Inheritance of Virulence and Uredial Color and Size in *Puccinia recondita tritici*

G. D. Statler and D. A. Jones

Professor and technician, respectively, Department of Plant Pathology, North Dakota State University, Fargo 58105
Journal Series Paper 1092.
Accepted for publication 22 November 1980.

ABSTRACT

Statler, G. D., and Jones, D. A. 1981. Inheritance of virulence and uredial color and size in *Puccinia recondita tritici*. Phytopathology 71:652-655.

Virulence, uredial length, width, area, and color, and chlorosis on wheat were recorded for each of 70 F_2 *Puccinia recondita tritici* cultures from a cross between cultures 70-197 and 71-112. These variables were compared to the F_1 and parents. *P. recondita* uredial size was normally distributed and did not fit discrete classes. Uredial length appeared to be under genetic control. Significant correlations were found between virulence and length, and virulence and area, on wheat with genotype *LrEG* and between virulence and pustule width, and virulence and color on wheat with *Lr23*. No other significant correlations existed for the variables studied. Chi-square tests did not indicate linkage between virulence, color, uredia size, or chlorosis. Uredia color was apparently controlled by a single dominant gene that was independent of genes for virulence. Single recessive genes conditioned virulence on genotypes *Lr1, Lr3, Lr11, Lr17, LrEG,* and experimental line 5534. The single recessive genes for virulence were inherited independently except for p^3 and *pEG* and for p^{17} and *pEG* for which the chi-square tests indicated linkage. The possibility of intermediate infection types caused by cultures in the heterozygous condition is discussed.

Additional key words: virulence genes, linkage, wheat leaf rust.

Several studies on the inheritance of virulence of *Puccinia recondita* Rob. ex Desm. f. sp. *tritici* have been conducted (2,3,9,10,12,13). However, little research has been done to study factors other than virulence to develop markers to aid in more detailed genetic studies (2). Green (4) indicated that color mutants had been reported in several rust species. Some genes controlling color have been linked to virulent genes in *P. recondita* (2) and *P. graminis* (4).

Pustule size has been associated with slow rusting of wheat cultivars infected with *P. recondita* (7). The genetics of the fungus undoubtedly plays a role in determining pustule size, but inheritance of pustule size has not been adequately studied. Ohm and Shaner (6) reported a correlation between latent period and pustule size.

This study was initiated to study the inheritance of size and color of uredia, associated chlorosis on wheat leaves, and of virulence in *P. recondita*. The objective was to establish genetic markers for detailed genetic studies.

MATERIALS AND METHODS

Cultures 70-197 and 71-112 of *P. recondita* were purified by three sequential single pustule isolations. Purity was evaluated on isogenic lines and differentials (Table 1). The infection types are listed in Table 1.

Teliospores were produced by injecting urediospores of each culture into culms of moderately resistant plants in the boot stage. Teliospores were induced to germinate by alternate wetting and drying. After several such cycles the telia were suspended over meadow rue (*Thalictrum speciosissimum* Loefl.), the alternate host of *P. recondita*. Honeydew and pycniospores were transferred from a pycnium of culture 70-197 to a pycnium of culture 71-112. Aeciospores resulting from the cross were used to inoculate Little Club wheat, and purity of the resulting urediospore culture labeled X65 was evaluated on the lines listed in Table 1.

Teliospores of the cross X65 were produced, conditioned to germinate, and suspended over meadow rue. Selfing to produce F_2 cultures was accomplished by separately transferring honeydew and pycniospores from one pycnium to another. Seventy cultures were developed from single aecial cultures resulting from selfing X65. These 70 cultures were used to inoculate isogenic lines and host cultivars listed in Table 1.

Inoculations and incubation were performed in moist chambers at 18.5 ± 2 C. Inoculated plants were held at approximately 100% relative humidity for 24 hr. After incubation, plants were returned to the greenhouse bench at 21 ± 4 C for the duration of the experiment.

The infection type expressed on each differential was classified on a standard scale (11) of 0–4 at 10–12 days after inoculation. Infection types 0 to 2 were classified as avirulent and types 3 and 4 as virulent (9). Chi-square tests were used to determine the

0031-949X/81/06065204/$3.00/0

probabilities of the segregations fitting hypothetical ratios. Chi-square tests for independence were used to determine if genes were independently inherited. The recombination values were estimated by the product method.

The length and width of each of four uredial pustules on each of five leaves of Little Club wheat were measured with an American Optical (Buffalo, NY 14215) binocular microscope at ×60 and expressed as millimeters. The variables analyzed were: length, width, the width-to-length ratio, and the area. The area of the uredia was calculated as: area = 3.14 (width/2) (length/2). A Kolmogorov-Smirnov D statistic (1) was used to test for normality of distribution of the above variables among the F_2 cultures. Variance of the F_2 cultures was compared with that of the F_1 and both parents.

Uredial color was visually compared to a Grumbacher color wheel (Grumbacher Inc., 460 W. 34th St., New York, NY 10001) and recorded as red-orange or orange. Color and size data for each culture were combined with virulence data for the same culture and the resulting set was analyzed by correlations according to the PROC CORR procedure of the SAS (Statistical Analysis Systems) computer program (1).

RESULTS

Data from pustule measurement variables did not fit discrete classes. The normality test indicated that these data were continuous and normally distributed ($P > 0.05$). The frequency distributions for length, width, and areas of uredial pustules caused by *P. recondita* are in Tables 2, 3, and 4, respectively. The fact that the F_2 frequency distribution transgresses both parents by several classes suggests that the F_2 population contains a number of unique gene recombinations not found in either parent. The variance for pustule length of the F_2 (0.0313) compared to that of the parents (0.0118 and 0.0080) indicated that pustule length may be under genetic control. The variance for pustule length of the F_1 was 0.0240. The fact that the variance for pustule length of the F_1 was larger than that of the parents does not support the data indicating that pustule length is under genetic control. The variances of pustule widths, width-to-length ratios, and pustule areas of the parents, F_1 cultures, and F_2 cultures suggested that width may not be under genetic control; either veins restrict width or there is little variation in width.

Means of pustule width, length, width/length ratio, and area of F_2 cultures were analyzed for correlation with color, chlorosis, and virulence for each single gene line. Significant correlations ($P = 0.05$) were observed between virulence and pustule length, and virulence and area on host genetype *LrEG* and between virulence and pustule width, and color and virulence on host genotype *Lr23*. No significant correlations were found between virulence and the above variables on any of the other differentials. A weak correlation ($r = 0.15$) was observed between chlorosis and width but none between chlorosis and length. However, chi-square tests indicated that uredial size and color were independent of virulence.

TABLE 1. Segregation of F_2 cultures derived from a cross (X65) of *Puccinia recondita* f. sp. *tritici* cultures 70-197 and 71-112

Host isogenic line or cultivar	Infection types of cultures[a]			Number of F_2 cultures with infection type or pathogenicity:								Expected ratio[b]	Goodness of fit
	70-197	71-112	X65	0 to 0;	0;1 to 0;2	1	2	3	4	Avirulent	Virulent		
Lr 1	01	4	0	47	7	0	0	0	16	54	16	3:1	$P > 0.50$
Lr 2a	4	4	4	0	0	0	0	15	55	0	70	HV	
Lr 2c	4	4	4	0	0	0	0	6	64	0	70	HV	
Lr 3	4	0;	01	9	23	20	4	9	5	56	14	3:1	$P > 0.25$
Lr 3ka	12	1	1	9	11	44	6	0	0	70	0	HA	
Lr 9	0;	1	0;	59	9	2	0	0	0	70	0	HA	
Lr 10	4	4	4	0	0	0	0	23	47	0	70	HV	
Lr 11	3	2−	2+	2	14	17	13	8	16	46	24	3:1	$P > 0.05$
Lr 16	12	2	2	0	5	36	29	0	0	70	0	HA	
Lr 17	12	4	12	0	13	25	13	5	14	51	19	3:1	$P > 0.50$
Lr 19	0;	0;	0	69	1	0	0	0	0	70	0	HA	
Lr 21	2+	2	2+	8	5	15	42	0	0	70	0	HA	
Lr 23	4	2+	2+	4	10	17	26	10	3	57	13	3:1	$P > 0.10$
Lr 24	01	01	12	21	24	18	7	0	0	70	0	HA	
Lr T	2+	02	12	8	14	31	17	0	0	70	0	HA	
Lr EG	2+	0;	2+	3	6	13	24	10	14	46	24	3:1	$P > 0.05$
5534	3−	2	2	8	10	8	31	11	2	57	13	3:1	$P > 0.10$
Transec	01	0;	12	22	33	12	3	0	0	70	0	HA	
Olaf	4	01	4	1	0	2	5	13	49	8	62	1:15	$P > 0.05$
Thatcher	4	4	4	0	0	0	0	0	70	0	70	HV	

[a] Avirulent = 0 to 2. Virulent = 3 to 4.
[b] HV = homozygous virulent. HA = homozygous avirulent.

TABLE 2. Frequency distributions for lengths of uredia of *Puccinia recondita* f. sp. *tritici*

Generation	0.3[a]	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8
Parent 1				2	7	8	1	2								
Parent 2				7	6	7										
F_1				4	7	5	1	1	2							
F_2	13	62	92	224	268	254	90	159	85	36	5	25	4	2	0	1

[a] Upper class limits in millimeters.

TABLE 3. Frequency distribution for widths of uredia of *Puccinia recondita* f. sp. *tritici*

Generation	0.20[a]	0.25	0.30	0.35	0.40	0.45	0.50	0.55	0.60	0.65	0.70	0.75	0.80	0.85	0.90	0.95
Parent 1			11	5	4											
Parent 2			9	10	1											
F_1			9	6	3	1	1									
F_2	28	78	262	366	349	52	87	77	5	10	3	1	1	1		

[a] Upper class limits in millimeters.

TABLE 4. Frequency distribution for areas of uredia of *Puccinia recondita* f. sp. *tritici*

Generation	0.04[a]	0.06	0.08	0.10	0.12	0.14	0.16	0.18	0.20	0.22	0.24	0.26	0.28	0.30	0.32	0.34	0.36	0.38	0.40	0.42	0.44	0.46	0.48	0.50	0.52	0.54	0.56
Parent 1					2	5	0	4	5																		
Parent 2					6	1	2	4	6	1																	
F_1					3	4	0	6	1	0	1	2	1	0	0	0	0	1	1								
F_2		2	19	41	80	95	109	87	146	100	103	63	109	55	80	33	61	24	18	32	19	16	7	9	1	7	2	2

[a]Upper class limits in square millimeters.

Parental culture 71-112 had orange uredia. Parental culture 70-197 and the F_1 culture X65 had red-orange uredia. There were 44 red-orange and 22 orange F_2 cultures that fit a 3:1 model for a single gene conditioning color ($P > 0.10$). The observed F_2 ratios and the uredia color of the F_1 indicated that red-orange color was dominant. Chi-square tests indicated that uredial color (orange or red-orange) was independent of virulence in the cultures segregating for virulence.

There were 18 cultures with chlorosis and 50 with no chlorosis on Little Club wheat. This distribution fit a 1:3 ratio for a single recessive gene conditioning chlorosis ($P > 0.50$). Neither parent nor the F_1 displayed chlorosis on Little Club.

The F_1 culture (X65) and all of the F_2 progenies were avirulent on lines with genotypes *Lr3ka, Lr9, Lr16, Lr19, Lr21, Lr24, LrT,* and cultivar Transec. The failure to observe virulent segregants among the F_2 cultures indicated that culture X65 was homozygous avirulent on these cultivars. A wide range of low infection types (type 0–2) was observed in the F_2 cultures, especially when the infection types on the parents were a 1 or 2.

The parental cultures, the F_1 culture and all of the F_2 cultures were virulent on lines with genotypes *Lr2a*, *Lr2c*, and *Lr10*. The failure to observe avirulent segregants on the lines with these genes indicated that the parental cultures and culture X65 are homozygous virulent on lines with genotypes *Lr2a*, *Lr2c*, and *Lr10*.

Segregation for virulence occurred among the F_2 cultures on genotypes *Lr1*, *Lr3*, *Lr11*, *Lr17*, *Lr23*, *LrEG*, and experimental line 5534. One parent was virulent, the other avirulent, and the F_1 avirulent on each of these lines in every case except for genotype *LrEG* in which case both parents were avirulent and the F_1 avirulent. Culture 70-197 and the F_1 was presumably heterozygous for virulence on *LrEG*.

The F_2 cultures segregated approximately three avirulent to one virulent on genotypes *Lr1*, *Lr3*, *Lr11*, *Lr17*, *Lr23*, *LrEG*, and experimental line 5534 when the ratings were grouped as avirulent (infection type 0 through 2) or virulent (infection type 3 or 4). Since the *P* values were all >0.05, single recessive genes for virulence were indicated (Table 1). Single recessive genes for virulence p^1, p^3, p^{11}, and p^{17} have been previously reported (3,9).

Most of the single recessive genes for virulence were inherited independently, but associations were found between p^3, and *pEG* ($P < 0.025$) and between p^{17} and *pEG* ($P < 0.005$). The recombination estimate was $22 \pm 11\%$ for p^3 and *pEG* and $37 \pm 11\%$ for p^3 and p^{17}. Linkage was not indicated between p^3 and p^{17} ($P > 0.20$).

Segregation on Olaf fit a ratio of one avirulent to 15 virulent and indicated segregation for two dominant genes ($P > 0.05$) for virulence. Dominant genes for virulence are not common in *P. recondita*, but they have been reported (9,10,12).

When infection types 1 and 2 are classified as intermediate, segregation on genotypes *Lr11*, *Lr17*, *Lr23*, and 5534 provided a good fit to a ratio of one avirulent to two intermediate to one virulent (*P* values all >0.05). This 1:2:1 ratio could be explained by incomplete dominance in which the heterozygous cultures produced an intermediate reaction. The F_1 cultures had intermediate virulence on genotypes *Lr11, Lr17, Lr23,* and 5534. The F_1 culture produced intermediate reactions on genotypes *Lr3* and *LrEG*, but the F_2 segregation ratios would not fit a 1:2:1 ratio because too few intermediate infection types were observed.

When the infection types 1 and 2 were classified as intermediate and 0; and 0;1 to 0;2 were classified as highly avirulent, observed F_2 segregation on genotypes *Lr3ka*, *Lr21*, and *LrT* fit the model of three intermediate to one highly avirulent (*Lr3ka*, $P > 0.25$; *Lr21*, $P > 0.10$; and *LrT* $P > 0.10$). This observation indicated segregation of a single recessive gene conditioning avirulent infection types. Parental cultures apparently did not differ for a gene conditioning intermediate infection types. Segregation on Transec fit a three avirulent to one intermediate ratio ($P > 0.50$).

DISCUSSION

Pustule size of *P. recondita* is important because it has been correlated with slow rusting (7). Large pustules obviously provide more inoculum than do small ones. Latent period has also been

correlated with pustule size (6). Variance data indicated that pustule length, but not width or area, showed genetic variability.

Previous workers have reported an association between color and pathogenicity of *P. recondita* (5). However, data from this study did not demonstrate linkage or correlation between color and virulence except for a correlation between color and virulence on host genotype *Lr23*. Segregation ratios of red-orange to orange uredia fit a model for a single dominant gene conditioning red-orange uredia in this study. Green (4), working with *P. graminis*, also reported a single dominant gene for virulence, which was linked to the gene for normal spore color. In 1949, Johnson (5) reported that red uredospore color was dominant to orange in *P. graminis avenae*.

Chlorosis was observed on 18 of 68 cultures on Little Club. A single recessive gene may condition chlorosis. On the other hand, variation in chlorosis could be caused by variation in light, temperature, or some other environmental factor. Further studies are necessary to determine the inheritance of chlorosis of wheat infected by *P. recondita*, and to clarify the lack of variability between the parents.

Single recessive genes apparently condition virulence on genotypes *Lr1*, *Lr3*, *Lr11*, *Lr17*, *Lr23*, *LrEG*, and experimental cultivar 5534. These single recessive genes for virulence were inherited independently except for p^3 and *pEG* and p^{17} and *pEG*.

In some cases, a wide range of infection types was observed (Table 1). Samborski (8) proposed that exceptions to the ideal situation, where only two infection types are found, may occur as a result of effects of temperature, nonallelic interaction, heterozygosity, and the possibility of alleles for virulence. He (8) further proposed that the simplest explanation is that the culture is heterozygous for virulence and that virulence is incompletely dominant. Incomplete dominance is not common in *P. recondita*.

Incomplete dominance could be used to explain the one avirulent (ratings 0 to 0;1) to two intermediate (ratings 1 to 2) to one virulent (ratings 3 to 4) infection types for *Lr11*, *Lr17*, *Lr23*, and 5534. Segregation on *Lr3ka*, *Lr21*, and *LrT* would fit a model of three intermediate to one avirulent. This may indicate a single recessive gene conditioning avirulent infection types over intermediate infection types. A more logical explanation is that the culture was heterozygous for intermediate infection types.

The ratio of three highly avirulent to one intermediate on Transec is difficult to explain since both parents of X65 were avirulent (infection types 0;1 and 0;). The infection type of X65 was intermediate. If genes in the heterozygous condition produce the intermediate response, one parent should be intermediate. Culture 70-197 on Transec could have been misclassified as a 0;1 instead of a 1. The results for Transec suggest that the separation of the resistant types into classes may be arbitrary and hence not readily explained by the genetical hypotheses. The range in infection types could be attributed to gene action influenced by modifiers in the genetic backgrounds of the host and pathogen as previously theorized (10).

LITERATURE CITED

1. Barr, A. J., Goodnight, J. H., Sall, J. P., Blair, W. H., and Chilko, D. M. 1979. SAS (Statistical Analysis Systems) User's Guide. Raleigh, NC. 494 pp.
2. Brown, A. M., and Johnson, T. 1949. Studies on variation in pathogenicity in leaf rust of wheat, *Puccinia triticina* Erickss. Can. J. Res., Ser. C27:191-202.
3. Dyck, P. L., and Samborski, D. J. 1968. Host-parasite interactions involving two genes for leaf rust resistance in wheat. Pages 245–250 in: Proc. Third International Wheat Genetics Symposium. Aust. Acad. Sci., Canberra.
4. Green, G. J. 1964. A color mutation, its inheritance, and the inheritance of pathogenicity in *Puccinia graminis* Pers. Can J. Bot. 2:1653-1664.
5. Johnson, T. 1949. Inheritance of pathogenicity and urediospore color in crosses between physiologic races of oat stem rust. Can. J. Res. 27:203-217.
6. Ohm, H. W., and Shaner, G. E. 1975. Segregation for slow leaf rusting in wheat. Agron. Abstr. 67:59.
7. Ohm, H. W., and Shaner, G. E. 1976. Three components of slow leaf-rusting at different growth stages in wheat. Phytopathology 66:1356-1360.
8. Samborski, D. J. 1963. A mutation in *Puccinia recondita* Rob ex. Desm. f. sp. *tritici* to virulence on Transfer, Chinese Spring ×*Aegilops umbellata* Zhuk. Can. J. Bot. 41:475-479.
9. Samborski, D. J., and Dyck, P. L. 1968. Inheritance of virulence in wheat leaf rust on the standard differential wheat varieties. Can. J. Genet. Cytol. 10:24-32.
10. Samborski, D. J., and Dyck, P. L. 1976. Inheritance of virulence in *Puccinia recondita* on six backcross lines of wheat with single genes for resistance to leaf rust. Can. J. Bot. 54:1666-1671.
11. Stakman, E. C., Stewart, D. M., and Loegering, W. Q. 1962. Identification of physiological races of *Puccinia graminia* var. *tritici*. U.S. Dep. Agric., ARS E617 (Rev.) 53 pp.
12. Statler, G. D. 1977. Inheritance of virulence of culture 73-47 *Puccinia recondita*. Phytopathology 67:906-908.
13. Statler, G. D. 1979. Inheritance of pathogenicity of culture 70-1, race 1 of *Puccinia recondita tritici*. Phytopathology 69:661-663.