

Genetic Control of Two Parasitic Fitness Attributes of *Helminthosporium maydis* Race T

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ABSTRACT

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Sets of 24 ascospores were isolated from crosses between seven isolates of *Helminthosporium maydis* race T. Conidial suspensions of ascospore cultures were used to inoculate quantitatively seedlings of two corn hybrids (RX404 and PA887 × B14) in Texas male-sterile cytoplasm. Disease efficiency (DE, the average number of lesions per plant) and sporulation capacity (SC, the average number of conidia produced per square millimeter of lesion) were measured and analyzed. The genotypic variance was calculated and used along with the progeny extreme values to estimate

the number of effective factors controlling these two parasitic fitness attributes. The effective factor estimates from the 24 ascospore analyses ranged from 2.8 to 6.7 for DE and from 4.0 to 7.5 for SC. Pooled estimates ranged from 5.8 to 7.9 and from 15.8 to 25.4 for DE and SC, respectively. These results, along with the relatively high heritabilities of DE (21–58%) and SC (23–52%), suggest that these parasitic fitness attributes could respond to selection. The utilization of “nonspecific” resistance may provide selection pressure toward increased parasitic fitness.

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

Race-nonspecific resistance (12) or horizontal resistance sensu Vanderplank (20) has gained considerable support as a means of managing highly variable pathogens. Race-nonspecific resistance is usually thought to be governed by many genes and functions epidemiologically to reduce the apparent infection rate and, thus, the terminal amount of disease (12). A major factor contributing to the support of nonspecific resistance is its increased stability in contrast to vertical resistance (20), which is usually controlled by one or few genes (12). This increased stability may be due, in part, to the decreased probability that a pathogen genotype could evolve with all the genes necessary to overcome nonspecific resistance. Some authors (3,14,15) believe that such resistance, governed by many genes, cannot be overcome by genetic changes in the pathogen; however, there is evidence (7) that the permanence of nonspecific resistance may be suspect.

Van der Zaag (21) identified the chance of infection, extension rate of mycelium in leaf tissue, rate of sporangium production, and number of sporangia produced per leaf lesion area as components of race nonspecific resistance in potato to late blight. Kuhn et al (8) reported that slow rusting in wheat also involves one or more of these components coupled with smaller pustules. One or more of these components are often cited in studies concerned with the mechanisms of nonspecific resistance. Shaner and Finney (16) demonstrated that an increase in certain components of race nonspecific resistance (slow rusting) resulted in a decreased r value. These components of nonspecific resistance seem to reduce measurably the apparent infection rate (r) of an epidemic (12).

The corresponding pathogen attributes influencing r are termed parasitic fitness attributes (9). Disease efficiency (DE) and sporulation capacity (SC), two relative parasitic fitness attributes, can be measured when isolates of a single pathogenic race are tested under the same environmental conditions on the same host genotype (7).

The expectation of genetic advance of a trait under selection depends upon the selection pressure, amount of genetic variation within the population, the magnitude of the differences between the

genotypic values, and heritability of the variation (1,11). Other factors may also be involved (11). Variation and relatively high heritability have been demonstrated for DE and SC of *Helminthosporium maydis* race T (7). The individual effect of a gene on genotypic values is greater when a certain amount of variation is controlled by a few, rather than many, genes (6).

The objective of this study was to determine the number of “effective factors” involved in controlling the relative parasitic fitness attributes of disease efficiency and sporulation capacity of *Helminthosporium maydis* Nisikado & Miyake (*Cochliobolus heterostrophus* Dreschler) race T.

MATERIALS AND METHODS

Five isolates of *H. maydis* race T were used 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 placing diseased leaf material in a petri dish lined with moist filter paper to induce sporulation. Single conidia were transferred to petri dishes containing potato-dextrose agar (PDA) and grown under continuous light at 21 C for 7 days. The isolates were mated in the following pairs: E8 × SWHC; MH237 × SP6; and MH237 × E9.

The crossing procedure and isolate storage were described in detail previously (7).

Four single-ascospore cultures were selected from the ascospores of the E8 × SWHC cross. The ascospore isolates and their mating types were I(A), II(a), III(A), and IV(a). The isolates were mated in the following pairs: I × II and III × IV. Four sets of three complete octads (24 ascospores) were isolated and cultured from the E8 × SWHC cross and three sets from the MH237 × SP6 cross. One set of three complete octads was obtained from each of the remaining crosses.

Corn (*Zea mays* L.) hybrid Asgrow RX404 (in Texas male-sterile [Tms] cytoplasm) was used in all experiments. One experiment also included the corn hybrid (PA 887P × B14), also in Tms cytoplasm. Four seeds per pot of each hybrid were planted in 10-cm-diameter plastic pots containing a sterilized mixture of soil, peat, and perlite (1:1:1, v/v) and maintained in the greenhouse. Plants were inoculated at the five-leaf stage. Conidia were washed from sporulating diseased leaf material with 0.5% water agar. Inocula

were calibrated to 1,000 conidia 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 (2.4 × 2.4 × 1.2 m) into which mist was sprayed 5 of every 15 min for 16 hr. Sporulation was induced after 5 days of colonization in the greenhouse by placing the plants into the mist chamber for 16 hr.

The average number of lesions per plant resulting from the standardized inocula was considered the DE. Conidia were collected by a vacuum method (13) from 15 previously marked and measured lesions per pot. The average number of conidia produced per square millimeter of lesion was calculated and considered the SC.

Each experiment (completely randomized design) consisted of four replications of inoculations made with each of the 24 progeny isolated (three complete octads) from one cross. One experiment consisted of two replications on Asgrow RX404T and two replications on (PA 887P × B14)T. The data obtained from each experiment for DE and SC were analyzed by a two-way analysis of variance (17). The analysis and components of variance (1) for DE from the E8 × SWHC cross are presented in Table 1. The genotypic variance ($V_g^2 = 208.88$) was calculated by subtracting the replication × progeny mean square from the among progeny mean square and dividing the difference by four (the number of replications) (1).

The number of effective factors (K) controlling DE and SC were estimated by dividing the square of the progeny extreme differences by four times the genotypic variance (2,11). The genotypic variation is multiplied by four because, in theory, the effect of one K can range from a plus to a minus depending on whether or not it is present in a particular genotype. The effect would be plus or minus one standard deviation, and thus, a range of two standard deviations per K . The two is squared to make it equitable with the components of variance (2).

The analyses of variance of three experiments for the E8 × SWHC cross were pooled as were those for the MH237 × SP6 cross (5) and effective factor estimates were calculated.

TABLE 1. The analysis and components of variance for disease efficiency of 24 *Helminthosporium maydis* ascospores isolated from the E8 × SWHC cross

Source of variation	df	ms	Components
Replication	3	140.49	$V_e^2 + g(V_g^2)$
Among progeny	23	891.14	$V_e^2 + r(V_g^2)$
Replication × progeny	69	55.65	V_e^2
Total	95		

TABLE 2. Components and effective factor estimates for disease efficiency (DE) and sporulation capacity (SC) of *Helminthosporium maydis* race T from three different sets of 24 ascospores isolated from crosses E8 × SWHC and MH237 × SP6

Component	DE ^a in sets:			SC ^b in sets:		
	1	2	3	1	2	3
E8 × SWHC cross						
High extreme	74.6	67.7	46.4	46.3	101.8	61.5
Low extreme	18.1	2.9	10.3	6.5	7.8	8.8
Mean	42.8	27.6	27.6	20.0	42.2	23.4
Genotypic variance	208.9	371.7	85.1	98.3	381.4	127.6
Standard deviation	6.5	6.8	5.3	8.7	19.0	15.4
Effective factors	3.8	2.8	3.8	4.0	5.8	5.4
MH237 × SP6 cross						
High extreme	46.7	66.2	78.5	146.1	62.8	255.3
Low extreme	14.8	13.4	24.0	11.4	9.4	101.8
Mean	29.6	43.1	56.5	63.9	27.5	188.1
Genotypic variance	57.9	170.5	174.3	659.9	153.1	969.8
Standard deviation	4.6	7.7	10.3	40.4	16.9	55.5
Effective factors	4.4	4.1	4.3	6.9	4.7	6.1

^a Average number of lesions per plant.

^b Average number of spores produced per square millimeter of lesion.

RESULTS

Three separate effective factor estimates of DE and SC derived from the crosses EB × SWHC and MH237 × SP6 are presented in Table 2. Different sets of 24 ascoprogeny were used in each experiment. Effective factor estimates of each cross derived from pooling the analyses of variance of the three separate experiments are presented in Table 3. Similar estimates for sets of ascoprogeny isolated from the crosses I × II, III × IV, MH237 × E9, and E8 × SWHC on RX404T and (PA 877P × B14)T are presented in Table 4. Effective factor estimates derived from single experiments ranged from 2.8 to 6.7 and 4.0 to 7.5 for disease efficiency and sporulation capacity, respectively. The estimates for the two pooled analyses are 5.8–7.9 for disease efficiency and 15.8–25.4 for sporulation capacity. There were no significant corn hybrid interactions in the experiment with the two host genotypes.

DISCUSSION

Effective factors (K units) are not a measure of the number of genes controlling a trait (11). An effective factor may be viewed as a segment of chromosome acting as a unit of inheritance (11) because more than one gene may be present in the segment. K units usually underestimate the number of genes involved (4,11). Analyses of succeeding generations usually, but not always, show an increase in effective factor estimates (11), probably due to crossing over and segregation. The reason for this discrepancy is that the following assumptions, necessary for analysis, may not always be true: K units show no interaction; K units are not linked; the effects of the K units are equal (10,11). The limitations and assumptions indicate that accuracy may be lacking; however, the techniques required to measure the small differences in a parasitic fitness trait virtually prohibit using other methods, such as back-cross analysis, due to restraints on time, space, and labor. Even though the effective factor estimate may be considered less precise, it does give a reasonable indication of the numbers involved. Effective factor

TABLE 3. Components and effective factor estimates for disease efficiency (DE) and sporulation capacity (SC) of *Helminthosporium maydis* race T from pooled analysis of variance of three sets of 24 ascospores isolated from the E8 × SWHC cross and also from the MH237 × SP6 cross

Component	E8 × SWHC		MH237 × SP6	
	DE ^a	SC ^b	DE	SC
High extreme	74.6	101.8	78.5	255.3
Low extreme	2.9	6.5	13.4	9.4
Mean	42.8	28.5	43.1	93.2
Genotypic variance	221.9	143.6	134.2	594.3
Standard deviation	6.1	14.4	7.5	37.6
Effective factors	5.8	15.8	7.9	25.4

^a Average number of lesions per plant.

^b Average number of spores produced per square millimeter of lesion.

TABLE 4. Components and effective factor estimates for disease efficiency (DE) and sporulation capacity (SC) of *Helminthosporium maydis* race T from 24 ascospores isolated from crosses I (E8 × SWHC) × II (E8 × SWHC), III (E8 × SWHC) × IV (E8 × SWHC), MH237 × E9, and E8 × SWHC (on two host genotypes)

Component	I × II		III × IV		MH237 × E9		E8 × SWHC ^c	
	DE ^a	SC ^b	DE	SC	DE	SC	DE	SC
High extreme	54.2	60.2	37.4	79.2	41.3	143.0	37.4	200.9
Low extreme	21.2	23.3	8.4	20.2	4.0	20.8	9.7	13.1
Mean	30.9	38.9	21.4	50.2	23.2	77.5	18.3	41.5
Genotypic variance	52.3	60.0	37.9	196.0	79.4	939.2	29.2	1,170.1
Standard deviation	7.2	13.8	5.5	25.4	7.6	29.1	7.5	24.1
Effective factors	5.2	5.7	5.5	4.5	4.4	4.0	6.6	7.5

^a Average number of lesions per plant.

^b Average number of spores produced per square millimeter of lesion.

^c On two host genotypes.

estimates can be useful in explaining and predicting population behavior of certain pathogens.

The higher K values in the pooled analyses may be due to the increased number of possible genotypes in the expanded progeny population. If the population has individuals closer to the ultimate extremes, the pooled estimate would be more accurate. However, the environmental variation between experiments may have had an effect on the genes controlling SC, increasing the total variation. This would result in a larger difference between the high and low extremes. The larger pooled estimate for SC may reflect both of these possibilities. Further studies involving larger populations are needed to resolve this question.

There is some evidence that quantitative traits are controlled by fewer genes than generally assumed. Thompson (19) observed that "... a large variance can be caused by a few genes just as it can be caused by the segregation of a large number." Thoday and Thompson (18) demonstrated that two or three loci can account for the major portion of variance of certain traits and that the distributions are essentially normal. Then it may not be surprising that so few K units seem to control the quantitative trait of DE. The relatively low number of effective factors conditioning DE, along with its high heritability, suggests that this fitness trait could change rapidly, given the mechanisms for genetic variation under which natural selection would be exercised. The high heritability, but larger number of effective factors conditioning SC, would indicate slower change than DE. The opportunity for advancement under selection should be higher for DE than for SC, if all other factors are equal.

An example of changing fitness (advancement under selection) occurred within race T of *H. maydis*. The race T isolates collected in the late 1950s and early 1960s had relatively low fitness, but because more Tms cytoplasm corn was grown, the fitness increased (12). The fitness peaked during the 1970 corn blight epidemic and steadily decreased with the return to normal cytoplasm corn. Another example of fitness improving over time could be race 15B of *Puccinia graminis* f. sp. *tritici* Eriks. & E. Henn. The race was identified many years before an epidemic occurred even though susceptible varieties were widely grown. It may be possible that the majority of isolates of the original populations of race 15B lacked parasitic fitness and that natural selection for the most fit resulted in the buildup of populations with traits capable of inducing epidemic losses.

Variability is known to exist in many other plant pathogen populations, but heritability estimates and K values of parasitic fitness attributes are not known. High heritabilities and low K values of parasitic fitness attributes could exist in other pathogen populations. With the recent emphasis on nonspecific resistance in breeding programs, and the incorporation of this resistance into commercial cultivars, the probability exists for an increase in parasitic fitness through natural selection.

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