# Differences Between Compatible Parasite/Host Genotypes Involving the Pm4 Locus of Wheat and the Corresponding Genes in Erysiphe graminis f. sp. tritici

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ABSTRACT

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Infection efficiency, defined as percent of *Erysiphe graminis* f. sp. *tritici* conidia applied to wheat leaves that produced secondary hyphae over 10 μm long, was determined for each of eight parasite/host genotypes. Near-isogenic lines of wheat with either *Pm4* or *pm4* were inoculated with cultures MS-1 *(P4)*, MS-2 *(p4)*, Kh×Cc$^7$ *(p4)*, and MS-3 *(p4)*. All cultures gave infection efficiencies of 76-78% and infection type 4 on wheat containing *pm4*. Isolate MS-1 *(P4)* inoculated on wheat containing *Pm4* gave an infection efficiency of 6% and an infection type 0. MS-2 *(p4)* inoculated on wheat containing *Pm4* gave 78% infection efficiency and an infection type 4, but primary infection proceeded more slowly and required 28 vs. 26 hours for completion. Kh×Cc$^7$ *(p4)* and MS-3 *(p4)* inoculated on wheat with *Pm4* gave an infection efficiency of 19 and 33%, respectively, but the successful primary infections developed into type 4 infections in 7 days. Although all compatible parasite/host combinations produced type 4 infections, none of the cultures with the *p4* gene conferred complete compatibility to the *p4*/*Pm4* parasite/host genotype in terms of infection efficiency or rate of development of primary infection. These results show how gene-for-gene relationships can contribute to the phenomenon known as horizontal resistance.

*Additional key words:* quadratic check, slow mildew development, development of fewer normal pustules.

Flor (2) found that the ability of *Melampsora lini* (Pers.) Len. to grow and produce symptoms on flax lines containing certain genes was determined by specific corresponding genes in the pathogen. The existence of one gene in the pathogen for each gene in the host led to the development of the gene-for-gene hypothesis (3, 12). The gene-for-gene hypothesis states that for every *R* gene in the host that conditions resistance there is a corresponding *P* gene in the parasite that conditions avirulence. The *P* gene interacts with the *R* gene in the host to determine incompatibility (low infection type). Incompatibility results only when a *P* gene in the parasite interacts with its specific *R* gene in the host *(P1/R1)*. Compatibility is specified with the other possible parasite/host genotypes *P1/r1*, *p1/R1*, and *p1/r1*. With two alleles at one locus in a host *(R* or *r)* and two at a corresponding locus in a parasite *(P* or *p)*, there are four possible interactions (Fig. 1). The basic scheme was proposed (14) as a biological test to study physiological and biochemical effects of disease development. By the use of different host genotypes in combination with various pathogen genotypes, a four-way or "quadratic check" is developed with which the observed phenomena can be associated. This test is useful for studying the disease development of powdery mildews on barley and wheat which are controlled by gene-for-gene interactions (7, 13).

The genetics of parasite/host interactions can be explained most satisfactorily if it is assumed that the gene-for-gene interactions have evolved following the evolution of a basic compatibility between parasite and host (1). If the assumption is made that parasite/host genotype *P/r* is the most primitive (see Fig. 1), then it is possible to rationalize a selective advantage for host plants with *R* if the parasite population contains *P*. The presence of *R* in a host population should give a selective advantage for parasites with *p* (11). Assuming that mutations of *P* to *p* have no deleterious effects unrelated to recognition of host gene *R*, any mutation of *P* which gives greater compatibility with host gene *R* should have a selective advantage over the parent genotype *P*. There is reason to believe, therefore, that some parasite/host genotypes, *p/R*, may not have the same degree of compatibility between host and parasite as *P/r*, but greater than *P/R*. The difference between *P* vs. *p* in the presence of *R* may not be as great as the difference between *R* vs. *r* in the presence of *P* for each parasite/host gene pair.

Using the criteria of final infection type, $^{35}S$ transfer rate from host to parasite, and the rate of development of elongating secondary hyphae (ESH) during primary

infection, the quadratic check was completed with the *Pl/Pm1* gene pair affecting wheat powdery mildew (16). The three compatible interactions *Pl/pm1, pl/Pm1,* and *pl/pm1* were indistinguishable by the criteria of percent ESH and final infection type, but a unique rate of $^{35}S$ transfer was observed for each of the three compatible genotypes. The differences observed may have been due to other gene differences between the two near-isogenic host lines or between the nonisogenic strains of the parasite. The quadratic check was also completed for *Pg/Mlg* gene pair affecting barley mildew using the same criteria (4). Identical final infection types, $^{35}S$ transfer rates, and percent ESH were reported for all compatible combinations of this gene pair; i.e., *Pg/mlg, pg/Mlg,* and *pg/mlg*. Thus, at least in one case (16), a difference between *p/R* and *P/r* has been reported.

The objectives of this study on powdery mildew of wheat were to use the criteria of final infection type (IT) and the percent of the applied parasites producing ESH during primary infection to determine if the three compatible parasite/host genotypes *(P4/pm4, p4/Pm4,* and *p4/pm4)* involving a single pair of corresponding genes in the parasite *(P4)* and the host *(Pm4)* are identical. If so, do all cultures which give high IT on plants with *Pm4* (which by definition contain *p4*) have identical primary infection kinetics on host lines with *Pm4?*

## MATERIALS AND METHODS

Inocula were obtained from cultures of *Erysiphe graminis* DC. f. sp. *tritici* E. Marchal maintained on wheat *(Triticum aestivum* L. 'Little Club'). Environmental conditions used to maintain inoculum were as described by Masri and Ellingboe (5, 6). Each culture was purified by single pustule isolations and was periodically checked for purity by scoring IT's 7 days after inoculation on a set of differential host lines (Table 1).

Near-isogenic wheat lines containing *pm4* or *Pm4* were grown in the greenhouse in 5-cm diameter pots. Single 5- to 6-day-old plants were inoculated with the appropriate culture on the lower (abaxial) leaf surface using the rolling technique (9). A set of inoculated plants, representing the four possible parasite/host genotypes involved with a single corresponding gene pair, was maintained in the same growth chamber under optimum environmental conditions necessary for synchronous

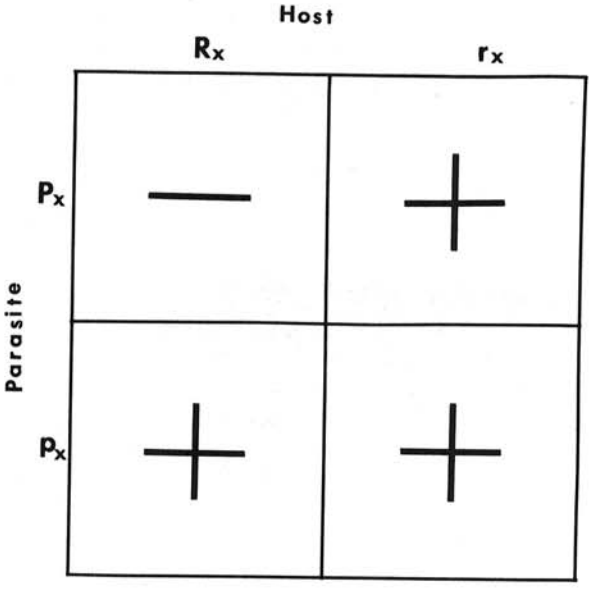


**Fig. 1.** The four possible parasite (*Erysiphe graminis* f. sp. *tritici*)/host (wheat) genotypes involving a single gene pair governing compatibility of host and parasite. Genes *Rx* and *rx* are alternate alleles in the host. Genes *Px* and *px* are alternate alleles in the parasite. The gene combination *Px/Rx* specifies incompatibility (−); whereas the combinations *Px/rx, px/Rx,* and *px/rx* specify compatibility (+).

TABLE 1. Infection type produced 7 days after inoculation of five near-isogenic wheat lines with four cultures of *Erysiphe graminis* f. sp. *tritici*

| | Infection type[a] | | | | |
|---|---|---|---|---|---|
| | Near-isogenic host line | | | | |
| Culture | (Chancellor) *pmx* | *Pm1* | *Pm2* | *Pm3b* | *Pm4* |
| MS-1 | 4 | 0 | 2 | 3 | 0 |
| MS-2 | 4 | 0 | 4 | 3 | 4 |
| MS-3 | 4 | 4 | 2 | 3 | 4 |
| Kh×Cc[7] | 4 | 0 | 2 | 3 | 4 |

[a]Infection type: 0 = no observable mildew development, no pustules; 2 = chlorosis, necrotic reaction; 3 = significant reduction in mildew development; 4 = abundant mildew development.

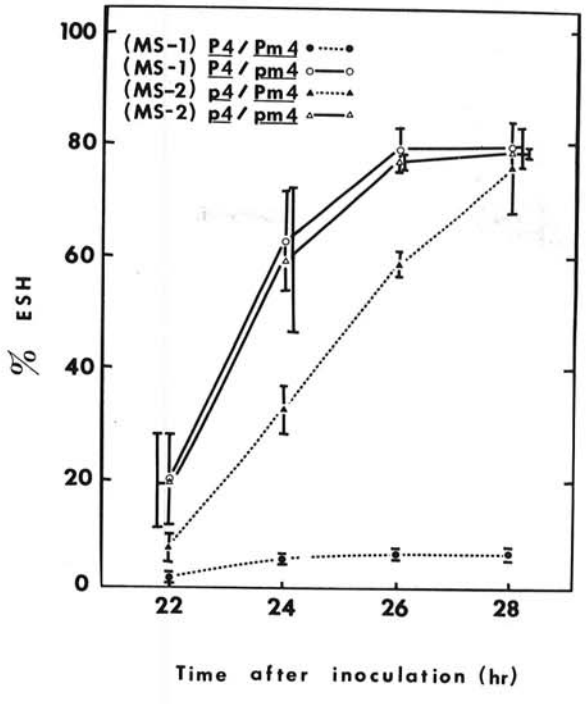


**Fig. 2.** Formation of elongating secondary hyphae (ESH) by *Erysiphe graminis* f. sp. *tritici* cultures MS-1 and MS-2 with the four possible parasite/host (wheat) genotypes involving the alternate alleles of *P4* and *Pm4*.

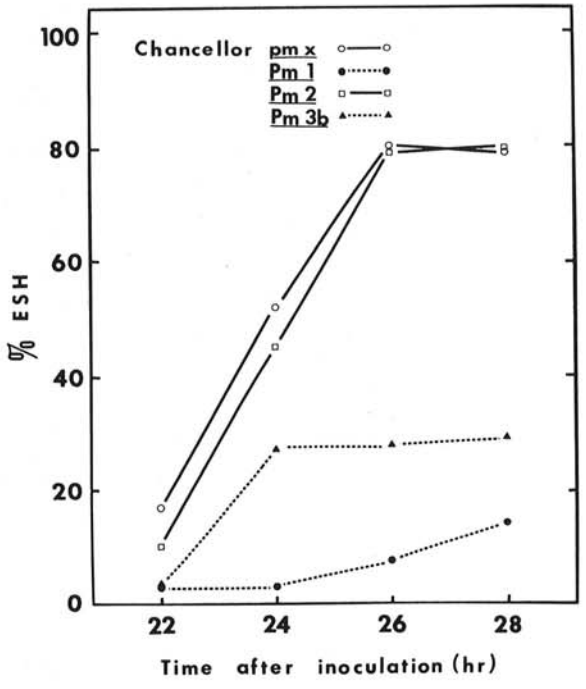


Fig. 3. Formation of elongating secondary hyphae by *Erysiphe graminis* f. sp. *tritici* culture MS-2 *(p4)* on four near-isogenic wheat lines, three of which contained a different dominant *Pm* gene.

development of the parasite during primary infection (5, 8, 10).

The development of the fungus was examined every 2 hours from 22 to 28 hours after inoculation. Counts were made of single isolated parasite units on 1- to 2-cm-long leaf sections by direct observations (×100). Fresh leaf sections were used at each sampling time. Parasite units with secondary hyphae longer than 10 $\mu$m were classified as ESH. The percent parasite units with ESH was calculated for each parasite/host genotype by counting 100-200 parasite units at each time. Experiments were repeated on three or four different days. The results are reported as averages, bracketed by the standard deviations.

Infection types were recorded for the various parasite/host genotypes 7 days after inoculation, as in Table 1.

## RESULTS

The IT's of the four possible genotypes involving *P4, Pm4,* and their alternate alleles, followed the expected pattern of the quadratic check when culture MS-1 was used for *P4* and culture MS-2 was used for *p4,* namely, *P4/Pm4* gave a IT 0, and *P4/pm4, p4/pm4,* and *p4/Pm4* gave IT 4. The quadratic check using the same cultures was then completed using the criterion of production of ESH. A slower rate of development of ESH with *p4/Pm4* than with the other two compatible genotypes was observed (Fig. 2). The same final percent of ESH (infection efficiency) was obtained but it was obtained

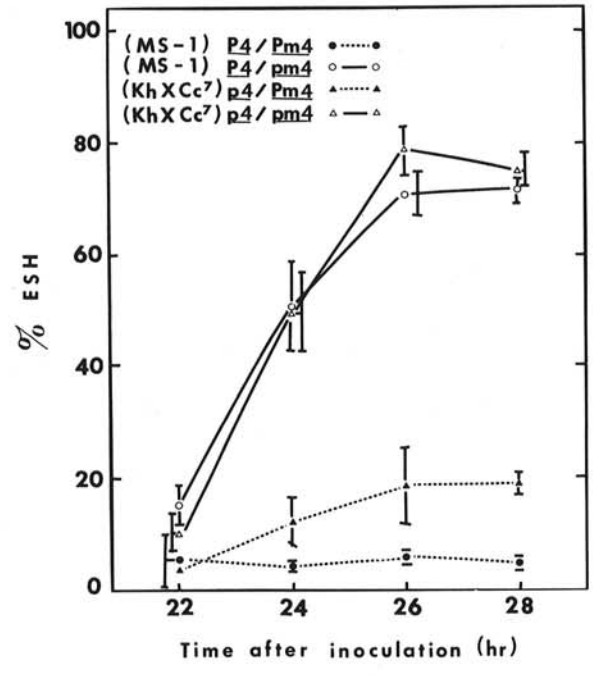


Fig. 4. Formation of elongating secondary hyphae by *Erysiphe graminis* f. sp. *tritici* cultures MS-1 and Kh×Cc$^7$ with the four possible parasite/host (wheat) genotypes involving the alternate alleles of *P4* and *Pm4*.

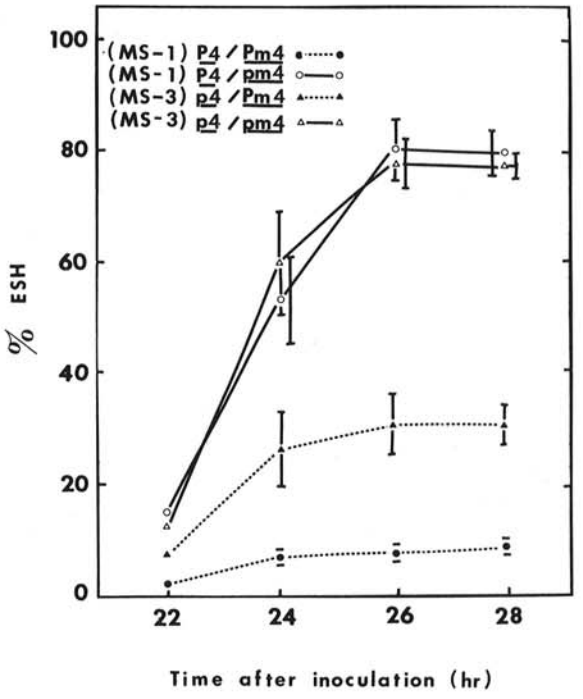


Fig. 5. Formation of elongating secondary hyphae by *Erysiphe graminis* f. sp. *tritici* cultures MS-1 and MS-3 with the four possible parasite/host (wheat) genotypes involving the alternate alleles of *P4* and *Pm4*.

approximately 2 hours later compared to the other compatible genotypes. The behavior of MS-2 *(p4)* cannot be due to nonspecific genes for slow growth because normal development of ESH was observed on the host line with *pm4*.

To further substantiate that culture MS-2 did not have genes that merely conditioned slow growth, the percent of MS-2 parasite units producing ESH was determined on near-isogenic lines containing *pmx, Pm1, Pm2,* and *Pm3b*. The production of ESH (Fig. 3) was similar to that reported for MS-1 *(P1, P2, P3b,* and *P4)* on the same host lines (15). Culture MS-2 had slow growth only on host plants containing *Pm4*.

The quadratic check was completed again for *P4* and *Pm4* except that culture Kh×Cc$^7$ *(p4)* was substituted for culture MS-2 *(p4)*. Kh×Cc$^7$ gave IT 4 seven days after inoculation on host plants with *Pm4* and, therefore, by definition contains *p4*. An infection efficiency of 19% was observed for the *p4/Pm4* genotype when Kh×Cc$^7$ was used as the source of *p4* (Fig. 4). Apparently the 19% of the parasite units that did produce successful primary infections continued to develop and produced high IT's. A third culture with *p4*, culture MS-3, gave an infection efficiency of 33% and a high IT when used to inoculate a host line with *Pm4* (Fig. 5).

## DISCUSSION

These data indicate that differences in compatibility among the three possible compatible parasite/host genotypes *(P4/pm4, p4/Pm4* and *p4/pm4)* can be detected. *P4/pm4* was distinguishable from *p4/Pm4* and *p4/pm4*. The latter two were not distinguishable. These data also indicate three cases in which the change from *P4* to *p4* in the parasite did not restore the same degree of compatibility found in the parasite/host genotype *P4/pm4*. One *p4/Pm4* genotype had a slower rate of mildew development. The slower rate was not due to nonspecific genes in the parasite which conditioned slow growth since development with *p4/pm4* was similar to the rate with *P4/pm4*. Two other *p4/Pm4* genotypes had a reduced number of parasite units which successfully completed primary infection. These two characteristics (i.e., slow mildew development and the development of fewer normal pustules) have always been associated with "horizontal" or "nonspecific" resistance. Our results show that in this study such differences were due to specific genotypes. "Horizontal" resistance may, in some cases, be the result of the presence and accumulation of *px/Rx* interactions which have not restored complete comptaibility to host-parasite relationships.

Whether the differences between the three cultures which give infection type 4 on wheat plants with *Pm4* is due to allelic differences at the *P4* locus in the parasite or to differences in modifiers of the gene-for-gene interaction is not yet known. The existence of an allelic series at the *P4* locus could be very useful in studies on the product of the *P4* locus and its function in host/parasite interactions. If the differences between the three cultures which give a high IT on wheat plants with *Pm4* is due to modifiers of the gene-for-gene interactions, it is important to determine if the modifiers act on the *P4/Pm4* or *p4/Pm4* interactions. These distinctions should give information pertinent to determining if there is specific recognition for compatible or incompatible interactions.

These results also show that *Pm4* in wheat cannot be considered to be a gene for low reaction to *E. graminis* f. sp. *tritici*. With culture MS-2, the presence of *Pm4* gives slow growth of the parasite. With cultures Kh×Cc$^7$ and MS-3, the presence of *Pm4* gives reduced numbers of infections. The phenotype of parasite/host interaction with *Pm4* is, therefore, determined by the genotype of the parasite. Slow mildewing and low numbers of infections are just as specifically controlled as final infection type.

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