated by lesion type identically to the controls (six full-sib families, each having one parent known to be heterozygous for MGR). The two lesion types were fleck, denoting an incompatible reaction, and yellow or red, denoting a compatible one. Segregants with the fleck phenotype were divided into two equal groups (41 seedlings each) and grown under continuous illumination to promote early secondary needle production (2). Each group was reinoculated in the autumn (6 mo after sowing) with either the AV or V inoculum. Seedlings not previously inoculated but known to be susceptible were added to each group.

After inoculation, seedlings were moved to a lathhouse for winter chilling for 3 mo (October–January), then returned to a greenhouse under continuous illumination. Foliar and bark symptoms on each seedling were recorded in March. Lesions were also counted, by type, on a sample of secondary needles from heavily infected seedlings representing each inoculum treatment.

RESULTS AND DISCUSSION

Seedlings with MGR clearly expressed differential reactions to the two inocula (Table 1). Of the seedlings exposed to AV, 61% developed only incompatible lesions and 3% developed compatible

Table 1. Foliar reactions of sugar pine seedlings resistant and susceptible to two sources of *Cronartium ribicola* inoculum

	Response to inoculum: number (%) of seedlings	
Lesion type[a]	**Avirulent**	**Virulent**
	Resistant genotypes[b]	
Fleck	24 (61)	0
Yellow/red	0	29 (71)
Fleck and yellow/red	1 (3)	3 (7)
None	14 (36)	9 (22)
Total	39	41
	Susceptible genotypes	
Fleck	0	0
Yellow/red	19 (95)	13 (92)
None	1 (5)	1 (8)
Total	20	14

[a] Fleck = resistant (hypersensitive); yellow/red = susceptible.

[b] Genotypes of resistant seedlings determined by previous inoculation of the same seedlings; genotypes of susceptible seedlings determined by previous inoculation of sibs.

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lesions; of those exposed to V, 71% developed only compatible lesions. Seedlings lacking MGR had only compatible lesions. Four seedlings—one inoculated with AV and three with V inoculum—developed both incompatible and compatible lesions. For unknown reasons, seedlings with no symptoms were much more common among resistant genotypes exposed to either inoculum (AV, 36%; V, 22%) than among susceptible genotypes (AV, 5%; V, 8%). Because resistant genotypes, by definition, had developed fleck symptoms in the previous inoculation, we assume that any lack of symptoms in the second inoculation was the result of escape.

The same differential reaction was shown by the frequency of individual lesion types on 13 needles sampled from AV and 17 from V inoculum treatments: all of 133 lesions induced by AV inoculum were fleck, whereas only 5 of 133 lesions induced by V inoculum were fleck. Bark symptoms developed only on seedlings having compatible needle lesions. These results unequivocally demonstrated the existence of a virulent race.

We were surprised at the low frequency of fleck reactions to V inoculum on MGR seedlings. Because the haploid basidiospores that infect pine needles are products of meiosis, the phenotype of each lesion denotes the genotype of the spore that induced it (assuming that virulence and avirulence are conditioned by alternative alleles at a single locus). It would thus follow that nearly all of the 15 dicaryotic aeciospore collections used to produce the V inoculum mix were homozygous for virulence. Therefore, these collections resulted either from selfmatings or from outcrosses among each other and like genotypes for virulence in the rust population. The latter alternative is less likely. Three times as many living, infected trees of susceptible rather than resistant genotypes were present on the test site in 1978, during pycnial fertilizations resulting in aeciospore production in 1979. The test plantation is also surrounded by many naturally regenerated and infected sugar pines.

Knowledge of sexuality in *C. ribicola* is meager. Controlled matings are technically difficult to make and few have been attempted. McDonald (6) reviewed the evidence, which included work by Hirt (1), earlier unpublished work by Pierson, and recent unpublished data by Lee, and concluded that the fungus exhibited heterothallic sexual behavior. This conclusion agreed with Pierson's interpretation but was contrary to Hirt's (1), which strongly favored homothallism. Lee's evidence, though indirect, may be the best. It is based on segregation ratios of 245 monoaeciospore lines isolated from a population of infected *P. monticola* seedlings, individuals of which exhibited either yellow, red, or both yellow and red needle lesion types. McDonald considered these types as marking alternative alleles at a single locus in the dicaryotic aeciospores from which Lee's inocula derived. Although genetic markers are validly used to infer sexual behavior, wide departure of observed from expected ratios left McDonald's interpretation inconclusive.

Our data did not prove that the differential reactions observed on seedlings with MGR were determined by alternative alleles in the pathogen, but the assumption is valid by analogy with other rust pathosystems studied. Accordingly, our data are more consistent with Hirt's interpretation of a predominantly homothallic mating system in *C. ribicola* (1). The argument is significant because mating behavior influences the rate of gene flow among subpopulations of the pathogen and thus has important implications for the spread and increase of virulence.

In either case, the ability to discern the genotype of individual spores by the phenotype of induced lesions will allow us to make rapid and direct estimates of the distribution and frequency of *C. ribicola* genes that are virulent to MGR in sugar pine.

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