The Evaluation of Relative Parasitic Fitness of Isolates of *Helminthosporium maydis* Race T

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ABSTRACT

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Results of previous research demonstrated that the relative parasitic fitness of isolates of *Helminthosporium maydis* race T could be reduced by serial passage on a resistant host. Three of these isolates were used to evaluate the consequences of reduced parasitic fitness on disease spread, disease severity, and relative parasitic survival under field conditions. Three original field isolates and the same isolates with reduced parasitic fitness after 10 serial passages on normal cytoplasm corn were used to initiate epidemics resulting from a point source of inoculum in field plots of corn cultivar PA887P × B14 T-cms. Late in the epidemics, whole-plot disease severity was significantly lower in plots inoculated with isolates having reduced parasitic fitness than in plots inoculated with original field isolates. Disease severities at point sources and at distances from a point source reflected similar trends. Use of orthogonal comparisons to test combinations of isolates revealed significant differences among field isolates and isolates conditioned on normal cytoplasm. Reductions in disease severity among comparisons of conditioned isolates most likely reflects the effect of a reduced sporulation capacity. This intraracial source of pathogen variability should be considered in disease management strategies involving host resistance and yield loss assessment.

Additional key words: southern corn leaf blight, corn, *Zea mays* L., maize.

Much research has been undertaken to identify new races among pathogen populations, but little emphasis has been placed on the evaluation of subpopulations within a race for changes in parasitic fitness. It is logical to assume that changes in parasitic fitness would influence disease development and that more fit members of a pathogen population would cause a greater disease severity and yield loss. This source of variability should be recognized in disease management strategies.

It has been demonstrated that the relative parasitic fitness of isolates of race T of *Helminthosporium maydis* Nisikado and Miyake can be altered by serial passage on a resistant host (7). After several cycles of disease on resistant corn (*Zea mays* L.) in normal cytoplasm, reductions were observed in relative disease efficiency, lesion size, and sporulation when isolates were evaluated on corn in Texas male-sterile cytoplasm (T-cms). The availability of isolates with these traits permitted an evaluation of decreased parasitic fitness on disease spread and severity and relative parasitic survival under field conditions, which is the subject of this paper.

MATERIALS AND METHODS

Plots were planted with corn cultivar PA887P × B14 T-cms on 7 May 1979 at Landisville, Pennsylvania. Experimental units were seven rows wide (0.9 m spacing) and 7 m long and bordered on all sides by at least 7 m of corn in normal cytoplasm. The plots were arranged in a randomized complete block design with four replications of each treatment. The treatments consisted of three isolates of race T of *H. maydis* collected in Pennsylvania in 1970 and the same three isolates with reduced parasitic fitness after 10 serial passages on normal cytoplasm corn (7) (Table 1). Isolates 7297N and 7280N displayed reductions in relative disease efficiency and lesion size while isolate 7080N showed reductions in the same traits and in sporulation when compared to their counterparts maintained on corn in T-cytoplasm.

Inoculum was prepared from cultures grown on potato dextrose agar for 7 days. A spore suspension containing 2.1×10^3 conidia per milliliter was obtained for each isolate and approximately 75 ml was applied at a point source to the four basal leaves of the center plant in each plot for the appropriate treatment. Inoculations were made shortly after anthesis on 17 July.

Twenty-four plants, not including the central inoculated plant, were marked for disease assessment radiating in eight directions about the center plant at approximately 0.95-m intervals. Actual distances of individual plants from the center inoculated plant were recorded for each plot. Disease assessments were made at weekly intervals beginning 2 August and recorded as the percent foliar tissue blighted on individual leaves of each plant for a total of 12 leaves per plant. Disease severity for the plant was calculated by multiplying disease severities by respective leaf areas, summing the actual blighted area, and dividing by the total leaf area of the plant (1).

The influence of differing levels of relative parasitic fitness were determined by analysis of variance of disease severities for the entire plot, center plant only, and at distances from the point source. Duncan's least significant difference was calculated for the comparisons of disease severities of original field isolates and their counterparts conditioned on normal cytoplasm (10). Orthogonal comparisons (8) were constructed to test significance among specific groups of isolates. Apparent infection rates (9) and disease gradients (2) were determined by regression.

RESULTS

Differences in relative parasitic fitness were observed in disease severities for the entire plots at the later dates of assessment (Table 2). The most dramatic difference observed was between isolates 7080T and 7080N, which were significantly different ($P = 0.05$) on 23 and 30 August and 7 September. There also was a significant difference between 7280T and 7280N, but this was not apparent until 30 August and 7 September.

A similar trend was observed when disease severities were analyzed using only the center inoculated plant as a simple variable for estimating relative parasitic fitness (Table 3). Isolates 7080T and 7080N were significantly different on 30 August and 7

September. Differences in other isolates were observed although these were not statistically not significant.

When distance was incorporated as a factor in the analysis, differences were significant for disease severities observed on 7 September at each interval from the point source for isolates 7080T and 7080N (Table 4). Differences in disease severities of isolates 7080T and 7080N were significant at distances proximal to the point source. Differences in disease severities of isolates 7280T and 7280N were significant at 1.2 and 1.8 m from the point source.

Disease severities for entire plots recorded on 7 September were analyzed by using orthogonal comparisons to test for significant differences among specific combinations of isolates (Table 5). A significant difference was observed between field isolates and their counterparts conditioned on normal cytoplasm. No differences were detected for comparisons involving only field isolates. There was a significant difference in the comparison of 7080N with 7297N and 7280N which most likely reflects the reduced sporulation capacity of isolate 7080N. There were no significant differences between 7297N and 7280N.

Apparent infection rates and disease gradients were computed, but variability in these parameters was large and resulted in no useful comparisons between isolates.

DISCUSSION

The approach taken for the evaluation of relative parasitic fitness was to allow differences among isolates to be compounded over several cycles of disease. Factors such as the rate of disease increase and magnitude of spread were indirectly reflected in whole-plot disease severities assessed toward the end of the season. It is interesting that reductions in parasitic fitness among isolates could

TABLE 1. Components of relative parasitic fitness of three isolates (T) of *Helminthosporium maydis* race T and the same three isolates with reduced parasitic fitness (N) after 10 serial passages on corn in normal cytoplasm (7)

Isolate	Components of relative parasitic fitness		
	Relative disease efficiency[a]	Lesion size (mm²)	Sporulation[b] (no./mm²)
7297T	22	9.2	29
7297N	8	5.6	26
7080T	22	13.3	158
7080N	9	7.7	54
7280T	12	16.1	162
7280N	7	8.7	156

[a] Relative disease efficiency is the number of successful infections resulting from a given amount of inoculum.
[b] Sporulation is the number of spores produced per lesion area at one point in time.

TABLE 2. Percent foliar tissue blighted of entire plots resulting from a point source inoculation with three field isolates (T) of *Helminthosporium maydis* race T and the same three isolates with reduced parasitic fitness (N) after 10 serial passages on normal cytoplasm corn

Isolate	Date of disease assessment					
	2 Aug	8 Aug	16 Aug	23 Aug	30 Aug	7 Sept
7297T	0.43[a] AB	0.75	1.44	3.82 A	14.65 AB	44.63 AB
7297N	0.51 A	0.93	1.26	2.60 A	10.77 B	36.18 BC
7080T	0.54 A	0.95	1.69	4.05 A	17.09 A	45.65 A
7080N	0.26 B	0.64	0.96	1.33 B	3.68 C	16.78 D
7280T	0.53 A	0.74	1.48	3.90 A	18.31 A	51.58 A
7280N	0.49 A	0.82	1.24	2.28 AB	10.32 B	35.87 C
DLSD	0.19	NS[b]	NS	2.02	5.02	8.05

[a] Values are an average of four replications with 25 observations in each replication. Means followed by the same letter are not significantly different ($P = 0.05$) by Duncan's least significant difference (DLSD) test.
[b] Values are not significant ($P = 0.05$).

TABLE 3. Percent incidence of foliar tissue blighted on the centrally located inoculated plants (one in each pot) after inoculation singly with three field isolates (T) of *Helminthosporium maydis* race T or the same three isolates with reduced parasitic fitness (N) after 10 serial passages on normal cytoplasm corn

Isolate	Date of disease assessment					
	2 Aug	9 Aug	16 Aug	23 Aug	30 Aug	7 Sept
7297T	9.85[a] AB	14.78	18.90	25.87	36.68 AB	63.31 A
7297N	12.78 A	20.68	23.10	25.86	34.60 AB	56.10 A
7080T	13.58 A	20.33	22.10	26.33	39.59 A	60.61 A
7080N	6.45 B	15.70	20.57	22.89	25.70 B	38.91 B
7280T	13.03 A	15.83	18.56	25.94	35.03 AB	63.62 A
7280N	12.23 A	18.70	21.25	25.39	28.95 AB	51.52 AB
DLSD	4.77	NS[b]	NS	NS	12.77	16.72

[a] Values are an average of four replications of observations made on the plant in each plot which was inoculated to initiate the epidemic. Means followed by the same letter are not significantly different ($P = 0.05$) by Duncan's least significant difference (DLSD) test.
[b] Values are not significant ($P = 0.05$).

TABLE 4. Percent foliar tissue blighted on 7 September at approximate distances from a point source of inoculum of three field isolates (T) of *Helminthosporium maydis* race T and the same three isolates with reduced parasitic fitness (N) after 10 serial passages on normal cytoplasm corn

Isolate	Distance from point source of inoculum (m)					
	0.9	1.2	1.8	2.6	2.7	3.8
7297T	53.79[a]	42.57	45.09	40.15	43.96	37.59
7297N	47.13	34.02	36.46	37.21	34.71	22.53
7080T	54.86	53.26	45.85	41.10	41.94	33.14
7080N	23.54	17.78	13.64	11.52	16.64	12.02
7280T	58.20	58.69	54.88	48.93	45.66	40.12
7280N	45.70	34.91	34.41	32.01	37.35	26.97
DLSD[b] = 19.63						

[a] Values are an average of four replications with four observations per replication.
[b] Duncan's least significant difference ($P = 0.05$) for comparison of all means.

TABLE 5. Orthogonal comparisons among groups of three field isolates (T) of *Helminthosporium maydis* race T and the same three isolates (N) with reduced parasitic fitness based on disease severities of whole plots assessed on 7 September

Analysis of variance Source	d.f.	Sum of squares	Mean square	F ratio
Isolates[a]	5	2,976.02	295.21	
T isolates vs N isolates	1	1,875.49	1,875.49	51.45**
7080T vs 7297T, 7280T	1	16.10	16.10	0.44
7297T vs 7280T	1	96.47	96.47	2.65
7080N vs 7297N, 7280N	1	987.78	987.78	27.10**
7297N vs 7280N	1	0.18	0.18	0.01
Error	15	546.75	36.45	

[a]7297T, 7080T, and 7280T were field isolates collected in Pennsylvania in 1970. 7297N and 7280N displayed reductions in relative disease efficiency and lesion size, while 7080N showed reductions in the same traits and in sporulation.

be differentiated by either disease severities on the inoculated plant, reflecting primarily rate of disease increase, or disease severities at distances from the point source, a function of disease spread. Whereas this approach does not allow for further analysis by mathematical models involving population genetics (4) it does in fact simply and effectively demonstrate differences in parasitic fitness. Similar methods have been proposed for the identification of the horizontal resistance of wheat to *Puccinia graminis* f. sp. *tritici* (3) that should be applicable to the identification of parasitic fitness.

This research identified an intraracial source of variability that has not been widely investigated. Previously the identification of races among pathogen populations served as a measure of pathogen variability for disease resistance and other disease management strategies. Changes in relative parasitic fitness may go virtually undetected in a screening program for races of a particular pathogen. As was observed here, changes in relative parasitic fitness resulted in differing levels of disease and therefore differing losses since percent yield loss is directly proportional to the severity of disease (1). Information concerning levels of relative parasitic fitness in pathogen populations should be incorporated into disease management strategies.

Finally, information gathered concerning relative parasitic

fitness may be relevant to breeding programs for horizontal resistance. Host attributes which reduce the rate of disease increase are manifest in traits such as relative disease efficiency, latent period, and sporulation. Parlevliet and Van Ommeren (5) attributed the primary expression of partial resistance in barley to *Puccinia hordei* to either the latent period or a correlation of latent period with other components of resistance. Rouse (6) concluded that the greater horizontal resistance of wheat cultivar Redcoat over Knox was a result of a reduction in sporulation of *Erysiphe graminis* f. sp. *tritici*. It is apparent in this research that the reduced sporulation of isolate 7080N was a primary factor involved in reduced spread and disease severity as detected by orthogonal comparisons. Reductions in lesion size and relative disease efficiency did result in lower disease severities, although the decrease was not as great as that attributable to decreased sporulation.

LITERATURE CITED

1. Gregory, L. V., Ayers, J. E., and Nelson, R. R. 1978. Predicting yield losses in corn from southern corn leaf blight. Phytopathology 68:517-521.
2. Gregory, P. H. 1968. Interpreting plant disease dispersal gradients. Annu. Rev. Phytopathol. 6:189-212.
3. MacKenzie, D. R. 1976. Application of two epidemiological models for the identification of slow stem rusting in wheat. Phytopathology 66:55-59.
4. MacKenzie, D. R. 1978. Estimating parasitic fitness. Phytopathology 68:9-13.
5. Parlevliet, J. E., and Van Ommeren, A. 1975. Partial resistance of barley to leaf rust, *Puccinia hordei* II. Relationship between field trials, micro-plot tests and latent period. Euphytica 24:293-303.
6. Rouse, D. I. 1979. Components of horizontal resistance to powdery mildew of wheat as related to disease progress and parasitic fitness. Ph.D. thesis. Department of Plant Pathology, The Pennsylvania State University, University Park. 92 pp.
7. Royer, M. H., and Nelson, R. R. 1981. The effect of host resistance on relative parasitic fitness of *Helminthosporium maydis* race T. Phytopathology 71:351-354.
8. Steel, R. G. D., and Torrie, J. H. 1960. Principles and Procedures of Statistics. McGraw-Hill, New York, Toronto, and London. 481 pp.
9. Vanderplank, J. E. 1963. Plant Diseases: Epidemics and Control. Academic Press, New York. 349 pp.
10. Waller, R. A., and Duncan, D. B. 1969. A Bayes rule for the symmetric multiple comparisons problem. J. Am. Statist. Assoc. 64:1484-1503.