The Residual Effects of Some "Defeated" Powdery Mildew Resistance Genes in Isolines of Winter Wheat

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ABSTRACT

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Six near-isogenic winter wheat lines (isolines), each possessing a different powdery mildew resistance gene, were evaluated for their potential residual effects against an isolate of *Erysiphe graminis* f. sp. *tritici* possessing all the virulence genes needed to overcome the six resistance genes. The infection efficiency, disease efficiency, and sporulation capacity of the isolate on each of the isolines were assessed relative to the susceptible recurrent parent, Chancellor winter wheat. The isolines with resistance genes *Pm*3c, *Pm*4, and a gene known as Michigan Amber (MA) demonstrated significant residual effects (relative to Chancellor). Mean number of sporulating colonies was dramatically less on the *Pm*3c, *Pm*4, and MA isolines than on Chancellor (32, 40, and 65% fewer spores, respectively). No statistically significant residual effects were obtained for resistance genes *Pm*2, *Pm*2+, and *Pm*5. Prevailing resistance theory assumes that "defeated" single, major resistance genes are of no value when confronted with a pathogen genotype possessing the matching virulence genes. The present study demonstrates that some defeated major resistance genes have measurable residual ability to restrict disease increase and disease severity.

Addtional key words: disease resistance.

Single-gene resistance has dominated plant disease control through breeding for nearly 75 yr, primarily because of the ease of incorporating single genes into acceptable plant types and the dramatic disease reduction achieved. Single resistance genes are usually effective against some but not all races and are commonly called race-specific. Their dramatic ability to confine incompatible races of the pathogen to very small infection sites probably explains why race-specific genes are described as "major" resistance genes.

Vanderplank (17,18) characterized disease resistance in epidemiologic terms. He described race-specific, major gene resistance as vertical resistance and identified the role of vertical genes as that of reducing the effective initial inoculum, termed X_0. Race-specific resistance genes play no role in moderating disease increase after infections have taken place (17,18). Vanderplank depicted nonrace-specific, "minor" gene resistance as horizontal

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resistance and characterized the role of horizontal resistance genes as that of reducing the rate of disease increase as measured by the apparent infection rate, termed *r*.

Flor's gene-for-gene concept (7) clearly identifies single, major resistance genes qualitatively as either extremely effective or totally ineffective. The specificity of major, qualitative resistance genes and the periodic arrival of pathogen populations with virulence genes to overcome them have sent many such genes into oblivion. The tendency to discard "defeated" resistance genes, presuming that they are no longer of any value at all, seems implicit in this approach. The relative ease, in most cases, of finding another effective resistance gene to replace the defeated gene has created a tendency to give little thought to the departed gene.

Nelson et al (14), with further elaboration by Nelson (11–13), proposed that race-specific, major gene resistance and nonrace-specific, minor gene resistance are conditioned by the same genes; ie, there are no major or minor genes. The two main kinds of disease resistance are not indications of the action of different genes, but rather expressions of different actions of the same genes in different genetic backgrounds or confronted by different pathogen genotypes. This theory implies that genes are characterized as major when the host response reveals a race-specific reaction, while the same genes are characterized as minor when the host response suggests a nonspecific or rate-reducing type of resistance.

Many wild species of plants and their pathogens exist in apparent genetic equilibrium, with the host reaction indicating a rate-reducing form of resistance. Nelson theorized (11–13) that such equilibrium was achieved through a stepwise accumulation of resistance genes and virulence genes over time and that the resistance genes functioned as race-specific genes until they were defeated by a new race of the pathogen. The archaic major genes then additively confer a nonspecific, rate-reducing resistance.

Recently, other researchers have had similar or related thoughts on the presumed singular role of race-specific, major genes for disease resistance. Riley (16) asked, "Do major genes for resistance that have been overcome by virulence changes in the fungus contribute to quantitative resistance?" Clifford (4) stated, "It has been suggested, for example, that polygenes are archaic major genes which have lost their large effect through the evolution of virulence in the pathogen but which have a residual or 'ghost' effect." Eenink (5) wrote, "Consequently, neither in genotypes nor in phenotypes can essential differences be shown to exist between uniform and differential resistance." Similar thoughts have been expressed by Hayes (8), Abdalla and Hermsen (1), Arnold and Brown (2), and Ellingboe (6).

Martin and Ellingboe (9) reported a most significant discovery regarding the quantitative effect of a qualitative gene. Working with the powdery mildew-winter wheat model, they demonstrated that a race-specific mildew resistance gene, *Pm*4, reduced the infection efficiency of a powdery mildew isolate with the matching virulence gene, P4, compared with the same isolate on a near-isogenic wheat line containing the recessive allele *pm*4.

We have been working with the winter wheat-powdery mildew system to evaluate the relative merits of genetic strategies designed to manage plant diseases at some acceptable level. Having access to the near-isogenic winter wheat lines developed by Briggle (3) in a Chancellor background, each containing a single, different powdery mildew resistance gene, we have bred two four-gene pyramids to test for the benefits of several defeated resistance genes. If the pryamid *Pm* resistance genes do cause a slow-mildewing relationship with a compatible pathogen genotype, each *Pm* gene will have to be evaluated individually to ascertain its contribution.

The objective of the present research was to evaluate six near-isogenic winter wheat lines with different *Pm* genes for residual effects against a powdery mildew isolate possessing all virulence genes needed to overcome the six *Pm* resistance genes.

MATERIALS AND METHODS

The pathogen. One single-spore isolate of *Erysiphe graminis* DC. f. sp. *tritici* E. Marchal, designated isolate 144 in our program, was used throughout. The susceptible reactions of the powdery mildew near-isogenic lines (isolines) revealed that isolate 144 possessed known virulence genes to overcome the resistance genes *Pm*2, *Pm*2+, *Pm*3c, *Pm*4, *Pm*5, and a resistance gene known as Michigan Amber (MA). The isolate was maintained at 2–4 C on infected Chancellor wheat plants growing in vermiculite in large test tubes closed with cotton plugs.

Inoculum was obtained by inoculating 10-day-old wheat seedlings growing in sterilized soil in clay pots. To protect the plants from contamination, lamp chimneys were placed in each pot at the time of planting and covered with filter paper. To give seeds and the resulting plants water, the pots were placed in a water-filled pan. The plants were inoculated by shaking infected leaves inside the chimneys. Inoculated plants were placed in a growth chamber at 20 ± 2 C for 7–8 days to obtain an ample amount of conidia for subsequent inoculation.

The host. Six near-isogenic lines carrying the resistance genes *Pm*2, *Pm*2+, *Pm*3c, *Pm*4, *Pm*5, or MA were used. Each isoline was produced from eight backcrosses to the recurrent parent Chancellor. The CI (USDA Cereal Introduction) number and the donor source of the resistance genes are as follows: *Pm*2 (CI 14118, Ulka); *Pm*2+ (CI 14119, CI 12632); *Pm*3c (CI 14122, Sonora); *Pm*4 (CI 14123, Khapli); *Pm*5 (CI 14125, Hope); and MA (CI 14033, Michigan Amber). To identify residual effects of these resistance genes, the reactions of the isolines were compared with that of Chancellor wheat.

Inoculation. Seeds from Chancellor and the six isolines were planted in 12 × 16.5 × 6 cm plastic trays containing a mixture of soil, perlite, and peat moss (1:1:1). Seeds were planted in a single row parallel to the longest side of the tray. For the relative disease efficiency studies, five seeds each of Chancellor and one of the isolines were planted in the row. For the sporulation studies, 10 seeds of Chancellor or one of the isolines were planted in the row.

The trays were placed in larger trays containing water and then in a growth chamber at 20 ± 2 C with 16 hr of light (861 lux) and 8 hr of darkness for 2 wk. The primary leaf of each 14-day-old seedling was draped over an 8 × 15 × 10 cm wire grid, exposing 8 cm of the leaf's adaxial surface. To hold the leaf in position, the tip of the leaf was weighted with a paper clip.

The trays were then placed in a simplified version of the Melching (10) settling or inoculation tower, and the plants were inoculated with conidia produced on Chancellor plants maintained under lamp chimneys. The quantity of inoculum was adjusted by placing leaves with varying numbers of sporulating colonies in the inoculation funnel. An attempt was made to synchronize the age of the conidia by shaking older conidia from the colonies 24 hr earlier. Short blasts of nitrogen gas (maximum 10 lb/in.^{2}) were used to blow the conidia from the colonies. Conidia were allowed to settle on the leaves of the test plants. During the inoculation process, the trays were rotated on a turntable at 4–5 m to permit a more uniform deposition of inoculum. Petri plates containing water agar were placed at fixed positions on the turntable to determine uniformity and rate of deposition. The viability of the conidia deposited in the plates was determined by counting the number of conidia deposited per square centimeter after 24 hr. The plants were then placed in a growth chamber at 20 ± 2 C for the duration of the particular study.

Relative disease efficiency. The mean numbers of sporulating colonies produced per leaf on Chancellor and the six isolines were recorded as a measure of relative disease efficiency. Each experiment had three replicates (trays containing both Chancellor and isoline plants).

Sporulation. Six days after inoculation, sporulation data were obtained daily by removing the conidia by vacuum suction from all colonies on the 8-cm section of adaxial leaf surface. A bent glass tube, functioning as a nozzle, was inserted through a rubber stopper into the collection tube. Another glass tube, functioning as an exhaust outlet, was inserted through the rubber stopper and connected to a vacuum pump with a rubber hose. The collection tube contained 5 ml of 1.0% NaCl solution containing 0.1% Tween 80. The resulting spore suspensions were adjusted to a volume of 50 ml with a 1.0% NaCl solution, and 0.5-ml aliquots were counted with a Model Zb Coulter counter (Coulter Electronics Industrial

Division, Franklin Park, IL 60131). The number of conidia per aliquot was converted to the mean number of conidia per colony per day. Spore harvests were discontinued after 8 days because secondary colonies formed around the primary colonies on Chancellor leaves.

RESULTS

Relative disease efficiency. The isolines with powdery mildew resistance genes *Pm*4, *Pm*3c, and MA sustained significantly fewer sporulating mildew colonies than Chancellor when inoculated with conidia of isolate 144 of *E. graminis* f. sp. *tritici* (Table 1). Powdery mildew resistance genes *Pm*2, *Pm*2+, and *Pm*5 were not statistically different from Chancellor in restricting colony formation.

Relative infection efficiency. During one of the early experiments of this research, we observed numerous minute yellow spots or points on the leaves of Chancellor and all the near-isogenic lines within 24 hr of inoculation. Most of the spots disappeared on the near-isogenic lines carrying resistance genes *Pm*4, *Pm*3c, and MA by the time sporulating colonies developed. However, the number of sporulating colonies on Chancellor and the near-isogenic lines *Pm*2, *Pm*2+, and *Pm*5 appeared to be similar to the number of yellow spots that had been observed several days earlier.

To investigate this phenomenon, several plants of Chancellor and some of the near-isogenic lines were inoculated with conidia of isolate 144. Several leaves of each line were excised when the yellow spots appeared. The leaves were cleared in boiling lactophenol plus cotton blue, rinsed in running water, and examined under the microscope. We observed that the germinated conidia had produced small, branched, elongating secondary hyphae. Martin and Ellingboe (9) considered the development of elongating secondary hyphae an indication of successful penetration and infection. Accordingly, in two later experiments designed to evaluate the relative infection efficiency of Chancellor and the near-isogenic lines, the appearance of the minute yellow spots was used to evaluate successful penetrations and infections (Table 2). The results suggest that powdery mildew resistance genes *Pm*3c, *Pm*4, and MA may activate some resistance mechanism shortly after the initial host-parasite interaction, although this is only speculation.

Sporulation. Four separate experiments were made to determine the total number of spores per lesion produced by isolate 144 on Chancellor and the six isolines (Table 3). Sporulation on the *Pm*2, *Pm*2+, and *Pm*5 isolines consistently resembled that on Chancellor. A statistical analysis of data using all four experiments as replicates showed that isolate 144 produced significantly fewer spores per lesion on isolines *Pm*3c, *Pm*4, and MA than on Chancellor. The fewest spores per lesion were consistently produced on the *Pm*4 isoline.

The sporulation experiments were discontinued after eight daily spore collections because small secondary lesions appeared around the initial colonies on leaves of Chancellor winter wheat. However, colonies on leaves of Chancellor appeared to consistently produce spores for a longer time than did colonies on isoline *Pm*4, particularly when Chancellor leaves sustained relatively few colonies.

The number of spores produced on the first day of sampling differed greatly between Chancellor and the MA isoline. In experiment 2, for example, no spores were obtained from colonies on the 16 leaves of the MA isoline on the first sampling date, while 15,278 spores were collected from 15 Chancellor leaves. In the three

TABLE 1. Mean number of sporulating colonies produced by isolate 144 of *Erysiphe graminis* f. sp. *tritici* on Chancellor winter wheat and five near-isogenic lines carrying known powdery mildew (*Pm*) resistance genes

Wheat line	Experiment 1			Experiment 2		
	Mean[a]	s.d.[b]	Mean difference[c]	Mean[a]	s.d.[b]	Mean difference[c]
Chancellor	27.6	13.2	6.7 *	63.8	6.0	14.4*
*Pm*4 isoline	1.7	1.8		9.5	2.8	
Chancellor	32.6	9.6	10.38*	59.4	7.1	15.6*
*Pm*3c isoline	2.8	4.0		5.6	3.1	
Chancellor	27.0	9.4	8.8 *	56.7	19.9	7.1*
MA[d] isoline	2.4	2.1	3.3	2.1		
Chancellor	18.1	9.0	0.1	62.0	7.5	0.3
*Pm*5 isoline	17.8	7.8		54.3	10.7	
Chancellor	26.0	14.5	0.9	58.1	6.2	0.3
*Pm*2 isoline	21.6	9.9		57.2	9.0	

[a] Average of three replicates, with four leaves per replicate.
[b] Standard deviation.
[c] Differences between means marked with an asterisk are significant at the 5% level by Student's paired *t*-test.
[d] Michigan Amber gene.

TABLE 2. Number of successful infections and resulting number of sporulating colonies produced by isolate 144 of *Erysiphe graminis* f. sp. *tritici* on Chancellor winter wheat and six near-isogenic lines carrying known powdery mildew (*Pm*) resistance genes

Wheat line	Experiment 1			Experiment 2		
	Infections	Colonies	Leaves	Infections	Colonies	Leaves
Chancellor	261	253	14	578	566	20
*Pm*2 isoline	29	24	4	226	221	8
*Pm*2+ isoline	406	381	12	966	940	21
*Pm*5 isoline	244	239	17	229	216	17
*Pm*4 isoline	201	96	11	241	96	20
*Pm*3c isoline	150	46	12	226	73	21
MA[a] isoline	129	56	14	203	79	24

[a] Michigan Amber gene.

TABLE 3. Mean number of spores produced per lesion by isolate 144 of *Erysiphe graminis* f. sp. *tritici* on Chancellor winter wheat and six near-isogenic lines carrying known powdery mildew (*Pm*) resistance genes[w]

Wheat line	Experiment[x]				Combined analysis[x]
	1	2	3	4	
Chancellor	72,521 abc	82,381 a	82,966 a	56,262 a	73,783 a
*Pm*2 isoline	92,600 a	71,474 ab	78,289 a	59,275 a	75,410 a
*Pm*5 isoline	87,191 ab	64,153 b	70,033 a	47,339 a	67,179 a
*Pm*2+ isoline	73,406 ab	54,637 bc	68,041 a	53,906 a	62,511 ab
*Pm*3c isoline	57,592 bc	51,654 bc	36,648 b	57,009 a	50,728 bc
MA[y] isoline	...[z]	46,689 c	40,531 b	47,490 a	44,903 c
*Pm*4 isoline	22,725 d	30,335 d	30,215 d	18,557 d	25,458 d

[w] Data are averages of three replicates. Spores were harvested from lesions on one leaf at a time. Daily spore collections were discontinued after 8 days because of the appearance of secondary lesions on Chancellor plants.
[x] Means within a column followed by a common letter are not significantly different at the 5% level, according to Duncan's multiple range test.
[y] Michigan Amber gene.
[z] Not included.

experiments investigating sporulation on MA, only 1,578 spores were collected from colonies on 43 leaves on the first sampling day, while 25,113 spores were collected from 39 Chancellor leaves. A total of 6,780 spores were collected from 36 leaves of isoline *Pm*4 on the first sampling date, and 8,201 spores were harvested from 32 leaves of isoline *Pm*3c. Forty of the 111 test leaves of isolines *Pm*3c, *Pm*4, and MA produced no spores on the first sampling date. Only 3 of 126 leaves of isolines *Pm*2, *Pm*2+, and *Pm*5 failed to produce spores on the first sampling date. Although no attempt was made to determine precisely the latent periods, these results suggest that isolate 144 may have a somewhat longer latent period on isolines *Pm*3c, *Pm*4, and MA than on Chancellor.

DISCUSSION

Vanderplank (17,18) states that race-specific, vertical resistance genes function against epidemic development of plant diseases by reducing the amount of effective initial inoculum (X_0) available for disease onset and have no influence on disease increased by races with virulence genes to match them. Mathematical analysis and modeling of plant disease epidemics make these assumptions concerning the role of vertical resistance genes. The dramatic effect of some vertical *Pm* genes on disease efficiency and sporulation by races with virulence genes to overcome them clearly demonstrates that race-specific resistance may also reduce the apparent infection rate (r)—a trait heretofore attributed solely to genes for horizontal resitance (17,18).

Researchers working with the genetic lines of winter wheat we used, but not knowing that the lines were isogenic for so-called major *Pm* genes, would have concluded quite logically that the lines carrying genes *Pm*3c, *Pm*4, or MA possessed some level of rate-reducing resistance. And yet, rate-reducing resistance, with few exceptions, is considered to be controlled by so-called minor genes or polygenes. The present research demonstrates that a gene may perform in a qualitative or quantitative manner, depending on the genotype of the pathogen it confronts. These results support the contention by Nelson (12,13) that there are no major or minor genes for disease resistance, but only genes for disease resistance. The sporulation studies suggest that combining the *Pm* genes with residual resistance in a breeding program could reduce total sporulation and thereby limit epidemic development of powdery mildew.

The gene-for-gene concept proposed by Flor (7) states that for every resistance gene in the host, there is a matching virulence gene in the pathogen. The present research in no way challenges the basic premise of this concept, but it suggests that the gene-for-gene concept might be restricted to a qualitative sense, such as a black or white reaction type of resistance or susceptibility, without reference to potential quantitative or residual effects of resistance genes.

Nelson theorized (11–13) that wild plant species and their pathogens have coevolved to genetic equilibrium through the gradual, stepwise accumulation of resistance genes and virulence genes and that the resistance genes at some point in the coevolutionary process conditioned race-specific reactions before being overcome by new and virulent strains of the pathogen. It seems logical now to speculate that the defeated genes were retained in host populations because their residual effects contributed something of biological value to the populations. If so, defeated resistance genes should be retained in modern cultivars rather than discarded.

The residual effects of defeated *Pm* genes we have demonstrated also suggest some speculative interpretations of some epidemiologic and biological phenomena. For example, multilines with a given amount of susceptible tissue consistently sustain a percentage of disease somewhat below the percentage of susceptible tissue. This phenomenon could be attributed to the expression of the buffering effects of multilines (15), provided the susceptible tissue is intentionally incorporated into the multiline. However, if a component line carrying a specific resistance gene were rendered susceptible by the advent of a pathogen genotype with a matching virulence gene, less-than-expected disease severity might reflect the residual effects of the defeated gene, as well as any buffering effects that may accrue. Theoretically, the latter type of multiline should sustain less disease than a multiline with a known susceptible component line.

The capacity of a resistance gene to express residual effects may depend on the background genotype of the pathogen. Martin and Ellingboe (9) demonstrated that the powdery mildew resistance gene *Pm*4 interacted differently with different isolates of *E. graminis* f. sp. *tritici* with respect to the gene's ability to restrict the development of elongating secondary hyphae. We are currently studying the interaction of each *Pm* isoline with a series of isolates each possessing at least the virulence gene needed to match the particular *Pm* gene.

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