Inheritance of Race-Specific Resistance to *Mycosphaerella graminicola* in Wheat

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ABSTRACT

McCartney, C. A., Brûlé-Babel, A. L., and Lamari, L. 2002. Inheritance of race-specific resistance to *Mycosphaerella graminicola* in wheat. *Phytopathology* 92:138-144.

Mycosphaerella graminicola causes Septoria tritici blotch of hexaploid and tetraploid wheat. The inheritance of high-level resistance to Septoria tritici blotch was studied in controlled environment experiments. Intraspecific reciprocal crosses were made between hexaploid wheat lines Salamouni, ST6, Katepwa, and Erik, and the tetraploid wheat lines Coulter and 4B1149. Parental, F₁, F₂, F₃, BC₁F₁, and BC₁F₂ populations were evaluated for reaction to isolates MG2 and MG96-36 of *M. graminicola*. Resistance was controlled by incompletely dominant nuclear

genes in all cases. Salamouni had three independent resistance genes to isolate MG2, two of which also controlled resistance to isolate MG96-36. ST6 had a single resistance gene to isolate MG2 and none to isolate MG96-36. The resistance genes in Salamouni and ST6 were not allelic. Two independent genes control resistance to isolate MG2 in Coulter, one of which also controlled resistance to isolate MG96-36. These data are consistent with a gene-for-gene interaction in the wheat-*M. graminicola* pathosystem.

Additional keywords: host-pathogen interactions, major gene, qualitative inheritance, *Triticum aestivum*, *T. turgidum* subsp. *durum*.

Septoria tritici blotch (STB) is a major leaf disease of bread and durum wheat (*Triticum aestivum* and *T. turgidum* subsp. *durum*) that is found in many wheat-producing areas of the world (3,14). STB is caused by the ascomycete *Mycosphaerella graminicola* (Fuckel) J. Schröt. in Cohn (anamorph: *Septoria tritici* Roberge in Desmaz.). Frequent rainfall and moderate temperatures favor the development of STB epidemics (23,24). Epidemics can result in yield losses of 25 to 50%, with the resulting seed being shriveled and unfit for milling (14,17,24,33). Incorporating genetic resistance into wheat cultivars is an economically and environmentally sound method of controlling this disease.

An understanding of the inheritance of STB resistance is needed to design an effective breeding program. However, studies of the inheritance of host resistance have produced conflicting results. These studies have reported STB resistance to be controlled by one or two dominant or partially dominant genes (15,20,26,31,32), two to three recessive genes (20,32), or by several genes with additive and dominant gene effects (9,25,28,29). Differences in the isolates of *M. graminicola*, methods of inoculation, rating scales, and environmental conditions make comparisons of these studies difficult.

Physiological specialization of *M. graminicola* has been reported by many authors (1,4,5,8,21) but remains controversial. A gene-for-gene interaction has been suggested in the wheat-*M. graminicola* pathosystem based on significant cultivar-isolate interactions (5,10,11). Recently, Kema et al. (13) found that avirulence in the pathogen was controlled by a single locus, which is also consistent with a gene-for-gene relationship. Despite evidence for cultivar-isolate interactions, no attempts have been made to understand the genetics of resistance in wheat to different races of *M. graminicola*. Western Canadian isolates of *M. graminicola* can be differentiated into two races based on the reaction of the

hexaploid wheat line ST6 (8). Race 1 is virulent on ST6, whereas race 2 is avirulent on ST6. The objective of this study was to determine the mode of inheritance of high level resistance derived from hexaploid wheat lines, Salamouni (University of Manitoba accession 6B359) and ST6 (cv. Estanzuela Federal), and a tetraploid cultivar, Coulter, to two races of *M. graminicola* from Manitoba.

MATERIALS AND METHODS

Population development. Intraspecific reciprocal crosses were made among the four hexaploid wheat lines and among the two tetraploid wheat lines shown in Table 1. Glassine bags were placed over emasculated wheat spikes for pollination control during crossing and to ensure complete self pollination of F₁, F₂, and BC₁F₁ plants. F₁ and F₂ generations were produced for all crosses. F₃, BC₁F₁, or BC₁F₂ generations were investigated depending on the number of genes segregating in a cross as determined by F₂ data. The susceptible parent was the recurrent parent in all backcrosses.

Seeds were germinated in the dark prior to planting (2 days 4°C, 2 days room temperature) to ensure uniform emergence and facilitate disease ratings. Five seedlings were planted per 15-cm-diameter pot containing a 2:1:1 soil mix (soil/sand/peat). Plants were fertilized weekly and watered as needed. Experiments containing F₂ or BC₁F₁ populations were kept in a growth room set at 21/19°C (day/night) with a 16-h photoperiod (250 µE m⁻² s⁻¹) prior to inoculation. Experiments containing F₃ or BC₁F₂ families were grown in a greenhouse prior to inoculation rather than in controlled environments because of space limitations in the latter. In the greenhouse, temperatures ranged from 25°C in the day to 18°C at night and supplemental lighting provided a 16-h photoperiod (260 µE m⁻² s⁻¹).

Due to space limitations of the humidity chamber, a total of 480 plants could be evaluated for reaction to *M. graminicola* in a single inoculation. Ten parental seedlings of all six wheat lines and 4 to 10 F₁ seedlings of the appropriate cross, and its reciproc-

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Publication no. P-2001-1217-02R

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cal, were included as inoculated controls during these inoculations. By this method, 400 to 410 seedlings of a segregating population were screened at a time. A single inoculation could test an entire F_2 population, more than one BC_1F_1 population, 20 F_3 families of 20 individuals, or 41 BC_1F_2 families of 10 individuals for reaction to a particular isolate of *M. graminicola*. For F_3 and BC_1F_2 family experiments, all the individuals of a particular family were challenged in a single inoculation to avoid variation between inoculations. However, different families of the same population were evaluated in different inoculations because of the space limitations stated previously. The inoculated controls verified the successful infection of the pathogen.

Inoculation. Two single-spore isolates of *M. graminicola* were used in the study, each being a representative member of the two races found in western Canada. Isolate MG96-36 was typical of race 1, and MG2 was typical of race 2 (8). Infected leaf tissue from Manitoba wheat fields was the original source of these cultures. Each isolate was derived from a single pycnidium (8). From each culture, single conidiospores were isolated from freshly inoculated yeast malt agar plates with a dissecting microscope. Inoculum was produced on yeast malt agar: 4 g of Difco yeast extract (Difco Laboratories, Detroit), 4 g of Difco malt extract, 4 g of sucrose (Fisher Scientific, Fair Lawn, NJ), 15 g of Difco agar, 250 mg of chloramphenicol (Sigma Chemical, St. Louis), and 1,000 ml of distilled water (6). Cultures were grown at room temperature under fluorescent lights for 7 days. Conidia were harvested by flooding cultures with sterile water and dislodging spores with a wire loop. The conidia suspension was poured through cheesecloth and adjusted to 10^7 spores per ml. Twenty drops of Tween 20 (polyoxyethylene sorbitan monolaurate) were added per liter of inoculum to reduce surface tension. Seedlings at the three-leaf stage were sprayed with the spore suspension until runoff with a DeVilbiss-type sprayer. Plants were incubated for 67 to 72 h under continuous leaf wetness provided by two ultrasonic humidifiers. Seedlings were then moved to a growth cabinet set at 21/19°C (day/night) with a 16-h photoperiod ($390 \mu E m^{-2} s^{-1}$) and relative humidity between 70 and 80%.

Disease assessment. Plants were evaluated for reaction to *M. graminicola* at 17 days after inoculation. At this time, there was maximum differentiation between the reactions of resistant and susceptible checks. Seedling reactions were scored by a qualitative disease rating scale developed by Rosielle (19), which was slightly modified. The modified scale accommodated the expanded chlorotic reactions of heterozygous individuals better than the original scale, which was developed to screen inbred wheat lines in the field. The modified scale is as follows: 0 = immune characterized by an absence of pycnidial formation, an occasional hypersensitive fleck, or no visible symptoms; 1 = highly resistant with hypersensitive flecking; 2 = resistant with small chlorotic or necrotic lesions, typically no pycnidial formation; 3 = intermediate characterized by coalescence of chlorotic or necrotic lesions normally evident toward the leaf tips and to a lesser extent elsewhere on the leaf blade, very light pycnidial formation; 4 = susceptible with moderate pycnidial formation, coalesced necrotic lesions; and 5 = very susceptible with large, abundant pycnidia, necrotic lesions extensively coalesced.

Reaction types 0 to 3 were considered resistant, whereas reaction types 4 and 5 were considered susceptible. Reaction type 3 was considered resistant because the growth and sporulation of the pathogen were severely restricted. The leaves of these plants had very few pycnidia and limited necrosis, two disease parameters commonly utilized to evaluate disease reaction (5,10,13). The chlorotic reaction of reaction type 3 was interpreted as a resistance reaction because of the similarity to the chlorotic blotches of reaction type 2. Chlorosis is recognized as a resistance reaction in other pathosystems such as the wheat-*Puccinia graminis* f. sp. *tritici* pathosystem (18). The large necrotic lesions with numerous pycnidia of reaction types 4 and 5 were considered susceptible. F_3

families were classified as homozygous resistant, segregating, or homozygous susceptible based on the reactions of 20 seedlings per family. BC_1F_1 -derived BC_1F_2 families were classified as segregating or homozygous susceptible based on 10 seedlings per family. The same BC_1F_2 families were evaluated for reaction to both isolates at the three-leaf stage. For a given BC_1F_2 family, 10 seedlings were inoculated with one isolate of *M. graminicola* and 10 different seedlings were inoculated with the other isolate. This allowed the investigation of the relationship between resistance genes to one isolate and the resistance genes to the other isolate. Data was tested for goodness of fit to specific genetic ratios using chi-square analysis (27). Yates correction factor was used where appropriate, and data from reciprocal crosses were tested for homogeneity prior to pooling data using a homogeneity chi-square test (27).

RESULTS

The reactions of the parental wheat lines are shown in Table 1. Salamouni had a reaction type of 0 in response to both isolates, indicated by the production of tiny flecks in response to isolates MG96-36 (Fig. 1A and G) and MG2 of *M. graminicola*. ST6 had a reaction type of 2 in response to inoculation with MG2, which was characterized by chlorotic blotches and no pycnidial formation (Fig. 1M). However, ST6 was rated as 5 when challenged by isolate MG96-36 (Fig. 1B). Coulter had a reaction type of 1, because it produced flecks in response to isolates MG2 (Fig. 1S) and MG96-36 that were slightly larger than the flecks of Salamouni. Katepwa, Erik, and 4B1149 were highly susceptible to both isolates and were scored as 5.

The reactions of F_1 populations to both isolates are shown in Table 2. F_1 seedlings of all resistant/susceptible crosses had a reaction type of 3. Such F_1 seedlings had coalesced, chlorotic lesions containing few pycnidia (Fig. 1C, I, O, and U). The large chlorotic lesions these F_1 seedlings produced were similar to the small chlorotic blotches on ST6 when it was inoculated with isolate MG2. These data suggested resistance is incompletely dominant because the resistant reaction of F_1 plants of resistant/susceptible crosses was not as strong as the resistant reaction of the resistant parent.

Reciprocal effects were not observed in the study allowing data from reciprocal crosses to be pooled. The F_2 populations of susceptible/susceptible crosses to a specific isolate did not segregate for disease reaction (Table 3). The disease reactions of the F_2 progeny of resistant/susceptible crosses included the reaction types of the parental lines and the reaction types between that of the parents. No progeny of a resistant/susceptible cross were more resistant than the resistant parent. Reaction type 3 was frequently observed in the segregating generations of resistant/susceptible crosses, whereas reaction type 4 was relatively infrequent relative to reaction types 3 or 5. Reaction types 2 and 3 were difficult to distinguish in resistant/susceptible F_2 populations because of the similarity of the symptoms. This was particularly true for crosses with more than one gene segregating. The homozygous resistant, segregating, and homozygous susceptible classes of F_3 and BC_1F_2 families were quite distinct. Resistance was controlled by incompletely dominant nuclear genes in all segregating crosses.

Salamouni. The reactions of parental, F_1 , and F_2 individuals of the crosses Salamouni/ST6 and Salamouni/Katepwa to isolate MG96-36 are shown in Figure 1A to F and G to L, respectively. The high level of resistance of Salamouni was recovered in the Salamouni/ST6 and Salamouni/Katepwa F_2 populations. Reaction type 3 in the F_1 and F_2 generations displayed chlorotic and necrotic lesions with very little pycnidial formation (Fig. 1C, E, I, and K). The susceptibility of ST6 and Katepwa to isolate MG96-36 was also recovered in the F_2 generation.

The F_2 populations from crosses between Salamouni and the different susceptible wheat genotypes tested with isolate MG2

could not be validly analyzed for goodness of fit because the number of susceptible individuals was too small (Table 3). When these crosses were backcrossed to the susceptible parent, the BC₁F₁ segregated in a 7:1 (resistant/susceptible) ratio for reaction to MG2 (Table 4), indicating three genes independently confer resistance to isolate MG2. The segregation ratios in the BC₁F₁-derived BC₁F₂ families fit a 7:1 (segregating/homozygous susceptible) ratio when inoculated with MG2 (Table 4), which is consistent with three incompletely dominant genes, each exhibiting dominant epistasis over the other resistance genes.

When inoculated with MG96-36, F₂ populations from the three Salamouni/susceptible crosses segregated in a 15:1 (resistant/susceptible) ratio (Table 3). This indicates Salamouni has two incompletely dominant genes displaying duplicate dominant epistasis to isolate MG96-36. The segregation ratios of the BC₁F₁ were 3:1 (resistant/susceptible) and the BC₁F₁-derived BC₁F₂ families were 3:1 (segregating/homozygous susceptible) with isolate MG96-36 (Table 4). This is also consistent with two independent resistance genes to isolate MG96-36.

The relationship between resistance genes to both isolates was investigated with BC₁F₂ families, which segregated in a 6:1:1 (segregating to MG2 and MG96-36/segregating to MG2, homozygous susceptible to MG96-36/homozygous susceptible to MG2 and MG96-36) ratio (Table 4). This indicates Salamouni has three resistance genes. Two of the resistance genes confer resistance to both isolates and the third gene is only effective against MG2.

ST6. The reactions of parental, F₁, and F₂ individuals of the cross ST6/Katepwa to isolate MG2 are shown in Figure 1M to R. Resistant and susceptible reaction types equivalent to that of the parental genotypes were observed in the F₂ generation. Reaction type 3 was observed in the F₁ and F₂ generations. The chlorotic reactions of reaction types 2 and 3 were similar (Fig. 1M and O to Q). Seedlings of these reaction types restrict the production of asexual fruiting bodies of the pathogen.

F₂ populations involving ST6 and a susceptible wheat genotype segregated in a 3:1 (resistant/susceptible) ratio when inoculated with isolate MG2 (Table 3), which is consistent with a single gene segregating in these crosses. This was supported by F₂-derived F₃ families segregating in a 1:2:1 (homozygous resistant/segregating/homozygous susceptible) ratio (Table 3). The backcrosses of the F₁ to the susceptible parents segregated in a 1:1 (resistant/susceptible) ratio for reaction to isolate MG2 (Table 4). These data indicate ST6 has a single incompletely dominant gene conferring resistance to isolate MG2.

The accuracy of the disease rating scale to correctly classify F₂ individuals was investigated by comparing the data of 310 F₃ families of the ST6/Katepwa and ST6/Erik populations to the corresponding rating of its F₂ parent. In total, 94.8% of F₂ plants rated 4 or 5 were confirmed homozygous susceptible by their corresponding F₃ families. In total, 96.6% of F₂ plants rated 2 or 3 were confirmed homozygous resistant or heterozygous based

on the reaction of their corresponding F₃ families. However, only 67.1% of F₂ individuals rated as 2 were homozygous resistant based on the F₃ family data. Therefore, the rating scale can accurately detect homozygous susceptible individuals in these F₂ populations but cannot accurately differentiate between homozygous resistant individuals and heterozygous individuals. These data support treating reaction type 3 as resistant for data analysis.

ST6 was susceptible to isolate MG96-36 and acted the same as the other susceptible parental genotypes in crosses tested for reaction to MG96-36 (Tables 3 and 4).

Coulter. Figure 1S to X displays the reactions of parental, F₁, and F₂ individuals of the cross Coulter/4B1149 to isolate MG2. The high level of resistance of Coulter was recovered in the F₂ generation, as was the susceptibility of 4B1149. Reaction type 3, characterized by large chlorotic and necrotic lesions with restricted pycnidial formation, was observed in the Coulter/4B1149 F₁ and F₂ generations.

The F₂ population of Coulter/4B1149 tested with isolate MG2 fit a 15:1 (resistant/susceptible) ratio (Table 3), indicating two genes confer resistance to MG2 and function independently of each other. For the backcross to 4B1149 tested with MG2, the BC₁F₁ fit a 3:1 (resistant/susceptible) ratio and the BC₁F₁-derived BC₁F₂ families fit a 3:1 (segregating/homozygous susceptible) ratio (Table 4). This indicates that two incompletely dominant resistance genes control resistance to isolate MG2 in Coulter.

When inoculated with isolate MG96-36, the Coulter/4B1149 F₂ population fit a 3:1 (resistant/susceptible) ratio (Table 3), indicating that a single gene controls resistance to isolate MG96-36. The BC₁F₁ segregated in a 1:1 (resistant/susceptible) ratio (Table 4), and the BC₁F₁-derived BC₁F₂ families fit a 1:1 (segregating/homozygous susceptible) ratio (Table 4). Therefore, one incompletely dominant gene controls resistance to MG96-36.

The relationship between the resistance genes to isolates MG2 and MG96-36 was investigated with progeny tests of the BC₁F₁. The BC₁F₁-derived BC₁F₂ families segregated in a 2:1:1 (segregating to MG2 and MG96-36/segregating to MG2, homozygous susceptible to MG96-36/homozygous susceptible to MG2 and MG96-36) ratio (Table 4). This indicates that a single gene confers resistance to both isolates MG96-36 and MG2, and a second gene confers resistance specific to isolate MG2 in Coulter.

Resistant/resistant. Salamouni/ST6 was evaluated for reaction to isolate MG2 to test for allelism of resistance genes in these wheat lines. The F₁ generation had a reaction type of 2 to 3 to isolate MG2 (Table 2). The F₂ population of this cross segregated in reaction to isolate MG2 because two susceptible individuals were identified (Table 3). Poor seed set did not allow for progeny tests. However, susceptible individuals were identified in the F₃ generation with available seed. Segregation in this cross indicates the three resistance genes in Salamouni cannot be allelic to the resistance gene in ST6.

TABLE 1. Reactions of parental wheat lines to isolates MG2 and MG96-36 of *Mycosphaerella graminicola*

Wheat line	Ploidy	MG2 ^a						MG96-36 ^a					
		0	1	2	3	4	5	0	1	2	3	4	5
Salamouni	Hexaploid	330 ^b	0	0	0	0	0	170	0	0	0	0	0
ST6	Hexaploid	0	0	324	6	0	0	0	0	0	0	3	167
Katepwa	Hexaploid	0	0	0	0	31	298	0	0	0	0	0	170
Erik	Hexaploid	0	0	0	0	31	299	0	0	0	0	30	140
Coulter	Tetraploid	0	327	0	0	0	0	0	166	1	0	0	0
4B1149	Tetraploid	0	0	0	0	34	296	0	0	0	0	19	151

^a Reaction types 0 to 5 (0 = immune characterized by an absence of pycnidial formation, an occasional hypersensitive fleck, or no visible symptoms; 1 = highly resistant with hypersensitive flecking; 2 = resistant with small chlorotic or necrotic lesions, typically no pycnidial formation; 3 = intermediate characterized by coalescence of chlorotic or necrotic lesions normally evident toward the leaf tips and to a lesser extent elsewhere on the leaf blade, very light pycnidial formation; 4 = susceptible with moderate pycnidial formation, coalesced necrotic lesions; and 5 = very susceptible with large, abundant pycnidia, necrotic lesions extensively coalesced) in which 0 to 3 are resistant and 4 and 5 are susceptible.

^b Number of individuals with corresponding reaction type.

DISCUSSION

The inheritance of race-specific resistance to *M. graminicola* in bread and durum wheat has not been previously reported. The data presented in this paper indicate that the number of genes controlling resistance to *M. graminicola* is dependent on the resistance source and the isolate of *M. graminicola* used to evaluate the cross. To our knowledge, the qualitative inheritance of resistance to *M. graminicola* has not been previously reported in *T. turgidum* subsp. *durum*. The present results are consistent with previous findings of qualitative inheritance of resistance to STB in *T. aestivum* (15,20,26,31,32). Other authors have reported STB resistance as a quantitative trait in bread and durum wheats (9,25, 28,29). Differing reports on the mode of inheritance of STB resistance are likely due to different resistance sources and disease assessment methods in these studies. In addition, some of the previous genetic studies may have been confounded by natural inoculum in field experiments or by the utilization of isolate mixtures as the inoculum to screen segregating populations. The results of this study illustrate this problem because both of the races of *M. graminicola* are common in western Canada (8).

The reaction types of the resistant parental wheat lines were between 0 to 2 in this study. However, the number of resistance genes in the host did not affect the degree of resistance expressed. Coulter displayed reaction types of 1 to isolates MG2 and MG96-36, even though Coulter has two resistance genes to isolate MG2 and only one to MG96-36. Likewise, Salamouni displayed reaction types of 0 to both isolates despite Salamouni having three resistance genes to isolate MG2 and two genes to MG96-36. The degree of resistance expressed is likely due to the resistance gene involved in the incompatible interaction, the genetic background in which the gene is present, or a combination of both.

F₁ plants of resistant/susceptible crosses displayed reaction type 3 and the resistant/resistant cross had a reaction type of 2 to 3. Reaction type 3 appears to be associated with heterozygous individuals, because the number of genetic loci conferring resistance does not appear to affect reaction type as discussed previously. Utilizing doubled haploid (DH) or recombinant inbred line (RIL) populations could simplify disease scoring in subsequent genetic studies of STB resistance in wheat. Because these populations consist of only homozygous individuals, they should be easy to score for disease reaction. If creating DH or RIL populations is impractical, F₁ plants should be included as inoculated controls when screening segregating populations with heterozygous individuals, particularly F₂ and BC₁F₁ populations. F₁ plants may not be needed as controls when conducting progeny tests, such as F₃ families. The reaction of an F₃ family is based on a group of plants, making scoring easier than using single F₂ individuals.

Kema and van Silfhout (12) found significant differences in the reactions of wheat lines at seedling and adult plant stages to two of three *M. graminicola* isolates, indicating differential expression of resistance at the seedling and adult plant stages. However, other studies have found good correlation between seedling and adult plant reactions (4,30). Somasco et al. (26) reported that the resistance gene *Stb4* is likely active at all plant stages because of high positive correlation between seedling and adult plant disease scores. Segregating populations were not evaluated at the adult plant stage in this study. However, the reactions of Salamouni, ST6, Erik, Coulter, and 4B1149 (Katepwa was not investigated) to isolates MG96-36 and MG96-W were the same at seedling and adult stages (8). Isolate MG2 was not investigated at the adult plant stage, however MG96-W and MG2 both belong to race 2 and were isolated from Manitoba fields. These data suggest that the resistance genes identified in this study may be effective at both plant stages.

TABLE 2. Reactions of F₁ populations to isolates MG2 and MG96-36 of *Mycosphaerella graminicola*

Cross ^a	MG2 ^b						MG96-36 ^b					
	0	1	2	3	4	5	0	1	2	3	4	5
Salamouni/ST6	0 ^c	0	34	16	0	0	0	0	0	40	0	0
Salamouni/Katepwa	0	0	0	60	0	0	0	0	0	59	0	0
Salamouni/Erik	0	0	0	58	0	0	0	0	0	60	0	0
ST6/Katepwa	0	0	0	119	0	0	0	0	0	0	0	20
ST6/Erik	0	0	0	88	0	0	0	0	0	0	0	20
Katepwa/Erik	0	0	0	0	8	32	0	0	0	0	0	40
Coulter/4B1149	0	0	1	15	0	0	0	0	0	24	0	0

^a Pooled data of reciprocal crosses.

^b Reaction types 0 to 5 (0 = immune characterized by an absence of pycnidial formation, an occasional hypersensitive fleck, or no visible symptoms; 1 = highly resistant with hypersensitive flecking; 2 = resistant with small chlorotic or necrotic lesions, typically no pycnidial formation; 3 = intermediate characterized by coalescence of chlorotic or necrotic lesions normally evident toward the leaf tips and to a lesser extent elsewhere on the leaf blade, very light pycnidial formation; 4 = susceptible with moderate pycnidial formation, coalesced necrotic lesions; and 5 = very susceptible with large, abundant pycnidia, necrotic lesions extensively coalesced) in which 0 to 3 are resistant and 4 and 5 are susceptible.

^c Number of individuals with corresponding reaction type.

TABLE 3. Segregation of F₂ populations and F₂-derived F₃ families for reaction to isolates MG2 and MG96-36 of *Mycosphaerella graminicola*

Cross ^a	Generation	MG2			MG96-36		
		Observed ^b	Expected ratio	$\chi^2(P)^c$	Observed ^b	Expected ratio	$\chi^2(P)^c$
Salamouni/ST6	F ₂	798:2	...	...	373:24	15:1	0.004 (0.95)
Salamouni/Katepwa	F ₂	399:1	...	...	380:19	15:1	1.26 (0.26)
Salamouni/Erik	F ₂	395:4	...	...	379:21	15:1	0.52 (0.47)
ST6/Katepwa	F ₂	297:103	3:1	0.08 (0.77)	0:399	0:1	...
ST6/Katepwa	F ₃	42:102:56	1:2:1	2.04 (0.36)	...	...	...
ST6/Erik	F ₂	302:97	3:1	0.07 (0.79)	0:400	0:1	...
ST6/Erik	F ₃	28:57:25	1:2:1	0.31 (0.86)	...	...	...
Katepwa/Erik	F ₂	0:400	0:1	...	0:400	0:1	...
Coulter/4B1149	F ₂	356:27	15:1	0.29 (0.59)	291:88	3:1	0.55 (0.46)

^a Pooled data of reciprocal crosses.

^b Resistant/susceptible for F₂ data; homozygous resistant/segregating/homozygous susceptible for F₃ data.

^c χ^2 corrected with Yates correction factor where appropriate. A fit to the expected ratio is accepted if $P > 0.05$.

Fig. 1. Reactions of different populations to isolate MG2 or MG96-36 of *Mycosphaerella graminicola*. **A to F**, Reaction of individuals in the Salamouni/ST6 population to isolate MG96-36. **A**, Reaction type 0 (0 = immune characterized by an absence of pycnidial formation, an occasional hypersensitive fleck, or no visible symptoms) of Salamouni. **B**, Reaction type 5 (5 = very susceptible with large, abundant pycnidia, necrotic lesions extensively coalesced) of ST6. **C**, Reaction type 3 (3 = intermediate characterized by coalescence of chlorotic or necrotic lesions normally evident toward the leaf tips and to a lesser extent elsewhere on the leaf blade, very light pycnidial formation) of Salamouni/ST6 F₁. **D, E, and F**, Reaction types 1, 3, and 5 of Salamouni/ST6 F₂ individuals, respectively. **G to L**, Reaction of individuals in the Salamouni/Katepwa population to isolate MG96-36. **G**, Reaction type 0 of Salamouni. **H**, Reaction type 5 of Katepwa. **I**, Reaction type 3 of Salamouni/Katepwa F₁. **J, K, and L**, Reaction types 0, 3, and 5 of Salamouni/Katepwa F₂ individuals, respectively. **M to R**, Reaction of individuals in the ST6/Katepwa population to isolate MG2. **M**, Reaction type 2 (2 = resistant with small chlorotic or necrotic lesions, typically no pycnidial formation) of ST6. **N**, Reaction type 5 of Katepwa. **O**, Reaction type 3 of ST6/Katepwa F₁. **P, Q, and R**, Reaction types 2, 3, and 5 of ST6/Katepwa F₂ individuals, respectively. **S to X**, Reaction of individuals in the Coulter/4B1149 population to isolate MG2. **S**, Reaction type 1 (1 = highly resistant with hypersensitive flecking) of Coulter. **T**, Reaction type 5 of 4B1149. **U**, Reaction type 3 of Coulter/4B1149 F₁. **V, W, and X**, Reaction types 1, 3, and 5 of Coulter/4B1149 F₂ individuals, respectively.

TABLE 4. Segregation of BC₁F₁ populations and BC₁F₁-derived BC₁F₂ families for reaction to isolates MG2 and MG96-36 of *Mycosphaerella graminicola*

Cross ^a	Generation	MG2			MG96-36			MG2 and MG96-36		
		Observed ^b	Expected ratio	$\chi^2(P)^c$	Observed ^b	Expected ratio	$\chi^2(P)^c$	Observed ^b	Expected ratio	$\chi^2(P)^c$
Salamouni/ST6//ST6	BC ₁ F ₁	...	...	...	151:49	3:1	0.01 (0.93)	...	...	...
Salamouni/Katepwa//Katepwa	BC ₁ F ₁	159:23	7:1	0.00 (1.00)	68:22	3:1	0.00 (1.00)	...	...	...
Salamouni/Katepwa//Katepwa	BC ₁ F ₂	68:14	7:1	1.18 (0.28)	58:24	3:1	0.59 (0.44)	58:10:14	6:1:1	1.58 (0.45)
Salamouni/Erik//Erik	BC ₁ F ₁	171:25	7:1	0.00 (1.00)	74:22	3:1	0.13 (0.72)	...	...	...
Salamouni/Erik//Erik	BC ₁ F ₂	75:7	7:1	0.84 (0.36)	60:22	3:1	0.07 (0.80)	60:15:7	6:1:1	3.27 (0.20)
ST6/Katepwa//Katepwa	BC ₁ F ₁	57:58	1:1	0.00 (1.00)	...	...	...	...	...	...
ST6/Erik//Erik	BC ₁ F ₁	47:52	1:1	0.16 (0.69)	...	...	...	...	...	...
Coulter/4B1149//4B1149	BC ₁ F ₁	54:23	3:1	0.73 (0.39)	36:33	1:1	0.06 (0.81)	...	...	...
Coulter/4B1149//4B1149	BC ₁ F ₂	35:17	3:1	1.26 (0.26)	28:24	1:1	0.17 (0.68)	28:7:17	2:1:1	4.15 (0.13)

^a Pooled data of reciprocal crosses.^b Resistant/susceptible for BC₁F₁ data; segregating/homozygous susceptible for BC₁F₂ data with two classes; and segregating to MG2 and MG96-36/segregating to MG2, homozygous susceptible to MG96-36/homozygous susceptible to MG2 and MG96-36 for BC₁F₂ data with three classes.^c χ^2 corrected with Yates correction factor where appropriate. A fit to the expected ratio is accepted if $P > 0.05$.

The present results are consistent with physiological specialization of *M. graminicola* and a gene-for-gene interaction in the wheat-*M. graminicola* pathosystem. In gene-for-gene systems, only a single incompatible interaction is required to provide the host resistance to the pathogen. The present data indicate that only a single resistance gene is needed to confer resistance to the host. Each resistance gene exhibited dominant epistasis over the other resistance genes effective against a particular isolate. In addition, in gene-for-gene systems, resistance genes are race-specific due to the presence or absence of avirulence genes in different races of the pathogen. In this study, three of the six resistance genes were effective against only one of the two races identified in Manitoba. The resistant/resistant cross, Salamouni/ST6, segregated for disease reaction, which can also be observed in gene-for-gene systems. Additionally, data from other authors are consistent with a gene-for-gene interaction. Kema et al. (13) demonstrated that avirulence is controlled by a single genetic locus in *M. graminicola* by crossing virulent and avirulent isolates. Changes in the virulence of field populations of *M. graminicola* have also been reported. The breakdown of resistance has occurred in cvs. Florence-Aurore in Israel (4) and Heron and Robin in Australia (1). Conversely, Etit 38 became resistant to field isolates after it was no longer commonly grown in Israel (4).

A solid understanding of host-pathogen interactions is critical to design an efficient breeding program for disease resistance. Incorporating the ST6 resistance gene into a Canadian variety would not provide effective resistance because a portion of the pathogen population is virulent on ST6 (8). Further efforts need to be taken to develop a differential set of wheat lines to survey *M. graminicola* populations for avirulence/virulence genes. This will allow wheat breeders to select appropriate resistance genes based on the avirulence/virulence genes present in the local *M. graminicola* population. The construction of a differential set will require genetic studies to elucidate the relationships between different resistance sources. Wilson (32) identified three unlinked loci for STB resistance in hexaploid wheat, *Stb1*, *Stb2*, and *Stb3*. A fourth gene, *Stb4*, has been proposed (26) based on independent assortment with *Stb1*, but its relationship with *Stb2* and *Stb3* is unknown. Efforts need to be taken to determine the relationship between these resistance genes and the four unlinked genes among Salamouni and ST6. The relationship between the resistance genes in *T. aestivum* and *T. turgidum* subsp. *durum* is also unknown.

Genetic studies of avirulence in the pathogen will be useful in understanding the interactions in the wheat-*M. graminicola* pathosystem. Kema et al. (13) reported that avirulence segregated as a single locus when evaluated on resistant lines Shafir, Kavkaz, and Veranopolis. More research is needed to determine whether all avirulence genes are located at this locus or if they are scattered throughout the genome. This is important because sexual

recombination of *M. graminicola* commonly occurs in the United States (16) and the sexual state has been identified on different continents (2,7,22). The development of new races through sexual recombination may be hindered if avirulence genes are linked, thereby prolonging the effectiveness of resistance genes.

The results of this study indicate that resistance to *M. graminicola* is qualitative in the bread and durum wheat resistance sources tested. Resistance was controlled by incompletely dominant genes, each exhibiting dominant epistasis over the other resistance genes. The number of resistance genes segregating in a cross depended on the resistance source and the race used to test for reaction to *M. graminicola*. Race-specific resistance genes were identified in this study. This is consistent with a gene-for-gene system.

ACKNOWLEDGMENTS

We thank the Natural Sciences and Engineering Research Council of Canada, Western Grains Research Foundation, Agriculture and Agri-Food Canada, and the Canadian Wheat Board for their financial support.

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