Components of Fitness Attributes in *Cochliobolus carbonum* Race 3

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ABSTRACT

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Three components of parasitic fitness of *Cochliobolus carbonum* (=*Helminthosporium carbonum*) race 3 were investigated in growth chamber experiments on 3-wk-old susceptible corn (*Zea mays*) inbred lines. Disease efficiency (DE), lesion length (LL), and sporulation capacity (SC) were studied. There were significant differences in DE, LL, and SC among isolates and among host × isolate interactions. Heritability estimates for DE, LL, and SC were 0.77, 0.87, and 0.60, respectively, indicating that it should be possible for natural selection to operate in favor of increased parasitic fitness of the pathogen.

Additional key words: epidemiology, genetics, horizontal resistance, maize, quantitative inheritance.

Leaf spot and ear infection caused by races 1 and 2 of *Cochliobolus carbonum* Nelson (=*Helminthosporium carbonum* Ullstrup) has been considered a minor disease of corn (*Zea mays* L.) in the United States (15,16). A third race (10) has been identified and isolated frequently from corn in annual disease surveys in Pennsylvania (1).

The three races can be differentiated according to the reaction of susceptible corn lines. Isolates of race 1 produce well-defined, zonate lesions with light-brown centers and dark-brown margins on corn inbred line Pr. Race 2 isolates produce chlorotic flecks on Pr. The difference in virulence between races 1 and 2 is controlled by a single gene (11). Race 3 isolates cause long, linear, grayish-tan lesions surrounded by a light- to dark-pigmented border (10). The production of linear lesions typical of race 3 is polygenically controlled (3,7).

Studies reported by Castor et al (2) indicated that corn inbred lines reacted differently with respect to disease severity and lesion type when inoculated with a mixture of isolates of *C. carbonum* race 3. Variation in lesion type on a single inbred suggests that genetic variation among isolates in the mixture may have been present. This hypothesis is based, in part, on preliminary evidence presented by Dalmacio (3) on the quantitative variation in virulence among progeny of crosses between races of the fungus.

Nelson (8) classified certain differences among isolates of a plant pathogen as differences in "fitness attributes." Some components of fitness attributes are: disease efficiency (DE), the number of lesions resulting from a given amount of inoculum; sporulation capacity (SC), the amount of inoculum produced per unit area of diseased tissue; and virulence, the relative ability to induce a given amount of disease on a particular host genotype.

When first identified (10), race 3 of *C. carbonum* was not considered a serious threat to corn production in Pennsylvania and surrounding states. However, reports (J. E. Ayers, *unpublished*) of increased frequency and severity over a wide area suggest that *C. carbonum* race 3 can significantly reduce corn yields and may threaten corn production.

The work described here was designed to study the extent of variation in fitness attributes among race 3 isolates of *C. carbonum*. A knowledge of the variability of DE, lesion length (LL) as a measure of virulence, and SC could be useful in breeding cultivars for disease resistance.

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MATERIALS AND METHODS

Twenty single-spore isolates of *C. carbonum* race 3, collected from corn leaf samples in Pennsylvania in 1975, 1976, and 1978, were cultured on potato-dextrose agar for 5 days and inoculated onto seedlings of the susceptible corn inbred line, Pa33. Leaves producing well-developed lesions were air-dried and stored as stock cultures.

Inoculum was prepared by incubating leaf sections of the stock cultures in petri dishes containing moistened filter paper at room temperature for approximately 5 days. Fresh inoculum was prepared for each inoculation date. Sporulating leaf tissue was washed with 0.05% water agar solution and the volume was adjusted to provide $1.5–3.0 \times 10^3$ spores per milliliter.

The susceptible corn inbred lines, Pa33 and Pa419P, were planted in 10-cm-diameter plastic pots (three seeds were planted per pot and the seedlings were thinned to one plant per pot prior to inoculation) and maintained in the greenhouse until the day of inoculation. A sterilized potting mixture of soil, peat, and perlite (1:1:1, v/v) was used. Inoculations were carried out when seedlings reached the five-leaf stage, approximately 3 wk after emergence. A split-plot experimental design with inbred lines as whole plots and isolates as subplots was used. Three inoculation dates were used as replications. In the statistical analyses, hosts were assumed to be fixed effects and isolates, random effects. Mean-square expectations for the model are shown in Table 12.10 of Steel and Torrie (14). Heritability was calculated as the ratio of the genotypic to the phenotypic variance.

DE was assessed by applying the spore suspension on the middle of the fifth leaf with a quantitative inoculator (13). After inoculation of each leaf, a known quantity of inoculum in 0.05% water agar was sprayed on 4% water agar in a petri dish to obtain an indirect estimate of the number and viability of conidia placed at the inoculation site on the leaf. An equal volume of 0.05% water agar without spores was used as a control. The plates were incubated at room temperature for 4–6 hr to allow germination of viable conidia. The proportion of conidia that germinated was used to estimate the number of viable conidia applied to the test seedlings. A conidium was considered germinated if its germ tube was twice the length of the conidium.

Inoculated plants were immediately transferred to the dew chamber (model DC 20, Percival Refrigeration and Mfg. Co., Boone, IA 50036), maintained at 21 C for 15 hr, then transferred to a growth chamber (locally constructed) and maintained at 23 C for 5 days (12 hr of light per day; 135 $\mu E \cdot m^{-2} sec^{-1}$). DE was calculated as:

$$DE\ (\%) = 100 \times (\text{no. of lesions})/(\text{no. of spores})$$

Comparisons of LL among isolates were made by spraying spore suspensions in 0.05% water agar onto the seedlings with a hand atomizer. No attempts were made to determine directly the number of spores applied to the test plants; however, during a preliminary study, inoculum containing $1.5–3.0 \times 10^3$ spores per milliliter produced the desired number of lesions on the seedlings. Inoculated plants were kept in the dew chamber for 15 hr at 21 C and transferred to the growth chamber at 23 C. All isolates produced grayish-tan oval-to-elongated lesions surrounded by a dark-pigmented border. Individual lesions reached maximum length 13–14 days after inoculation. The first 10 individual lesions from the tip of the fifth leaf were measured on the 14th day. If an insufficient number of lesions was produced on the fifth leaf, lesions on the fourth leaf were used. The lengths of 10 lesions were used to calculate the mean LL for a particular host-isolate combination.

Lesion areas of the same 10 mature lesions used for the LL study were estimated as the product of the length and width at the middle of each lesion.

In a preliminary trial, sporulation of two highly virulent and two less virulent (on the basis of LL) isolates of *C. carbonum* race 3 was tested on lines Pa33 and Pa419P. Sporulation in individual lesions began within 2–4 days after seedlings with mature lesions were placed in the dew chamber at 21 C. In the study to determine SC, seedlings with matured lesions were kept in the dew chamber for 4 days at 21 C under a 12-hr light/dark (195 $\mu E \cdot m^{-2} \cdot sec^{-1}$) regime to induce sporulation; after 4 days the leaves tended to deteriorate rapidly. At the end of the period, the leaves were allowed to dry in the dew chamber for 2 hr and the spores were harvested.

Spores from individual lesions were collected with a vacuum pump (at 34 kPa) into test tubes containing 25 ml of 0.5% $CuSO_4$ plus 0.05% agar solution. The $CuSO_4$ was used to inhibit spore germination. A 2% water agar block was placed on a microscope slide and 0.1 ml of the spore suspension was pipetted onto the agar block. The total number of conidia present in 0.1 ml of spore suspension was counted. Determinations of the number of conidia in the spore suspension were repeated five times. The mean of these values was used to estimate the total number of spores present in the original suspension. Sporulation capacity of each isolate-host combination was calculated as:

$$SC = 250 \times (\text{mean no. conidia per 0.1 ml})\ /\ (\text{area of lesion in cm}^2)$$

RESULTS

The overall mean DE was slightly higher on Pa419P than on Pa33 (Table 1). Similarly, the range of DE was greater on Pa419P than on Pa33. With few exceptions, isolate rankings on both inbreds were about the same. For example, isolate TL-1 was the least compatible on both host genotypes. However, isolate RS419-3 had the highest DE on Pa33 and was less than the mean of all the isolates on Pa419P.

Differences among isolate genotypes and the interaction of isolate and host genotypes for DE were significant (Table 2). The heritability estimate for DE, calculated from the ratio of genetic variance to phenotypic variance, was 0.77. The isolate mean square was about 4.5 times as large as the interaction mean square.

Contrary to the results for DE, LL on Pa33 was greater than on Pa419P (Table 3). Similarly, the range in LL was greater on Pa33 than on Pa419P. With few exceptions, isolate rankings on both inbreds were about the same. For example, isolate NL-2 was the least compatible on both host genotypes. Despite the continuous distribution in LL among the isolates on both inbred lines, isolates

TABLE 1. Mean disease efficiency for 20 isolates of *Cochliobolus carbonum* race 3 determined 5 days after inoculation on corn inbreds Pa33 and Pa419P

	Inbred lines	
Fungal isolates	Pa33	Pa419P
RS419-3	16.7 A[a]	5.8 DE
RS33-3	15.1 A	18.8 A
RS64-3	14.0 AB	16.7 A
TS75-14/2	13.6 AB	13.2 B
TL-18	13.2 AB	12.6 BC
NL-5a	11.7 BC	6.6 CDE
TL-4	10.3 BC	12.8 BC
NL-10	10.0 BC	9.1 C
TL-16	8.9 C	8.6 CD
TS75-1/6	7.2 CD	7.1 CDE
TX-8	6.7 CD	8.9 C
TL-17	6.0 CD	9.1 C
NS75-30/2	5.6 CD	9.6 C
TL-14	4.9 CD	5.5 DE
NL-11	4.5 CD	5.0 E
NL-2	4.5 D	8.8 C
TL-6	4.2 D	7.4 CDE
TL-9	4.2 D	5.2 E
TL-12	3.5 D	8.1 CDE
TL-1	2.6 D	1.6 F
Mean	8.3	9.0

[a] Values are expressed in percent (see text for calculation) and are the average of three replications. Means followed by the same letter are not statistically different ($k = 100$; approximately $P = 0.05$) as determined by Duncan's (Bayesian) modified least significant difference test.

with similar magnitude appeared to belong to two distinct populations; the means for isolates RS419-3, NS-75-30/2, RS64-3, and RS33-3 were greater than for the remaining group of isolates.

Differences in LL among isolate and host genotypes were statistically significant (Table 2). Similarly, the isolate × host genotype interaction was also significant. The heritability estimate for LL was 0.87. The mean square for isolates was nearly eight times the interaction mean square.

Average SC on Pa33 was higher than on Pa419P, but the difference between the highest and the lowest SC values was greater on Pa419P (Table 4). With few exceptions (NL-5a, TS75-14/2, TL-4, TL-17, TL-16), isolates ranked about the same on both hosts.

The analysis of variance indicated that differences among hosts and among isolates were statistically significant (Table 2). Similarly, the interaction of isolate by host genotypes was significant and smaller than the mean squares for isolates. Heritability for SC was estimated as 0.60.

DISCUSSION

The results obtained from this study on three fitness attributes indicate considerable variation within race 3 isolates of *C. carbonum*. The DE estimates reported in this study were generally higher than those described in other host-pathogen models (6,12,17). In those experiments, DE was measured by depositing a standardized, but unknown, quantity of viable inoculum on test plants. Much inoculum was probably deposited on surfaces other than leaves. Thus, the proportion of inoculum that actually produced lesions could not be determined. The procedure for estimating DE in our study, which used a precision inoculator, measured the actual number of lesions produced from a known amount of viable inoculum placed at a specific spot on the leaf. The earlier studies give a reliable relative measure of DE, whereas our technique yields an absolute measure that may or may not be comparable to field conditions.

An isolate of a plant pathogen can be considered to have higher fitness if it has a high DE, produces large lesions, and has a high SC on a given host genotype. Two other components, not considered in this study, latent period and infectious period, may be of equal importance in parasitic fitness. Estimation of the latent period for plant pathogens that require dew for sporulation would be difficult. Similarly, determinations of infectious period could not be achieved satisfactorily due to premature senescence of foliage under continuously moist conditions. Differences in latent period and infectious period were not considered; hence, the combination of DE and SC might not reflect the actual differences in parasitic fitness among the isolates.

Nelson (9) emphasized that an increased capacity in one or more components of fitness attributes is an indication of a potential crop production threat posed by the parasite. The occurrence of such a phenomenon was clearly demonstrated in the epidemic of southern corn leaf blight caused by *Helminthosporium maydis* race T (6). Increased prevalence of *C. carbonum* race 3 could initiate a similar trend.

Components of parasitic fitness can also be viewed as the components of resistance among corn lines (9). Host lines that reduce DE, LL, and SC a substantial amount should be valuable in breeding for disease resistance. Parasitic fitness in *C. carbonum* race 3 as measured in terms of DE, LL, and SC was highly heritable. Each of these fitness attributes appeared to be independently inherited and their high heritability values suggest that it is possible for natural selection to operate in favor of isolates with increased fitness.

Resistance (based on reducing DE, LL, and SC) to *C. carbonum* race 3 in corn inbreds appears to be quantitative in nature and

TABLE 2. The mean squares from the analysis of variance tables for disease efficiency (DE), lesion length (LL), and sporulation capacity (SC) of 20 isolates of *Cochliobolus carbonum* race 3 on corn inbreds Pa33 and Pa419P

Source	df	Mean squares[a] DE	LL	SC
Replications (R)	2	8.9	0.1	2,272
Hosts (H)	1	13.9	67.9**	254,657**
R × H	2	3.2	0.7	1,053
Isolates (I)	19	89.0**	38.8**	105,809**
H × I	19	19.6**	4.9**	68,223**
Error	76	1.2	0.2	537

[a]Asterisks (**) indicate statistical significance, $P = 0.01$.

TABLE 3. Mean lesion length (LL) for 20 isolates of *Cochliobolus carbonum* race 3 determined 14 days after inoculation on corn inbreds Pa33 and Pa419P

Fungal isolates	LL on inbred lines[a] Pa33	Pa419P
RS419-3	12.9 A	6.2 B
NS75-30/2	11.7 B	7.2 AB
RS64-3	10.6 C	7.8 A
RS33-3	10.0 C	6.5 B
NL-5a	6.7 D	3.2 D
TL-9	4.9 E	3.2 D
NL-10	4.7 EF	3.6 CD
TL-4	4.4 EFG	2.5 D
TX-8	4.0 FGH	2.7 D
TS75-14/2	3.7 FGH	2.9 D
TL-12	3.7 FGH	3.6 C
TL-17	3.7 GH	3.3 D
TL-1	3.7 GH	3.6 D
TL-6	3.5 GHI	3.0 D
TS75-1/6	3.3 HI	3.0 D
TL-14	3.3 HI	3.1 D
NL-11	3.3 HI	3.2 D
TL-16	3.2 HI	3.2 D
TL-18	3.1 HI	2.6 D
NI-2	2.7 I	2.4 D
Mean	5.4	3.8

[a]Values are expressed in millimeters (see text for calculation) and are the average of three replications. Means followed by the same letter are not statistically different ($k = 100$; approximately $P = 0.05$) as determined by Duncan's (Bayesian) modified least significant difference test.

TABLE 4. Mean sporulation capacity (SC) for 20 isolates of *Cochliobolus carbonum* race 3 inoculated on corn inbreds Pa33 and Pa419P

Fungal isolates	SC on inbred lines[a] Pa33	Pa419P
NL-5a	861 A	129 J
TL-12	610 B	430 D
TS75-14/2	549 BC	214 I
NL-10	532 C	504 C
TS75-1/6	531 C	802 A
TL-6	521 C	548 B
TL-4	478 CD	181 J
TL-14	434 D	549 B
TL-17	430 D	115 J
NL-11	421 DE	393 E
NL-2	420 DE	397 E
TL-1	352 EF	309 FG
TL-9	343 EF	373 EF
RS419-3	315 FG	287 FG
TL-16	308 FG	115 J
TX-8	291 FGH	202 I
RS64-3	245 GH	278 G
RS33-3	223 H	276 H
TL-18	220 H	241 HI
NS75-30/2	217 H	124 J
Mean	415	323

[a]Values are expressed in spores per square centimeter (see text for calculation) and are the average of three replications. Means followed by the same letter are not statistically different ($k = 100$; approximately $P = 0.05$) as determined by Duncan's (Bayesian) modified least significant difference test.

consists predominantly of additive gene action (5). A further study suggests that breeding programs utilizing these fitness attributes should use a wide range of pathogen genotypes (4).

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