Inheritance of Resistance to *Aspergillus* Ear Rot and Aflatoxin Production of Corn from CI2

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ABSTRACT

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This study determined the types and magnitude of gene action, estimated heritabilities, and predicted gain from selection for resistance to *Aspergillus* ear rot and aflatoxin production in the cross of resistant corn inbred CI2 to susceptible inbred B73 in 1998 and 1999. The warm, dry summer of 1998 favored aflatoxin production, whereas the conditions of 1999 did not. Resistance to ear rot was mainly controlled by additive gene action. Aflatoxin values were analyzed by individual years (environments) because of the highly significant generation $\times$ environment interaction. Resistance to aflatoxin production was mainly controlled by epistasis in 1998 and by additive gene action in 1999. Heritabilities for ear rot and aflatoxin production were higher in the F_3 generation than in the BCP₁-selfed generation. In 1998, Spearman's correlation coefficients between *Aspergillus* ear rot ratings and aflatoxin values for the F_3 and the BCP₁-selfed families were not significant ($P > 0.05$). In 1999, both were highly significant ($P < 0.01$), but low at 0.41 and 0.17 for the F_3 and BCP₁-selfed generations, respectively. We found that CI2 is not an acceptable source of resistance due to lower heritabilities and disease resistance compared to other sources of resistance.

Additional keywords: *Aspergillus flavus*, maize, mycotoxin

Aspergillus ear rot of corn was first reported in 1920 (27); however, it was not considered to be economically important until some strains of the causal organism, *Aspergillus flavus* Link:Fr., were found to produce aflatoxin. The severity of *Aspergillus* ear rot of corn and the amount of aflatoxin produced are greatly affected by the environment. The highest amounts of aflatoxin occur in growing seasons when the temperatures are above average and rainfall is below average. The greatest potential for disease development exists with hot and dry environments, insect damage to corn ears, and low levels of host resistance (17,18,21,28). The consumption of aflatoxin-contaminated grain by animals can have detrimental effects on health (1,9,16). Corn grain containing aflatoxin at more than 20 ng/g (ppb) cannot be sold in interstate commerce, and some countries will not buy grain with aflatoxin levels above 10 ppb (20).

Although possible control methods of *Aspergillus* ear rot include irrigation and

insect control, the most desirable and economical control strategy in corn is the use of resistant hybrids (21). Resistance to *Aspergillus* ear rot and aflatoxin production from previously studied sources of resistance is quantitatively inherited. Several studies have been done to determine the gene action involved in resistance to *Aspergillus* ear rot and aflatoxin production. Diallel mating designs have been used extensively (7,10,11,29,30). Relatively high coefficients of variation that reflect the impact of the environment on aflatoxin production have been found with most studies. The predominant result from the diallel mating designs is that general combining ability (indicating additive genetic effects) is more important for resistance to aflatoxin production than specific combining ability (indicating dominance genetic effects).

Previous research at the University of Illinois developed a pinboard inoculation technique that has been used in subsequent research for determining susceptibility to ear rot and toxin production (4). In 1991, 1,189 inbreds crossed with Mo17 and 978 inbreds crossed with B73 were evaluated for *Aspergillus* ear rot resistance (5). The inheritance of resistance to *Aspergillus* ear rot was evaluated in nine of the most resistant inbreds crossed with the susceptible inbreds B73, Mo17, or both (6).

When the F_1 hybrids with Mo17 or B73 were evaluated using the pinboard inoculation method (5), CI2 $\times$ B73 was ranked as

one of those most resistant to ear rot and aflatoxin production. In the same study, the inbred CI2 did not differ significantly from the most resistant inbred, Tex6, for aflatoxin production in 1992 and 1993 (5). However, when evaluated in Mississippi in 1993 under drought conditions as an F_1 hybrid with B73, also using the pinboard inoculation method, CI2 had high aflatoxin production (5). Brown et al. (2) ranked the inbred CI2 as one of the most resistant inbreds in studies done in the laboratory when the kernel was not wounded, but as a susceptible inbred when the kernel was wounded. Hence, an intact pericarp appears to contribute to the resistance of CI2. Campbell and White (6) evaluated the cross B73 $\times$ CI2 in 1992 and 1993 to determine the types and magnitudes of gene action for resistance to *Aspergillus* ear rot but not aflatoxin production in the P_1 , P_2 , F_1 , F_2 , and BCP₁ generations. In that study, CI2 had high levels of resistance to *Aspergillus* ear rot and aflatoxin production in the F_1 cross with B73 and as an inbred per se. Resistance to ear rot was primarily additive (85.3%), with the remainder due to epistasis. Inferences from the previous study apply only to ear rot, because *Aspergillus* ear rot and aflatoxin production are not highly correlated (4,5,6,13). Due to this poor relationship, it is necessary to determine the inheritance of resistance to aflatoxin production per se in addition to ear rot.

The true value of an inbred line as a useful source of resistance in pragmatic corn improvement programs is known only after it has been evaluated in segregating generations of a cross to an inbred related to commercially used inbreds. The objective of this research was to determine the value of CI2 as a stable source of resistance in a breeding program for resistance to aflatoxin production. In this study, in order to calculate the epistatic parameters, the F_3 , BCP₂, and BCP₁-selfed generations were evaluated in addition to the P_1 , P_2 , F_1 , F_2 , and BCP₁ generations evaluated as in a previous study on ear rot. Additionally, the number of plants or families for each generation was increased to estimate more precisely the genetic effects. The types and magnitudes of gene action for resistance to both ear rot and aflatoxin production were determined, heritabilities were estimated, and the predicted gain from selection was calculated in segregating generations from the B73 $\times$ CI2 cross.

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MATERIALS AND METHODS

The generations evaluated for the B73 × CI2 cross in 1998 and 1999 were the P₁ (B73), P₂ (CI2), F₁, F₂, F₃, BCP₁, BCP₂, and BCP₁-selfed generations. The F₁, F₂, F₃, BCP₁, BCP₂, and BCP₁-selfed generations were produced by crossing the parental lines, self-pollinating the F₁ plants, self-pollinating the F₂ plants, crossing the F₁ plants to the susceptible and resistant parent, respectively, and self-pollinating the BCP₁ generation. The experiment was a randomized complete block design with two replications of all generations. Each replication consisted of 15 rows of the P₁ (B73), P₂ (CI2), F₁, F₂, BCP₁, and BCP₂ generations, 162 rows of F₃ families, and 273 rows of BCP₁-selfed families. For each replication, the rows or families within each generation were randomized, with all rows or families grouped together by generation to minimize competition. Experiments were planted 24 April 1998 and 1 May 1999. Plants were grown in single-row plots 5.34 m long and 0.76 m apart, with approximately 24 plants per row.

Isolates used, inoculum preparation, and inoculation technique were the same as previously reported (3). Inoculum consisted of an equal mixture of conidia from four isolates of *A. flavus*. Three isolates (6535, 6539, and 6540) were obtained from the Northern Regional Research Laboratory, Peoria, IL, and one was isolated from corn grain from Illinois in 1988. Individual ears were inoculated approximately 14 days after midsilks. Ears were hand harvested approximately 50 days after inoculation, rated for ear rot, and dried in a forced air dryer for 3 days. Ear rot was visually rated on an equal increment scale of 1 to 10, with 1 being no visible ear rot and 10 being fully rotted at the inoculation site. The few poorly inoculated ears, as determined by the absence of evidence of kernel wounding, or poorly pollinated ears were discarded. However, all ears produced by P₂ (CI2) were poorly pollinated; therefore, all ears from this generation were retained.

Ears from each row were bulked and shelled. Grain from individual rows was ground, and a 1-g sample was assayed for quantity of aflatoxin by an indirect competitive enzyme-linked immunosorbent assay (ELISA) modified from Campbell and White (5). Samples with nondetectable aflatoxin were recorded as 2 ng/g because this is the lower detection limit in this procedure. The 1-g samples were analyzed in triplicate and averaged.

Ear rot means were calculated for each row and then averaged by generation within each replication. Aflatoxin values were log_e transformed to homogenize the variances between generations. The transformed aflatoxin values of bulked ears from individual rows were averaged by generation within each replication. The

SAS general linear model procedure (PROC GLM; SAS Institute Inc., Cary, NC) was used to analyze the effect of years (environments), blocks, generations, and their interactions. Data were analyzed by individual years, then combined to test the effects of years. Blocks (replications) were considered random effects, whereas generations and years were considered fixed effects. Years were considered to be a fixed effect because of the small number of years, the variability within the data, and the large effect of environment on ear rot and aflatoxin production. A *t* test was used to test for significant differences between the F₁ generation and the midparent for both ear rot and aflatoxin values.

Generation mean analysis followed Mather and Jinks' notation (19) and used Hayman's coefficients (14,15). The means of the generations for individual years were fitted to the additive-dominance genetic model $Y = m + ax + b_1d + b_2h$, in which Y = generation mean, m = intercept value, x = block effect, d = pooled additive gene effects (pooled effects of homozygous loci), and h = pooled dominance effects (pooled effects of heterozygous loci). The data were fit to the model using the SAS regression procedure (PROC REG). This additive-dominance model was tested by using an *F* test to determine if the deviations from the model were significant. If the deviations were not significant, then the model was considered adequate. If the deviations were significant, then a model was tested that included epistasis in the form of digenic interactions. The equation used in this regression analysis was $Y = m + ax + b_1d + b_2h + b_3i + b_4j + b_5l$, where Y = generation mean, m = intercept value, x = block effect, d = pooled additive effects, h = pooled dominance effects, i = additive-additive (homozygote-homozygote) interactions, j = additive-dominance (homozygote-heterozygote) interactions, and l = dominance-dominance (heterozygote-heterozygote) interactions. Deviations from

this model are considered evidence of trigenic or higher-ordered epistasis. Subsequent models testing for higher-ordered epistasis were not tested because of the insufficient number of generations.

When the data were combined to test the effect of years, the data were fitted to the equation $Y = m + cy + a_1x_{(1998)} + a_2x_{(1999)} + b_1d + b_2h$, where Y = generation mean, m = intercept value, y = year effect, $x_{(1998)}$ = block effect in 1998, $x_{(1999)}$ = block effect in 1999, d = pooled additive effects, and h = pooled dominance effects. Blocks were nested within years and years were considered fixed. This additive-dominance model was tested by using an *F* test to determine if the deviations from the model were significant. If the deviations were significant, then the data were fitted to a model that included digenic epistasis. The equation used in this regression analysis was $Y = m + cy + a_1x_{(1998)} + a_2x_{(1999)} + b_1d + b_2h + b_3i + b_4j + b_5l$, where Y = generation mean, m = intercept value, y = year effect, $x_{(1998)}$ = block effect in 1998, $x_{(1999)}$ = block effect in 1999, d = pooled additive effects, h = pooled dominance effects, i = additive-additive interactions, j = additive-dominance interactions, and l = dominance-dominance interactions.

Broad-sense heritability estimates (ratio of genotypic variance to phenotypic variance) were calculated and expressed as percentages for both the F₃ and BCP₁-selfed generations for ear rot and aflatoxin production. Broad-sense heritability estimates for the F₃ generation were calculated using the equation $h^2(\text{broad}) = \sigma^2_{gF3} / (\sigma^2_{gF3} + \sigma^2_e / ry + \sigma^2_{gF3})$, where σ^2_{gF3} is the variance among the F₃ family means, σ^2_e is the pooled error variance, r is the number of replications, and y is equal to the number of years (12). Broad-sense heritability estimates for the BCP₁-selfed generation were calculated by replacing σ^2_{gF3} with $\sigma^2_{gBCP1S1}$ in the above equation. Additive variances, narrow-sense heritabilities, and predicted gain from selection were calculated using formulas

Table 1. Aspergillus ear rot and aflatoxin means of generations from the cross B73 × CI2 evaluated in Urbana, IL, in 1998 to 1999

Generation	Ear rot severity ^a		Aflatoxin (ng/g) ^b	
	1998	1999	1998	1999
P ₁ (B73) ^c	6.5	6.3	377	285
P ₂ (CI2) ^d	4.5	3.7	366	39
F ₁	5.2	4.5	342	139
F ₂	5.4	5.0	464	114
BCP ₁	5.5	5.1	419	135
BCP ₂	5.0	5.0	499	154
F ₃	5.6	5.1	556	178
BCP ₁ S ₁	5.7	5.4	435	200
Midparent ^e	5.5	5.0	371	162
F ₁ -midparent	-0.2	-0.5	-29	-23

^a Individual ears were rated on a scale of 1 to 10, with 10 being the most susceptible.

^b Measured by indirect competitive enzyme-linked immunosorbent assay (ELISA). Samples were run in triplicate. Aflatoxin values are the mean of two replications.

^c P₁ = susceptible parent.

^d P₂ = resistant parent.

^e Midparent value = (P₁ + P₂)/2.

provided by J. W. Dudley (*personal communication*) derived from Hallauer and Miranda (12), where additive genetic variance (σ_A^2) equals $2(\sigma_{gF_3}^2 - \sigma_{gBCP1S1}^2)$; narrow-sense heritability (h^2 ; ratio of additive genetic variance to phenotypic variance) for the F_3 generation equals $\sigma_A^2/(\sigma_e^2/ry + \sigma_{gF_3}^2)$; narrow-sense heritability for the BCP₁-selfed generation equals $\frac{1}{2}\sigma_A^2/(\sigma_e^2/ry + \sigma_{gBCP1S1}^2)$; predicted gain from selection (Δ_G) in the F_3 generation equals $k[3/2]\sigma_A^2/\sigma_p$, where k is a constant based on selection intensity and σ_p is the phenotypic variance within the F_3 families; and predicted gain from selection

(Δ_G) in the BCP₁-selfed generation equals $(k[1/2]\sigma_A^2)/\sigma_p$, where σ_p is the phenotypic variance within the BCP₁-selfed families. For these calculations, a 20% selection intensity ($k = 1.3998$) was used (12).

Spearman's correlation coefficients were calculated between Aspergillus ear rot ratings and aflatoxin values for the F_3 and the BCP₁-selfed families for 1998 and 1999 individually. Spearman's correlation coefficients were calculated using the SAS correlation procedure (PROC CORR SPEARMAN). Each generation mean was calculated as an average of two replications.

Table 2. Analysis of variance of Aspergillus ear rot and $\log_e$ transformed aflatoxin production in the generations of the cross B73 $\times$ CI2 evaluated in Urbana, IL, in 1998 to 1999

Source of variation	df	MS	F	P > F ^a
Ear rot 1998 to 1999				
Environment ^b	1	0.80	11.05	0.0050
Block (environment)	2	1.37	19.02	0.0001
Generation	7	1.34	18.50	0.0001
Generation $\times$ environment	7	0.05	0.71	0.6645
Pooled error (CV ^c = 5.12)	14	0.07		
Aflatoxin 1998 to 1999				
Environment	1	13.41	638.34	0.0001
Block (environment)	2	0.00	0.02	0.9823
Generation	7	0.66	31.44	0.0001
Generation $\times$ environment	7	0.58	27.40	0.0001
Pooled error (CV = 2.81)	14	0.02		
Aflatoxin 1998				
Block	1	0.00	0.02	0.8813
Generation	7	0.07	4.68	0.0296
Pooled error (CV = 3.67)	7	0.01		
Aflatoxin 1999				
Block	1	0.00	0.01	0.9071
Generation	7	1.17	42.37	0.0001
Pooled error (CV = 2.07)	7	0.03		

^a Probabilities associated with individual F tests.

^b Environment = years.

^c CV = coefficient of variation.

Table 3. Least squares estimates of the parameters of the simple additive-dominance model^a used in the generation mean analysis of the B73 $\times$ CI2 cross evaluated for Aspergillus ear rot^b in Urbana, IL, in 1998 to 1999

Parameter	Parameter estimate	Standard error	T for H0: parameter = 0	P > T
m	5.14	0.06	89.60	0.0001
y	0.16	0.05	2.99	0.0061
$x_{(1998)}$	-0.07	0.07	-0.91	0.3737
$x_{(1999)}$	0.41	0.07	5.46	0.0001
d	0.83	0.09	9.16	0.0001
h	-0.47	0.17	-2.72	0.0116

^a The simple additive-dominance model fitted was $Y = m + cy + a_1x_{(1998)} + a_2x_{(1999)} + b_1d + b_2h$. The parameters of the equation are m = midparent and intercept value, y = year effect, $x_{(1998)}$ = block effect in 1998, $x_{(1999)}$ = block effect in 1999, d = pooled additive effects (pooled effects of homozygous loci), and h = pooled dominance effects (pooled effects of heterozygous loci). There were no significant deviations from the additive-dominance model, hence no other models were evaluated.

^b Individual ears were rated on a scale of 1 to 10.

Table 4. Percent variation in ear rot and $\log_e$ transformed aflatoxin production among generation means accounted for by additive, dominant, and residual gene effects

Source	Percent gene effect		
	Additive	Dominant	Residual
1998 to 1999 ear rot	82.7	7.1	10.3
1998 aflatoxin	2.6	0.7	96.7
1999 aflatoxin	64.8	3.2	32.0

RESULTS

In 1998, temperatures were warmer than average in August and September and rainfall was less than average (24,25). The P₁ (B73) generation had the highest mean ear rot rating, and the P₂ (CI2) generation had the lowest mean ear rot rating (Table 1). The aflatoxin values were very high in 1998 and the P₁ (B73) generation was not significantly different from the P₂ (CI2) generation (Waller-Duncan, k -ratio = 100). The F_3 generation had the highest mean aflatoxin value and the F_1 generation had the lowest mean aflatoxin value in 1998. Ear rot and aflatoxin values for the F_1 generation were not significantly different from the midparent value.

In August and September 1999, the daily temperatures were cooler than average (22,23) and ear rot and aflatoxin values were generally lower than those observed in 1998 (Table 1). There was a better separation of parental ear rot ratings, ranging from 3.7 to 6.3, with P₁ having the highest average rating and P₂ having the lowest average rating (Table 1). The aflatoxin values of the two inbred parents were significantly different (Waller-Duncan, k -ratio = 100), and the mean aflatoxin values were lower than the 1998 values for all generations. Ear rot and aflatoxin values for the F_1 generation were not significantly different from the midparent value.

In the combined analysis of ear rot data from 1998 and 1999, the effect of environments, blocks within environments, and generations were significant, whereas the generation $\times$ environment interaction was not significant (Table 2). Deviations from the model $Y = m + cy + a_1x_{(1998)} + a_2x_{(1999)} + b_1d + b_2h$ were not significant, indicating that epistasis was not operating. The pooled additive gene effects were highly significant at $P = 0.0001$ accounting for 83% of the variation among generation means (Tables 3 and 4). The pooled dominance effects were significant at $P = 0.01$, accounting for 7% of variation (Tables 3 and 4). Residual effects accounted for 10% of the variation. The parameter for the dominant gene effect was negative, indicating dominance for resistance.

In the combined analysis for aflatoxin production, environments, generations, and generation $\times$ environment were significant (Table 2). Because of the highly significant ($P = 0.0001$) generation $\times$ environment interaction, data were analyzed by individual years. Deviations from the model $Y = m + ax + b_1d + b_2h$ were significant for both 1998 and 1999; hence, epistasis was indicated. A digenic epistasis model, $Y = m + ax + b_1d + b_2h + b_3i + b_4j + b_5l$ was tested (Table 5). In 1998, there were no significant parameter estimates when the parental means did not separate. Additive gene effects accounted for 3% of the variation among generation means, whereas dominant gene effects accounted for 1% of variation (Table 4). Residual effects ac-

counted for 97% of the variation. In 1999, when the parental means did separate, only the additive-dominance interaction parameter estimate j was significant at $P = 0.001$ (Table 5). Additive gene effects accounted for 65% of the genetic variation and dominant gene effects accounted for 3% of the genetic variation (Table 4). Residual effects accounted for 32% of the variation.

The broad-sense heritability estimates for resistance to ear rot of F_3 families and BCP₁-selfed families were 48 and 37%, respectively (Table 6). The narrow-sense heritability estimates for the F_3 families and BCP₁-selfed families were 39 and 25%, respectively. The ear rot average would be expected to decrease by 0.60 for the F_3 families and 0.23 for the BCP₁-selfed families after one cycle of selection.

The broad-sense heritability estimate of aflatoxin production for F_3 families and BCP₁-selfed families was 32 and 26%, respectively (Table 6). The narrow-sense heritability estimate for F_3 families and BCP₁-selfed families was 25 and 17%, respectively. Aflatoxin production would be expected to decrease by 0.27 for the F_3 families and 0.10 for the BCP₁-selfed families after one cycle of selection.

The relationship between ear rot severity and aflatoxin production varied in the different years. In 1998, there were no significant relationships (Table 7). However, in 1999, both the F_3 generation and the BCP₁-selfed generation had a low but statistically significant Spearman's correlation coefficient (Table 7).

DISCUSSION

Weather conditions in 1998 and 1999 had a large impact on aflatoxin production. The aflatoxin production data of 1998 did not have the separation of generations as expected. The quantity of aflatoxin in the P_1 and P_2 generations was high in 1998 when environmental conditions favored aflatoxin production, indicating that CI2 may not be as resistant as previously believed. In 1999, when temperatures were below average and rainfall was above average, the resistance of CI2 was again evident with the mean of inbred CI2 significantly less than the mean of inbred B73.

In addition to environmental effects, there are other potential sources for variation. In both years, the inbred CI2 pollinated very poorly because the tassels shed much of their pollen before silks were produced. The poor seed set on the ear made rating of ear rot difficult and may have resulted in an inadequate sample for aflatoxin determinations. Generation mean analysis is based upon significantly different parental values; therefore, when there is a poor estimation of one parent, results of generation mean analysis may lead to incorrect conclusions. Hayman's coefficients (14,15) reduce, but do not eliminate,

this problem by basing generation mean analysis on the F_2 generation rather than the P_1 and P_2 generations.

Another potential source of variation is the inoculation technique used in this study. The pinboard inoculation technique is beneficial in that it wounds the kernels without excessive damage to the husk of the developing ear. Wounding the kernel destroys the aleurone and pericarp, circumventing possible mechanisms of resistance that include an intact pericarp or the aleurone layer. Brown et al. (2) demon-

strated that wounding the kernel of CI2 during inoculation increased the amount of aflatoxin produced, thus changing its classification from resistant to susceptible. In the current study, however, although inbred CI2 did not appear to be resistant in 1998, it was resistant in 1999, and the F_1 generation was resistant in both years. In addition, in the previous screenings in the field, all inoculations were by the pinboard method and CI2 appeared to be resistant (5,6).

Previous studies have reported low or nonsignificant correlations between ear rot

Table 5. Least squares estimates of the parameters of the model^a used in the generation mean analysis of the B73 × CI2 cross evaluated for aflatoxin production^b in Urbana, IL, in 1998 to 1999

Year	Parameter	Parameter estimate	Standard error	T for H0: parameter = 0	P > T
1998	m	5.97	0.05	111.51	0.0001
	x	0.00	0.04	-0.13	0.9020
	d	-0.15	0.14	-1.10	0.3014
	h	-0.21	0.21	-0.98	0.3515
	i	-0.20	0.21	-0.97	0.3568
	j	-0.12	0.16	-0.72	0.4897
	l	-0.97	0.49	-1.98	0.0797
1999	m	4.63	0.11	42.43	0.0001
	x	0.01	0.07	0.07	0.9480
	d	-0.18	0.29	-0.64	0.5372
	h	-0.03	0.44	-0.06	0.9525
	i	-0.43	0.42	-1.02	0.3362
	j	-1.50	0.33	-4.57	0.0014
	l	0.00	1.00	0.00	0.9972

^a The model fitted was $Y = m + ax + b_1d + b_2h + b_3i + b_4j + b_5l$. The parameters of the equation are Y = generation mean, m = intercept value, x = block effect, d = pooled additive effects (pooled effects of homozygous loci), h = pooled dominance effects (pooled effects of heterozygous loci), i = additive × additive (homozygote × homozygote) interactions, j = additive × dominance (homozygote × heterozygote) interactions, and l = dominance × dominance (heterozygote × heterozygote) interactions.

^b Values based on $\log_e$ transformed data from indirect competitive enzyme-linked immunosorbent assay. Samples were run in triplicate.

Table 6. Heritability estimates and expected gain from selection for resistance to *Aspergillus* ear rot and aflatoxin production in the cross B73 × CI2 evaluated in Urbana, IL, in 1998 to 1999

Trait	Family	h^2 ^a	h_n^2 ^b	ΔG ^c
Ear rot	F_3	48.40	39.38	-0.60
	BCP ₁ S ₁	36.93	25.33	-0.23
Aflatoxin ^d	F_3	32.00	25.06	-0.27
	BCP ₁ S ₁	26.27	16.91	-0.10

^a Broad-sense heritabilities (h^2) were calculated for both F_3 and BCP₁S₁ families for comparison. Broad-sense heritabilities were calculated using the equation $h^2(\text{broad}) = \sigma_g^2 / [\sigma_e^2 / ry + \sigma_g^2]$, and expressed as a percent.

^b Narrow-sense heritabilities (h_n^2) were calculated for both F_3 and BCP₁S₁ families for comparison. Narrow-sense heritabilities were calculated using the equation $h^2(\text{narrow}) = \sigma_A^2 / [\sigma_e^2 / ry + \sigma_{gF3}^2]$ for the F_3 generation, and the equation $h^2(\text{narrow}) = 1/2 \sigma_A^2 / [\sigma_e^2 / ry + \sigma_{gBCP1S1}^2]$ for the BCP₁S₁ generation, and expressed as a percent.

^c ΔG is the expected gain from selection. The expected gain was calculated using the equation $\Delta G = [k(3/2)\sigma_A^2] / \sigma_p$ for the F_3 generation, and the equation $\Delta G = [k(1/2)\sigma_A^2] / \sigma_p$ for the BCP₁S₁ generation.

^d Values based on $\log_e$ transformed data.

Table 7. Relationship between *Aspergillus* ear rot and aflatoxin production in the cross B73 × CI2 evaluated in Urbana, IL, in 1998 to 1999

Year	Population ^a	No. of families	Spearman's correlation
1998	F_3	162	0.07
	BCP ₁ S ₁	273	-0.04
1999	F_3	162	0.41*** ^b
	BCP ₁ S ₁	273	0.17**

^a Within each generation analyzed, the year average for each family was used in the correlation analysis for both ear rot and aflatoxin production.

^b **, *** = significant at probability level $P = 0.01$ and 0.001 (F test).

and aflatoxin production (4,5,6,13). Therefore, in this study, the types and magnitude of gene action for resistance to aflatoxin production as well as ear rot were examined. The environment (years) had a larger effect on aflatoxin production than on ear rot, as indicated by the significant generation $\times$ environment interaction present for aflatoxin production but not present for ear rot. In the warm and dry conditions of 1998, the mean aflatoxin value of the P₁ generation was not significantly different from the P₂ generation, and all other generations averaged close to the parents. Generation mean analysis is based upon the assumption that the parental values will be significantly different, and that the genes for resistance are located in the resistant parent, whereas none are in that susceptible parent. Because there was not a significant difference between the parents, the analysis of generation means provided inconclusive results in 1998. In 1999, the aflatoxin values for each generation were much lower and the parents were significantly different. The results from the 1999 data indicate that a large portion of genetic variability is due to epistasis.

In the F₃ and the BCP₁-selfed families, transgressive segregation was observed for aflatoxin production. In both populations, especially in 1998, there were families that were more susceptible to aflatoxin production than the susceptible parent. This is also evidence that not all of the resistance genes are located in the resistant parent CI2, and not all of the susceptibility genes are located in the susceptible parent B73, as assumed for generation mean analysis.

The ear rot results of this study are similar to those previously reported. Campbell and White reported a similar separation of ear rot values for P₁ (B73) and P₂ (CI2) in 1992 and 1993 and a similar level of additive genetic effects (6). However, they reported no significant variation due to dominance genetic effects. The broad-sense heritability estimate of 48% for ear rot in the F₃ generation is similar to the 50% previously reported (6).

Ear rot and aflatoxin production were not correlated in 1998 when the environment was favorable for aflatoxin production. The significant 1999 correlation coefficients were very low and are comparable to results previously reported (6). Because the relationship between ear rot and aflatoxin appears to be inconsistent across environments, selection for resistance to aflatoxin production should not solely be based on resistance to ear rot.

The inbred CI2 has limited possibilities when incorporated into a breeding program. If CI2 is used in areas with very favorable conditions for disease development, then CI2 may not provide adequate resistance. This is indicated by the 1998 data, where higher levels of ear rot and aflatoxin production occurred in the resistant parent when the weather was more

favorable. Although the inbred CI2 was not resistant in 1998, the F₁ generation has maintained resistance in multiple environments. Therefore, although resistance is present in the cross B73 $\times$ CI2, the inbred CI2 is not a very useful source of resistance for breeding programs. The resistance of CI2 was not stable in multiple environments and the heritabilities for B73 $\times$ CI2 are lower than for other potential sources.

Other identified sources of resistance have proven to be more reliable than CI2 during years with environmental conditions favorable to aflatoxin production. Inbred Tex6 was resistant to aflatoxin production in 1995, when temperatures favored aflatoxin production (13). Resistance to aflatoxin production in Tex6 is primarily controlled by additive gene effects. The broad-sense heritability estimate for the B73 cross was 65% and the narrow-sense heritability estimate was 45%, which is much greater than the heritability estimates of CI2, therefore making Tex6 a more favorable source of resistance for breeding programs. A second identified source of resistance, inbred LB31, also was resistant to aflatoxin production in segregating generations over multiple environments (3). Resistance to aflatoxin production in LB31 is primarily controlled by additive gene effects, and the broad-sense heritability of the F₃ generation was estimated at 66%, also making LB31 a more favorable source of resistance for breeding programs. An additional inbred, Mp313E, had a consistently low percentage of corn kernels infected by *A. flavus* in multiple environments (26). Quantitative trait loci for resistance have been identified in this inbred, including one locus that accounts for 36.4% of the variation between the resistant and susceptible parent, thus making Mp313E potentially a very useful source of resistance (8).

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