Genetic Variation of Epidemiological Fitness Traits Among Single-Aeciospore Cultures of *Cronartium ribicola*

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ABSTRACT

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Single aeciospores of *Cronartium ribicola* (cause of white pine blister rust) were isolated from single aecial blisters produced by artificially inoculating a wild population of *Pinus monticola* seedlings with a wild population of basidiospores. Aeciospores were isolated and cultured on detached leaves of a single *Ribes hudsonianum* var. *petiolare* clone. Cultures stratified by color of needle lesion on the infected pines showed no significant variation. But, stratification by individual blisters showed highly significant variation in four epidemiological fitness traits: culture size 30 days after inoculation, uredial sorus density 30 days after inoculation, percent of successful uredial subcultures per aeciospore line, and percent of uredial subcultures per aeciospore line producing teliospores by 35 days. Quantitative genetic analysis suggested strong genetic control in the wild rust pathogen population of all four traits. Results are discussed in relation to the analysis of quantitative genetic variation in *C. ribicola.*

Additional key words: detached leaf culture, quantitative genetic variation, rust fertilization mechanics.

The ability of pathogens to adapt to a genetically changed host population is an important consideration in plant disease resistance breeding programs (6,19,20,23,26). Resistance to *Cronartium ribicola* J. C. Fisch. ex Rabenh. in *Pinus monticola* Dougl. has been the subject of investigation and development for 30 yr, and resistant cultivars have been developed. We are studying variation in *C. ribicola* by genetically analyzing single-aeciospore cultures grown on detached leaves of *Ribes hudsonianum* var. *petiolare.* Basidiospores from these cultures were used to inoculate *P. monticola* seedlings. Our initial results indicated that *C. ribicola* outcrossed extensively. Wide genetic variation can be expected among aeciospore populations derived from single blisters caused by *C. ribicola* (12).

Neither quantitative nor qualitative genetic analyses of the rust pathogens can be accomplished with confidence until the processes of fertilization that lead to aecial development are clarified; genetic analyses depend on the predictable probabilities associated with parental contributions typical of sexual recombination in higher organisms. The multitude of possible patterns of nuclear, chromosomal, and gene assortment coupled with unpredictable nuclear replication, not to mention possible cytoplasmic inheritance in all phases of the life cycles of rust pathogens, also compound the problem. Establishment of homokaryotic lines (1N basidiospore) would provide the ideal experimental control for genetic analyses, but reproducible single-basidiospore lines of *C. ribicola* cannot be obtained consistently with present methods. Therefore, we have concentrated on developing single-aeciospore lines homozygous for certain genes.

Needle lesions produced by *C. ribicola* basidiospores are usually yellow. However, a red-lesion type was observed in 1964 on *P. monticola* seedlings that were exposed to artificial inoculation with a wild population of *C. ribicola.* This type has since increased markedly in frequency in wild populations (15). An analysis of distribution of lesion types and their frequencies (15) led to the hypothesis that trees showing only red spots are resistant to basidiospores that cause yellow spots and trees showing only yellow spots are resistant to those that cause red spots. Trees with both red and yellow spots are susceptible to both, and the trees with neither are resistant to both (15). Both red and yellow needle spots have since been reported on *Pinus pinaster* Ait. after inoculation with *Cronartium flaccidum* (Alb. & Schw.) Wint. (21) and on 13 of 17 other *Pinus* spp. from sections Cembrae, Strobi, and Parrya artificially inoculated with *C. ribicola* (11). First attempts to establish homozygous red and yellow lesion producing lines of *C. ribicola* were not successful (12), but genetic control of this polymorphism in the rust was strongly indicated. Therefore, we continued attempts to develop single-aeciospore lines homozygous for red and yellow lesion type.

This paper deals with the variation observed in single-aeciospore cultures isolated on detached leaves of a single clone of *R. hudsonianum* var. *petiolare* as the first step toward development of the homozygous lines. To gain additional information about the genetics of *C. ribicola,* we isolated aeciospores from a natural population of the rust pathogen in such a way as to give preliminary information about the lesion color frequency shift in the blister rust system, and to provide data about quantitative genetic variation in *C. ribicola* through the application of quantitative genetic analysis techniques (8,16).

The dramatic increase in frequency of red-needle lesions led us to ask a first question, whether lesion color on pine may be associated with a readily observable epidemiological fitness trait on *Ribes* that could account for the increase in frequency. Thus, the single-aeciospore isolates were characterized as to lesion type on *P. monticola* as well as fitness on *Ribes.*

The second question concerned the presumed quantitative genetic control of growth rate and urediospore pustule density in aeciospore-derived-cultures, urediospore subculture success, and teliospore initiation in *C. ribicola.* Single aeciospores and the infections they produce can be thought of as functional diploid individuals genetically equivalent to the seeds and seedlings of higher plants. If *C. ribicola* is homothallic as claimed by Hirt (10), or all the products within a single blister are the result of one fertilization event as claimed by Zimmer et al (27) for a single aecial cup of *Puccinia coronata* Cda. then one would expect all variation within a population of aeciospores obtained from a single blister to be due to responses to environmental effects and variation among blisters from different cankers to be of genetic origin. If this is so, then a classic quantitative genetic analysis could be applied to quantitative traits in *C. ribicola* (5). Use of different blisters on the same canker, as Powers (18) has done in the case of *C. quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme* would not meet the unique individual test because different blisters on the same canker could conceivably be genetic copies of the contents of each other. To be

sure that our aecial blisters were genetically unique, we isolated aeciospores from only one blister per canker and only one canker per tree.

MATERIALS AND METHODS

The source of aeciospore isolates used in this study was a group of 12,000 inoculated seedlings growing in the Priest River Experimental Forest nursery. The seedlings, which had been grown for another study, were derived from open-pollinated seed from 500 female parents obtained from 10 stands (50 per stand) that exhibited 5–90% mortality due to rust. Seed production was the only individual tree selection criterion. The trees were artificially inoculated in September 1973 by the method of Bingham (4). Inoculum consisted of a wild population of teliospores growing on several hundred wild *Ribes hudsonianum* var. *petiolare* bushes obtained from three different collection areas in northern Idaho. A total of 11,400 leaves from all plants were placed randomly on screens suspended over the seedlings. Lesion number and type were recorded in June 1974. One aecia-bearing canker was collected from each of 44 trees in mid-April 1977; 13 of the trees had red needle spots only, 16 trees had both red and yellow spots, and 15 trees had yellow spots only (15). The trees were a random sample within each color class. Each canker was sealed in a jar, transported back to the laboratory, and stored at 5 C in a refrigerator. Between 14 April and 3 May, fine glass needles were used to individually transfer 100 single aeciospores from a single unopened blister on each of the cankers to the centers of 100 15-mm-diameter *R. hudsonianum* var. *petiolare* leaf disks. Leaf disks were cut from fresh (stored for not more than 3 days) leaves of clone 1 with a cork borer.

Clone 1 was obtained from the West Fork of Hobo Creek in the St. Joe National Forest and was kept actively growing in a greenhouse for >2 yr. Leaves used in this study were detached when 2 to 3 wk old and immediately immersed in tap water for a 3-hr wash. They were then either promptly used or stored in a covered dish held at 5 C not more than 3 days. Inoculated leaf disks were placed (25 per petri dish) on wet filter paper and then covered with another wet filter paper supported on 4-mm-high sections of 40.6-mm (1.6-in.) flexible PVC tubing. Each dish was placed in a growth chamber held at 21 ± 1 C and daily 12-hr light (43 hlx from cool-white fluorescent bulbs) and dark periods. The relatively warm temperature was used to speed development of the aeciospore-derived cultures and to retard development of teliospores (25). Inoculated disks were inspected daily for the next 21 days. At first appearance of symptoms of an infection and before formation of urediospores, infected disks were transferred individually to wet filter paper in 35-mm-diameter petri plates and inspected under a stereoscopic microscope at approximately 7-day intervals (the length of time required to inspect all cultures) until the leaf disk either became necrotic, the infection regressed, or 35 days had elapsed. Following that, the cultures were inspected at 7- to 14-day intervals for an additional 35 days. At each inspection, up to 28–35 days, the area of the infection was estimated by measuring length and width and making a sketch of the infected area. The number of uredial sori also were recorded. At each inspection during both 35-day periods the vigor of each infection was noted, numbers of telial columns were recorded, and water was added as necessary to keep the filter paper moist.

Lesion area at 30 days after inoculation and uredial sorus density (sori per square millimeter of infected area) at 30 days after inoculation were determined for each aeciospore-derived culture. Two to 16 urediospore subcultures were obtained for each single-aeciospore culture by inoculating new leaf disks of Clone 1 with urediospores from the aeciospore-derived cultures at each inspection. Thus, two additional traits (urediospore subculture inoculation success and presence of teliospores) in 35-day-old urediospore subcultures could be analyzed. Each data set was stratified by lesion type, blisters within lesions, and aeciospores within blisters (one blister per canker) and analyzed with a nested-random ANOV (22) (Table 1).

The data on pustule density and culture growth were adjusted to a common development period because the inspection schedule resulted in measurements being made within as few as 23 days and as many as 34 days after inoculation. A daily rate was calculated for each and then multiplied by 30 days. Since data on the success of urediospore subcultures and urediospore subcultures producing teliospores by 35 days were recorded as percentages, they were subjected to the arc sine transformation to compensate for the tendency toward a binomial distribution (22). Further adjustments were made to the proportions to reduce fluctuation caused by small sample sizes according to Bartlett (2).

Bartlett's test of homogeneity of variance was applied to all four data sets (22). Components of variance due to individuals within blisters (σ_W^2), lesion type (σ_A^2), and blisters (σ_B^2) were calculated (22) for aeciospore-derived lesion size at 30 days; urediospore sorus density on aeciospore-derived lesions at 30 days after inoculation; urediospore subculture inoculation success; and teliospore formation by urediospore subcultures by 35 days. Quantitative genetic analysis was completed by using the methods and notations of Falconer (8).

TABLE 1. Model of analysis of variance of single-aeciospore cultures and urediospore subcultures of *Cronartium ribicola* (random sources)

Source of variation		d.f.	Expectation of mean squares
(A)	Lesion type	$a-1$	$\sigma_w^2 + n_0 \sigma_B^2 + (nb)_0 \sigma_A^2$
(B)	Blisters/lesion type	$\sum_{i=1}^{a} (b_i-1)$	$\sigma_W^2 + n_0 \sigma_B^2$
(W)	Aeciospore/blister/lesions	$\sum_{i=1}^{a} \sum_{j=1}^{b_i} (n_{ij}-1)$	σ_W^2

$n_0 = [n_{..} - \Sigma n_{ij}^2/n_{..}]/[b-1]$ $\quad b$ = number of blisters

$(nb)_0 = [n_{..} - \Sigma n_{i.}/n_{..}]/[a-1]$ $\quad a$ = number of lesion types

RESULTS

Data for the three seedling types are summarized in Tables 2–4. Several features are evident in all three tables:

First, the success of single-aeciospore inoculations varied greatly from blister to blister, and no significant relationship between lesion type and probability of infection by a single aeciospore (not necessarily virulence-related in an all-or-nothing sense) placed on Clone 1 (Heterogeneity $\chi^2 = P$ of $> \chi^2 = 0.20$; d.f. = 2) was evident. Even though the isolations took about 20 days to complete, blister storage time apparently did not influence aeciospore inoculation success as shown by the following data. Average infection successes per blister stored for 1, 2, 6, 7, 8, 9, 13, 14, 15, 16, 19, and 20 days were 4, 8, 4.5, 10.5, 3, 5.5, 9.0, 8.29, 11.25, 7.63, 9.8, and 7%, respectively.

Second, an occasional aeciospore-derived culture (six out of 351 that lived more than 30 days) produced teliospores in less than 30 days. These six early teliospore producers were confined to three blisters and one blister yielded three such cultures. Tables 2, 3, and 4 show that the blisters yielded single-aeciospore lines that varied widely in ability to produce teliospores by 60 days. Of the blisters with five or more lines, the proportion producing telia varied from 38–100%. The chi-square value for lesion-type versus teliospore production heterogeneity was not significant (P of $> \chi^2 = 0.020$; d.f. = 2).

Fifteen of 367 cultures were characterized by very slow growth (0.09 mm^2/day compared to 0.3 mm^2/day average for all remaining cultures), fewer uredial sori (0.07 sori per square millimeter per day compared to 0.11 for remaining cultures), and in all cases death by 30 days. All the weak cultures were associated with nine blisters, which produced a total of 90 lines. Two of the nine blisters produced seven weak cultures of 33 cultures total.

Several of the cultures (51 of 351 that lived more than 30 days) died early. These cultures were typical in growth rate, urediospore

production, and probability of successful subculture. They were developing along with all the cultures at 28–34 days except those that had died by 30 days, but at the 49 day all were dead. Five blisters produced 29 "early-death" cultures. Weak-culture blisters were not associated with "early-death" blisters, since only three of the 20 "early-death" blisters also produced weak cultures. The chi-square value for lesion-type versus "early-death" heterogeneity was significant (P of $> \chi^2 = 0.05$; d.f. = 2). Twenty-one percent of the cultures from red-only plants died early, whereas only 9% of the yellow-only and 16% of the mixed-source cultures died early.

Tables 2, 3, and 4 give the average infected surface area of single-aeciospore cultures at 30 days after inoculation. Analysis of variance of this trait showed blisters to be a highly significant source of variation (Table 5). Each lesion type produced about the same average size of infection over all blisters-within-lesion type (Tables 2, 3, and 4), but the red and yellow class (Table 4) showed the largest range (3.5 mm^2–21.6 mm^2) of average size for all cultures from a blister. The coefficient of variation shows much within-blister variation for all lesion types.

The average pustule density (uredial sori per square millimeter) at 30 days after inoculation (Tables 2, 3, and 4) also showed blisters to be a highly significant source of variation (Table 5) even though within-blister variation was high. Again, lesion types did not contribute significantly to the variation. The remaining traits,

TABLE 2. Performance of single-aeciospore cultures of *Cronartium ribicola* obtained from *Pinus monticola* seedlings that exhibited only yellow needle lesions and grown on leaf disks from *Ribes hudsonianum* var. *petiolare* INT Clone 1

			Within-blister averages and among-aeciospore-lines-within-blister c.v.[a]					
			Aeciospore cultures		Urediospore subcultures			
	Percent of aeciospore lines per blister				Subculture success		Telia in 35 days	
Blister designation	Infections[b]	Produced telia in 60 days[c]	Size 30 days (mm^2)	Pustule density (No./mm^2)	Number inoculated	Percent infected	Number available	Percent with telial columns
1-2-14	6	50	4.7 ± 0.67	3.8 ± 0.38	27	89 ± 0.10	16	18 ± 0.72
1-7-14	10	86	5.7 ± 0.59	5.8 ± 0.40	63	75 ± 0.30	29	49 ± 0.44
1-23-15	19	68	12.0 ± 0.42	1.6 ± 0.41	114	59 ± 0.40	50	25 ± 0.94
1-28-14	4	75	10.2 ± 0.53	1.1 ± 0.88	30	67 ± 0.46	14	30 ± 0.74
1-138-14	10	100	7.7 ± 0.45	2.0 ± 0.70	90	89 ± 0.16	55	13 ± 0.81
1-160-14	13	75	9.5 ± 0.39	3.3 ± 0.38	109	67 ± 0.34	46	17 ± 0.23
1-163-15	10	78	5.7 ± 0.68	5.4 ± 0.30	56	89 ± 0.18	34	65 ± 0.35
1-183-15	11	91	7.1 ± 0.56	3.7 ± 0.35	65	66 ± 0.39	20	5 ± 0.61
2-10-13	9	88	6.1 ± 0.73	4.0 ± 0.26	68	69 ± 0.31	26	3 ± 0.67
3-57-14	6	60	6.1 ± 0.55	4.5 ± 0.53	42	55 ± 0.27	16	...
3-80-15	4	75	8.9 ± 0.76	2.9 ± 0.22	34	82 ± 0.13	18	61 ± 0.51
3-86-15	17	86	11.8 ± 0.34	2.4 ± 0.38	113	65 ± 0.26	42	18 ± 0.77
3-106-15	2	100	...	...	...	...	...	...
3-120-15	16	67	5.6 ± 0.63	2.9 ± 0.35	98	83 ± 0.22	45	69 ± 0.46
3-199-15	0	...	...	...	...	...	...	...
$\bar{x}$ ± c.v.	8.9 ± 0.64	78 ± 0.18						

[a] c.v. = coefficient of variation.
[b] Number of infections of 100 attempts.
[c] Number of aeciospore cultures per blister with telial columns divided by the number of aeciospore cultures per blister living past 45 days while cultured at constant 21 ± 1 C and 12-hr days × 100.

TABLE 3. Performance of single aeciospore cultures of *Cronartium ribicola* obtained from seedlings of *Pinus monticola* that exhibited only red needle lesions and grown on leaf disks from *Ribes hudsonianum* var. *petiolare* INT Clone 1

			Within-blister averages and among-aeciospore-lines-within-blister c.v.[a]					
			Aeciospore cultures		Urediospore subcultures			
	Percent of aeciospore lines per blister				Subculture success		Telia in 35 days	
Blister designation	Infections[b]	Produced telia in 60 days[c]	Size 30 days (mm^2)	Pustule density (No./mm^2)	Number inoculated	Percent infected	Number available	Percent with telial columns
1-3-15	16	82	10.3 ± 0.62	2.9 ± 0.45	104	68 ± 0.29	47	14 ± 0.79
1-31-15	3	33	8.6 ± 0.34	1.9 ± 0.37	16	62 ± 0.31	...	...
1-34-15	10	56	4.8 ± 0.44	3.3 ± 0.56	63	84 ± 0.21	26	11 ± 0.66
1-37-14	18	83	13.4 ± 0.34	2.7 ± 0.41	140	78 ± 0.16	68	12 ± 0.85
1-40-14	3	33	6.1 ± 0.74	4.3 ± 0.39	24	87 ± 0.17	11	24 ± 0.62
1-46-15	1	100	...	...	...	...	...	...
1-50-15	19	89	12.4 ± 0.42	2.5 ± 0.43	129	69 ± 0.20	49	20 ± 0.78
1-64-14	0	...	...	...	...	...	...	...
2-9-4	7	50	6.0 ± 0.34	2.3 ± 0.33	42	69 ± 0.29	15	10 ± 0.49
3-21-15	6	67	8.3 ± 0.65	5.2 ± 0.34	39	77 ± 0.21	18	35 ± 0.84
3-58-15	0	...	...	...	...	...	...	...
3-71-15	8	50	9.5 ± 0.70	1.4 ± 0.44	37	67 ± 0.22	18	30 ± 0.62
3-175-15	13	100	8.3 ± 0.30	2.1 ± 0.30		72 ± 0.20		6 ± 0.92
$\bar{x}$ + c.v.	8 ± 0.84	64 ± 0.37						

[a] c.v. = coefficient of variation.
[b] 100 attempts.
[c] Number of aeciospore cultures per blister with telial columns divided by the number of aeciospore cultures per blister living past 45 days while cultured at constant 21 ± 1 C and 12-hr days × 100.

TABLE 4. Performance of single-aeciospore cultures of *C. ribicola* obtained from *P. monticola* seedlings exhibiting both red and yellow needle lesions and grown on leaf disks of *R. hudsonianum* var. *petiolare* INT Clone 1

			Within-blister averages and among-aeciospore-lines-within-blister c.v.[a]					
			Aeciospore cultures		Urediospore subcultures			
	Percent of aeciospore lines/blister		Size 30 days	Pustule density	Subculture success		Telia in 35 days	
Blister designation	Infections[b]	Produced telia in 60 days[c]	(mm^2)	(no./mm^2)	Number inoculated	Percent infected	Number available	Percent with telial columns
1-138-15	0	...	...	...	...	...	...	...
1-144-15	13	91	5.6 ± 0.47	2.2 ± 0.58	93	68 ± 0.22	37	17 ± 0.73
1-150-15	12	70	16.3 ± 0.66	2.8 ± 0.39	77	70 ± 0.56	20	6 ± 0.35
1-179-15	7	43	7.1 ± 0.40	2.3 ± 0.13	42	76 ± 0.25	9	...
1-198-15	8	80	8.9 ± 0.61	3.7 ± 0.40	47	72 ± 0.24	15	11 ± 0.61
2-46-14	6	50	4.3 ± 0.46	4.1 ± 0.22	38	90 ± 0.18	19	3 ± 0.57
2-250-15	8	100	6.8 ± 0.59	3.7 ± 0.32	58	90 ± 0.13	23	24 ± 0.72
3-4-15	8	86	3.5 ± 0.37	5.1 ± 0.38	57	78 ± 0.25	23	26 ± 0.78
3-88-14	8	38	10.5 ± 0.45	3.9 ± 0.78	57	74 ± 0.29	29	45 ± 0.47
3-98-15	6	80	21.6 ± 0.37	1.6 ± 0.28	37	54 ± 0.37	14	4 ± 0.50
3-101-15	6	50	5.8 ± 0.54	3.1 ± 0.21	32	62 ± 0.11	13	4 ± 0.35
3-102-15	3	100	11.2 ± 0.60	3.8 ± 0.52	18	72 ± 0.54	...	...
3-104-14	13	56	7.5 ± 0.71	3.1 ± 0.51	107	70 ± 0.25	39	27 ± 0.87
3-109-15	15	38	6.0 ± 0.60	4.9 ± 0.87	105	81 ± 0.19	50	13 ± 1.34
3-114-14	5	60	3.5 ± 0.52	4.5 ± 0.45	23	78 ± 0.21	10	56 ± 0.22
3-117-15	11	82	10.1 ± 0.70	2.0 ± 0.38	69	81 ± 0.23	25	26 ± 0.77
$\bar{x}$ ± c.v.	8.1 ± 0.49	68.3 ± 0.32						

[a] c.v. = coefficient of variation.
[b] 100 attempts.
[c] Number of aeciospore cultures per blister with telial columns divided by the number of aeciospore cultures per blister living past 45 days when cultured at a constant 21 ± C and 12-hr days × 100.

TABLE 5. Analysis of variance (nested-random) table for teliospore formation, inoculation success, infection size, and pustule density of single-aeciospore cultures of *Cronartium ribicola* obtained from red only, yellow only, and red and yellow lesion-type *Pinus monticola* seedlings and cultured at a constant temperature of 21 C ± 1 C on *Ribes hudsonianum* var. *petiolare* INT Clone 1

	Aeciospore cultures 12 hr artificial light/day for 30 days			Urediospore cultures (natural light)			
	Infection size		Pustule density[a] mean square	Teliospore formation[a]		Inoculation success	
Source	d.f.	Mean square ($\sqrt{x}$)	($\sqrt{x+1}$)	d.f.	Mean square	d.f.	Mean square
Lesion type	2	2.66	.54	2	464.76	2	372.1
Blisters per lesion type	32	3.34***[b]	.74***	34	831.32***	32	422.0***
Error	296	.60	.11	307	169.62	312	139.2

[a] Nonhomogenous variance after transformation.
[b] *** significant, $P = 0.01$.

urediospore-subculture-success and teliospore-production also exhibited high levels of within-blister variation (Tables 2, 3, and 4), but they still had highly significant variation associated with blister source (Table 5). Variance component analysis of the four traits shows large amounts of the total variation is associated with the blisters-within-lesion-type source (Table 6).

DISCUSSION

The weak cultures observed could indicate the existence of specific hypersensitive reactions as typified by the gene-for-gene concept (9). Others have observed a range of infection types after mass inoculation of *C. ribicola* aeciospores on plants of greenhouse-grown *ribes* plants (1,24). When these aeciospore cultures were subcultured to other clones of *R. hudsonianum,* a differential interaction was observed (14).

The results of this study do not explain the large increase in frequency of red-lesion types, because the epidemiologic fitness features (17) studied were not associated with lesion type. The variance component analysis (Table 6) shows no variation associated with lesion type for any of the four traits. The negative variance components (σ_A^2) are small and only one falls outside the range of their standard errors. According to Snedecor (22), such negative components are not unusual and should be set equal to 0.0. The occasional culture that showed weak growth on Clone 1 did not appear to be related to lesion type.

Probability of early death was associated with red lesions, but this trait would tend to reduce the frequency of the red-lesion types rather than increase them. Further, one-half of the early deaths found in the red-lesion type occurred among aeciospore isolates from a single blister (blister 1-50-15 in Table 3).

In conclusion, it seems obvious that either other epidemiological fitness traits or some mechanism other than epidemiological fitness is involved in the shift of the *C. ribicola* population from yellow- to red-lesion type.

Before beginning to discuss quantitative genetic variation in *C. ribicola,* a brief comparison of the life cycle of *C. ribicola* and a generalized life cycle of diploid organisms should be helpful. Most diploid species produce four male gametes or one female gamete per meiotic event. In contrast, *C. ribicola* produces four initial gametes (basidiospores) for each meiotic event, and each can multiply indeterminately before producing functional gametes which presumably are pycniospores, individual hyphal cells, and "2-legged-basal cells" within one leg of which the dikaryon is established (10). If we assume that all basidiospores have an equal probability of multiplying equally (unbiased segregation) (5), then *C. ribicola* could be considered equivalent to a diploid in the production of gamete pools.

Most diploids are characterized by gamete union restricted to the body of the female or female organ. In contrast, gamete union in *C. ribicola,* which produces the "two-legged cells," more than likely is not thus restricted. In theory, all gametes (basidiospores) could unite randomly without regard to sex, which would be equivalent to the random mating assumption for diploid organisms. A problem could arise in *C. ribicola* if each union produced a variable number of aeciospores (zygotes). But if each produced an equal number, then a sample of aeciospores from a single blister could be regarded as an estimator of the zygote population of the sampled blister. In addition, even if + and − mating types occur, we cannot assume that − and −, and + and + combinations of nuclei in

aecisopores will not happen. Such − and −, and + and + combinations are not known to occur in other rust pathogens (6).

A significant source of variation likely to be encountered in *C. ribicola* and not present in diploids is the possible occurrence of somatic, nuclear, chromosomal, and cytoplasmic recombinations during rust pathogen development on *Ribes* as is known to occur in other rusts (6). This means that zygotes formed by the union of gametes could be unstable when allowed to develop in the company of other zygotes. It also means that urediospore cultures obtained from a single aeciospore should not be considered as clonal propagules of the aeciospore because some chromosomal and/or gene recombinations might have occurred. Thus, only two avenues to clonal reproduction of zygotes are available—production of homokaryotic aeciospores from a known single haploid infection and isolation of a chain of aeciospores produced by a single "two-legged" basal cell. Both paths appear to be burdened with difficulties.

Since individual zygotes (two-legged basal cells) can multiply indeterminately before fusion occurs, each individual nuclear pairing (plasmogamy) can result in from none to many possibly different meiotic events. Thus, a genetic combination can be unstable between nuclear pairing and meiosis (discussed above). But, if the nuclear combination could be stabilized (isolation) until production of new gametes, *C. ribicola* could be made equivalent to a diploid. In conclusion, single aeciospore lines of *C. ribicola* isolated from the wild can be considered equivalent to a diploid organism for purposes of some aspects of a quantitative genetic analysis. Also, procedures could be developed to insure random pairing of gametes to produce populations equivalent to full-sib families of higher organisms. The amounts of additive and interaction genetic variance and maternal and environmental variance would be estimable making most, if not all, of the theory of quantitative genetics applicable to the study of *C. ribicola* or other rust pathogens.

Different modes of "fertilization" should lead to definable patterns of genetic variation. Infections by several 1N basidiospores on different pines with subsequent production of aeciospores after self-fertilization would lead to a population of homokaryotic aeciospores within each blister. The only source of genetic variation within blisters would be mutation. This material would yield the quantitative genetic equivalent of a clonal or maternal twin test in diploid organisms. The variance component within blisters (σ_W^2) would measure only environmental variance (V_E) and the variance component between blisters (σ_B^2) would measure only genetic variance ($V_G = V_A + V_D + V_M$ in which V_A = additive genetic variance, V_D = dominance and interaction genetic variance, V_M = maternal variance). This is the pattern expected if *C. ribicola* is either homothallic (10) or heterothallic and the aecial hymenium formed in each blister is homogenous as Zimmer et al (27) claim for single aecial cups of *Puccinia coronata*. Thus, according to some prevailing ideas we can expect $\sigma_W^2 = V_E + 0.0\ V_G$ and $\sigma_B^2 = V_G$. Under an assumption of minimum genetic variation, each trait is shown to be strongly inherited (Table 6). In the case of aeciospore culture growth rate, variance component analysis shows that *at least* 33% of the variation is genetic (Table 6). Of particular interest is the finding that 30% of the variation in early teliospore formation by urediospore subcultures is genetic. This result and the previously reported (13) result that both spore stage (aeciospore or urediospore) and temperature as well as aeciospore genotype influence production of teliospores, indicate that a complex interaction of genes and environment controls the expression of this trait.

The blister-to-blister variation in period from inoculation to production of teliospores (Tables 2, 3, and 4) also indicates genetic control. Uredial sorus density could be a very significant epidemiological fitness trait that shows 38% of the variation due to genetic causes. Finally, urediospore inoculation success shows 17% genetic variation. One can clearly see how the interaction of these four traits could produce large variations in the ability of a given *C. ribicola* aeciospore to produce basidiospores.

The character of the genetic variation remains unknown. We do not know the relative contribution of V_A, V_D, and V_M to V_G. For example: a genetic system characterized by a genetically heterogeneous hymenium formed from obligate outcrossing by way of functional pycniospores, fertilization by aeciospores or urediospores and anastomosis of hyphae from multiple basidiospore infections could conceivably yield a population of aeciospores from one blister that would be equivalent to a one-seed-per-tree stand collection of tree seed. That is, no or very little common descent. Under these circumstances σ_B^2 would estimate mostly V_M and σ_W^2 would estimate mostly V_E. The amount of V_G would be very small. The actual level of V_E remained unknown in this experiment, even though it was designed to minimize V_E by using one clone of *Ribes*, uniform leaf age (± 7 days), and uniform growth chamber environment (± 1C) because a given rust genotype was not deliberately replicated. In other words V_E and V_G in σ_W^2 are totally confounded. We must also realize that other sources of environmental variation such as leaf location on the *Ribes* plants, leaf storage time, and the source location of a disk within the leaf could be present. Minor variation in light and temperature from place to place in the growth chamber might also add variation. The result is that under this interpretation σ_B^2 would be due to a maternal effect and no additive genetic variance would be present. Thus, the demonstrated amounts of genetic variation could be due to maternal effects.

A more likely situation is something between the two extremes. According to previous results (10) some degree of outcrossing occurs in *C. ribicola* and the results of Dinoor et al (7) indicate that *P. coronata* Cda. f. sp. *avenae* may behave in a similar fashion. *Melampsora lini* (Ehrenh.) Lev. likewise produces heterogenous aeciospore populations within a single aecium (6). Under an assumption of one haploid infection being fertilized by a large population of genetically heterogeneous pycniospores, one-half of the genetic material would have a common ancestry and this experiment would have a genetic interpretation comparable to a full-sib progeny test in diploids. Thus, $\sigma_B^2 = (1/2)\ V_A + (1/4)\ V_D + V_M$ and $\sigma_W^2 = (1/2)\ V_A + (3/4)\ V_D + V_E$ in which V_A = additive genetic variance, V_D = dominance and interaction genetic variance, V_M = common environmental or maternal variance and V_E = general environmental variance (8). Under this interpretation for this experiment we can not separate V_D and V_M from V_A in σ_B^2. But, we can assume that σ_B^2 is mostly V_A plus all the V_M and that σ_W^2 has an equal amount of V_A and all of the V_E component. The upper limit on proportion of variation due to genetic causes can be expressed by the narrow-sense heritability [$h^2 = 2\sigma_B^2/(\sigma_W^2 + \sigma_B^2)$] (8). This value will be double the presumed clonal values for estimates of the percentage of variation in fitness traits that is genetically controlled.

There is no direct evidence of a significant V_M component as one would suspect if σ_B^2 were larger than σ_W^2. However, this proposition and other questions about the nature of the observed variation could be explicitly tested by creating a "full-sib"

TABLE 6. Variance components and standard errors associated with four epidemiological fitness traits obtained from a population of single-aeciospore cultures of *Cronartium ribicola* grown on detached leaves of *Ribes hudsonianum* var. *petiolare* INT Clone 1

Variance components	Aeciospore cultures		Urediospore cultures	
	Infection size in 30 days	Urediospore sorus density at 30 days	Teliospore formation by 35 days	Urediospore inoculation success
Lesion type (σ_A^2)	−0.01 ± 0.019	−0.02 ± 0.004	−3.27 ± 3.41	−0.44 ± 2.51
Blisters/lesion types (σ_B^2)	0.29 ± 0.086	0.067 ± 0.019	71.27 ± 21.25	28.68 ± 10.44
Aeciospores/blister/lesion type (σ_W^2)	0.60 ± 0.035	0.11 ± 0.079	169.62 ± 9.68	139.20 ± 9.68

equivalent progeny test using a factorial mating design.

The "full-sib" equivalent test would require isolation of single aeciospores to use as "parents." Upon production of basidiospores (gametes) isolated single basidiospore infections could be crossed in a pattern that would yield the desired number of "full-sib" and "half-sib" families in accordance with a factorial mating design (16). Each resulting zygote (blister) would contain a homogenous population of aeciospores (all derived from the crossing of two 1N infections or gametes). At least one aeciospore would be cultured to obtain the measurements of desired characteristics. Then one could calculate a "female" component (σ_f^2), a "male" component (σ_m^2), a "male × female interaction" (σ_{mf}^2), and a within-family component (σ_w^2); so that $\sigma_m^2 = (1/4)\ V_A + (1/16)\ V_{AA} + (1/64)\ V_{AAA}$, $\sigma_f^2 = (1/4)\ V_A + (1/16)\ V_{AA} + (1/64)\ V_{AAA} + V_M$, $\sigma_{mf}^2 = (1/4)\ V_D + (1/8)\ V_{AA} + (1/8)\ V_{AD} + (3/32)\ V_{AAA}$, and $\sigma_w^2 = (1/2)\ V_A + (3/4)\ V_D + (3/4)\ V_{AA} + (7/8)\ V_{AD} + (15/16)\ V_{DD} + (56/64)\ V_{AAA} + V_E$(3). It follows that V_M could be estimated by the difference $\sigma_f^2 - \sigma_m^2$ and the difference $\sigma_m^2 - \sigma_{mf}^2$ could estimate the interaction and dominance genetic components. Also by replicating aeciospores from a single "full-sib" blister one could measure σ_E^2. Control of plasmogamy would produce a standard diploid progeny test.

The results from this study indicate quantitative inheritance of some presumed epidemological fitness traits of *C. ribicola* on *Ribes* and suggest that successful study of both the classic qualitative and quantitative genetics of *C. ribicola* could follow development of methods for the establishment and crossing of lines to simulate the genetic situation of higher plants. Matings that meet most, if not all, of the requirements of quantitative genetic analysis will then be possible. The logical next steps in the study of the genetics of *C. ribicola* are to develop procedures for the culture, in isolation, of these specified lines of rust through their complete life cycle and to pursue further definition of both qualitative and quantitative traits reported here.

LITERATURE CITED

1. Anderson, R. L., and French, D. W. 1955. Evidence of races of *Cronartium ribicola* on *Ribes.* For. Sci. 1:38-39.
2. Bartlett, M. S. 1947. The use of transformations. Biometrics 3:39-52.
3. Becker, W. A. 1964. Manual of Procedures in Quantitative Genetics. 1st ed., Washington State Univ. Press, Pullman. 70 pp.
4. Bingham, R. T. 1972. Artificial inoculation of large numbers of *Pinus monticola* seedlings with *Cronartium ribicola.* Pages 357–372 in: Biology of rust resistance in forest trees. Proceedings of a NATO-IUFRO Advanced Study Institute, August 17–24, 1969. U.S. Dep. Agric. Misc. Publ. 1221. 681 pp.
5. Caten, C. E., and Jinks, J. L. 1976. Quantitative genetics. Pages 93–111 in: Genetics of Industrial Microorganisms. K. D. MacDonald, ed. Academic Press. London, New York, and San Francisco.
6. Day, P. R. 1974. Genetics of Host-Parasite Interaction. W. H. Freeman and Company. San Francisco. 238 pp.
7. Dinoor, A., Khain, A. J., and Fleischmann, G. 1968. Pathogenic variability and the unit representing a single fertilization in *Puccinia coronata* var. *avenae.* Can. J. Bot. 46:501-508.
8. Falconer, D. S. 1960. Introduction to Quantitative Genetics. Ronald Press. New York. 365 pp.
9. Flor, H. H. 1971. Current status of the gene-for-gene concept. Annu. Rev. Phytopathol. 9:275-296.
10. Hirt, R. R. 1964. *Cronartium ribicola,* its growth and reproduction in tissues of eastern white pine. N.Y. State Coll. For. Syracuse Univ. Tech. Bull. 86. 30 pp.
11. Hoff, R., Bingham, R. T., and McDonald, G. I. 1980. Relative blister rust resistance of white pines. Eur. J. For. Pathol. 10:307-316.
12. McDonald, G. I. 1978. Segregation of "red" and "yellow" needle lesion types among monoaeciospore lines of *Cronartium ribicola.* Can. J. Genet. Cytol. 20:313-324.
13. McDonald, G. I., and Andrews, D. S. 1980. Influence of temperature and spore stage on production of teliospores by single aeciospore lines of *Cronartium ribicola.* U.S. Dep. Agric. For. Serv., Res. Pap. INT-256. 9 pp.
14. McDonald, G. I., and Andrews, D. S. 1981. Genetic interaction of *Cronartium ribicola* and *Ribes hudsonianum* var. *petiolare.* For. Sci. 27:758-763.
15. McDonald, G. I., and Hoff, R. J. 1975. Resistance to *Cronartium ribicola* in *Pinus monticola:* an analysis of needle-spot types and frequencies. Can. J. Bot. 53:2497-2505.
16. Namkoong, G. 1979. Introduction to quantitative genetics in forestry. U.S. Dep. Agric. For. Serv. Tech. Bull. 1588. 348 pp.
17. Nelson, R. R. 1973. Pathogen variation and host resistance. Pages 40–48 in: Breeding Plant for Disease Resistance Concepts and Application. R. R. Nelson, ed. Penn. State Univ. Press, University Park and London.
18. Powers, H. R. 1980. Pathogenic variation among single-aeciospore isolates of *Cronartium quercuum* f. sp. *fusiforme.* For. Sci. 26:280-282.
19. Powers, H. R., Jr., and Matthews, F. R. 1979. Interaction between virulent isolates of *Cronartium quercuum* f. sp. *fusiforme* and loblolly pine families of varying resistance. Phytopathology 69:720-722.
20. Powers, H. R., Jr., Matthews, F. R., and Dwinnell, L. D. 1977. Evaluation of pathogenic variability of *Cronartium fusiforme* on loblolly pine in the Southern USA. Phytopathology 67:1403-1407.
21. Raddi, P. 1976. Indagine suitipidi macchie foliani e sul lono significato in discendenzedi *Pinus pinaster* infette da *Cronartium flaccidum.* Riv. Patol. Veg. 4:91-98. (Abstr. from Rev. Plant Pathol. 56:944)
22. Snedecor, G. W. 1956. Statistical Methods Applied to Experiments in Agriculture and Biology. The Iowa State University Press, Ames.
23. Snow, G. A., Dinus, R. J., and Kais, A. G. 1975. Variation in pathogenity of diverse sources of *Cronartium fusiforme* on selected black pine families. Phytopathology 65:170-175.
24. Spaulding, P., and Gravatt, G. F. 1917. Inoculation on *Ribes* with *Cronartium ribicola.* Science 46:243-244.
25. Van Arsdel, E. P., Riker, A. J., and Patton, R. E. 1956. The effects of temperature and moisture on the spread of white pine blister rust. Phytopathology 46:307-318.
26. Vanderplank, J. E. 1975. Principles of Plant Infection. Academic Press. New York. 216 pp.
27. Zimmer, D. E., Shafer, J. F., and Patterson, F. I. 1965. Nature of fertilization and inheritance of viruluence in *Puccinia coronata.* Phytopathology 55:1320-1324.