Effect of Host Genotype on Estimating Relative Parasitic Fitness Among Populations of *Helminthosporium carbonum* Race 3

L. V. Gregory, J. E. Ayers, and R. R. Nelson

Research assistant, professor, and Evan Pugh professor, respectively, Department of Plant Pathology, Pennsylvania State University, University Park 16802.

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ABSTRACT

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Changes in the relative parasitic fitness of populations of *Helminthosporium carbonum* (= *Cochliobolus carbonum*) race 3 were studied using four populations of the fungus. Three populations, 10 isolates each, were collected in Pennsylvania in 1970, 1974, and 1979. The fourth population also consisted of 10 isolates and was collected from Illinois in 1979. Ten cultivars of greenhouse-grown corn (*Zea mays*) were inoculated at the three- to four-leaf stage with these 40 isolates. After 15 days, lesion length as a measure of parasitic fitness was determined. From the interaction of isolates and cultivars, it was evident that parasitic fitness was specific and that statistically significant differences among populations were dependent on the cultivar used to evaluate parasitic fitness. Variances of mean lesion length of the 1970 Pennsylvania and the Illinois populations were generally lower than the variances of the 1974 and 1979 Pennsylvania populations although there was some dependence on cultivar. The data suggest that choice of cultivar can be critical to the evaluation of parasitic fitness.

Additional key words: Helminthosporium leaf spot, maize.

In recent years, an increase in the severity of a leaf spot on corn (*Zea mays* L.) caused by *Helminthosporium carbonum* Ullstrup (= *Cochliobolus carbonum* Nelson) race 3 prompted a comparative study of potential changes in relative parasitic fitness of populations of the pathogen. Race 3 was first described in 1973 (13) and differentiated from races 1 and 2 on the basis of lesion type. Lesions produced by race 3 are long and linear to oval in contrast to the necrotic flecks produced by race 2 and the large, circular lesions produced by race 1. Isolates of *H. carbonum* race 3 were observed as early as 1971 (16) and since that time have been observed throughout the northeast (1) and the corn belt in the United States (9). Resistance to race 3 has been identified in inbreds and hybrids (2,6,8) and probably is best characterized as rate-reducing (12). Yield reductions attributed to race 3 were minor in the corn belt (3), but recent observations in Pennsylvania suggest possible yield losses at high severities (J. E. Ayers, *unpublished*). The frequent isolation of race 3 and increasing severities observed in the field may indicate this pathogen is more of a problem on corn than originally projected.

Information on variability of fitness attributes within pathogen populations would be pertinent to the question of the stability of resistance to this pathogen. Rate-reducing resistance (12) is measured, in part, as the influence of the host on the components of parasitic fitness of the pathogen (14). Therefore, parameters used to evaluate resistance could be utilized to evaluate variability within pathogen populations. Changes in parasitic fitness may occur within as well as among races since racial notation reflects the presence or absence of genes for virulence rather than parasitic fitness. Variation in parasitic fitness of several pathogens has been demonstrated to occur naturally (7,10,11,16).

In this research, lesion size was used as an attribute of parasitic fitness (7) to evaluate variation in four populations of *H. carbonum* race 3 and to assess the influence of host genotype on the ability to evaluate this measure of parasitic fitness.

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MATERIALS AND METHODS

Isolates chosen for use in these experiments were based on the results of related experiments (4). Ten isolates of *H. carbonum* race 3 were selected at random from each of three Pennsylvania populations of the pathogen. The 1970 population was isolated from diseased corn leaves collected in Pennsylvania in 1970–1971. The 1974 and 1979 populations consisted of isolates collected from corn during disease surveys in Pennsylvania in 1974–1975 and 1979, respectively. The final population, also consisting of 10 isolates, originated from diseased corn leaves collected in Illinois in 1979. Preliminary studies (4) and observations (J. E. Ayers and R. R. Nelson, *unpublished*) suggested that a shift in some parasitic fitness attributes had occurred over this period of time. The three Pennsylvania populations were chosen to represent the range of this time span. The Illinois population was used because it represented a sample of the pathogen from a different geographical region. All isolates were confirmed to be *H. carbonum* and inoculated on corn plants in the greenhouse to confirm the race 3 designation. Dried leaf material from these inoculations was used to preserve all isolates in a stable condition until needed.

Inoculum was prepared by placing dried leaf material on moistened filter paper in a petri dish and transferring single spores to potato-dextrose agar (5 g of dextrose per liter). After 7 days, 5-mm diameter mycelial plugs were cut from the perimeter of each culture and suspended in 10 ml of water in a glass test tube. Each tube was placed on a vortex mixer and stirred at a high rate for ~5 sec to dislodge the spores. Inoculum concentration was adjusted to $\sim 1.0 \times 10^3$ conidia per milliliter.

Susceptible hybrids were Doebler's 56X, Funk's G-4141A, Funk's G-4252, Agway 595S, Agway 425X, and Eastland 3X405. Resistant hybrids were Doebler's 64XA, Agway 849X, and Funk's G-4646. Corn inbred, Pa33, was included as a susceptible entry (2). In preliminary studies, entries were evaluated as resistant or susceptible based on disease severities resulting from natural infection of *H. carbonum* race 3 in the field in 1979.

Inoculations were performed in the greenhouse with a hand-held

atomizer connected to the exhaust port of a vacuum pump operated at 0.35 kg/cm^2. Five milliliters of inoculum from each isolate were applied to 10 pots (each a separate genotype) of 3-wk-old corn seedlings (three- to four-leaf stage). Each pot contained three plants. The experiment was replicated one time for a total of six plants per genotype. After inoculation, plants were placed in a mist chamber and maintained at 100% RH for ~16 hr. The chamber was opened, and the plants were allowed to dry and then they were transferred to a greenhouse bench.

The length (millimeters) of six lesions was recorded 15 days after inoculation. One lesion from the fourth leaf of each of the six plants was sampled whenever possible. Individual lesions were considered as replications. The data were subjected to analysis of variance procedures and orthogonal comparisons were used to determine significant differences between populations. Comparisons were made by individual host genotype since an isolate × genotype interaction was expected (5). Individual analyses were performed for populations, isolates, and genotypes to determine variances for lesion size within those factors.

TABLE 1. Analysis of variance of lesion lengths produced by 40 isolates of *Helminthosporium carbonum* race 3 representing three populations from Pennsylvania and one population from Illinois as measured on 10 corn genotypes

Source	df	Mean square	*F*-ratio[a]
Isolates (I)	39	701.04	293.98*
Populations	3	4,128.69	1,731.35*
Isolates within populations	36	415.40	174.20*
Genotypes (G)	9	99.94	41.91*
I × G	351	9.04	3.79*
Error	2,000	2.38	

[a] Asterisk (*) denotes significance, $P \leq 0.05$.

TABLE 2. Mean length of lesions produced by 10 isolates of *Helminthosporium carbonum* race 3 in each of three populations from Pennsylvania and one population from Illinois as measured on 10 corn genotypes 15 days after inoculation

Genotypes	Pennsylvania populations			Illinois population
	1970	1974	1979	
Pa33	7.6[a]	6.3	7.9	5.9
Doebler's 56X	10.0	5.0	8.6	2.9
Doebler's 64XA	6.1	3.6	6.6	1.9
Agway 425X	8.0	5.8	8.6	3.1
Agway 849X	8.1	4.4	8.9	2.8
Funk's G-4141A	8.6	5.8	8.1	3.0
Funk's G-4252	8.1	4.6	7.6	2.0
Funk's G-4646	8.1	4.4	7.9	2.7
Eastland 3X405	8.4	4.7	8.3	2.5
Agway 595S	8.1	4.5	8.9	2.6

[a] Values represent a mean of six lesions of 10 isolates within each population.

RESULTS

There was a significant interaction between isolates and genotypes as expected from the results of preliminary experiments and other research (Table 1) (5). Lesion length observed within and among populations varied over the 10 genotypes used (Table 2). Isolates from the Illinois population initiated significantly smaller lesions than Pennsylvania isolates on all genotypes (Table 3). Lesions from isolates of the 1974 population were significantly

TABLE 3. Orthogonal comparisons by genotype of lesion length produced by 40 isolates of *Helminthosporium carbonum* race 3 representing three populations from Pennsylvania and one population from Illinois as measured on 10 corn genotypes

Genotype	Mean square[a]		
	Illinois vs. Pennsylvania populations	Pennsylvania populations	
		1970, 1979 vs. 1974 populations	1970 vs. 1979 populations
Pa33	88.20*	84.10*	2.70
Doebler's 56X	1,056.09*	667.88*	28.03*
Doebler's 64XA	576.02*	300.67*	8.01
Agway 425X	862.42*	263.51*	10.80*
Agway 849X	906.76*	261.80*	6.08
Funk's G-4141A	1,020.07*	440.01*	7.50
Funk's G-4252	779.17*	537.78*	1.20
Funk's G-4646	961.42*	506.47*	3.01
Eastland 3X405	849.34*	545.14*	0.41
Agway 595S	875.61*	702.80*	33.08*

[a] Asterisk (*) denotes significance, $P \leq 0.05$.

TABLE 4. Variance in lesion length within 10 isolates of *Helminthosporium carbonum* race 3 in each of three populations from Pennsylvania and one population from Illinois as determined on 10 corn genotypes

Genotypes	Variance in lesion length			
	Pennsylvania populations			Illinois population
	1970	1974	1979	
Pa33	22.64	22.18	15.16	24.59
Doebler's 56X	47.00	73.15	73.60	36.98
Doebler's 64XA	36.60	65.75	50.38	31.72
Agway 425X	38.79	79.16	50.85	56.82
Agway 849X	29.52	101.67	61.60	51.39
Funk's G-4141A	45.63	90.74	50.19	28.74
Funk's G-4252	44.21	55.55	46.71	37.83
Funk's G-4646	29.34	95.56	50.85	39.83
Eastland 3X405	32.38	61.11	75.96	25.97
Agway 595S	16.41	79.45	100.96	37.93

TABLE 5. Mean and variance length of lesions caused by 40 isolates of *Helminthosporium carbonum* race 3 from four populations averaged across 10 corn genotypes

Pennsylvania populations									Illinois population lesion length (mm)		
1970			1974			1979					
	Lesion length (mm)			Lesion length (mm)			Lesion length (mm)				
Isolate	Mean	Variance	Isolate	Mean	Variance	Isolate	Mean	Variance	Isolate	Mean	Variance
1	8.1	21.41	1	1.9	7.08	1	9.3	8.15	1	2.7	7.13
2	9.8	4.77	2	7.7	7.67	2	9.5	6.08	2	2.2	8.33
3	9.3	10.79	3	8.9	6.47	3	8.8	6.49	3	1.5	8.51
4	2.5	4.91	4	2.6	10.86	4	8.8	10.13	4	1.9	3.78
5	8.5	13.74	5	2.5	19.99	5	9.4	7.98	5	2.1	12.08
6	7.7	10.29	6	2.2	6.16	6	9.3	10.70	6	2.2	15.96
7	8.9	4.71	7	8.4	7.46	7	10.1	15.12	7	2.3	9.48
8	8.1	7.85	8	1.9	4.31	8	3.1	10.45	8	9.5	9.36
9	7.9	12.67	9	9.8	16.41	9	10.2	21.29	9	2.9	31.52
10	10.0	28.03	10	3.2	19.37	10	2.5	20.00	10	1.9	5.07

smaller than those of the 1970 and 1979 populations on all genotypes. A significant difference was observed between isolates of the 1970 and 1979 populations in three cultivars only. Isolates of the 1970 population caused larger lesions on Doebler's 56X, whereas isolates from the 1979 population caused the larger lesions on Agway 425X and Agway 595S. There were no significant differences between the two populations on other genotypes.

Overall, variances of mean lesion length for the 1970 and Illinois populations were lower than for the 1974 and 1979 populations, but this was dependent on genotype (Table 4). Variances were low for the 1970 population on Agway 595S and highest for the 1979 population. In contrast, the variance of the 1974 population was greatest on Agway 849X.

Mean lesion size and variance among the 10 genotypes also was dependent upon individual isolates (Table 5). Mean lesion length ranged from 1.5 to 10.2 mm with variances ranging from 3.78 to 31.52. There was no correlation between lesion length and variance.

DISCUSSION

Host genotype had a great influence on the evaluation of parasitic fitness. In cases for which differences in lesion length were large (eg, the 1974 and Illinois populations versus the 1970 and 1979 populations), comparisons were significant and consistent over all genotypes. When differences were small (eg, the 1970 versus the 1979 population), significance was not observed on susceptible genotypes such as Pa33 and Eastland 3X405. Similarly, resistant genotypes, Doebler's 64XA and Funk's G-4646, were ineffective in differentiating populations. Less-susceptible genotypes such as Doebler's 56X, Agway 425X, and Agway 595S were more effective in detecting differences between populations. Given the three distinct outcomes in comparisons of the 1970 and 1979 populations, it is obvious that one genotype was not sufficient to evaluate parasitic fitness. For example, an investigator assessing potential or actual population shifts on only one of these host genotypes might have reached an entirely different conclusion if another host genotype had been used.

Since parasitic fitness in the pathogen may be analogous to rate-reducing resistance in the host, there may be a range in fitness among isolates just as there are levels of resistance in the host. An isolate with increased fitness may be detected by the larger lesion it produces. The inherent problem with *H. carbonum* is that the designation of race 3 was based on lesion type, specifically lesion length (13), which may be considered a parasitic fitness trait. In some cases, it would be difficult to discern an isolate of race 3 with reduced fitness (small lesion) from an isolate designated as race 2 (necrotic fleck). This is further complicated because *H. carbonum* race 3 was named without the use of a standard cultivar for identification; likewise, there are no standards for evaluating parasitic fitness.

The aim of monitoring parasitic fitness is to establish a method for detecting pathogen population shifts towards increased fitness in order to avoid any potentially destructive epidemics. A primary factor in monitoring parasitic fitness is the variation of the pathogen population but, as was shown here, that variability is dependent upon host genotype. For this reason, it may be appropriate to evaluate parasitic fitness by screening isolates on genotypes commonly grown during the period the isolates were collected. In this way, both sources of variation, host and pathogen, are included in comparisons of pathogen populations. This will improve the chances of detecting large differences as well as subtle changes.

As stated previously, the effectiveness of rate-reducing resistance is dependent upon pathogen variability. Previous research has suggested the possibility of erosion of this type of resistance (5–7,10,11,15). There was ample variation within populations of *H. carbonum* race 3 used in this research to suggest that a shift to pathogen genotypes with increased parasitic fitness is possible.

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