Resistance

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


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ABSTRACT


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 Pm3c, Pm4, 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 Pm3c, Pm4, and MA isolines than on Chancellor (32, 40, and 65% fewer spores, respectively). No statistically significant residual effects were obtained for resistance genes Pm2, Pm24, and Pm5. 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.

Additional key words: disease resistance.

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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.

Fior'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 inherited by the same genes; i.e., 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 genomes. 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 resistance change 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 genomes 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 Hermes (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, Pm4, 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 pM4.

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 Briggie (3) in a Chelaniller 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 pyramid 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 Pm2, Pm2+, Pm3c, Pm4, Pm5, 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.

**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 (600 lux) and 8 hr of darkness or 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 0-5 rpm 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 isolate 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 isolones with powdery mildew resistance genes Pm4, Pm3c, and MA maintained 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 Pm2, Pm2+, and Pm5 were not statistically different from Chancellor in restricting colony formation.

Relative infection efficiency. During 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 Pm4, Pm3c, and MA by the time sporulating colonies developed. However, the number of sporulating colonies on Chancellor and the near-isogenic lines Pm2, Pm2+, and Pm5 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 I44. 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 Ellingson (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 Pm3c, Pm4, 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 I44 on Chancellor and the six isolones (Table 3). Sporulation on the Pm2, Pm2+, and Pm5 isolones consistently resembled that on Chancellor. A statistical analysis of data using all four experiments as replications showed that isolate I44 produced significantly fewer spores per lesion on isolones Pm3c, Pm4, and MA than on Chancellor. The fewest spores per lesion were consistently produced on the Pm4 isolone.

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 isolone Pm4, 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 isolone. In experiment 2, for example, no spores were obtained from isolones on the 16 leaves of the MA isolone on the first sampling date, while 15,278 spores were collected from 15 Chancellor leaves. In the three

<table>
<thead>
<tr>
<th>Wheat line</th>
<th>Experiment 1</th>
<th>Mean ± s.d.</th>
<th>Mean difference†</th>
<th>Experiment 2</th>
<th>Mean ± s.d.</th>
<th>Mean difference†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chancellor</td>
<td>27.6 ± 13.2</td>
<td>6.7 *</td>
<td></td>
<td>27.0 ± 9.0</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Pm4 isolate</td>
<td>1.7 ± 1.8</td>
<td></td>
<td></td>
<td>1.9 ± 2.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chancellor</td>
<td>32.6 ± 9.6</td>
<td>10.38 *</td>
<td></td>
<td>27.0 ± 9.4</td>
<td>8.8 *</td>
<td></td>
</tr>
<tr>
<td>Pm3c isolate</td>
<td>2.8 ± 4.0</td>
<td></td>
<td></td>
<td>2.4 ± 2.1</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>Chancellor</td>
<td>18.1 ± 9.0</td>
<td>7.1 *</td>
<td></td>
<td>18.1 ± 9.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pm5 isolate</td>
<td>17.8 ± 7.8</td>
<td></td>
<td></td>
<td>21.6 ± 9.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Mean number of sporulating colonies produced by isolate I44 of *E. graminis f. sp. tritici* on Chancellor winter wheat and five near-isogenic lines carrying known powdery mildew (Pm) resistance genes.

<table>
<thead>
<tr>
<th>Wheat line</th>
<th>Experiment 1</th>
<th>Infections</th>
<th>Colonies</th>
<th>Leaves</th>
<th>Infections</th>
<th>Colonies</th>
<th>Leaves</th>
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<tr>
<td>Chancellor</td>
<td>261</td>
<td>253</td>
<td>14</td>
<td></td>
<td>578</td>
<td>566</td>
<td>20</td>
</tr>
<tr>
<td>Pm2 isolate</td>
<td>29</td>
<td>24</td>
<td>4</td>
<td></td>
<td>226</td>
<td>221</td>
<td>8</td>
</tr>
<tr>
<td>Pm2+ isolate</td>
<td>406</td>
<td>381</td>
<td>12</td>
<td></td>
<td>966</td>
<td>940</td>
<td>21</td>
</tr>
<tr>
<td>Pm5 isolate</td>
<td>244</td>
<td>239</td>
<td>17</td>
<td></td>
<td>229</td>
<td>216</td>
<td>17</td>
</tr>
<tr>
<td>Pm4 isolate</td>
<td>201</td>
<td>96</td>
<td>11</td>
<td></td>
<td>241</td>
<td>96</td>
<td>20</td>
</tr>
<tr>
<td>Pm3c isolate</td>
<td>150</td>
<td>46</td>
<td>12</td>
<td></td>
<td>226</td>
<td>73</td>
<td>21</td>
</tr>
<tr>
<td>MA′ isolate</td>
<td>129</td>
<td>56</td>
<td>14</td>
<td></td>
<td>203</td>
<td>79</td>
<td>24</td>
</tr>
</tbody>
</table>

Table 2. Number of successful infections and resulting number of sporulating colonies produced by isolate I44 of *E. graminis f. sp. tritici* on Chancellor winter wheat and six near-isogenic lines carrying known powdery mildew (Pm) resistance genes.

<table>
<thead>
<tr>
<th>Wheat line</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Combined analysis†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chancellor</td>
<td>72,521 ab</td>
<td>82,381 a</td>
<td>82,966 a</td>
<td>56,262 a</td>
<td>73,783 a</td>
</tr>
<tr>
<td>Pm2 isolate</td>
<td>92,600 a</td>
<td>71,474 ab</td>
<td>78,289 a</td>
<td>59,275 a</td>
<td>75,410 a</td>
</tr>
<tr>
<td>Pm3 isolate</td>
<td>87,191 ab</td>
<td>64,153 b</td>
<td>70,033 a</td>
<td>47,339 a</td>
<td>67,179 a</td>
</tr>
<tr>
<td>Pm2+ isolate</td>
<td>73,406 ab</td>
<td>54,637 bc</td>
<td>68,041 a</td>
<td>53,906 a</td>
<td>62,511 ab</td>
</tr>
<tr>
<td>Pm3c isolate</td>
<td>57,592 bc</td>
<td>51,654 bc</td>
<td>36,668 b</td>
<td>57,009 a</td>
<td>50,728 bc</td>
</tr>
<tr>
<td>MA′ isolate</td>
<td>46,689 c</td>
<td>40,531 b</td>
<td>47,490 a</td>
<td></td>
<td>44,903 c</td>
</tr>
<tr>
<td>Pm4 isolate</td>
<td>22,725 d</td>
<td>30,335 d</td>
<td>30,215 d</td>
<td>18,557 d</td>
<td>25,458 d</td>
</tr>
</tbody>
</table>

Table 3. Mean number of spores produced per lesion by isolate I44 of *E. graminis f. sp. tritici* on Chancellor winter wheat and six near-isogenic lines carrying known powdery mildew (Pm) resistance genes.

*Data are averages of three replications. 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.
†Means within a column followed by a common letter are not significantly different at the 5% level, according to Duncan's multiple range test.
‡Michigan Amber gene.
§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 isolate Pm4 on the first sampling date, and 8,201 spores were harvested from 32 leaves of isolate Pm3c. Forty of the 111 test leaves of isolates Pm3c, Pm4, and MA produced no spores on the first sampling date. Only 3 of 126 leaves of isolates Pm2, Pm2+, and Pm5 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 Pm3c, Pm4, 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 (Xo) 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 resistance (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 Pm3c, Pm4, 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 Pm4 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 isolate with a series of isolates each possessing at least the virulence gene needed to match the particular Pm gene.

**LITERATURE CITED**