Genetic Selection and Adaptation of *Cochliobolus heterostrophus* to Corn Hosts with Partial Resistance

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ABSTRACT

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The capacity of *Cochliobolus heterostrophus* race O to adapt to quantitative resistance in corn was evaluated in three cycles of recurrent selection for increased length of lesions induced on leaves of inbred line 316 derived from the open-pollinated cultivar Jarvis. Mean lesion length for selected progeny increased gradually through the selection generations. Estimated realized heritability for lesion length on inbred line 316 was 0.27. Progeny from the third generation of selection also produced significantly larger lesions than the parental isolates on inbred lines W64A, B37, and MO17 and hybrid B73 × MO17 as well as on inbred line 316. A significant cultivar × generation effect was also detected, with the greatest increase in lesion length occuring on inbred 316, the cultivar on which the selection took place. The results demonstrate that *C. heterostrophus* has the genetic capacity to increase both general and specific virulence and thus, potentially, to reduce the effectiveness of polygenic resistance. The results also illustrate the inherent weakness of attempts to classify resistance as vertical or horizontal on the basis of preexisting phenotypic variation among pathogen isolates before there has been ample opportunity for the pathogen population to adapt to the resistance.

Additional key words: durability of resistance, specific resistance, specific virulence, *Zea mays*.

Vanderplank's (17) definition of horizontal resistance stated that the combined additive effects of resistance in the host and aggressiveness in the pathogen determine the measure of disease severity. This definition precluded any specific host cultivar × pathogen isolate interaction. Quantitative disease resistance has often been assumed to be horizontal, because clearly defined pathogenic race specificity has not been demonstrated for it. Robinson (15) stated that all polygenic disease resistance is horizontal and is conferred by mechanisms beyond the pathogen's capacity for adaptation. However, significant cultivar × isolate interactions involving quantitative resistance that appeared to be polygenically inherited have been found in some host-pathogen systems (2,3,5,14).

With *Cochliobolus heterostrophus* Drechsler on corn, Jenns et al (10) found that resistance, as determined by mean length of lesions, was normally distributed among inbred lines derived from the open-pollinated cultivar Jarvis. This suggested that lesion length, which was significantly correlated with sporulation per lesion (9,10), may be polygenically inherited in inbreds from Jarvis. Jenns et al (10) found statistically significant inbred line × isolate interactions in six of six individual trials of 10 inbreds inoculated in all combinations with 10 isolates, but the interaction term was not significant in the combined analysis over all trials in their experiment. They concluded that the isolates of *C. heterostrophus* and the corn inbreds may interact more strongly with environmental conditions than with each other. Such environmental interactions would blur the already debatable distinction between horizontal and vertical resistance. Therefore, we will disregard these distinctions and instead will refer to general and specific components of resistance and virulence as they are expressed in a particular environment.

This study was undertaken to evaluate the capacity of *C. heterostrophus* to adapt to quantitative resistance in corn and to test whether the adaptation, if it did occur, involved selection of genes for general or specific virulence or both. By subjecting a population of *C. heterostrophus* isolates to recurrent selection for increased lesion length on an inbred from Jarvis, we evaluated the genetic capacity of the fungus to adapt to increased virulence. Through tests of selected and unselected isolates on other corn cultivars, we determined the host specificity of the increased virulence.

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MATERIALS AND METHODS

General procedures. The experiments were based on a sequence of three cycles of selection for increased virulence as expressed in length of lesions produced by isolates of *C. heterostrophus* on inbred line 316 derived from the open-pollinated corn cultivar Jarvis. Inbred 316 had previously been shown to have an intermediate level of resistance to isolates of *C. heterostrophus* (10).

Isolates used in this study were derived from race O isolates collected from diseased corn in North Carolina in 1974 and 1975 and used in the experiments of Jenns et al (10). Isolates were grown on potato-dextrose agar (PDA) containing 10 g of dextrose per liter and stored on PDA slants at 4 C. After isolates were tested for virulence, the corn leaves infected with each isolate were collected, dried, and stored at 4 C. Cultures for subsequent tests were established from conidia obtained from lesions on the stored leaves after they had been incubated for 3 days in petri dishes lined with damp filter paper.

Inoculum was prepared from cultures grown on ground corn leaf agar (GCLA) (16) for 2 wk with alternating 12-hr periods of fluorescent light and darkness at room temperature. Conidial suspensions were prepared by flooding the GCLA plates with a solution containing one drop of Tween 20 per 100 ml of distilled water and scraping to release conidia. Conidial suspensions were filtered twice through two layers of cheesecloth, and spore concentrations were determined with a Coulter Counter (Coulter Electronics, Hialeah, FL 33010). Spore concentrations were standardized for isolates within each trial. Concentrations used for different trials ranged from 10,000 to 25,000 spores per milliliter of water.

Corn seedlings for inoculation were grown in 10-cm clay pots filled with Metro Mix (W. R. Grace, Cambridge, MA 02138) in a greenhouse at 18–30 C. At 21 days after planting, eight 10-μl drops of standardized conidial suspension were applied about 2 cm apart to the middle portion of the fourth leaf of each plant. Inoculated plants were incubated in a mist chamber for 16 hr, then placed on a greenhouse bench. Four days after inoculation, the lengths of the resultant lesions were measured with a ruler to the nearest millimeter.

Crosses among isolates of *C. heterostrophus* were carried out by pairing isolates of compatible mating types on 1-cm-diameter disks of senescent corn leaves on modified Sachs agar (7) in petri dishes. Small (2–4 mm diameter) mycelial plugs from 4-day-old cultures of sexually compatible isolates grown on PDA were placed opposite of each other across the autoclaved, senescent corn leaf disks in the Sachs agar petri dishes. Crosses were incubated in the dark at 24 C for 21 days. Fertile perithecia that developed on the corn leaf disks were crushed in a drop of sterilized water on a sterile microscope slide, and the contents were washed onto an agar surface to separate the ascospores. Ascospores were transferred to 10 g of PDA in petri dishes, and mycelial plugs from 3-day-old cultures were transferred to PDA slants for storage at 4 C.

Selection for virulence. The population of *C. heterostrophus* for selection was initiated from 10 parental race O isolates, including conidial isolates collected in 1974 and 1975 and used by Jenns et al (10) and first-generation ascospore progeny obtained from crosses of such isolates. The 10 parental isolates were mated in all sexually compatible combinations, and 25 ascospore progeny, which have been described previously (11), were isolated. The 10 parents and the 25 initial-generation isolates (generation 0) were inoculated on inbred 316 and evaluated for lesion length in two separate trials at different times with four replicates (four plants with eight lesions each for each isolate) in each trial. Seven generation 0 isolates that produced lesions of greater than average length in both trials were selected as parents for the next generation. Isolates selected by this criterion were not necessarily those with the greatest average lesion length; however, the selected isolates produced the most consistently long lesions over both trials.

The seven generation 0 isolates selected for high virulence were mated in all compatible combinations, and 25 ascospore progeny were obtained. These 25 isolates comprised the first selection generation (generation 1). Selection for lesion length was carried out in this manner for two additional generations. All selection generations had about 25 isolates. Each selection generation was evaluated with the parental generation in two separate trials with four replicates (four plants with eight lesions each for each isolate) for each trial.

Evaluation of selection generations in a common environment. After three generations of selection, the 10 original parents and the selected isolates from each of the three selection generations were evaluated together for virulence on inbred 316 in two separate trials at different times with four replicates each. Lengths of lesions produced by isolates of each generation were averaged over both trials. This test was conducted to compare the virulence of the parents and the selected progeny of each generation in a common environment to confirm that the increase in mean lesion length over generations observed in separate tests had resulted from genetic rather than environmental changes.

Specificity of virulence. An additional test was conducted to determine whether the observed increases in virulence were specific for inbred 316 or involved general virulence equally effective against other corn genotypes. The 10 parental isolates and the selected isolates from the third generation of selection (generation 3) were inoculated on inbred corn lines 316, W64A, B37, and MO17 and hybrid B73 × MO17 in all possible combinations. The test consisted of two separate trials at different times with six replicates in each (six plants of each corn cultivar with eight lesions each for each isolate). Results of the two trials were combined for analysis.

Effect of crossing without selection on virulence. To check on the effect of repeated cycles of crossing without selection for virulence, a population of ascospore isolates from a separate study was also tested for virulence. In that study (11), a population derived from the same 10 parental isolates used in this study was selected for fertility (number of fertile perithecia produced per cross) without regard to virulence on corn. Progeny from the third and sixth cycle of selection for fertility and the initial generation of ascospore isolates were inoculated onto seedlings of the hybrid B73 × MO17 and evaluated for lesion length as described.

RESULTS

The mean length of lesions produced on inbred 316 by isolates in the unselected progeny of the 10 original parental isolates (generation 0) did not differ significantly from the mean of the parental isolates (parental generation), but those of the three selection generations (generations 1–3) were significantly greater than those of the parental generation and generation 0 (Table 1). In this comparison, no statistically significant differences were detected among the selected isolates in lesion length relative to the parental isolates after the first generation of selection.

The selected isolates from each selection generation showed a nearly linear increase in lesion length when tested on inbred 316 together in a common environment (Fig. 1). The selected isolates

TABLE 1. Mean lesion lengths (mm) and standard errors produced by isolates[a] of *Cochliobolus heterostrophus* in the parental and selection generations on corn inbred line 316

Generation of selection	Trial	Mean lesion length (mm)		
		Parental generation	Selection generation[b]	Selected progeny[c]
0	A	5.60 ± 0.007	5.23 ± 0.035	···
0	B	4.02 ± 0.032	4.12 ± 0.160	5.73 ± 0.055
1	A	7.38 ± 0.183	8.19 ± 0.087	···
1	B	5.55 ± 0.084	6.03 ± 0.055	9.06 ± 0.680
2	A	7.18 ± 0.081	7.78 ± 0.100	···
2	B	8.15 ± 0.093	9.28 ± 0.140	9.45 ± 0.300
3	A	6.09 ± 0.072	6.85 ± 0.048	···
3	B	5.73 ± 0.081	6.38 ± 0.050	···

[a] Isolates were selected for lesion length on line 316.
[b] Mean lesion length over all isolates in selection generations 1–3 were significantly greater ($P<0.05$) than the mean for the parental generation.
[c] Mean lesion lengths over two trials for those progeny selected for mating to produce the next selection generation.

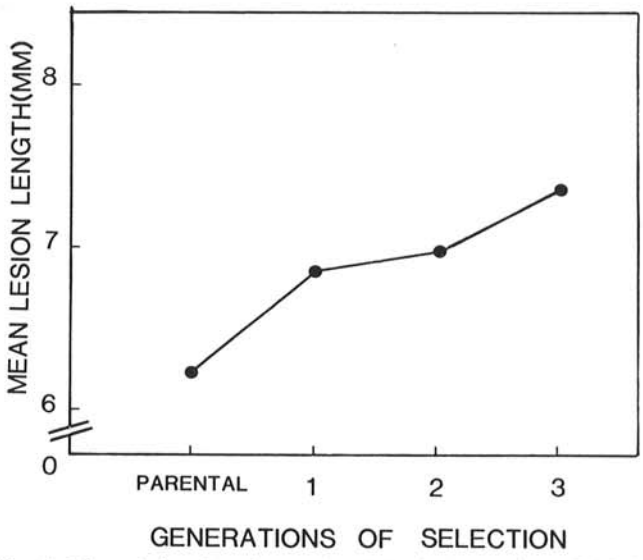

Fig. 1. Mean lesion lengths produced by isolates of *Cochliobolus heterostrophus* in the parental and selection generations 1, 2, and 3 in a common environment on inbred 316. Isolates were selected for lesion length on inbred 316. Isolates in the three selection generations had significantly ($P<0.05$) longer lesions than the isolates in the parental generation according to LSD.

from each generation produced significantly longer lesions than those of the parental generation in this test (Fig. 1) as well as in the individual generation tests. A realized heritability estimate of 0.27 was determined by the regression of the mean lesion length for each generation on inbred 316 on the accumulated selection differential (4). The regression coefficient was significant at $P = 0.12$; however, with only two degrees freedom in the regression error term, the F value required for significance at $P < 0.05$ is high.

Selected isolates from the third generation of selection produced lesions significantly longer than those of the parental generation on all five corn genotypes tested (Fig. 2). Analysis of variance (Table 2) for lesion length showed that the effects of generations, isolates within generations, cultivars, trials, generations × cultivars, and isolates within generations × trials were significant at $P < 0.10$. The proportions of the variance attributable to generations, cultivars, generations × cultivars, and trials were 11.2, 85.6, 2.6, and 0.4%, respectively.

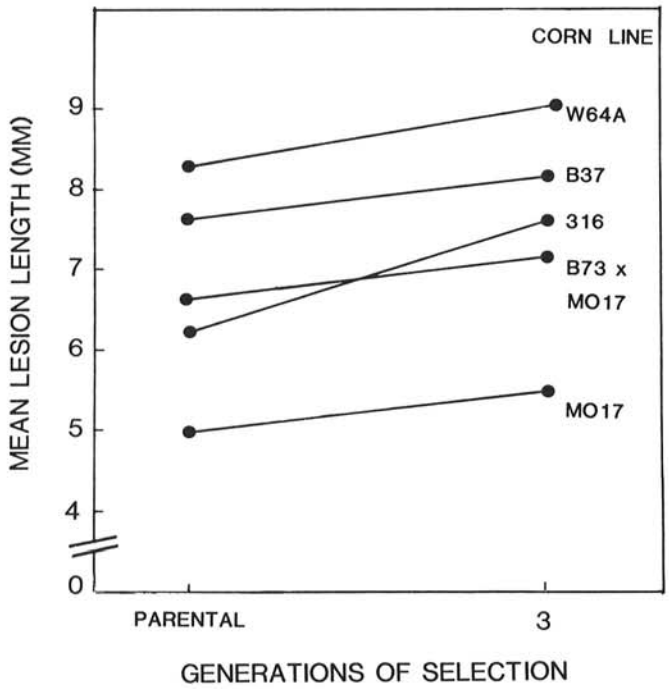

Fig. 2. Mean lesion lengths produced by isolates of *Cochliobolus heterostrophus* in the parental and selection generation 3 on five cultivars of corn. Isolates were selected for lesion length on inbred 316. Isolates in selection generation 3 were significantly greater ($P < 0.05$) than the parental generation isolates on all corn cultivars according to LSD.

TABLE 2. Analysis of variance of lesion length produced by isolates[a] of *Cochliobolus heterostrophus* in the parental and selection generation 3 on five corn cultivars

Source[b]	df	Mean square	F	$P > F$
Generations	1	99.42	4.51[c]	0.0600
Isolates (generation)[d]	13	22.01	3.46[e]	0.0190
Cultivars	4	291.26	71.91[f]	0.0008
Trial	1	5.57	3.21	0.0700
Generation × cultivar	4	5.86	3.39[g]	0.0095
Cultivar × trial	4	4.05	2.34	0.0540
Isolate (generation) × trial	14	6.36	3.67	0.0001
Generation × cultivar × trial	4	1.55	0.89	0.4660
Error[h]	768	1.73	...	...

[a] Isolates were selected for lesion length on line 316.
[b] Generations, isolates, and cultivars are fixed; trial is random.
[c] Error term was isolates (generation) mean square.
[d] Isolates were nested within generations.
[e] Error term was isolates (generation) × trial mean square.
[f] Error term was cultivar × trial mean square.
[g] Error term was generation × cultivar × trial mean square pooled with error mean square.
[h] Differences in degrees of freedom for error are due to missing values.

Selection for increased fertility for six generations in a population derived from the same 10 original parental isolates did not result in increased virulence. The mean lesion lengths produced were 7.20, 7.41, and 7.20 mm for the initial, third, and sixth generation, respectively. Lesions produced by the initial generation were not significantly different in length from those produced by isolates in two advanced fertility generations.

DISCUSSION

When isolates from all three selection generations and the parental generations were evaluated together in a common environment, a progressive increase in lesion length over generations of selection was apparent. Slow selection gains such as this are commonly observed in selection experiments for polygenic traits with low heritability (4).

Isolates from the initial, third, and sixth generations of selection for fertility in *C. heterostrophus* did not differ significantly when tested for virulence on the hybrid B73 × MO17. Selection for fertility, without regard to virulence, had no effect on the virulence of the isolates in the advanced selection generations. The absence of any detectable change in virulence during selection for fertility indicates that the process of repeated crossing and isolation of ascospore isolates would not by itself measurably increase the virulence of a population of the fungus. Although the sexual stage of the fungus is not commonly found in nature, ascospore isolates of *C. heterostrophus* with high levels of fertility would appear to be equally as fit as wild-type, conidial isolates.

It was apparent that uncontrolled variation in conditions between trials affected the relative ranking of isolates for lesion length. Some isolates that produced relatively long lesions in the first trial produced relatively short lesions in the second. This is indicated by the significant isolates within generation × trial interaction term in the analysis of variance (Table 2). The significant cultivar × trial interaction term indicates that cultivars also differed in their relative resistance between trials.

Jenns et al (10) also found significant isolate × trial and inbred × trial interactions in greenhouse tests of 10 conidial isolates of race O of *C. heterostrophus* inoculated in all combinations on 10 inbred lines of corn. Jenns and Leonard (9) found significant inbred × temperature but not isolate × temperature interactions for length of lesions produced by *C. heterostrophus* in growth chamber tests. The inbred × illuminance interaction was significant at $P = 0.10$. Inbreds differed in the extent to which their resistance was diminished by increased temperature or decreased illuminance. In that controlled-environment study, the inbred × trial and isolates × trial interactions within temperature and illuminance treatments were still highly significant, indicating a source of uncontrolled variation that affected the ranking order of inbreds and isolates in different trials.

The low heritability and the genotype × environment interactions may explain why comparisons of individual selection generations with the parental generations in separate tests did not show a measurable increase in the difference between parents and the selection generations after the first generation. Another, less likely explanation might be the early fixation of alleles affecting lesion length. In selecting for fertility in *C. heterostrophus*, which had a heritability of 0.74, we observed a linear decline in the proportion of additive genetic variance over six generations of selection intensity equivalent to that employed in selection for increased lesion length (11). It seems unlikely that a major portion of additive variation for lesion length would have been exhausted in the first generation of selection.

Our estimate of the realized heritability for length of lesions produced by isolates of *C. heterostrophus* is considerably lower than the estimate of 0.87 determined for heritability of length of lesions produced by isolates of *C. carbonum* race 3 (6). That heritability estimate was calculated by the ratio of genotypic to phenotypic variances from an experiment in which two inbred lines

of corn were inoculated in all combinations with 20 isolates of *C. carbonum* race 3. Heritability measured as a ratio involving total genetic variance would include possible variance (in the numerator) attributable to genetic interactions that would not be inherited by progeny and therefore would not be reflected in a heritability estimate based on response to selection (4). Our estimate of heritability was calculated using the responses to selection for lesion length (Fig.1) and the cumulative selection differentials from the parental and selection generations (Table 1). Heritabilities determined from genetic responses and multiple environments are more realistic estimates than values determined by an analysis of variance method, which uses fewer environments and limited numbers of phenotypes.

Hill and Nelson's (8) estimate of heritability for length of lesion produced by race T of *C. heterostrophus* on corn lines with T cytoplasm was only 0.05. This is extremely low, but it also may not be directly comparable to our estimate. There is probably little genetic variation among race T isolates for length of lesions produced on T cytoplasm corn because a single gene controls toxin production in the fungus (13). Genetic variation in other aspects of virulence that would be important sources of variation in race O may have little effect on size of lesions produced by race T. Furthermore, there is evidence that there may be only limited genetic variation among isolates of race T, particularly isolates from the northern areas of the United States (12). With limited genetic variation present in the fungus, the ratio of genetic variance to phenotypic variance would be predictably low.

Our method of selection increased both general and specific virulence. The increase in general virulence is illustrated by the nearly uniform, significant increase in length of lesions produced by selected isolates on four cultivars of corn that are not closely related to inbred 316. Comparisons of virulence of isolates from the parental and third selection generations on the five corn cultivars revealed a significant culivar × generation interaction. The difference in virulence between third-generation isolates and parental isolates was much greater on inbred 316 than on the other cultivars. The specificity of third-generation isolates for inbred 316 is easily seen in the comparison of virulence on inbred 316 and the hybrid B73 × MO17 (Fig. 2). Parental isolates were more virulent on the hybrid than on inbred 316, whereas the reverse was true for the isolates from the third selection generation on inbred 316. The specificity of virulence of the third-generation isolates to inbred 316 is also apparent in comparisons with inbreds both more resistant (MO17) and more susceptible (W64A) than inbred 316. These results are similar to those of Clifford and Clothier (3) and Caten (2), who found that isolates of *Puccinia hordei* and *Phytophthora infestans* had some specific virulence on host cutivars from which they were isolated.

Variation attributable to the generation × cultivar and isolates within generation × cultivar terms was relatively small compared with effects attributable to cultivars, generations, and isolates within generations. This illustrates the difficulty in detecting specific interactions between host and pathogen in this disease. Previous studies using statistical tests to detect specificity indicated an absence of specificity or were inconclusive (9,10). However, our results (Table 2, Fig. 2) show that genes for specific virulence occur in *C. heterostrophus* and that the level of specificity can be enhanced by repeated cycles of crossing and selection.

Burnette and White (1) found that resistance to race O of *C. heterostrophus* in 12 families from crosses of nine resistant corn inbreds and three susceptible inbreds was quantitatively inherited, with additive effects accounting for most of the genetic variation. Transgressive segregation in three of the families suggested that the susceptible inbreds chosen as parents may carry some genes for resistance. Thus, the evidence indicates polygenic rather than oligogenic inheritance of resistance to race O of *C. heterostrophus*.

Evidence from Jenns et al (10) also strongly suggests that the resistance of inbred lines from the open-pollinated cultiver Jarvis to race O of *C. heterostrophus* is polygenic. The range of lesion lengths among 51 inbred lines from Jarvis that Jenns et al (10) tested was about five times greater than the 95% confidence intervals for lesion lengths of inbred 316 or other individual inbred lines. The frequency distribution of inbreds in different lesion size catagories resembled a normal distribution. Because the inbred lines are essentially homozygous, such a wide range and normal distribution of lesion sizes among the 51 inbreds is indicative of polygenic control.

The ability to select and enhance specificity in virulence against a polygenically resistant host renders the distinction between vertical and horizontal resistance less valid (15,17). Specific interactions between host and pathogen populations can be difficult to detect by standard statistical techniques. These analyses may be inadequate to distinguish between specfic and nonspecific virulence and resistance.

The durability of effective disease resistance depends on the ability of the pathogen population to develop matching virulence to the resistant host genotypes. By artificial selection in the greenhouse, we increased both the general and specific components of virulence in *C. heterostrophus*. The rate of increase, however, was relatively slow. Natural selection in the field would be less intense, and the rate of increase in general and specific virulence would probably be even slower. Nevertheless, our results indicate that this pathogen may be capable of developing field populations with specific virulence to hybrids with quantitative, partial resistance and should serve as a caution against over reliance on the durability of quantitative resistance in monoculture.

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