The Heritability of Three Parasitic Fitness Attributes of *Helminthosporium maydis* race T

J. P. Hill and R. R. Nelson

Assistant professor, Department of Botany and Plant Pathology, Colorado State University, Fort Collins 80523 — formerly research aide, Department of Plant Pathology, The Pennsylvania State University; and Evan Pugh Professor, Department of Plant Pathology, The Pennsylvania State University, University Park 16802.

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ABSTRACT

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Nine sets of 24 ascoprogeny were isolated from complete octads obtained from five crosses of isolates of *Helminthosporium maydis* race T. Conidia of the ascoprogeny were used to quantitatively inoculate RX404 Tms corn hybrid seedlings. Each experiment included four replications of inoculations with the 24 ascoprogeny isolates and the parents. Disease efficiency (DE), the average number of lesions per plant; lesion size (LS), the average lesion area; and sporulation capacity (SC), the average number of conidia produced per square millimeter of lesion was measured. The data obtained from each experiment for each parasitic fitness attribute were analyzed by a two-way analysis of variance, and genotypic and phenotypic variances were calculated. Epistasis was estimated by subtracting the progeny mean from the parental mean. Narrow-sense heritability, the ratio of the additive genetic variance to phenotypic variance, was calculated if epistasis was not significant, and broad-sense heritability, the ratio of genotypic variance to phenotypic variance, was calculated if epistasis was significant. The heritability estimates ranged from 21 to 58% for DE, 23 to 53% for SC, and 0 to 6% for LS. These results indicate that the fitness attributes of DE and SC are subject to selection. Increased parasitic fitness of DE and SC may result in a reduced effectiveness of nonspecific resistance.

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

Heritability may be defined simply as the proportion of observed variability due to heredity (1) and can be calculated as the ratio of genetic variance to phenotypic (genetic plus environmental) variance. Theoretically, heritability for any variation may range from 0 to 100%, depending on the influence of environmental effects on the measured trait.

The apparent infection rate (r) is influenced both by the level of horizontal resistance in the host (10) and the corresponding parasitic fitness in the pathogen (8). The relative parasitic fitnesses of isolates are compared by measuring the differences in r for the isolates when they are tested under the same environmental conditions and on the same host (8). Parasitic fitness attributes such as disease efficiency, lesion size, sporulation capacity, latent period, and generation time are components of relative parasitic

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fitness that can be measured separately (10). Differences in these attributes explain why, for a given plant population, some fungal isolates are capable of causing more disease than others even though all isolates are compatible with the host.

The heritabilities of some quantitative traits of haploid organisms have been measured (9), but to our knowledge there is no published information on heritability measurements of parasitic fitness attributes of haploid pathogenic fungi. The objective of this study was to determine the heritabilities of disease efficiency, lesion size, and sporulation capacity of *Helminthosporium maydis* Nisikado & Miyake (*Cochliobolus heterostrophus* Drechsler) race T.

MATERIALS AND METHODS

Five isolates of *H. maydis* race T were utilized in this study. Their mating types, and sources of origin were: E8 (A, England); MH237 (A, Mexico); SP6 (a, Spain); SWHC (a, Switzerland); and E9 (a, England). The isolates were obtained by transferring a single conidium from sporulating leaf material to petri dishes containing potato-dextrose agar (PDA). Cultures were grown at 21 C under continuous light for 7 days. The isolates were mated in the following pairs: E8 × SWHC; MH237 × SP6; and MH237 × E9.

The crosses were prepared by placing four 20 × 20-mm sections of autoclaved senescent corn leaf in plastic petri dishes containing Sachs' agar. Small mycelial plugs of each isolate in the pair were placed on opposite edges of the leaf sections before incubating the dishes in paper bags at 21 C. Approximately 3 wk after mating, the perithecia were crushed, the asci removed, and complete octads of ascospores were isolated. Each ascospore was transferred to a PDA plate and cultured for 7 days at 21 C under continuous light. The sporulating cultures were rinsed with distilled water to form a spore suspension, which was atomized onto the leaves of Texas male-sterile (Tms) corn seedlings at the five- to six-leaf stage.

The inoculated plants were placed in a Percival Model DC 20 dew chamber (Percival Refrigeration and Mfg. Co., Boone, IA 50036) for 16 hr at 24 C to induce infection. Those plants were then transferred to an Isco Model E2 Growth Chamber (Instrumentation Specialties Co., Inc., Lincoln, NE 68504) at 28 C for 7 days. The diseased leaf material was collected, dried, and stored in paper bags. This procedure was performed separately with each isolate to avoid contamination. The preserved leaf material became the inoculum source for subsequent studies.

Seeds of corn (*Zea mays* L.) hybrid Asgrow RX404 Tms were planted in 10-cm-diameter plastic pots (six seeds per pot) and grown in the greenhouse. A sterilized soil mixture of soil, peat, and perlite (1:1:1, v/v) was utilized. The plants were later thinned to four plants per pot. Plants were inoculated at the five-leaf stage.

Inoculum was obtained by incubating pieces of diseased corn leaves in petri dishes lined with moistened filter paper for 4 days at 21 C to induce sporulation. The sporulating leaf material was washed with 0.05% water agar. Inocula were calibrated to 1,000 spores per milliliter in 0.05% water agar and atomized onto plants at the rate of 0.6 ml per plant. The inoculated plants were placed in a transparent plastic chamber (2.44 × 2.44 × 1.22 m) into which mist was injected for 5 of every 15 min for 16 hr. Colonization proceeded in the greenhouse for 5 days.

Each experiment included four replications of inoculations with the two parents and 24 ascoprogeny isolates representing three complete octads from one cross. Three experiments, each with a different set of 24 ascoprogeny, were conducted for crosses E8 × SWHC and MH237 × SP6; one experiment was conducted for MH237 × E9.

Disease efficiency (DE) was considered to be the average number of lesions per plant resulting from a given number of conidia. The leaves were numbered from the bottom to the top of the plant and the lesions were counted on leaves two through five.

Lesion size (LS) was determined by measuring the length and width of 15 lesions on the fourth leaf of one plant per pot. All lesions were measured starting from the base of the leaf and progressing to the tip, until 15 were recorded. If less than 15 lesions were present, the fourth leaf of another plant in the same pot was utilized to complete the set of 15 lesions. Length and width measurements were converted to area in square millimeters by reference to a previously assembled table (11).

Sporulation capacity (SC) was determined by collecting conidia from 15 lesions on the fourth leaves of four plants per pot. Lesions were selected at random, labeled with a permanent marker pen, and measured. Sporulation was induced by placing the plants in the mist chamber for 16 hr. Spores were collected by a vacuum method immediately after the leaves dried (6). The collected spores were placed in a test tube containing 5.0 ml of 0.05% water agar with 0.5% copper sulfate, the latter added to inhibit spore germination. The contents of each tube were agitated and a 0.1-ml aliquot of the spore suspension was evenly spread on a 10 × 30-mm strip of water agar placed on a glass slide. The spores were counted in four 0.1-ml aliquots per test tube under 30X power. The total number of spores collected per test tube was calculated and the data converted to the number of spores produced per square millimeter of lesion. The converted data constituted SC.

In addition to the three crosses already described, four ascoprogeny from the E8 × SWHC cross were mated; I(A) × II(a) and III(A) × IV a). Twenty-four ascoprogeny from each cross were utilized in the same method described earlier in order to estimate the heritability of the three parasitic fitness attributes.

The data obtained from each experiment for each parasitic fitness attribute were analyzed by a two-way analysis of variance (AOV) (13). The heritability of each trait was determined by calculating the genotypic (V_g^2) and phenotypic (V_p^2) variances from the mean squares of the AOV (1,2). For crosses with three experiments, the combined analysis of variance of the three experiments was used (3).

The analysis and components of variance (1) for SC from the MH237 × E9 cross are presented in Table 1. The genotypic variance (V_g^2 = 939.23) was calculated by subtracting the replication × progeny mean square from the between progeny mean square and dividing the difference by four (the number of replications). V_g^2 for DE and LS was calculated in the same manner. For SC the phenotypic variance (V_p^2 = 1,788.2) is the sum of the V_g^2 and the replication × progeny mean square. For DE and LS the V_p^2, because of the in-cells component of the AOV, is the sum of V_g^2, the replication × progeny mean square minus the in-cell mean square,

TABLE 1. The analysis and components of variance for sporulation capacity of *Helminthosporium maydis* of three sets of 24 ascoprogeny isolated from the E8 × SWHC cross

Source of variation	df	ms	Components
Replication	3	1,859.18	$V_e^2 + g(V_r^2)$
Between progeny	23	4,605.88	$V_e^2 + r(V_g^2)$
Replication × progeny	69	848.97	V_e^2
Total	95		

TABLE 2. Heritability estimates for disease efficiency, lesion size, and sporulation capacity of *Helminthosporium maydis* from pooled analyses of variance of three sets of 24 ascoprogeny isolated from the E8 × SWHC cross

	Disease efficiency	Lesion size	Sporulation capacity
High extreme	74.63	8.68	101.79
Low extreme	2.90	2.18	6.49
Mean	42.81	6.81	28.52
Parental mean	40.79	7.06	28.25
Harmonic mean	3.74	15.00	...[a]
Between-progeny mean square	938.66	4.78	799.08
Rep × progeny mean square	51.17	1.07	224.75
In-cell mean square	41.01	1.01	...[a]
Genotypic variance	221.87	0.93	143.58
Phenotypic variance	385.41	16.14	368.33
Heritability (%)	57.57	5.76	38.98
Standard deviation	6.16	0.89	14.37
Epistasis	2.02	0.25	0.27

[a] In-cell mean square and harmonic mean were not calculated in analysis of variance for sporulation capacity.

and the in-cell mean square multiplied by the harmonic mean (3). Heritability is the ratio of the genotypic variance to the phenotypic variance (1).

Haploid organisms do not have intra-allelic interactions and, therefore, their genotypic variance consists of only additive and epistatic effects (2). Broad-sense heritability is the ratio of the genotypic variance to phenotypic variance. Narrow-sense heritability is the ratio of the additive genetic variance to the phenotypic variance. We used broad-sense heritability when epistasis was significant and narrow-sense heritability when it was nonsignificant. Epistasis was estimated by subtracting the progeny mean from the average of the parental means (2). When the absolute value of epistasis was less than the standard deviation of the progeny, the epistatic effect was assumed to be nonsignificant and the genotypic variance was due to only additive effects. The experimental design requiring backcrosses to derive estimates of epistasis partitionable from the genetic variance was beyond the scope of this study.

RESULTS

Estimates of heritabilities of DE, LS, and SC from analysis of progeny from the crosses E8 × SWHC, MH237 × SP6, and MH237 × E9 are presented in Tables 2, 3, and 4, respectively. Similar estimates from two crosses between ascoprogeny from E8 × SWHC are presented in Tables 5 and 6. Heritabilities of 21–58% were estimated for DE, 0–6% for LS, and 23–52% for SC. Epistasis was significant for SC in two analyses and for disease efficiency in one.

DISCUSSION

The evidence presented herein demonstrates that certain components of parasitic fitness of *H. maydis* race T are heritable. There is evidence that certain parasitic fitness attributes in other pathogens are heritable (4,5), but quantitative estimates of these heritabilities were not made. Although similar evidence for other plant pathogens is lacking, it now seems reasonable to assume parasitic fitness per se is a heritable genetic trait, since there is no reason to believe that *H. maydis* is unique among parasitic fungi.

The heritabilities of DE and SC are relatively high; the heritability of LS is low. The pooled values from crosses E8 × SWHC and MH237 × SP6 should, in theory, yield more accurate estimates due to the increased number of genotypes represented in the progeny population. These more accurate estimates may be somewhat offset by the environmental variation between experiments due to greenhouse conditions (temperature, humidity, and light) varying daily as well as seasonally.

The heritability values obtained were in the range expected since previous studies comparing isolates of *H. maydis* race T have consistently demonstrated significant differences in DE and SC, but not LS (6). In an evolutionary aspect, the LS of *H. maydis*

TABLE 3. Heritability estimates for disease efficiency, lesion size, and sporulation capacity of *Helminthosporium maydis* from pooled analyses of variance of three sets of 24 ascoprogeny isolated from the MH237 × SP6 cross

	Disease efficiency	Lesion size	Sporulation capacity
High extreme	78.48	12.70	255.33
Low extreme	13.44	3.74	9.43
Mean	43.07	7.70	93.21
Parental mean	44.96	7.32	58.50
Harmonic mean	3.81	15.00	...[a]
Between-progeny mean square	620.11	3.02	4,044.23
Rep × progeny mean square	83.06	1.22	1,667.08
In-cell mean square	66.37	1.00	...[a]
Genotypic variance	134.24	0.45	594.29
Phenotypic variance	403.80	15.67	2,261.37
Heritability (%)	33.24	2.87	26.78
Standard deviation	7.53	0.95	37.60
Epistasis	1.89	0.38	34.71

[a] In-cell mean square and harmonic mean were not calculated in analysis of variance for sporulation capacity.

TABLE 4. Heritability estimates for disease efficiency, lesion size, and sporulation capacity of *Helminthosporium maydis* of 24 ascoprogeny isolated from the MH237 × E9 cross

	Disease efficiency	Lesion size	Sporulation capacity
High extreme	41.25	10.23	143.01
Low extreme	4.00	7.62	20.80
Mean	23.19	9.01	77.46
Parental mean	33.65	9.19	125.52
Harmonic mean	3.17	14.68	...[a]
Between-progeny mean square	395.16	2.06	4,605.88
Rep × progeny mean square	77.74	1.07	848.97
In-cell mean square	43.05	2.09	...[a]
Genotypic variance	79.36	0.25	939.23
Phenotypic variance	250.52	29.91	1,788.20
Heritability (%)	31.68	0.83	52.52
Standard deviation	7.64	0.90	29.14
Epistasis	10.46[b]	0.18	48.06[b]

[a] In-cell mean square and harmonic mean were not calculated in the analysis of variance (AOV) for sporulation capacity.
[b] Epistasis is considered significant if the absolute value is larger than the standard deviation from the progeny AOV.

TABLE 5. Heritability estimates for disease efficiency, lesion size, and sporulation capacity of 24 ascoprogeny of *Helminthosporium maydis* isolated from the E8 × SWHC I × E8 × SWHC II cross

	Disease efficiency	Lesion size	Sporulation capacity
High extreme	54.19	12.90	60.16
Low extreme	21.17	9.71	23.32
Mean	30.87	10.93	38.92
Parental mean	37.00	11.88	33.74
Harmonic mean	3.33	14.94	...[a]
Between-progeny mean square	278.90	2.43	431.30
Rep × progeny mean square	69.89	3.88	191.35
In-cell mean square	52.75	1.99	...[a]
Genotypic variance	52.25	0.00	59.99
Phenotypic variance	245.05	31.62	251.34
Heritability (%)	21.32	0.00	23.87
Standard deviation	7.24	1.71	13.83
Epistasis	6.13	0.95	−5.18

[a] In-cell mean square and harmonic mean were not calculated in analysis of variance for sporulation capacity.

TABLE 6. Heritability estimates for disease efficiency, lesion size, and sporulation capacity of 24 ascoprogeny of *Helminthosporium maydis* isolated from the E8 × SWHC III × E8 × SWHC IV cross

	Disease efficiency	Lesion size	Sporulation capacity
High extreme	37.35	10.10	79.24
Low extreme	8.38	8.49	20.17
Mean	21.41	9.14	50.24
Parental mean	23.79	8.56	94.86
Harmonic mean	3.74	15.00	...[a]
Between-progeny mean square	191.35	1.14	1,426.72
Rep × progeny mean square	39.83	1.55	642.66
In-cell mean square	21.64	1.47	...[a]
Genotypic variance	37.88	0.00	196.02
Phenotypic variance	137.00	22.13	642.66
Heritability (%)	27.65	0.00	23.37
Standard deviation	5.47	1.08	25.35
Epistasis	21.38[b]	−0.58	44.62[b]

[a] In-cell mean square and harmonic mean were not calculated in analysis of variance (AOV) for sporulation capacity.
[b] Epistasis considered significant if the absolute value is larger than the standard deviation from the progeny AOV.

would be less subject to changes within races than DE and SC. Furthermore, evidence (6) does point to a situation where the pathogen has the ability to cause a lesion and the host genotype determines the size. In any case, LS does not seem to be a suitable attribute to utilize when investigating epidemiological (or parasitic fitness) differences within race T of *H. maydis*.

Epistasis estimates were usually nonsignificant, and this suggests that only additive effects are responsible for the genotypic variance of the parasitic fitness attributes. Under these circumstances a gene-for-gene relationship may exist between parasitic fitness attributes and nonspecific resistance in the host. A greater opportunity would exist for a single gene to contribute toward increased parasitic fitness of the pathogen or increased resistance in the host. The presence of significant epistasis estimates in some crosses indicates the need for further studies utilizing backcrosses to accurately estimate epistasis in a form that can be partitioned from the additive effects.

Given the assumption of some that nonspecific resistance is stable and long-lasting, it may be significant that this form of resistance is known to function in several ways, two of which are to reduce the number of infections and the amount of inoculum for subsequent generations of disease. Increased parasitic fitness for these two traits, thus, could result in an increased level of disease for a given level of nonspecific resistance. It is not known to what extent parasitic fitness can increase to overcome the effects of nonspecific resistance.

Riley (12) has stated that nonspecific resistance is not permanent and the potential for the evolution of increased parasitic fitness exists. Leonard (7) has also expressed this concern. Evidence that two parasitic fitness traits of *H. maydis* are heritable, and thus subject to evolution through natural selection, warrants concern that nonspecific resistance may not be as stable as many believe.

Measuring parasitic fitness of isolates over time could provide information on the long-term effectiveness of nonspecific resistance. If the parasitic fitness of isolates remains constant or virtually so over time, greater confidence in the stability of nonspecific resistance may be warranted. However, detection of increasing parasitic fitness may provide advanced warning of the need to add resistance genes before cultivars are overcome. These attributes can also be used to more efficiently select for nonspecific resistance in the host—currently a most challenging task. The search for and the utilization of new nonspecific resistance genes should be a continuing process if improved parasitic fitness is commonplace.

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