Virulence Spectrum of the *Erysiphe graminis* f. sp. *tritici* Population in New York

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ABSTRACT

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Isolates of *Erysiphe graminis* f. sp. *tritici* collected from mildewed wheat leaves in central and western New York in 1984 were tested for virulence against differential lines and cultivars of wheat with major genes for powdery mildew resistance (*Pm* genes). A high percentage of the 116 isolates tested had virulence to *Pm*2, *Pm*3c, *Pm*4, *Pm*6, and *Pm*8. A few isolates had virulence to *Pm*1 and *Pm*3a. Isolates were found that could overcome one or more of all but two, *Pm*3b and *Pm*9, of the known *Pm* genes in wheat. About 40 and 46% of the isolates from commercial fields and from breeding plots had zero and one virulence gene, respectively. The most complex isolates detected in commercial fields and breeding plots had three and five virulence genes, respectively. Virulence gene pairs corresponding to wheat genes *Pm*4 and *Pm*8 occurred in close association.

Powdery mildew, induced by *Erysiphe graminis* DC. ex Merat. f. sp. *tritici*, is an important disease of wheat (*Triticum aestivum* L.) worldwide. Yield reductions as great as 45% in the United Kingdom, New Zealand, and India (5,8,13,14) and as great as 35% in the United States have been attributed to powdery mildew (6,7). Powdery mildew has long been considered one of the most important diseases of soft white winter wheat in New York. Recently, it has also been shown to be prevalent and severe on hard red spring wheat in New York (4).

Satisfactory control of powdery mildew can be achieved with fungicides. In many wheat production situations, however, foliar fungicide application is uneconomical or inconvenient and is not used extensively. Varietal resistance is an effective, safe, and economically feasible alternative for powdery mildew control. Rational decisions on which resistance genes to incorporate into cultivars should be based on surveys of virulence patterns in the pathogen population. Yet, in the United States, the only comprehensive virulence survey reported was done in Pennsylvania (12). A similar survey was recently reported from Ontario (11). This paper reports the results of the first survey for virulence in the New York population of *E. graminis* f. sp. *tritici*. The objectives were to determine the virulence spectrum and relative frequency of virulence genes in fungal isolates collected from the predominant wheat-growing areas of New York and to evaluate the independence of the virulence genes in those isolates.

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MATERIALS AND METHODS

Collection of isolates. During May and June 1984, mildewed wheat plants were collected from various wheat fields throughout central and western New York. The counties included were those with the largest wheat acreage in 1983 (*New York Crops and Livestock Statistics*, 1984). Samples were collected from commercial fields of soft white winter wheat, of which the cultivars Houser and Frankenmuth predominated, as well as from breeding plots maintained by Cornell University. One to five commercial fields were sampled per county, and only one isolate was taken from each field. Sixty isolates were recovered from the commercial fields, whereas 56 were recovered from Cornell breeding plots. A 5-cm-long section of diseased leaf was cut from each sample and was dropped onto seedlings of Chancellor wheat growing in a test tube 25 × 250 mm. The test tubes were covered with germination paper to prevent contamination and were kept in a growth chamber maintained at 21 C, relative humidity of at least 70%, and illumination of 180 $\mu E/m^2/sec$ with a 12-hr photoperiod. Under these conditions, mildew developed and the fungus sporulated profusely within 7 days of inoculation.

Single-colony isolation and maintenance of isolates. A single-colony isolate of the pathogen was obtained from each sample. A few conidia from a single colony of the pathogen were removed with a disposable pipette, and the conidia were inoculated onto new Chancellor seedlings. Isolations were made three or four times successively to increase the probability of eliminating isolate mixture in the culture. The resulting single-colony isolates were cultured and maintained on Chancellor seedlings in test tubes 25 × 250 mm under the same growth chamber conditions described previously. Isolates were transferred to new seedlings every 3 wk.

Production of inoculum. Inoculum of the isolates was produced on either Chancellor or Powell seedlings grown in 4-in.-diameter plastic pots containing Cornell soil mix composed primarily of peat and Vermiculite (1). A clear plastic tube covered with a petri dish cover was placed over each pot at planting to avoid contamination of the seedlings.

Inoculations were done by tapping the tube containing the isolate culture over the Chancellor seedlings to allow the conidia to fall onto the leaf surfaces. Inoculum produced in this manner was usually ready for use within 7 or 8 days of inoculation. As much as possible, this source of inoculum was used only once, but whenever it was necessary to reuse it, the pot containing the inoculum was shaken 24–28 hr before reusing it to remove old conidia from the plants.

Identification of virulence genes. The virulence of each isolate was characterized using nine lines and cultivars of wheat known to have major genes for powdery mildew resistance (Table 1). This list includes seven of the near-isogenic lines for powdery mildew resistance developed by Briggle (3). CI 14125, a near-isogenic

line for *Pm5*, was included in the test, but because of its questionable identity, results from that test were not included in this paper. The source of *Pm7* was not included in this study because the seeds we received were not pure lines. Chancellor served as the susceptible check cultivar.

Ten seeds of each line or cultivar were sown in radial rows in an 8-in. shallow plastic pot containing Cornell soil mix, then the top of the pot was partially covered (leaving a small opening for aeration) with a plastic bag supported on the sides with four stakes. Seedlings were maintained throughout the test in a growth chamber with a light intensity of about 250 $\mu E/m^2/sec$, a 16-hr photoperiod, relative humidity of 80% or higher, and night and day temperatures of 18 and 20 C, respectively. Test seedlings were inoculated at the one-leaf stage or about 1 wk after sowing, except seedlings of CI 14122 and CI 14033, which were inoculated at the three- to four-leaf stage. Each pot of seedlings was tested with one isolate of the pathogen. The inoculation procedure used in this study was similar to that described by Briggle (2) and involved shaking the inoculum source over the seedlings being inoculated. One week after inoculation, seedling reactions to pathogen isolates were rated on an individual-plant basis on a scale of 0–9, where 0 = immune (no visible signs or symptoms); 1–3 = highly resistant (tiny necrotic spots, little to no mycelium evident); 4–6 = moderately resistant (moderate-sized necrotic spots to no necrosis, moderate amount of mycelium, and sporulation evident); and 7–9 = susceptible (increasing in amount of mycelium and conidia production to a completely compatible relationship with the pathogen).

A score of 0–6 indicated a resistant reaction of the host, whereas a score of 7–9 indicated susceptibility. Therefore, an isolate that caused a rating of 7–9 on a particular test line or cultivar was interpreted as possessing the virulence gene matching the resistance gene in that line. On the other hand, an isolate that caused a rating of 0–6 was considered as having no virulence gene that could match the resistance gene in that line. The virulence test for each isolate was done at least three times, and the average rating was used as a basis for interpreting the results.

Table 1. Cereal investigation (CI) numbers and gene symbols of near-isogenic lines and cultivars of wheat used to differentiate virulence genes in *Erysiphe graminis* f. sp. *tritici*

Designation	CI	Gene[w]
Chancellor	12333	–[x]
Axminster/ 8*Chancellor	14114	*Pm1*
Ulka/8*Chancellor	14118	*Pm2*
Asosan/8*Chancellor	14120	*Pm3a*
Chul/8*Chancellor	14121	*Pm3b*
Sonora/8*Chancellor	14122	*Pm3c*
Khapli/8*Chancellor	14123	*Pm4*
Michigan Amber/ 8*Chancellor	14033	*Pm6*
Kavkaz	361879[y]	*Pm8*
Amigo[z]	17609	*Pm9*

[w] Following gene designation by Briggle (2) and McIntosh (10).
[x] No known gene for powdery mildew resistance.
[y] Plant investigation number.
[z] Reported as having a single dominant gene, temporarily designated as *Pm9*, which is closely linked to *Pm*a locus (7).

RESULTS AND DISCUSSION

One hundred sixteen isolates of *E. graminis* f. sp. *tritici* were recovered from mildewed wheat leaves collected in New York in 1984. The test for virulence of these isolates on wheat differential lines resulted in reactions ranging from 0 to 9 on our rating scale. In most cases, resistant reactions of the seedlings were within the range of 0–3, whereas the susceptible ones were within the range of 7–9. When tests were repeated, highly consistent results were obtained in most of the isolates, except for a few where an additional two to three tests had to be made.

Tests for virulence of the isolates revealed 27 virulence gene combinations (Table 2). The actual frequency of some virulence groups deviated from the expected frequency based on independent gene occurrence and lack of differential selection for genes or gene combinations. A virulence gene pair matching wheat genes *Pm4* and *Pm8* occurred in close association. The reason for such an association is not clear, but possibly, these genes are linked or have formed an adaptive complex relative to the host.

In collections both from commercial fields and breeding plots, a high frequency of the isolates had virulence to *Pm2*, *Pm3c*, *Pm4*, *Pm6*, or *Pm8* (Fig. 1). None of the breeding plot isolates had virulence to either *Pm3b* or *Pm9*, and none of the commercial field isolates had virulence to *Pm1*, *Pm3a*, *Pm3b*, or *Pm9*. A low frequency (3 and 2%) of the breeding plot isolates had virulence to *Pm1* and *Pm3a*, respectively. If a single *Pm* gene were to be used in a cultivar, the choice of *Pm1*, *Pm3a*, *Pm3b*, or *Pm9* would result in the greatest protection against the powdery mildew isolates revealed in this survey. Combining two or more of these genes in the same cultivar, however, would likely result in more lasting crop protection.

About 40 and 46% of the isolates from commercial wheat fields and from breeding plots had zero and one virulence gene, respectively (Fig. 2). A low percentage had combinations of two or three virulence genes, and very few had combinations of four or five virulence genes. In general, the frequency of the isolates decreased as the number of virulence genes increased; however, the isolates recovered from the breeding plots are comparatively more complex than those from commercial field isolates, which had no more than three virulence genes. Isolates from the

Table 2. Frequency of *Erysiphe graminis* f. sp. *tritici* races collected in New York in 1984

Culture number	Virulence Group[y]	Frequency (%)[z]	
		Observed	Expected
1	1,2,3a,3b,3c,4,6,7,9/	26.72	26.24
2	2,3a,3b,3c,4,6,8,9 /1	1.72	0.89
3	1,3a,3b,3c,4,6,8,9 /2	6.03	6.14
4	1,2,3a,3b,4,6,8,9 /3c	11.20	11.66
5	1,2,3a,3b,3c,6,8,9 /4	9.47	8.68
6	1,2,3a,3b,3c,4,8,9 /6	6.90	7.56
7	1,2,3a,3b,3c,4,6,9/8	7.76	4.57
8	2,3a,3b,3c,4,8,9 /1,6	1.72	2.70
9	1,3a,3b,4,6,8,9 /2,3c	2.60	2.90
10	1,3a,3b,3c,6,8,9 /2,4	4.31	2.13
11	1,3a,3b,3c,4,8,9 /2,6	0.86	1.88
12	1,2,3a,3b,6,8,9 /3c,4	2.60	2.81
13	1,2,3a,3b,4,8,9 /3c,6	2.60	4.80
14	1,2,3a,3b,3c,8,9/4,6	1.72	2.63
15	1,2,3a,3b,3c,6,9 /4,8	2.60	1.60
16	1,2,3a,3b,3c,4,9/6,8	0.86	1.38
17	1,3b,3c,4,8,9 /2,3a,6	0.86	0.03
18	1,3a,3b,4,8,9 /2,3c,6	0.86	0.87
19	1,3a,3b,4,6,9 /2,3c,8	0.86	0.53
20	1,2,3a,3b,8,9 /3c,4,6	0.86	1.23
21	1,2,3a,3b,6,9 /3c,4,8	0.86	0.74
22	1,2,3a,3b,4,9 /3c,6,8	2.60	0.65
23	1,3a,3b,8,9 /2,3c,4,6	0.86	0.29
24	1,3a,3b,6,9 /2,3c,4,8	0.86	0.19
25	1,3a,3b,4,9 /2,3c,6,8	0.86	0.14
26	1,2,3a,3b,9 /3c,4,6,8	0.86	0.23
27	1,3b,8,9 /2,3a,3c,4,6	0.86	0.005

[y] Described as virulence formula (effective/ineffective *Pm* genes).
[z] Sample size = 116; actual frequency observed and expected frequency based on independence of virulence genes.

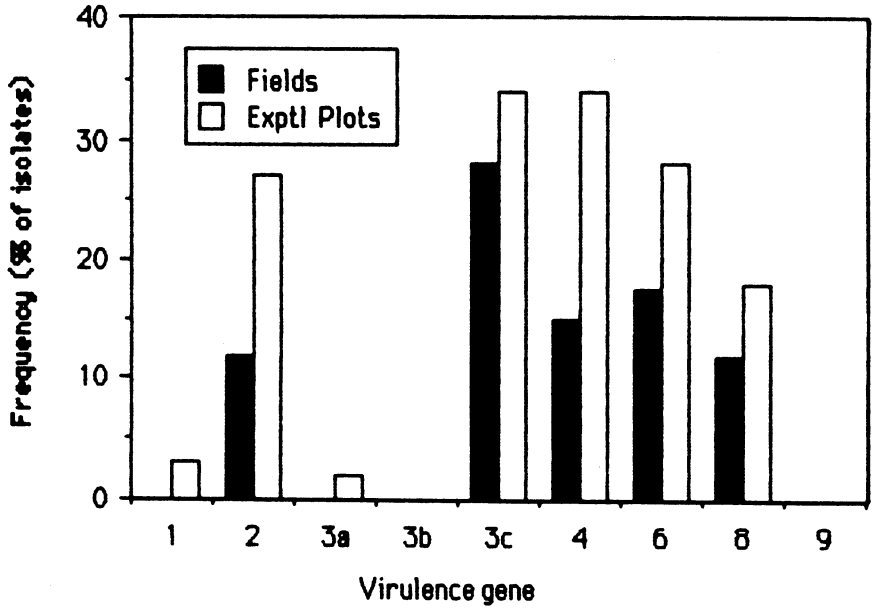

Fig. 1. Virulence gene frequencies of *Erysiphe graminis* f. sp. *tritici* collected in New York in 1984.

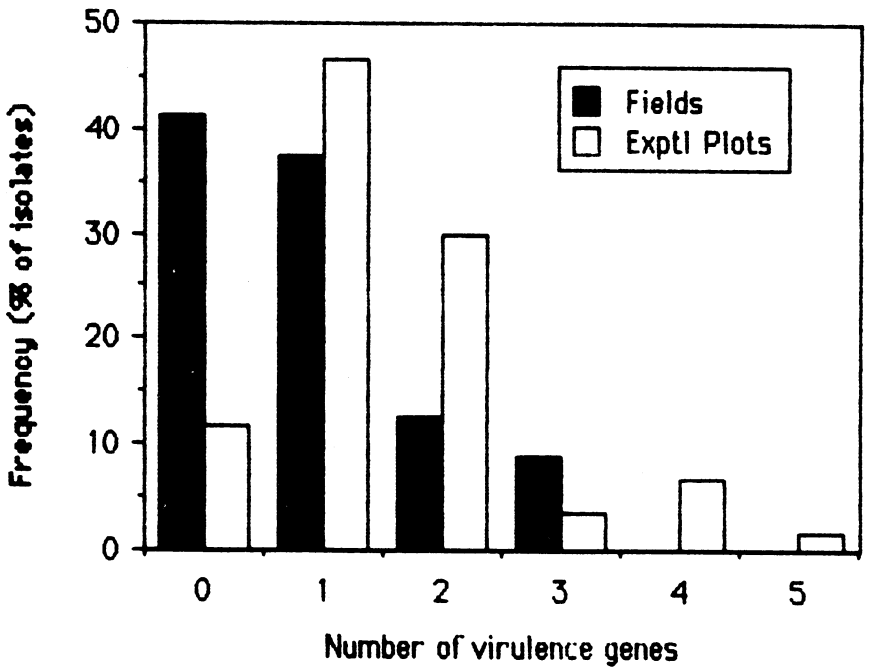

Fig. 2. Frequency of isolates of *Erysiphe graminis* f. sp. *tritici* collected in New York in 1984 having various numbers of virulence genes corresponding to *Pm* resistance genes in wheat.

breeding plots had as many as five virulence genes. In the breeding plots, more variable wheat genotypes are being grown every year. Undoubtedly, these genotypes differ in major genes for mildew resistance and collectively may exert selection pressure on the pathogen population in favor of more complex types. The cultivars Houser and Frankenmuth accounted for most of New York's commercial winter wheat acreage in 1984. Both of these cultivars lack major genes for powdery mildew resistance, but both show rate-reducing (nonspecific or horizontal) resistance to powdery mildew when compared with very susceptible cultivars such as Chancellor or Powell. Several other wheat cultivars of undocumented powdery mildew resistance are grown on a small percentage of New York's total wheat acreage. In the absence of differential selection pressure, simple races of the pathogen predominated in the population. This may explain why the percentage of isolates with no virulence gene in New York commercial wheat fields reached 40% compared with 13% in Ontario (11) and 8–11% in Pennsylvania (12). We do not know exactly how many and which *Pm* genes were present in the commercial cultivars and breeding lines from which the isolates were sampled. Thus, it is not possible to draw any definite conclusion regarding the resistance genes in the host and the virulence genes in the pathogen.

The data from this study are useful in deciding which *Pm* genes should be used in the Cornell breeding program. The virulence survey should be continued in conjunction with data on cultivars and *Pm* gene deployment. Such data are needed to monitor shifts in the virulence spectrum of the pathogen population and will give valuable guidance to wheat breeders as they seek to maintain adequate levels of powdery mildew resistance in wheat cultivars adapted to New York.

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