

The Effect of Host Resistance on Relative Parasitic Fitness of *Helminthosporium maydis* Race T

M. H. Royer and R. R. Nelson

Graduate assistant and Evan Pugh Professor, respectively, Department of Plant Pathology, The Pennsylvania State University, University Park 16802.

Contribution 1170, Department of Plant Pathology, The Pennsylvania Agricultural Experiment Station, University Park. Authorized for publication 16 April 1980 as Journal Series Paper 5958.

Portion of a thesis submitted by the senior author in partial fulfillment of the requirements for the M.S. degree.

Accepted for publication 11 August 1980.

ABSTRACT

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.

Eight isolates of *Helminthosporium maydis* race T were passed through one conidial generation on the susceptible Texas male sterile corn hybrid PA887 × B14 T-cms, followed by six serial conidial generations on the resistant, normal (N) cytoplasm hybrid Pioneer Brand 3306 N. A part of each generation was preserved in dry leaf material. Relative disease efficiency (number of lesions per unit of inoculum), lesion size, and sporulation of the first (T_1) and seventh (N_6) generations of each isolate were compared on the T-cms hybrid. Significant decreases occurred in at

least one of the fitness attributes for each isolate. The effects of two generations (T_2) on the T-cms hybrid after two (N_2) or 10 (N_{10}) generations on the N hybrid were tested for five isolates. Some isolates exhibited a significant decrease in relative disease efficiency after only two passages on the resistant host. Relative disease efficiency lost during 10 generations on the N hybrid was partially restored in some isolates after two passages on the T-cms hybrid ($N_{10}-T_2$).

The widespread epidemic of southern corn leaf blight in the United States in 1970 was incited by race T (7) of *Helminthosporium maydis* Nisikado and Miyake. Race T is highly virulent on hybrids of corn (*Zea mays* L.) produced in Texas male-sterile cytoplasm (T-cms) and T-cms hybrids occupied approximately 90% of the 1970 acreage (3). Hybrids produced in normal (N) cytoplasm are resistant to race T, and the frequency of the race decreased dramatically with the agricultural use of resistant hybrids (3).

Relative parasitic fitness has been defined as the relative ability of parasitic genotypes or populations to persist successfully over time (4). Relative parasitic fitness is a measure of reproductive success and survival. Populations of plant parasites that increase in frequency relative to other populations possess certain fundamental assets, some of which are characterized in a general way as "fitness attributes" (4). Some fitness attributes are: relative disease efficiency, a relative measure of the number of successful infections resulting from a given amount of inoculum; sporulation, the amount of inoculum generated per unit area of diseased tissue; and lesion size.

Although race T was described during the 1970 epidemic of southern corn leaf blight, Nelson et al (5) reported that isolates of race T had existed throughout many areas of the world for many years before the epidemic. Comparative studies with an isolate of race T collected in 1955 and maintained in preserved leaf material and representative isolates of the 1970 race T population revealed some substantial differences in certain fitness attributes (3). The 1955 isolate induced fewer, smaller lesions, and produced substantially fewer spores per square millimeter of lesion area. Because of this and other criteria, Nelson (3) concluded that the 1970 population was more fit to cause an epidemic and, furthermore, that the 1955 isolate probably would not have been able to cause an epidemic even if susceptible T-cms hybrids had been grown over vast acreages at that time.

Nelson (6) speculated that the increasing use of T-cms hybrids in the southeastern USA during the 1960s enabled existing populations of race T to increase in parasitic fitness until populations were capable of generating an epidemic. The implication was that populations of race T lacked parasitic fitness

prior to the development of T-cms hybrids or that their fitness was impaired through many years of residence on resistant hybrids.

The objective of this research was to determine the effect of host resistance on the asexual stage of parasitic fitness by using race T of *H. maydis* and T-cms and N cytoplasm corn hybrids.

MATERIALS AND METHODS

Eight isolates of *H. maydis* race T, collected in 1971 were used in this study. The isolates originally had been single-spored and increased on potato-dextrose agar (PDA) to obtain inoculum to inoculate T-cms corn plants. The resulting diseased leaves were collected, dried, and stored under dry conditions in order to avoid the genetic changes that could occur during prolonged *in vitro* culturing. When the present studies were initiated, portions of the stored leaf material of the eight isolates were placed in petri dishes lined with moistened filter paper and incubated in darkness at 21 C. When the fungus in the leaf tissue began to sporulate, single spores were transferred to petri dishes containing PDA. After 7 days at 21–23 C the sporulating colonies were harvested by pouring water, which contained 0.05% agar to facilitate adherence of the spores to the leaf surface, over the cultures and scraping the cultures with a microscope slide. Male-sterile corn plants in the sixth-leaf stage were inoculated by atomizing the spore suspension onto the leaves and into the whorls of the plants. The inoculated plants were placed in a mist chamber for 16 hr and then transferred to greenhouse benches. The resulting diseased leaves were collected 7 days later, dried in paper bags, and subsequently used as the source of inoculum to initiate the serial passage studies.

Pioneer Brand 3306 N and PA 887P × B14 T-cms were the resistant and susceptible hybrids, respectively, used throughout the studies. All plantings were made in the greenhouse by placing four seeds in a sterilized potting mixtures of Hagerstown silty clay loam, peat, and perlite (1:1:1, v/v) in 800 cm³ pots.

Serial passage. Serial passage studies were initiated by taking some of the stored diseased leaves of each of the eight isolates, designated here as T_1 , and inducing sporulation by the process previously described. Spores were harvested directly from the sporulating fungus in the leaf material by agitating the leaves in 0.05% water agar. Unquantified spore suspensions were atomized onto N cytoplasm plants that had grown to the sixth leaf stage. Inoculated plants were incubated in a mist chamber for 16 hr and transferred to greenhouse benches. Infected leaves were collected 4

TABLE 1. Comparison within isolates of fitness attributes of *Helminthosporium maydis* race T isolates before (T₁) and after residence on a normal cytoplasm corn hybrid for six consecutive generations (N₆)

Isolate number and generation	Fitness attributes ^a		
	DE ^b	LS ^c	S ^d
7297 T ₁	20 A	2.6 A	6 A
N ₆	7 B	1.8 B	2 B
7193 T ₁	61 A	4.4 A	21 A
N ₆	38 B	1.9 B	9 B
7177 T ₁	102 A	2.9 A	9 A
N ₆	56 B	1.4 B	<1 B
7080 T ₁	92 A	3.2 A	16 A
N ₆	50 B	2.0 B	2 B
7280 T ₁	15 A	7.9 A	10 A
N ₆	8 B	5.6 B	3 B
7275 T ₁	31 A	4.1 A	14 A
N ₆	23 B	4.9 B	11 B
7298 T ₁	20 A	2.4 A	4 A
N ₆	17 A	1.9 B	2 A
7198 T ₁	37 A	6.9 A	9 A
N ₆	25 B	6.7 A	10 A

^a Means of fitness attributes for each isolate followed by same letter are not statistically significant, $P=0.05$, according to the Behrens-Fisher unpaired *t*-test.

^b Mean number of lesions on four plants for each of eight pots (replicates).

^c Average lesion size of 56–128 lesions.

^d Average sporulation per square millimeter from 56–128 lesions.

TABLE 2. Comparison within isolates of fitness attributes of five *Helminthosporium maydis* race T isolates on a Texas male sterile (T-cms) corn hybrid after various serial passages

Isolate number and generation ^b	Fitness attributes ^a		
	DE ^c	LS ^d	S ^e
7177 T ₁	21 A	13.0 A	129 A
N ₂	18 AB	10.3 B	103 AB
N ₁₀	14 B	8.8 C	66 BC
N ₂ -T ₂	21 A	11.0 B	122 A
N ₁₀ -T ₂	15 B	8.7 C	46 C
7297 T ₁	22 A	9.2 A	29 A
N ₂	21 A	7.9 AB	29 A
N ₁₀	8 B	5.6 C	26 A
N ₂ -T ₂	22 A	8.2 AB	37 A
N ₁₀ -T ₂	16 A	6.7 BC	25 A
7080 T ₁	22 A	13.3 A	158 A
N ₂	16 B	9.9 B	91 B
N ₁₀	9 C	7.7 C	54 C
N ₂ -T ₂	16 B	10.8 B	142 AB
N ₁₀ -T ₂	14 B	7.4 C	30 D
7280 T ₁	12 A	16.1 A	162 A
N ₂	9 AB	13.9 A	108 B
N ₁₀	7 B	8.7 B	156 AB
N ₂ -T ₂	9 AB	14.5 A	155 AB
N ₁₀ -T ₂	9 AB	9.7 B	140 AB
7298 T ₁	19 A	12.8 A	117 A
N ₂	13 B	11.0 A	120 A
N ₁₀	13 B	7.5 B	96 A
N ₂ -T ₂	11 B	11.6 A	114 A
N ₁₀ -T ₂	12 B	7.4 B	118 A

^a Means of a fitness attribute for each isolate followed by same letter are not statistically significant, $P=0.05$, using Duncan's modified range test. Data were transformed to \log_{10} for analyses.

^b T₁ = original isolate before serial passage; N₂ and N₁₀ = second and tenth serial generation, respectively, on the normal hybrid; N₂-T₂ and N₁₀-T₂ = two generations of N₂ and N₁₀ on the T-cms hybrid.

^c Disease efficiency (DE) = mean number of lesions on four plants for each of eight pots (replicates).

^d Lesion size (LS) = mean of 10 lesion measurements on a plant, in each pot (replicate).

^e Sporulation (S) = mean sporulation per square millimeter of 10 lesions per plant (replicate), one plant per pot.

to 6 days after lesions were first observed. The infected leaf material constituted the first serial passage on N cytoplasm and was designated as N₁.

Portions of the diseased leaf material of the N₁ isolates subsequently were induced to sporulate, and the harvested spores were atomized onto N cytoplasm plants. Diseased leaves were harvested subsequently and constituted the second serial passage on N cytoplasm, designated as N₂. The process was repeated in a similar manner for five isolates through 10 serial passages on N cytoplasm plants. Some diseased leaf material from each of the 10 transfers was preserved for further studies.

Fitness attributes. When the 10 serial passages on N cytoplasm plants were completed, the fitness of the original isolates, T₁, were compared with the isolates from the sixth serial passage on N cytoplasm plants, N₆. Subsequent studies compared the original isolates with other generations of serial passage as indicated in the Results portion of this paper. Additionally, five isolates passed for two (N₂) and 10 (N₁₀) transfers on N cytoplasm plants were transferred serially for two generations on T-cms cytoplasm plants, N₂-T₂ and N₁₀-T₂, respectively, to determine if any loss of fitness incurred on a resistant host could be restored in two generations on a susceptible host.

Relative disease efficiency. Inoculum of the T₁ isolates and designated generations of serial passage on N was harvested directly from the fungus in the leaf material by agitating the leaf tissue in 0.05% water agar. A 0.1-ml sample of the spore suspension was placed onto agar blocks, 10 × 15 × 5 mm, that were set upon a microscope slide, and spores were counted at ×30 (6). Inoculum was adjusted to 100 spores per 0.1 ml of 0.05% water agar in the initial studies, but subsequently was reduced to 50 spores per 0.1 ml. The spore suspension was sprayed onto T-cms plants that had grown to the five- to six-leaf stage. Two sets of 16 plants, four plants per pot, were each inoculated with 10 ml of inoculum. Inoculated plants were incubated in a mist chamber for 16 hr and transferred to greenhouse benches. All lesions on the 32 plants were counted 2 days after they were first evident.

Lesion size. Lesion size was estimated by measuring the length and width of 7 to 16 lesions, for a given experiment, on the third leaf of a plant chosen at random from each pot. Lesions were chosen, starting from the base of the leaf and proceeding towards the tip, until a given number of distinct lesions was measured. If less than that number were present, another plant in the same pot served to complete the set of lesions, chosen in an identical manner. A table of average lesion area for a given length and width, to 0.5 mm, was used to convert the measurements into area in square millimeters (6).

Sporulation. Sporulation was assessed by counting the number of spores produced per square millimeter of lesion area. The lesions measured were delimited with a permanent marker pen, and the plants were placed in the mist chamber for 16 hr to induce sporulation. As soon as the leaves dried, spores were harvested by suction into a test tube containing 5 ml of 0.05% water agar and 0.5% CuSO₄ to inhibit germination (6). Four samples of 0.1 ml each were taken from the spore suspension in each test tube, and the total number of spores per test tube was calculated. Sporulation per square millimeter was estimated from the total lesion area from which the spores were collected.

The data comparing the fitness attributes of isolates before and after six serial transfers on the resistant host were analyzed by using the Behrens-Fisher unpaired *t*-test. The remaining data pertaining to multiple comparisons of fitness attributes were transformed to \log_{10} to normalize variance and analyzed with a completely randomized design and Duncan's modified multiple range test (9).

RESULTS

Serial passage. The changes in parasitic fitness of eight isolates of *H. maydis* race T during six serial transfers on a resistant host are presented in Table 1. Five of the eight isolates exhibited significant decreases in relative disease efficiency (DE), lesion size (LS), and sporulation (S). A sixth isolate exhibited a significant decrease only in DE, and a seventh isolate only in LS. The eighth isolate, isolate

7275, exhibited a significant decrease in DE and S, but also exhibited a significant increase in LS. However, no significant difference in LS was detected in a second test of isolate 7275, nor was there a significant difference in LS at the tenth serial transfer on the resistant host. This suggests that the increase in LS shown previously may reflect some unknown variation in experimental technique.

Further studies with five isolates were used to compare the original isolates to the recovered isolates after two and 10 generations on the resistant host, and after the latter generations from the resistant host had been passed on to the susceptible host for two generations. The mean comparisons are presented in Table 2 and specific fitness comparisons are summarized in Table 3. The important observations are as follows: all five isolates exhibited decreased DE and LS and three isolates exhibited decreased S, after serial passage on the resistant host (comparisons 1 and 2); there is evidence of a gradual or continued decrease in fitness when comparisons are made between the second and 10th generations of serial passage on the resistant host (comparison 3); no significant differences occurred for any attribute when the second generation of passage on the resistant host was returned to the susceptible host for two generations (comparison 4); and two generations on the susceptible host of the 10th generation from the resistant host produced an increased DE, but not LS or S (comparison 5). No fungal generation on the resistant host was passed for more than two generations on the susceptible host.

The differences between fitness attributes in Table 1 and 2 for any particular isolate reflect the seasonal changes in the greenhouse environment as well as the quantity of inoculum. It is apparent that environmental differences did not alter the relationship between any comparison in either table and only their numerical values changed.

DISCUSSION

Evidence from the study supports the theory that host resistance is deleterious to parasitic fitness. Significant decreases in at least one fitness attribute occurred for each race T isolate that was serially passed on the resistant hybrid.

Relative disease efficiency and sporulation are highly heritable traits in *H. maydis* race T (2). If isolates residing on N-cytoplasm hybrids exhibit reduced fitness in either of these traits under natural conditions, as these studies suggest, rather significant changes in the population dynamics of the species should occur. Hill (2) estimated that the number of effective factors (genes) conditioning relative DE ranged from three to eight factors, whereas the number conditioning sporulation ranged from four to 25 factors. The larger number of factors conditioning sporulation may explain why fewer and more subtle changes were observed for sporulation. Frequent changes were observed for DE, a fitness trait controlled by relatively fewer factors.

The effect of decreased fitness on field behavior and epidemiological competence over time was investigated by Gregory et al (1).

If natural populations of race T change in fitness due to residence on resistant hosts, this study may offer some explanation as to why race T isolates existing prior to the widespread planting of T-cms hybrids (5) did not have the fitness to cause an epidemic (3). The increased use of T-cms hybrids in the southeastern USA may have allowed an increase in fitness of certain race T subpopulations on T-cms. The continued increase of more fit populations, coupled with favorable climatic conditions, may have caused the 1970 epidemic of southern corn leaf blight.

It was not our objective in this study to identify the mechanism(s) responsible for the observed changes in parasitic fitness. However, some speculation may be warranted. Enzymatic adaptation is considered to be temporary and reversible (8). Some of the race T isolates exhibited a significant decrease in parasitic fitness after a brief residence (two serial passages) on a resistant host, and the DE of some of the isolates was restored partially after two passages on a susceptible host; this could be interpreted as support for the theory of enzymatic adaptation. However, some race T isolates did not increase in fitness after two passages on the susceptible host, and

their behavior appears to be contradictory to the enzymatic adaptation theory. Several of the isolates exhibited decreased fitness after six or more passages on the resistant host. This may suggest that a comparable number of passages on a susceptible host may be necessary to cause an increase in fitness in certain isolates.

Decreased parasitic fitness relative to the susceptible host may have resulted through a process of selection for mutations that may have existed or occurred at random within the isolates. This would appear to be a less likely explanation than enzymatic adaptation for two reasons. At the conclusion of each serial passage on the resistant host, all of the isolates were collected in leaf tissue and induced to sporulate to obtain inoculum for the next serial passage. If a mutation had occurred at a locus involved with parasitic fitness, it would be unlikely that it could increase in frequency enough to significantly affect the population after two serial passages. The mutational theory also fails to adequately explain fitness traits that are under quantitative control (2). Finally, the partial restoration of DE after two passages on a susceptible host would theoretically require either back-mutations at the locus or loci in question or mutations at a different locus or loci that produce this effect.

Results of our study have shown that DE is readily affected by particular host genotypes, whereas consistent changes in sporulation were not detected. Since DE increased after a brief serial passage on the susceptible host, the benefit of reducing DE through residence on the resistant host may be short-lived. A host genotype with the ability to reduce only the DE of a pathogen population may then appear less valuable than one that would reduce only the sporulation. However, a host genotype that briefly reduces the DE may have value in the case of alloinfection, particularly as applied to multiline cultivars. Since sporulation is controlled by more genes than DE, it may be less likely to change through mutations and may be a more stable attribute to examine in a breeding program. A strategy that reduced the size of the pathogen population would reduce the probability of selection for mutants. This could be achieved by accompanying host genes that reduce DE with genes that reduce sporulation.

These results indicated that isolates that are taken from the resistant genotype and inoculated on to the susceptible genotype may increase in DE after only two infection cycles. Therefore, single tests, such as in race-typing, of the resistance of particular cultivars to isolates that have been taken from other cultivars may overlook the pathogen's potential to change over several generations. It may be a good practice to look for any change in the fitness attributes of individual physiological race isolates when testing the resistance of cultivars.

The serial-passage effect could be used as a tool, along with studies to determine the number of genes or effective factors controlling fitness attributes in pathogens, to reveal which particular fitness attributes are the most subject to change.

Based on this study, host resistance decreased parasitic fitness. It remains to be seen if the results observed in this study apply to other pathogen-host interactions. The application of this knowledge may prove useful in studying population shifts in pathogens and in breeding resistant cultivars.

TABLE 3. Significant changes in fitness attributes for five *Helminthosporium maydis* race T isolates during various serial passages on corn

Comparison ^a	Number of isolates that exhibited significant differences between comparisons of fitness attributes		
	Relative disease efficiency	Lesion size	Sporulation
1. T ₁ vs N ₂	two decreased	two decreased	two decreased
2. T ₁ vs N ₁₀	five decreased	five decreased	two decreased
3. N ₂ vs N ₁₀	two decreased	five decreased	one decreased
4. (N ₂ -T ₂) vs N ₂	no changes	no changes	no changes
5. (N ₁₀ -T ₂) vs N ₁₀	two increased	no changes	no changes

^aT₁ = original isolate before serial passage; N₂ and N₁₀ = second and tenth serial generation, respectively, on the normal hybrid; N₂-T₂ and N₁₀-T₂ = two generations of N₂ and N₁₀ on the T-cms hybrid.

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