An Evaluation of the Independence of Certain Virulence Genes of *Erysiphe graminis* f. sp. *tritici*

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ABSTRACT

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Single-colony isolates of *Erysiphe graminis* f. sp. *tritici* were collected in Pennsylvania during 1976–1978 and 1980. Races were distinguished by using nine near-isogenic lines of the winter wheat cultivar Chancellor with known powdery mildew resistance (*Pm*x) genes. The occurrence of virulence on lines carrying *Pm*1, *Pm*3a, and *Pm*3b was rare relative to virulence on lines carrying *Pm*2, *Pm*3c, *Pm*4, *Pm*5, and a line carrying a gene from cultivar Michigan Amber (MA). Nonindependent occurrences of the following virulence gene (*p*) pairs were found: (*p*2,*p*5), (*p*3c,*p*4), and (*p*3c,*p*MA). Genotype *p*2,*p*5 appeared to be more prevalent in the 1976–1978 collection, but the apparently greater frequency of genes *p*3c and *p*MA in combination with genotype *p*2,*p*5 may have produced an increase in racial complexity in 1980. These hypotheses emphasize areas of future research that may be of interest to plant pathologists who plan and interpret plant pathogen racial surveys.

The purposes of plant pathogen racial surveys may be to identify shifts in pathogen populations that may reflect "breakdowns" in existing resistance, to detect hazard areas for the deployment of resistance genes, and to detect possible alternative disease management strategies.

Approaches that have been used to measure plant pathogen population virulence shifts include plotting changes in race frequency over time (10), regressing virulence gene frequencies on time (16), and analyzing departures from the expected frequency for combinations of virulence genes (16,25,31).

The first method gave an appreciation of historical population shifts but produced no statistical test of changes from year to year. The second method allowed comparison of relative rates of change of gene frequencies and produced prediction equations. One shortcoming of the second method (16) was the unreliability in the amount of variability that could be explained with each model (R^2 = 1 to 87%). Also, the applicability of extrapolations of the prediction equation to dates beyond those upon which the model was constructed was uncertain. The third method included attempts to test for departures from the expected frequencies based on the assumption of independence, often without testing for gene independence.

The purpose of this investigation was to compare virulence gene frequencies within and between two collections of *Erysiphe graminis* DC. f. sp. *tritici* obtained during different years in Pennsylvania. The virulence gene frequencies were to be tested for independence to indicate which resistance genes may be deployed with a relatively low probability of being exposed to compatible races of *E. graminis*. The information obtained from the different collections is presented to indicate possible shifts in virulence gene frequencies that may have occurred between the collection dates.

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MATERIALS AND METHODS

Virulence gene identification. One hundred and fifty-six single colony isolates were randomly collected in several Pennsylvania counties during the spring of 1980: Erie, 11 isolates; Mercer, 8; Lawrence, 12; Clinton, 2; Centre, 49; Union, 12; Columbia, 9; Dauphin, 10; York, 9; Lancaster, 13; and Bucks, 21. This sampling pattern was chosen so that the isolates would have been collected from the major wheat producing areas in Pennsylvania. One hundred and one isolates were previously collected in the same counties in approximately the same proportions during 1976–1978 (V. Elliott, University of California, Berkeley, *personal communication*).

Two seeds of cultivar Chancellor winter wheat (*Triticum aestivum* L.) were planted in vermiculite in 25 × 200-mm test tubes plugged with sterile cotton. The plants were inoculated at the one- to two-leaf stage with field isolates of *E. graminis* f. sp. *tritici* by dropping 2- to 5-cm-long infected leaf sections into the test tubes. The test tubes were then plugged with cotton and placed in a cooler. All tubes were transferred to a growth chamber within 10 hr, and were maintained at 20 ± 2 C with illumination at the top of the tubes at 80 $\mu E \cdot m^{-2} \cdot sec^{-1}$ and a photoperiod of 12 hr. At least two cycles of single-colony isolations were performed on Chancellor to increase the probability of eliminating isolate mixtures in stock cultures. All cultures were maintained at 3 ± 1 C on infected Chancellor plants growing in test tubes. All isolates were transferred every 5–6 wk.

Inoculum was obtained by inoculating 7-day-old Chancellor seedlings growing in a sterilized soil mixture of Hagerstown silty clay loam, peat, and sand (2:1:1, v/v) in 5-cm-diameter plastic pots. Glass lamp chimneys were placed over each pot at the time of planting and closed with two layers of tissue paper to prevent contamination. Inoculated plants were placed in a growth chamber for 9 days to increase conidia for further inoculations. Plants that

were used as inoculum sources were shaken at 12 hr prior to use to remove old conidia.

Races were identified by inoculating plants of nine near-isogenic lines of cultivar Chancellor (developed by Briggle [1]) at the two- to three-leaf stage. The CI (USDA Cereal Investigation) number and the source of each powdery mildew (*Pm*) resistance gene follows: *Pm*1 (CI14114, Axminster); *Pm*2 (CI14118, U1ka); *Pm*2(+) (CI14119, CI12621); *Pm*3a (CI14120, Asosan); *Pm*3b (CI14121, Chul); *Pm*3c (CI14122, Sonora); *Pm*4 (CI14123, Khapli); *Pm*5 (CI14125, Hope); and the resistance gene from MA (CI14033, Michigan Amber).

The seed was sown at two to three seeds per hole in 16.5 ×12 × 6-cm plastic flats containing the soil mixture described above. Inoculations were performed by shaking infected Chancellor plants over the flats in a 1.2 × 0.5-m cylindrical settling tower. The flats were then covered with plastic bags and placed in a growth chamber under the environmental regime outlined previously. The bags were removed after 2 days and the flats were moved closer to the lights; illumination at the top of the canopy was at 170 $\mu E \cdot m^{-2} \cdot sec^{-1}$. Infection types were identified 8 days after inoculation by using the 0–4 scale (8). Isolates causing type 3– to 4 reactions on a particular host line were interpreted as possessing the virulence gene (*p*x) matching the resistance gene in the host.

Statistical methods. Since the haploid stage of *E. graminis* was the one of interest in this study, the analyses were performed with the assumption that phenotype frequencies were equivalent to genotype frequencies with respect to virulence genes. All expected race frequencies were calculated as the product of the virulence gene frequencies for the genes in a given race times the complement of the virulence gene frequencies that were not in a given race. The assumption was that the virulence genes occurred independently and were selectively neutral.

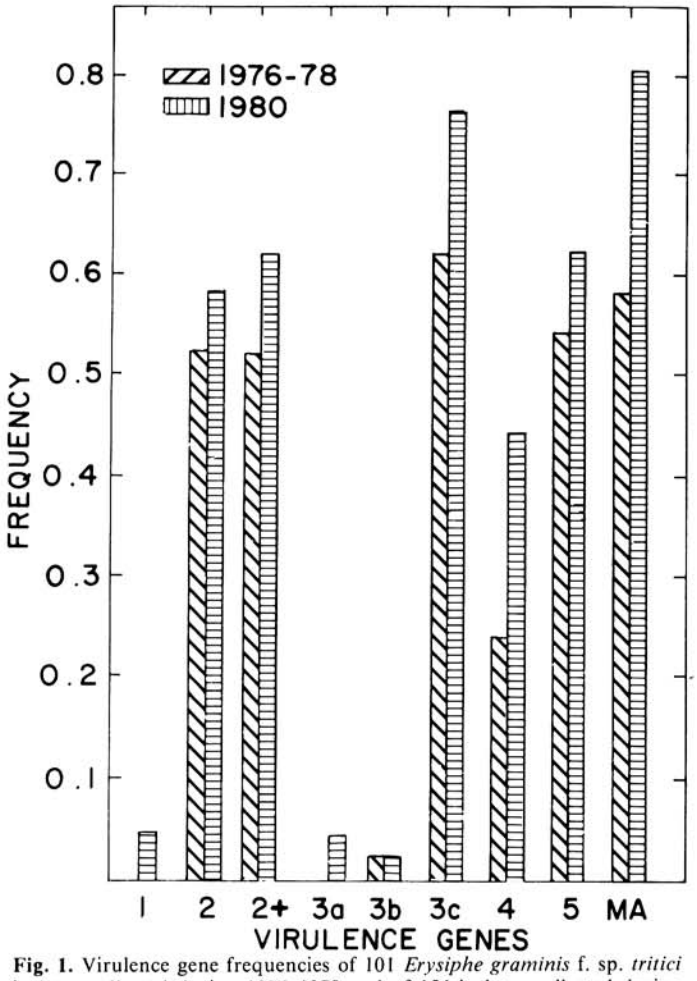

Fig. 1. Virulence gene frequencies of 101 *Erysiphe graminis* f. sp. *tritici* isolates collected during 1976–1978 and of 156 isolates collected during 1980 in Pennsylvania.

The hypothesis of independent occurrences of virulence genes was tested with pairwise comparisons by using the 2 × 2 contingency table, corrected for continuity, to compute chi-square. If an expected value was less than five, chi-square was not interpreted. Four possibilities exist in correctly testing for the independence of the occurrence or nonoccurrence of two events, and no assumptions need to be tested regarding the accuracy of the estimated frequencies of the two events (9,29).

RESULTS

The frequencies of pairs of virulence genes were compared to the frequencies expected based on independence for each year. Significant departures were common to both collections (Table 1). Virulence on lines with *Pm*2 and *Pm*2(+) occurred in near absolute association. This was expected since these resistance genes were considered identical with respect to *E. graminis* f. sp. *tritici* specificity as determined by infection type (1), and further analyses treated these virulences as the same.

Since the 2^n possible races (for which n = nine virulence gene categories for each collection) were reduced to 2 × 2 tables to test for overall gene independence by pairs, it was possible that apparent nonindependences may have been caused by the masking of independences due to the compression of the 2^n categories. However, expected frequencies of races for each collection were calculated to aid the interpretation of the data, although the frequencies were not testable due to the lack of orthogonality.

Seventeen races were identified in the 1976–1978 collection and 26 were identified in the 1980 collection (Table 2). Races of *E. graminis* f. sp. *tritici* with genes *p*1, *p*3a, or *p*3b were found in 1980 but were not detected in 1976–1978. Race *p*3c,*p*MA appeared to have occurred more frequently than expected in the 1976–1978 collection, although it was not testable statistically. Race *p*3c,*p*4,*p*MA appeared to have occurred with a greater than expected frequency in the 1980 collection.

The overall virulence gene frequencies for each collection are presented in Fig. 1. There is an apparent trend in Fig. 1 with higher virulence gene frequencies in the 1980 than in the 1976–1978 collection. It is therefore expected that the frequency of races with relatively more virulence genes would also be greater in the 1980 collection. This is illustrated in Fig. 2 by comparing the actual number of isolates with a particular number of virulence genes in the 1976–1978 and 1980 collections.

Races with two genes appeared to be more prevalent in the earlier collection, and the three- or four-gene races appeared to have occurred more often in the 1980 collection. Specifically, race *p*2,*p*5 occurred at a frequency of 0.18 in the 1976–1978 collection but was at 0.02 in the later collection. This was the race frequency that differed the most between collections. Race *p*3c,*p*MA occurred at a frequency of 0.21 and 0.10 in the 1976–1978 and 1980 collection,

TABLE 1. Virulence genes of *Erysiphe graminis* f. sp. *tritici* that did not occur independently in a 1976–1978 collection and in a 1980 collection in Pennsylvania

Virulence gene		Virulence pairs[a]				
A	B	A & B	A, not B	B, not A	not A & B	Chi-square statistic[b]
1976–1978[c]						
*p*2	*p*2(+)	52	0	0	49	97.06
*p*2	*p*5	52	0	2	47	89.48
*p*2(+)	*p*5	52	0	2	47	89.48
*p*3c	*p*4	21	41	5	34	4.50
*p*3c	*p*MA	51	11	6	33	40.88
1980						
*p*2	*p*2(+)	92	1	3	60	136.60
*p*2	*p*5	91	2	6	57	120.86
*p*2(+)	*p*5	93	1	4	58	131.98
*p*3c	*p*MA	104	13	20	19	23.12

[a] Observed numbers from 2 × 2 contingency table.
[b] Adjusted for continuity, $P < 0.05$ at $\chi^2 > 3.84$.
[c] N = 101 for 1976–1978 and N = 156 for 1980.

respectively. Race *p*2,*p*3c,*p*5,*p*MA occurred at a frequency of 0.11 and 0.24 in the 1976–1978 and 1980 collection, respectively. It is possible that races *p*2,*p*5, and *p*3c,*p*MA may have joined through recombination to produce an increase in race *p*2,*p*3c,*p*5,*p*MA in 1980.

Significantly greater frequencies of *p*3c, *p*4, and *p*MA were present in the 1980 collection relative to the 1976–1978 collection (Table 2). The genes that occurred in the lowest frequency in both collections (Fig. 1) tended to be associated with the more frequently occurring genes (Table 3). For example, in the 1976–1978 collection, *p*1 occurred with *p*3c and *p*MA in one race, with *p*2, *p*4, and *p*5 in another race, and with *p*2, *p*3c, *p*5 and *p*MA in a third race. In the 1976–1978 collection, gene *p*1 did not occur singly or with the relatively rare genes *p*3a or *p*3b as a "two-gene" race.

DISCUSSION

In this study, the consistently higher frequency of virulence genes *p*2, *p*3c, *p*4, *p*5, and *p*MA relative to *p*1, *p*3c, and *p*3b may be partially explained by the possible occurrence of identified resistance genes in commercial cultivars.

In only a few cases was the cultivar from which *E. graminis* was isolated known. Extension agents were contacted in the sampled counties and asked for their best estimate of the percentage of wheat acreage occupied by each cultivar in 1979. Four major seed wheat suppliers also shared sales information for each cultivar in 1979. Both sources of information estimated percentages that did not differ significantly (correlation of 96%): Hart and Redcoat were estimated to be in equal frequency on 60% of the Pennsylvania acreage sown to wheat, followed by Abe at 15–22%, and Pennoll, Arthur 71, and Pioneer S-76 at 2–3%.

A survey (2) of 672 Pennsylvania farms reported different percentages for the total wheat acreage occupied by each cultivar in 1979: Redcoat (56.2%), Hart (9.4%), Abe (8.0%), Arthur 71 (6.6%), Pennoll (5.2%), and Pioneer S-76 (4.5%).

Several of these cultivars appear to carry identified *Pm*x genes according to their lineage: Redcoat may carry *Pm*5 from Hope (12,13,23), Arthur 71 and Abe may carry *Pm*2 and *Pm*6 from Wisconsin CI12633 and *Pm*5 from Hope (13,22). However, neither Arthur, Arthur 71, nor Abe succumbed to mildew until virulence to *Pm*2 was detected in the differentials (G. Shaner, Purdue University, *personal communication*). Cultivar Hart (28) appears to carry no known *Pm*x genes. None of the virulence gene frequencies in the 1980 collection could be related to *Pm*x genes without a detailed genetic analysis of the above cultivars.

According to Table 4 from Sebastian et al (27), neither Hart nor Arthur carry *Pm*1, *Pm*2, *Pm*3a, *Pm*3b, *Pm*4, *Pm*5, or the resistance

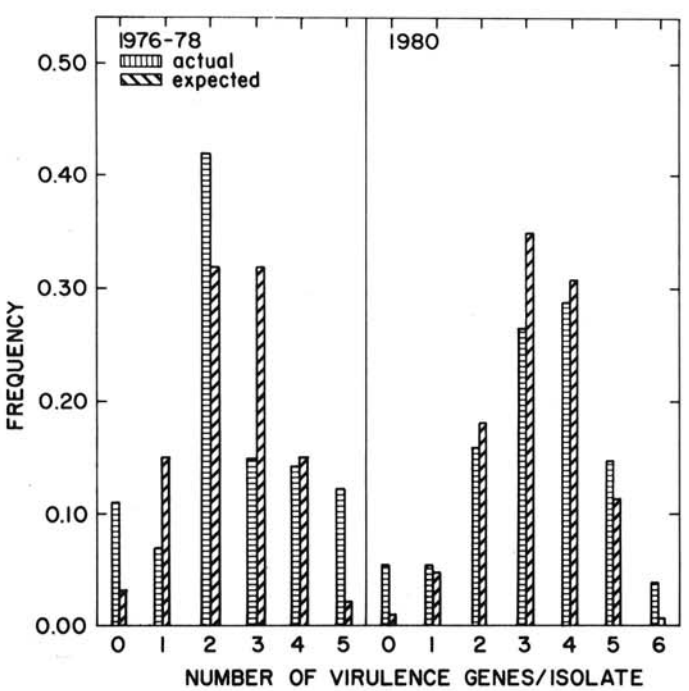

Fig. 2. Actual and expected frequencies of *Erysiphe graminis* f. sp. *tritici* genotypes, by number of virulence genes per isolate, collected during 1976–1978 and 1980 in Pennsylvania.

TABLE 2. Observed and expected frequencies of races of *Erysiphe graminis* f. sp. *tritici* collected during 1976–1978 and 1980 in Pennsylvania[a]

Race[b]	Observed	Expected[c]
1976–1978		
...	11	2.8
3c	4	4.5
MA	3	3.7
3b,3c	1	0.0
3c,4	2	1.6
3c,MA	21	5.8
2,5	18	3.4
3c,4,MA	5	2.0
3c,5,MA	1	6.7
2,5,MA	2	4.5
2,4,5	4	1.2
2,3c,5	3	5.5
3c,4,5,MA	1	2.3
2,3c,5,MA	11	7.1
2,4,5,MA	1	1.5
2,3c,4,5	1	1.9
2,3c,4,5,MA	12	2.5
1980		
...	8	0.6
MA	4	2.4
4	1	0.5
3c	3	1.9
4,MA	2	1.8
3c,MA	15	7.2
3c,4	3	1.4
3a,MA	1	0.1
2,MA	1	3.8
2,5	3	1.6
1,3c,MA	1	0.2
3c,5,MA	2	11.8
3c,4,MA	19	5.4
2,5,MA	10	6.3
2,4,MA	1	2.9
2,4,5	5	1.2
2,3c,5	3	4.9
3c,4,5,MA	1	8.9
2,3c,5,MA	37	18.9
1,2,4,5	2	0.0
2,4,5,MA	1	4.7
2,3c,4,5	4	3.7
1,2,3c,5,MA	1	0.5
2,3c,4,5,MA	22	14.2
2,3b,3c,4,5,MA	1	0.1
2,3a,3c,4,5,MA	5	0.6

[a] Sample size $N = 101$ for 1976–1978 and 156 for 1980, respectively.
[b] Designated with virulence genes present in the race. Virulence genes tested for were *p*l, *p*2, *p*3a, *p*3b, *p*3c, *p*4, *p*5, and *p*MA.
[c] Expected race frequencies were calculated as the product of the frequencies of virulence genes in a given race and the complement of the frequencies of genes not in that race.

TABLE 3. Virulence genes of *Erysiphe graminis* f. sp. *tritici* that significantly differed in frequency between a 1976–1978 collection and a 1980 collection from Pennsylvania

Virulence gene (*p*x)	*p*x present[a]		*p*x absent		Chi-square statistic
	1976–1978	1980	1976–1978	1980	
*p*3c	62	117	39	39	4.76
*p*4	26	67	75	89	7.13
*p*MA	57	124	44	32	14.55

[a] Observed numbers from 2 × 2 contingency table. $N = 101$ for 1976–1978 and 156 for 1980.
[b] Adjusted for continuity, $P < 0.05$, at $\chi^2 > 3.84$.

gene from MA. It could not be determined from that study if these two cultivars possessed *Pm*3c, *Pm*6, *Pm*7, or other *Pm*x genes. Several other researchers have been unable to explain all virulence gene frequencies on the basis of known resistance genes in the host population (17–32). These information gaps have made it difficult to address the theory of selection favoring only the alleles necessary for virulence on particular hosts (30,31).

Wild hosts (7) as well as commercial cultivars (33) may have selected for specific virulence genes and host-nonspecific fitness genes. An alternative hypothesis may be that random genetic drift was responsible for certain virulence gene frequencies.

Population shifts in plant pathogens may be more efficiently identified if random genetic drift can be separated from directional selection for the more fit phenotypes. The current models for random genetic drift include many assumptions (11). Their value lies in the reference to ideal populations in which each subpopulation produces a large number of gametes, a small number of which randomly form the next generation. In the above example, drift results from sampling error. In this study, drift may result from the chance event of compatible genotypes arriving on particular hosts after the sexual cycle each season. A large number of subpopulations from the same original population would have to be examined simultaneously to obtain an estimation of drift.

Selection is also difficult to measure in practice and as many phenotypes as possible should be examined to ensure that the genetic background will average out to be equal in all genotypes (14). A logical approach is to relate changes in race frequencies to the frequency with which commercial cultivars were grown.

Evidence is accumulating that the effects of virulence genes are not independent (4,25,31,34). Linkage may occur (4,14,25,30,32,34), and selection for fitness in addition to virulence genes also may occur (3,5,6,15,17–20,24,26,32,33).

Proper analysis and sampling is important to make meaningful comparisons of population fluctuations over several samples. More research is needed to test the relevance of comparing samples obtained from different epidemics both spatially and temporally (34). The plotting (10) and regression (16) of virulence gene frequencies over time did not take full advantage of the discrete nature (9) of racial classification data. The contingency table approach to test whether gene frequencies are the same from year to year is another way to detect population shifts.

The sample size is important in survey work and should be wisely considered. It will influence the accuracy of the estimation of each gene's frequency which can be expressed as the confidence limits of a proportion (24), the probability of detecting all possible races, and the precision with which we can detect differences between frequencies. A sample of size $N = 498$ would be needed to obtain at least one isolate of a race with a frequency of $X = 0.006$ with a probability of $P = 0.95$. The equation is expressed as $N = \log(1-P)/\log(1-X)$. It may also be impractical to collect enough isolates to detect small changes in gene frequencies between sample dates (29). "Mobile nurseries" (35) may be very useful in detecting rare virulence genes and races if the resistance genes in the test plants are combined to detect these pathogen phenotypes. If the sample size is large enough, differences between even the smallest frequencies become significant. Therefore, it is important to limit the sample size to test pertinent hypotheses concerning differences between frequencies, or to use confidence limits to test these differences (29).

The basic question of what methodology is required to obtain an accurate representation of a pathogen population must be answered so that statistical tests are meaningful. Another researcher should reach the same conclusions from a determined sample size needed to detect a shift in gene frequency within a defined sampling space and time.

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