Evaluation of the MI82 Corn Line as a Source of Resistance to Aflatoxin in Grain and Use of BGYP as a Selection Tool

L. M. Maupin, M. J. Clements, and D. G. White, Department of Crop Sciences, University of Illinois, Urbana 61801

ABSTRACT

Maupin, L. M., Clements, M. J., and White, D. G. 2003. Evaluation of the MI82 corn line as a source of resistance to aflatoxin in grain and use of BGYP as a selection tool. *Plant Dis.* 87:1059-1066.

Our objectives were to determine if the corn (*Zea mays*) inbred MI82 has alleles for resistance to *Aspergillus* ear rot (caused by *Aspergillus flavus*) and aflatoxin accumulation in grain that can be transferred to commercially used inbreds, and to determine the types and magnitudes of gene action, heritabilities, and gain from selection for low levels of bright greenish-yellow fluorescence (BGYP), aflatoxin, and ear rot with MI82. Also, we hoped to determine if selection against BGYP would substantially reduce the concentration of aflatoxin in grain. Primary ears and ground grain from inbred MI82 (P₁), the susceptible inbred B73 (P₂), and the F₁, F₂, F₃, BCP₁S₁, and BCP₂S₁ generations developed from these inbreds were evaluated for BGYP, concentration of aflatoxin in grain, and severity of *Aspergillus* ear rot in 2000 and 2001. Dominance was the most important gene action associated with low levels of BGYP and a low concentration of aflatoxin in grain. Heritabilities for low levels of BGYP (83.5%), aflatoxin (74.1%), and ear rot (62.8%) were high. Correlation coefficients between aflatoxin and BGYP were high in both years ($r = 0.75$ and 0.79 for 2000 and 2001, respectively). Unlike aflatoxin, BGYP was not affected by the year in which plants were grown. Selection for low levels of BGYP prior to selection based on aflatoxin concentration is as effective as selection for either factor alone. MI82 has value in programs designed to improve the resistance of commercially used corn inbreds.

Additional keywords: maize, mycotoxin

Aspergillus ear rot is an economically important disease of corn (*Zea mays* L.) worldwide. The fungus that causes *Aspergillus* ear rot, *Aspergillus flavus* Link:Fr., produces aflatoxins—a group of mycotoxins that are carcinogenic to humans and many animal species. Severity of *Aspergillus* ear rot and aflatoxin accumulation in grain are affected greatly by environmental conditions (1,13,23,24,36,41,45,46). High temperatures (36 to 38°C), drought, and other conditions that increase plant stress predispose plants to infection and favor fungal growth and aflatoxin accumulation in grain. Interstate commerce of aflatoxin-contaminated grain is restricted by the U.S. Food and Drug Administration at an “Action” level of 20 ng/g (ppb) (34). Disrup-

tions in grain markets brought about by aflatoxin occur infrequently in the central United States; however, disruptions are common in the southern United States, where the environment favors disease development in most years.

Bright greenish-yellow fluorescence (BGYP) has been used as a visual marker to identify corn and other commodities that may be contaminated with *A. flavus* and aflatoxin. *A. flavus* typically produces kojic acid, a secondary metabolite that is not directly related to aflatoxin (32). Heat labile peroxidases associated with kojic acid undergo oxidation and fluoresce under long wave (365 nm) ultraviolet light. The presence of BGYP is used as a presumptive test in grain elevators to identify corn samples that should be examined with a determinative test for aflatoxin. Positive correlations ($r = 0.6$ and 0.85) between BGYP and aflatoxin in corn grain (27,28) have been reported. However, grain samples exhibiting fluorescence with no detectable aflatoxin also have been reported (26,29,40,43).

Researchers evaluating corn samples from a grain elevator determined that aflatoxin levels could not be predicted from the weight of whole and cracked kernels that fluoresced under ultraviolet light (25). In another experiment, researchers determined that BGYP was not a precise quanti-

tative estimator of the concentration of aflatoxin in grain when whole or partial kernels were evaluated (40). Lillehoj et al. (31) determined that the relative concentration of aflatoxin in grain could be evaluated more effectively by rating finely ground samples for fluorescence rather than by counting individual kernels and particles that fluoresce. The positive relationship between incidence of fluorescing kernels and aflatoxin has been used by several researchers to screen for aflatoxin-contaminated samples (2,15,30,39).

Scott (38) evaluated the feasibility of using BGYP to select genotypes with low levels of aflatoxin. Grain samples were selected based on the number of fluorescing particles, and samples with the lowest levels of fluorescence were analyzed for the concentration of aflatoxin. Scott concluded that the number of fluorescing particles could be used to select genotypes with resistance to aflatoxin accumulation in grain, and that a large number of susceptible genotypes could be discarded prior to determinative tests for aflatoxin. This would reduce the cost and time spent evaluating genotypes for resistance to aflatoxin accumulation in grain.

Although irrigation and insect management may help to reduce the concentration of aflatoxin in grain and severity of *Aspergillus* ear rot, planting resistant hybrids is the most effective strategy for controlling disease development. Inheritance of resistance to *Aspergillus* ear rot and aflatoxin accumulation in grain has been studied with diallel mating designs (12,17,19,47,48) and generation means analyses (6,8,21,43). Diallel analyses indicate that general combining ability (indicating additive genetic effects) (12,47,48) and specific combining ability (indicating dominance genetic effects) (17,19) can be important in resistance to aflatoxin accumulation in grain. Generation means analyses of specific inbreds support the importance of additive gene effects (6,21,43) and the possibility for dominant gene effects (6) for resistance to aflatoxin accumulation in grain.

F₁ crosses of the inbreds MI82 and B73 are highly resistant to aflatoxin accumulation in grain and *Aspergillus* ear rot following inoculation (9). MI82 is an unreleased inbred developed by D. G. White from a hybrid commercially used in India. In one laboratory study, kernels from MI82 supported minimal fungal growth and a low concentration of aflatoxin when endo-

Corresponding author: D. G. White
E-mail: d-white@uiuc.edu

Current address for L. M. Maupin: Pioneer Hybrid International, Inc., 18285 County Rd. 96, Woodland, CA 95695.

Current address for M. J. Clements: USDA-ARS Corn Host Plant Resistance Research Unit, Mississippi State, MS 39762.

Accepted for publication 5 April 2003.

Publication no. D-2003-0623-03R
© 2003 The American Phytopathological Society

sperms were not wounded by inoculation (4). In further laboratory studies, kernels from MI82 supported minimal fungal growth and a low (4) to moderate (5) concentration of aflatoxin when endosperms were wounded by inoculation. Kernels from MI82 supported a high rate of fungal growth and a high concentration of aflatoxin when embryos were wounded by inoculation (5). A 14-kDa protein has been quantified in kernels of MI82 at a concentration of 22%—the highest percent from 13 resistant and susceptible genotypes evaluated in a study in 1998 (10). The 14-kDa protein limits the growth of *A. flavus* by inhibiting α -amylase production in the kernel endosperm (11,14) and may aid in minimizing the concentration of aflatoxin in grain.

Although several sources of resistance to *Aspergillus* ear rot and aflatoxin accumulation in grain have been identified, and inheritance of resistance is known with some, no commercially used hybrids have a level of resistance that is high enough to acceptably minimize aflatoxin in all environments. Very high levels of resistance could be developed by incorporating various genes for resistance from diverse sources into commercially used inbreds. Numerous inbreds must be evaluated to identify novel genes that improve the resistance of commercial materials. The objectives of this study were to determine the value of MI82 as one source for improving the resistance of commercial corn hybrids; to determine the types and magnitudes of gene action, heritabilities, and gain from selection for BGYP, aflatoxin accumulation in grain, and ear rot with MI82; and to determine if selection against BGYP would substantially reduce the concentration of aflatoxin in grain. Also, we wanted to develop phenotypic data for a large number of backcross to the susceptible parent-selfed families that will be used in future studies to identify molecular markers associated with resistance.

MATERIALS AND METHODS

Seed of inbred MI82 (P_1), inbred B73 (P_2) and the F_1 , F_2 , F_3 , backcross to P_1 -selfed (BCP_1S_1), and backcross to P_2 -selfed (BCP_2S_1) generations produced from these inbreds were planted at the University of Illinois Crop Science Research and Education Center on 28 April 2000 and 27 April 2001. The F_1 , F_2 , F_3 , BCP_1S_1 , and BCP_2S_1 generations were produced in previous years by crossing the parental lines, self-pollinating the F_1 , self-pollinating the F_2 , crossing F_1 plants to the susceptible parent (B73) and self-pollinating, and crossing F_1 plants to the resistant parent (MI82) and self-pollinating, respectively.

The experiment was a randomized complete block design with two replicates per year. Replicates included 15 rows each of the P_1 (B73), P_2 (MI82), F_1 , F_2 , and

BCP_2S_1 generations, 30 rows of individual F_3 families, and 447 rows of individual BCP_1S_1 families. Experimental units were single rows that were 5.3 m long, spaced 0.8 m apart, and included approximately 18 plants. All rows or families were grouped together by generation to minimize plant competition. Generations were randomized within replicates.

Inoculum was prepared and ears were inoculated with the same isolates and technique that have been used successfully to differentiate resistant and susceptible germ plasm in studies at the University of Illinois since 1991 (6–9,21,43). Inoculum was produced from three isolates of *A. flavus* (6536, 6539, and 6540) that were obtained from the Northern Regional Research Laboratory (NRRL), Peoria, IL, and one isolate of *A. flavus* (Western IL-88) that was obtained from corn grain collected in 1988. Isolates from the NRRL were originally collected by Don Wicklow from corn grain harvested in North Carolina. The Western IL-88 isolate was collected by D. G. White in Illinois. Selection of *A. flavus* isolates was based on high levels of virulence and aflatoxin accumulation in grain during a 1990 isolate virulence study (D. G. White, unpublished).

Researchers have demonstrated that isolates of *A. flavus* differ in their capacity to infect corn kernels (50), fluoresce under ultraviolet light (16), induce BGYP in corn kernels (44), produce kojic acid (22), and produce aflatoxins (3,16,22,35,44); therefore, inoculum was prepared by blending an equal number of cultures of the four isolates with deionized water and adjusting the resulting suspension with deionized water to a concentration of 2×10^6 spores per ml. The inoculation device consisted of a 16×4.5 cm concave aluminum bar to which 161 No. 2 steel sewing pins and a hollow stainless steel needle were mounted. The pinboard assembly was mounted to a MeterJet spray gun (Spraying Systems Co., Wheaton, IL) and a backpack sprayer (Solo Co., Newport, VA). The device wounds the ear, and inoculum is delivered to the center of the wounded area. Ten milliliters of the spore suspension was injected through the hollow needle and beneath the husk leaves of the primary ear on all plants at approximately the R3 (milk) growth stage. Details of the inoculation device and the inoculation process are described by Campbell and White (7).

All primary ears from an experimental unit were harvested approximately 50 days postinoculation, dried with forced air at 32°C for 7 days, and visually rated for severity of *Aspergillus* ear rot as the percentage of the inoculated area with symptoms. Ratings were expressed on a 0 to 10 scale, with 0 being 0% to 10 being 100% of the inoculated area rotted. Similar rating systems have been used by researchers at the University of Illinois (7,8,9,21,43).

Grain was shelled from cobs, bulked by experimental unit, and ground with a Romer grinding/subsampling mill (model 2A, Romer Labs, Inc., Union, MO) to pass through a 1-mm mesh. A 2-g sample of ground grain from each experimental unit was assayed for concentration of aflatoxin B_1 with an indirect-competitive enzyme-linked immunosorbent assay (ELISA) developed by Campbell and White (9). Samples were analyzed in triplicate, and the data were averaged. A 25-g sample of ground grain from each experimental unit was distributed evenly into a 100×15 mm plastic petri plate. Samples were visually rated for percent surface area of ground grain that fluoresced under ultraviolet light (365 nm). Samples were thoroughly stirred and rated again. The two ratings were averaged, and the average rating was expressed as percent BGYP.

Generation means for severity of ear rot, concentration of aflatoxin, and percent BGYP were calculated by averaging data from all rows within a generation. Aflatoxin concentration in grain and percent BGYP were transformed with a $\log_e$ function and a square root function, respectively, to homogenize variances between generations. Effects of years, replicates (blocks), generations, and the interaction of these factors on ear rot, aflatoxin, and BGYP were analyzed with the general linear models procedure (37) of Statistical Analysis System software (SAS Institute, Cary, NC). Years and blocks nested within years were considered random effects, and generations were considered fixed.

Generation means analysis followed Mather and Jinks' notation and method (33). Parental lines were removed from the analyses due to poor pollination and poor grain set on ears or MI82 and/or possible susceptibility of MI82 with our inoculation technique. Means of the remaining generations were fit to the additive-dominance genetic model:

$$Y = m + cy + a_1x_{(2000)} + a_2x_{(2001)} + b_1d + b_2h$$

where Y = generation mean, m = intercept value, y = year effect, $x_{(2000)}$ = block effect in 2000, $x_{(2001)}$ = block effect in 2001, d = pooled additive gene effects, and h = pooled dominance effects. Data were fit to the model with the regression procedure (37) of SAS software. Deviations from the additive-dominance model were determined with an F test. If deviations were not significant, then the model was considered adequate. If deviations were significant, then a model that included digenic interactions (digenic model) was tested. The equation for the digenic model was

$$Y = m + cy + a_1x_{(2000)} + a_2x_{(2001)} + b_1d + b_2h + b_3i$$

where Y = generation mean, m = intercept value, y = year effect, $x_{(2000)}$ = block effect in 2000, $x_{(2001)}$ = block effect in 2001, d = pooled additive gene effects, h = pooled dominance effects, and i = additive-

additive interaction. The number of generations evaluated in this experiment precluded testing of more involved models. Potence ratios were calculated by dividing the dominant parameter estimate by the additive parameter estimate for both square root-transformed fluorescence and log_e-transformed aflatoxin concentration in grain (20).

Heritabilities for resistance to ear rot, BGYF, and aflatoxin accumulation in grain were estimated with the equation

$$h^2 = \sigma_{\text{BCP}_1\text{S}_1}^2 / [(\sigma_e^2/r) + (\sigma_{ge}^2/y) + \sigma_{\text{BCP}_1\text{S}_1}^2]$$

where $\sigma_{\text{BCP}_1\text{S}_1}^2$ = variance among the BCP₁S₁ families, σ_e^2 = pooled error variance, r = number of replications, y = number of years, and σ_{ge}^2 = generation by year variance (20). Variance components for the effects of years, blocks within years, families, and families–years were estimated with the variance components procedure (37) of SAS software. All effects were considered random.

Predicted gain from selection was calculated with the formula: $\Delta_G = [k(1/2)\sigma_A^2]/\sigma_p$, derived from Hallauer and Miranda (20), where k is a constant based on selection intensity, σ_A^2 is the additive genetic variance, and σ_p is the square root of the phenotypic variance among BCP₁S₁ families. Genetic gain was calculated with a 20% selection intensity ($k = 1.3998$) (20). The small number of F₃ families evaluated in this experiment did not provide an adequate estimate of additive genetic variance for the calculation of genetic gain; therefore, genetic variance among BCP₁S₁ families was substituted for additive variance in the equation. Because of this substitution, our estimation of genetic gain may be slightly higher than an estimation using the additive variance component.

Predicted response on aflatoxin concentration in grain when selection is based on ear rot severity was calculated with the formula: $CR_y = k c h_x h_y r_{gxy} \sigma_{py}$, where k is the selection intensity, c is the recombination unit, h_x is the square root of heritability for resistance to ear rot, h_y is the square root of heritability for resistance to aflatoxin accumulation in grain, r_{gxy} is the genetic correlation between ear rot and aflatoxin concentration in grain, and σ_{py} is the phenotypic standard deviation for aflatoxin concentration in grain. Predicted response on aflatoxin concentration in grain when selection is based on percent BGYF was calculated by replacing ear rot values with BGYF values in the above equation. Predicted response for both equations was calculated with a 20% selection intensity ($k = 1.3998$) and a recombination unit of 1/2 (20). The genetic correlation between ear rot and aflatoxin concentration in grain ($r_{gxy} = 0.97$) was calculated with the formula: $r_{gxy} = \text{cov}_{gxy} / \sigma_{gx} \sigma_{gy}$, where cov_{gxy} is the genetic covariance between ear rot and aflatoxin concentration, σ_{gx} is the genetic standard deviation for ear rot,

and σ_{gy} is the genetic standard deviation for aflatoxin concentration. The genetic correlation between BGYF and aflatoxin concentration in grain ($r_{gxy} = 1.01$) was calculated by replacing ear rot values with BGYF values in the above equation.

Pearson's correlation coefficients were calculated between treatment means of ear rot severity, log_e-transformed aflatoxin concentration in grain, and square root-transformed percent BGYF for the BCP₁S₁ families evaluated in 2000 and 2001. Coefficients were calculated with the Pearson correlation procedure (37) of SAS software.

Frequency distributions of the BCP₁S₁ families were generated for percent BGYF and aflatoxin concentration in grain. Families were divided among 10 equal-increment classes for percent BGYF in 2000 and 2001. Families were divided among 20 equal-increment classes for aflatoxin concentration in grain in 2000 and 2001.

Climatological data for Urbana, IL, in 2000 and 2001 were obtained from the Illinois State Water Survey, Division of the Office of Scientific Research and Analysis, Illinois Department of Natural Resources, University of Illinois.

RESULTS

Temperature and precipitation were below average in the summer of 2000. Temperature did not reach 32°C until 9 August—the latest date since temperature records began in 1888. Temperature was above average, and precipitation was below average in 2001. July marked the fifth successive month with below average precipitation. Precipitation was 16.8 cm below average for the year by the end of September.

Aflatoxin concentration in grain was affected significantly by the environment in which plants were grown and the generations of MI82 and B73 (Table 1). Genera-

tion means for aflatoxin concentration in grain ranged from 11 to 59 ng/g in 2000 and 187 to 809 ng/g in 2001 (Table 2). In 2001, generation means for aflatoxin concentration in grain were the highest values recorded at the University of Illinois since research on aflatoxin resistance began in 1990. Grain samples from the F₁ generation had the lowest aflatoxin concentration in both years (Table 2). Grain samples from the BCP₁S₁ generation had the greatest aflatoxin concentration in both years (Table 2). Distributions of BCP₁S₁ families were similar for aflatoxin concentration in grain in 2000 and 2001 (Figs. 1 and 2); however, the range of aflatoxin values was 10-fold greater in 2001 than in 2000. Although aflatoxin concentration in grain from inbreds MI82 and B73 was not significantly different ($P < 0.05$), aflatoxin concentration in grain from generations produced with B73 and MI82 was significantly different (Table 2). Gene action associated with resistance to aflatoxin accumulation in grain was determined with generation means analysis. Inbred parents were not included in generation means analyses of aflatoxin accumulation in grain.

Deviations from the additive–dominance model were not significant for aflatoxin concentration in grain; therefore, epistasis was not an important factor in resistance to aflatoxin accumulation. Pooled additive and dominant gene effects were highly significant (Table 3). The parameter for the dominant genetic effect was negative, indicating dominance for resistance. Additive and dominant genetic effects accounted for 16.9 and 77.1% of the variation among generations, respectively. Residual effects accounted for 6.0% of the variation among generations. The potence ratio for aflatoxin concentration in grain was -2.33 , suggesting that overdominance serves some function in resistance (Table 3).

Table 1. Analysis of variance for 2 years, two replicates (blocks) per year, and seven generations. B73, MI82, and five generations produced with B73 and MI82 were evaluated for aflatoxin concentration in grain, percent bright greenish-yellow fluorescence (BGYF), and severity of Aspergillus ear rot in 2000 and 2001^a

Source of variation	df	Aflatoxin ^b (mean square)	BGYF ^c (mean square)	Ear rot ^d (mean square)
Year	1	53.48***	1.61	4.17*
Block (year)	2	0.04	0.65*	0.03
Generation ^e	6	1.22***	1.98**	0.06
Generation × year	6	0.03	0.21	0.18
Pooled error	12	0.02	0.13	0.11
Coefficient of variation		2.99	8.49	5.87

^a *, **, *** indicate significance at $\alpha = 0.05$, 0.01, and 0.001, respectively.

^b Measured by indirect-competitive enzyme-linked immunosorbent assay. Samples were analyzed in triplicate, and values were averaged. Data were log_e-transformed prior to statistical analysis.

^c Ground samples were rated on a percent scale, with 100 being most susceptible. Samples were visually rated twice, and values were averaged. Samples were stirred between ratings. Data were square root-transformed prior to statistical analysis.

^d Primary ears from all plants were visually rated for percentage of inoculated area affected by Aspergillus ear rot. Ratings were expressed on a 0 to 10 scale (0 = 0%, 10 = 100% rotted).

^e P₁ (B73) and P₂ (MI82) were not evaluated due to difficulties with inbred MI82. The F₁, F₂, and F₃, backcrosses to P₁ selfed (BCP₁S₁), and backcrosses to P₂ selfed (BCP₂S₁) generations were evaluated.

Differences between years accounted for a greater proportion of the variation in aflatoxin concentration in grain than did differences between BCP₁S₁ families, blocks nested within years, and the family-year interaction (Table 4). The BCP₁S₁ family variance component was higher than the variance component for blocks nested within years and the family-year interaction. Heritability for resistance to aflatoxin accumulation in grain from inbred MI82 was 74.1%. Selection against aflatoxin accumulation in grain should decrease the log_e-transformed concentration of aflatoxin by 0.39 after one cycle of selection in the BCP₁S₁ generation.

Percent BGYP was not affected significantly by the year in which plants were grown, but was affected significantly by generations of MI82 and B73 (Table 1). Generation means for percent BGYP ranged from 11.3 to 28.1% when data were averaged across years (Table 2). Grain samples from the F₁ generation had the lowest percent BGYP across years (Table 2). Grain samples from the F₂ generation were not significantly different ($P > 0.05$) than grain samples from the F₁ generation for percent BGYP. Grain samples from the BCP₁S₁ generation had the greatest percent BGYP across years (Table 2). Grain samples from inbreds B73 and MI82 were not significantly different ($P > 0.05$) from grain samples of the BCP₁S₁ generation for percent BGYP. Distributions of BCP₁S₁ families were similar for percent BGYP in 2000 and 2001 (Fig. 3). Gene action associated with resistance to percent BGYP was determined with generation means analysis. Inbred parents were not included in generation means analyses of percent BGYP.

Fig. 1. Frequency distribution of 447 backcrosses to the susceptible parent (B73) selfed families developed from inbreds B73 and MI82 and evaluated for aflatoxin concentration in grain in 2000.

Fig. 2. Frequency distribution of 447 backcrosses to the susceptible parent (B73) selfed families developed from inbreds B73 and MI82 and evaluated for aflatoxin concentration in grain in 2001.

Table 2. Aflatoxin concentration in grain, percent bright greenish-yellow fluorescence (BGYP), and severity of *Aspergillus* ear rot for B73, MI82, and five generations produced with B73 and MI82 in 2000 and 2001

Generation ^a	Aflatoxin ^b			Antilogarithm ^f expressed in ng aflatoxin/g ground grain			BGYP ^c		Ear rot ^d	
	Natural log ^e of ng aflatoxin/g ground grain									
	2000	2001	Average ^g	2000	2001	Average	Average ($\sqrt{[\%]}$) ^h	Average (%) ⁱ	2000	2001
P ₁ (B73)	3.7	6.3	5.0	40	556	147	4.8	23.3	5.7	5.8
P ₂ (MI82)	3.9	6.5	5.1	52	634	179	5.0	25.2	5.9	5.2
F ₁	2.4	5.2	3.8	11	187	45	3.4	11.3	6.2	4.8
F ₂	2.7	5.7	4.2	15	312	68	3.8	14.2	5.9	5.0
F ₃	3.3	6.1	4.7	27	464	113	4.2	17.6	6.0	5.3
BCP ₁ S ₁	4.1	6.7	5.4	59	809	217	5.3	28.1	6.0	5.1
BCP ₂ S ₁	3.3	6.1	4.7	28	444	109	4.1	16.8	5.8	5.0
LSD _{$\alpha=0.05$} ^j	... ^k	...	0.2	...	...	...	0.6	...	...	...

^a The F₁ is the cross B73 × MI82. F₂ and F₃ generations were produced by self pollinating F₁ and F₂ plants, respectively. The BCP₁S₁ and BCP₂S₁ generations were produced by crossing F₁ plants with B73 and MI82, respectively.

^b Measured by indirect-competitive enzyme-linked immunosorbent assay. Samples were analyzed in triplicate, and values were averaged.

^c Ground samples were rated on a percent scale, with 100 being most susceptible. Samples were visually rated twice, and values were averaged. Samples were stirred between ratings.

^d Primary ears from all plants were visually rated for the percentage of inoculated area affected by *Aspergillus* ear rot. Ratings were expressed on a 0 to 10 scale (0 = 0%, 10 = 100% rotted).

^e Data were transformed to the natural log for analyses.

^f Geometric means (antilogarithm of logarithmic means) were rounded to the nearest whole number.

^g Average of data from 2000 and 2001.

^h Data were transformed to the square root for analyses.

ⁱ Means were transformed back to original percent scale.

^j Fisher's protected least significant difference test.

^k Not an appropriate test, as determined by ANOVA, or not evaluated on untransformed data.

Deviations from the additive–dominance model were significant for percent BGYP; therefore, data were fit to the digenic model. Pooled additive and dominant gene effects were highly significant ($P < 0.01$), and the additive–additive interaction was significant ($P < 0.05$) (Table 3). The parameter for dominant genetic effects was negative, indicating dominance for resistance. Additive and dominant genetic effects accounted for 31.5 and 55.8% of the variation among generations, respectively. Residual effects accounted for 12.7% of the variation among generations. The potence ratio for BGYP was -0.98 , suggesting partial dominance for resistance (Table 3).

Differences between BCP₁S₁ families accounted for a greater proportion of the variation in percent BGYP than did differences between years, blocks nested within years, and the family–year interaction (Table 4). Heritability for resistance to BGYP from inbred MI82 was 83.5%. Selection against BGYP should decrease the square root-transformed percent BGYP and the log_e-transformed aflatoxin concentration in grain by 0.83 and 0.42, respectively, after one cycle of selection in the BCP₁S₁ generation.

Severity of *Aspergillus* ear rot was affected significantly by the year in which plants were grown, but not by the generations of MI82 and B73 (Table 1). Generation means for ear rot severity ranged from 5.7 to 6.2 in 2000, and from 4.8 to 5.7 in 2001 (Table 2). Since differences between generation means were not significant, gene action associated with resistance to ear rot was not determined in this study.

Differences between BCP₁S₁ families accounted for a greater proportion of variation in ear rot than did differences between years, blocks nested within years, and the family–year interaction (Table 4). Heritability for resistance to ear rot from the inbred MI82 was 62.8%. Selection against ear rot severity should decrease the severity of *Aspergillus* ear rot and the log_e-transformed aflatoxin concentration in grain by 0.39 and 0.35, respectively, after one cycle of selection in the BCP₁S₁ generation (Table 4).

Correlation coefficients between severity of *Aspergillus* ear rot, aflatoxin concentration in grain, and percent BGYP were lower in 2000 than in 2001 (Table 5). All relationships were highly significant ($P < 0.001$) and positive in both years. Correlation coefficients for percent BGYP and aflatoxin concentration in grain were high in both years of this study (Table 5). The correlation coefficient for percent BGYP in 2000 (a low aflatoxin year) and aflatoxin concentration in grain in 2001 (a high aflatoxin year) was $r = 0.63$ ($P < 0.001$) (Table 5).

DISCUSSION

Hamblin and White (21) reported that additive gene action was the most impor-

tant component of resistance to aflatoxin accumulation in grain of the cross of B73 × Tex6. Walker and White (43) reported that epistasis was associated with resistance to aflatoxin accumulation in grain of the cross of B73 × CI2. Campbell et al. (6) determined that additive and dominant gene actions were important in the inheritance of resistance to aflatoxin accumulation in grain of the cross of B73 × LB31. Additive

and dominant genetic effects were important in inheritance of resistance to aflatoxin accumulation in grain from crosses developed with the inbreds MI82 and B73. Overdominance also served some function in resistance to aflatoxin accumulation in grain. Epistatic effects were not associated with resistance to aflatoxin accumulation in grain from generations produced with MI82 and B73.

Table 3. Least squares estimates for the parameters of the model^a used in generation means analyses of aflatoxin concentration in grain and percent bright greenish-yellow fluorescence (BGYP) in 2000 and 2001^b

Parameter	Aflatoxin ^c			BGYP ^d		
	Parameter estimate	Standard error	T for H0: Parameter = 0	Parameter estimate	Standard error	T for H0: Parameter = 0
<i>m</i>	4.85	0.08	60.87***	4.27	0.21	20.43***
<i>y</i>	-1.49	0.04	-34.46***	0.30	0.08	3.96**
<i>x</i> ₍₂₀₀₀₎	-0.10	0.06	-1.60	0.16	0.11	1.48
<i>x</i> ₍₂₀₀₁₎	0.02	0.06	0.35	-0.03	0.11	-0.31
<i>d</i>	0.54	0.14	3.96**	1.01	0.24	4.19**
<i>h</i>	-1.26	0.15	-8.46***	-0.99	0.32	-3.15**
<i>i</i>	...			1.97	0.75	2.62*
Potence ratio ^e	-2.33			-0.98		

^a The model fit to aflatoxin data was $Y = m + cy + a_1x_{(2000)} + a_2x_{(2001)} + b_1d + b_2h$. The model fit to BGYP data was: $Y = m + cy + a_1x_{(2000)} + a_2x_{(2001)} + b_1d + b_2h + b_3i$. The parameters of the equations are Y = generation mean, m = intercept value, x = block effect, d = pooled additive effects, h = pooled dominance effects, and i = additive–additive interaction. Parental lines were not included in the analyses.

^b *, **, *** indicate significance at $\alpha = 0.05$, 0.01, and 0.001, respectively.

^c Values based on log_e-transformed data.

^d Values based on square root-transformed data.

^e The potence ratio was calculated by dividing the dominance parameter estimate by the additive parameter estimate.

Table 4. Variance component estimates, heritability estimates, expected gain from selection, and correlated response for aflatoxin concentration in grain, percent bright greenish-yellow fluorescence (BGYP), and severity of *Aspergillus* ear rot from 447 BCP₁S₁ families evaluated in 2000 and 2001

Variance component	Aflatoxin ^b	BGYP ^c	Ear rot ^d
Year	4.44	0	0.44
Block (year)	0	0.02	0.01
Family	0.42	1.69	0.49
Family × year	0.04	0.09	0.17
Pooled error	0.51	1.16	0.83
Heritability (h^2) ^e	74.08	83.52	62.75
Expected gain from selection (Δ_G) ^f	0.39	0.83	0.39
Correlated response on aflatoxin ^g		0.42	0.35

^a Backcross to the susceptible parent (B73) selfed once.

^b Measured by indirect-competitive enzyme-linked immunosorbent assay. Samples were analyzed in triplicate, and values were averaged. Values are based on log_e-transformed data.

^c Ground samples were rated on a percent scale, with 100 being most susceptible. Ground grain samples were visually rated twice, and values were averaged. Samples were stirred between ratings. Values are based on square root-transformed data.

^d Primary ears from all plants were visually rated as the percentage of inoculated area affected by *Aspergillus* ear rot. Ratings were expressed on a 0 to 10 scale (0 = 0%, 10 = 100% rotted).

^e Heritabilities were calculated with the equation: $h^2 = \sigma^2_{BCP1S1} / ((\sigma^2_d / ry) + (\sigma^2_e / y) + \sigma^2_{BCP1S1})$, where σ^2_{BCP1S1} is the variance among BCP₁S₁ families, σ^2_e is the pooled error variance, r is the number of replications, y is the number of years, and σ^2_{ge} is the generation by year variance. Heritabilities were expressed as a percent.

^f Gain from selection was calculated with the equation $\Delta_G = [k(1/2) \sigma^2_A] / \sigma_p$, where k is the constant 1.3998 (20% selection intensity), σ^2_A is genetic variance among BCP₁S₁ families, and σ_p is the square root of the phenotypic variance among BCP₁S₁ families.

^g Correlated response on aflatoxin concentration is based on selection for ear rot or BGYP. The response was calculated with the equation $CR_y = k c h_x h_y r_{gyx} \sigma_{py}$, where k is the constant 1.3998 (20% selection intensity), c is the recombination unit (1/2), h_x is the square root of heritability for resistance to ear rot, h_y is the square root of heritability for resistance to aflatoxin accumulation, r_{gyx} is the genetic correlation between ear rot and aflatoxin, and σ_{py} is the phenotypic standard deviation for aflatoxin concentration in grain. Predicted response on aflatoxin concentration in grain when selection is based on percent BGYP was calculated by replacing ear rot values with BGYP values in the above equation.

Researchers at the University of Illinois have evaluated F_1 crosses of numerous inbreds and the inbred B73 (6,9,21,43). F_1 crosses were evaluated in these studies because inbred plants often have agronomic qualities that make inoculation and evaluation difficult. B73 was chosen as a parental line because it was developed through improvement of the Stiff Stalk Synthetic population that has produced many of the commercial inbreds used for hybrid development in the United States (42). The purpose of this and other studies has been to identify inbreds that contribute novel genes for resistance to B73 and possibly to other Stiff Stalk Synthetic-related lines. Since dominant genetic effects from inbred MI82 were a major component of resistance to aflatoxin accumulation in grain, and grain from several BCP_1S_1 families produced with MI82 and B73 was highly resistant to aflatoxin accumulation, alleles from MI82 should improve the resistance of an inbred derived from Stiff Stalk Synthetic. MI82 should be used in

pragmatic breeding programs directed toward improving commercially used inbreds in the United States.

This study is the first to evaluate the relationship between BGYP and aflatoxin concentration in grain from a large group of BCP_1S_1 families over 2 years. Previous experiments have characterized the positive relationship between BGYP and aflatoxin concentration in grain, and have predicted aflatoxin concentration based on the weight of fluorescing kernels and kernel fragments (25,28,31,40). Many of these experiments evaluated corn from grain elevators, so samples were from different hybrids and different locations. All of these experiments evaluated naturally infected grain; therefore, results were dependent on levels of infection that may or may not have been great enough to adequately determine relationships between dependent variables.

Only one study has reported that BGYP may be used as a marker for selecting germ plasm that is resistant to aflatoxin accumu-

lation in grain (38). In that experiment, Scott concluded that genotypes with a high level of resistance to aflatoxin accumulation in grain could be identified as samples with a low number of fluorescing particles. Grain samples with a large number of fluorescing particles could be discarded to reduce cost associated with determinative aflatoxin analyses.

Significant variation due to the environment has complicated studies of aflatoxin accumulation in grain (12,17,18,36,49). Variation in percent BGYP was not associated with environmental differences in this study; however, variation in aflatoxin accumulation in grain was affected greatly by environmental differences. Unlike aflatoxin accumulation in grain, percent BGYP does not appear to be dependent upon environmental conditions. A moderate to high correlation ($r = 0.63$) between percent BGYP in 2000 (a low aflatoxin year) and aflatoxin concentration in grain in 2001 (a high aflatoxin year) suggests that samples can be evaluated for BGYP in years when aflatoxin accumulation is not favored.

Quantifying aflatoxin concentration in corn grain is expensive and time consuming; thus, the number of samples that can be screened each season is limited. By comparison, evaluation of percent BGYP is rapid and inexpensive. In this experiment, samples were finely ground for aflatoxin analyses; therefore, the relationship between BGYP and aflatoxin could be evaluated without a significant increase in time or labor. Selection of genotypes with resistance to aflatoxin accumulation in grain may be expedited in breeding programs by discarding genotypes with a high percentage of fluorescing grain particles, and quantifying aflatoxin concentration in grain only from those genotypes with a low percentage of fluorescing particles. Although some genotypes that are susceptible to kojic acid accumulation in grain and resistant to aflatoxin accumulation in grain may be missed with this system of selection, this system would significantly reduce the time and cost associated with determinative tests for aflatoxin.

Heritability of resistance to percent BGYP was higher than heritability of resistance to aflatoxin accumulation in grain from the BCP_1S_1 generation. Aflatoxin concentration in grain was affected at a slightly greater rate by direct selection for resistance to aflatoxin accumulation than by selection for resistance to percent BGYP. Since the difference in progress between the two selection methods was very small, progress from selection against BGYP should be nearly as effective as selection against aflatoxin concentration in grain.

An example of progress from selection can be drawn from the results of this experiment.

Suppose that BCP_1S_1 families were selected when aflatoxin accumulation in

Fig. 3. Frequency distributions of 447 backcrosses to the susceptible parent (B73) selfed families developed from inbreds B73 and MI82 and evaluated for percent bright greenish-yellow fluorescence (BGYP) in 2000 and 2001.

Table 5. Relationships between aflatoxin concentration in grain, percent bright greenish-yellow fluorescence (BGYP), and severity of *Aspergillus* ear rot for 447 BCP_1S_1 families developed from the cross of inbreds B73 and MI82 and evaluated in 2000 and 2001^b

	Pearson's correlation coefficient			
	2000		2001	
	BGYF ^c (%)	Aflatoxin ^d (ng/g)	BGYF (%)	Aflatoxin (ng/g)
2000				
Ear rot (%) ^e	0.57	0.48	0.49	0.41
BGYF (%)		0.75		0.63
2001				
Ear rot (%)	0.61	0.59	0.74	0.64
BGYF (%)		0.67		0.79

^a Backcross to the susceptible parent (B73) selfed once.

^b All values were significant at $\alpha = 0.001$.

^c Ground samples were visually rated on a percent scale, with 100 being most susceptible. Ground grain samples were visually rated twice, and values were averaged. Samples were stirred between ratings. Data were square root-transformed prior to statistical analysis.

^d Aflatoxin concentration in grain was measured by indirect-competitive enzyme-linked immunosorbent assay. Samples were analyzed in triplicate, and values were averaged. Data were log_e-transformed prior to statistical analysis.

^e Severity of *Aspergillus* ear rot was rated as the percentage of inoculated area affected and expressed on a 0 to 10 scale (0 = 0%, 10 = 100% rotted).

grain was not favored (e.g., in 2000), and that selected materials were re-evaluated when aflatoxin accumulation in grain was favored (e.g., in 2001). If 30% of the 447 BCP₁S₁ families had been selected for low percentage of BGYP and 50% of the resulting families had been selected for low aflatoxin concentration in grain, then 66 families would have been identified with resistance to aflatoxin accumulation in grain. The average aflatoxin concentration in grain of the 66 selected families would have been 380 ng/g in 2001.

In contrast, if 15% of the BCP₁S₁ families had been selected for low percent BGYP only, then the average aflatoxin concentration in grain of the 66 selected families would have been 435 ng/g in 2001. Moreover, if 15% of the BCP₁S₁ families had been selected for low aflatoxin concentration in grain only, then the average aflatoxin concentration in grain of the 66 selected families would have been 405 ng/g in 2001. A similar selection intensity against *Aspergillus* ear rot alone would have resulted in 66 selected families with an average aflatoxin concentration in grain of 625 ng/g.

If selection in the above example had been based on aflatoxin concentration in grain alone, then 447 samples would have been analyzed for aflatoxin concentration in grain. If selection had been based on BGYP prior to aflatoxin analyses, then only 131 samples would have been analyzed for aflatoxin concentration in grain. Aflatoxin concentration in grain was reduced significantly from the original population mean of 809 ng/g by all four systems of selection. Selection against both percent BGYP and aflatoxin concentration, however, yielded a result that was comparable to selection against aflatoxin concentration alone. Selection against BGYP and aflatoxin concentration in grain minimized the number of determinative tests needed for aflatoxin and validated identification of the most resistant materials through determinative testing. This example suggests that genotypes with resistance to aflatoxin accumulation in grain can be identified by selection against BGYP prior to selection against aflatoxin concentration. Selection against both factors should be less expensive than selection against aflatoxin alone.

Severity of our inoculation technique combined with the poor agronomic qualities of inbred MI82 demonstrate two of the difficulties associated with evaluating inbred lines for resistance to ear rot diseases. Generation means analysis is typically based upon differences between two parental lines; however, this assumes that the susceptible parent is very susceptible, i.e., lacking the genes necessary for resistance, and that the resistant parent has all of the major genes necessary for resistance. Since resistance to aflatoxin accumulation in grain is often confounded by heat, drought, and other forms of abiotic stress

(1,13,23,24,36,41,45,46), "resistant" inbred lines may perform poorly when grown in environments in which they are not adequately adapted. The quantity and quality of grain samples obtained from inbred MI82 were substantially impaired by severe lodging and poor pollinations brought about by drought conditions during flowering in 2000 and 2001. Inbred B73, a line developed from the Stiff Stalk Synthetic population in Iowa, was affected less by drought conditions than MI82. Relatively large, high-quality grain samples were obtained from B73. When evaluated as an inbred, MI82 was not noticeably resistant to disease development. As a result, aflatoxin concentration in grain and percent BGYP were not significantly different between the inbred parents MI82 and B73. Disparities in kernel size and grain sample size between B73 and MI82 may have affected severity of wounding produced by our inoculation technique and may explain why aflatoxin concentration was not different between grain of B73 and grain of MI82 in 2000 and 2001.

Since differences between parental lines were not apparent in this study, we chose to determine the genetic effects associated with resistance to aflatoxin accumulation and BGYP using the F₁, F₂, F₃, BCP₁S₁, and BCP₂S₁ generations. This reduced the number of generations in the analysis and decreased the number of parameters that could be estimated. Hence, the most complex model included only pooled additive gene effects, pooled dominance effects, and the additive-additive interaction.

Disease development that results from natural infection of corn ears by *A. flavus* is rarely consistent between years and locations in the midwestern United States. Typically, researchers evaluating genotypes for resistance rely on inoculation to offset variability associated with the environment. The inoculation technique used by researchers at the University of Illinois since 1991 (7) damages endosperm and often embryo tissue of developing corn kernels. Researchers have demonstrated that resistance to aflatoxin accumulation is apparent from kernels of MI82 when the endosperm is and is not wounded (4,5), but that resistance is not apparent when the embryo is wounded (5). Kernels of inbred MI82 may have a mechanism of resistance that is independent of the pericarp (4,5,10,14), or that limits fungal access to the embryo when the embryo is not wounded (5,14); however, wounding of the embryo may diminish resistance attributed to MI82 when MI82 is evaluated as an inbred (5).

ACKNOWLEDGMENTS

This material is based on work supported by USDA-CSRS grants AG-58-3620-0-125 and AG-58-3620-0-003. Any opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect the views of USDA-CSRS. We

thank J. Dudley for assistance with genetic equations and G. Bollero for assistance with statistical analyses. We also thank C. Kleinschmidt, S. Phillips, S. Sipp, and R. Walker for assistance with laboratory and field work.

LITERATURE CITED

1. Aldrich, S. R., Scott, W. O., and Hoeft, R. G. 1986. Modern Corn Production. 3rd ed. A&L Publications, Champaign, IL.
2. Barabolak, R., Colburn, C. R., Just, D. E., Kurtz, F. A., and Schleicher, E. A. 1978. Apparatus for rapid inspection of corn for aflatoxin contamination. Cereal Chem. 55:1065-1067.
3. Bayman, P., and Cotty, P. J. 1993. Genetic diversity in *Aspergillus flavus*: Association with aflatoxin production and morphology. Can. J. Bot. 71(1):23-31.
4. Brown, R. L., Cleveland, T. E., Payne, G. A., Woloshuk, C. P., Campbell, K. W., and White, D. G. 1995. Determination of resistance to aflatoxin production in maize kernels and detection of fungal colonization using an *Aspergillus flavus* transformant expressing *Escherichia coli* β -glucuronidase. Phytopathology 85:983-989.
5. Brown, R. L., Cleveland, T. E., Payne, G. A., Woloshuk, C. P., and White, D. G. 1997. Growth of an *Aspergillus flavus* transformant expressing *Escherichia coli* beta-glucuronidase in maize kernels resistant to aflatoxin production. J. Food Prot. 60(1):84-87.
6. Campbell, K. W., Hamblin, A. M., and White, D. G. 1997. Inheritance of resistance to aflatoxin production in the cross between corn inbreds B73 and LB31. Phytopathology 87:1144-1147.
7. Campbell, K. W., and White, D. G. 1994. An inoculation device to evaluate maize for resistance to ear rot and aflatoxin production by *Aspergillus flavus*. Plant Dis. 78:778-781.
8. Campbell, K. W., and White, D. G. 1995. Inheritance of resistance to *Aspergillus* ear rot and aflatoxin in corn genotypes. Phytopathology 85:886-896.
9. Campbell, K. W., and White, D. G. 1995. Evaluation of corn genotypes for resistance to *Aspergillus* ear rot, kernel infection, and aflatoxin production. Plant Dis. 79:1039-1045.
10. Chen, Z. Y., Brown, R. L., Lax, A. R., Guo, B. Z., Cleveland, T. E., and Russin, J. S. 1998. Resistance to *Aspergillus flavus* in corn kernels is associated with a 14-kDa protein. Phytopathology 88:276-281.
11. Chen, Z. Y., Brown, R. L., Russin, J. S., Lax, A. R., and Cleveland, T. E. 1999. A corn trypsin inhibitor with antifungal activity inhibits *Aspergillus flavus* α -amylase. Phytopathology 89:902-907.
12. Darrah, L. L., Lillehoj, E. B., Zuber, M. S., Scott, G. E., Thompson, D., West, D. R., Widstrom, N. W., and Fortnum, B. A. 1987. Inheritance of aflatoxin B₁ levels in maize kernels under modified natural inoculation with *Aspergillus flavus*. Crop Sci. 27:869-872.
13. Davis, N. D., and Diener, U. L. 1983. Some characteristics of toxigenic and nontoxigenic isolates of *Aspergillus flavus* and *Aspergillus parasiticus*. In: Aflatoxin and *Aspergillus flavus* in Corn. U. L. Diener, R. L. Asquith, and J. W. Dickens, eds. Auburn Univ. So. Coop. Series Bull. 279.
14. Fakhoury, A. M., and Woloshuk, C. P. 1999. Amy1, the α -amylase gene of *Aspergillus flavus*: Involvement in aflatoxin biosynthesis in maize kernels. Phytopathology 89:908-914.
15. Fennell, D. I., Bothast, R. J., Lillehoj, E. B., and Peterson, R. E. 1973. Bright greenish-yellow fluorescence and associated fungi in white corn naturally contaminated with aflatoxin. Cereal Chem. 50:404-414.

16. Fente, C. A., Jaimez Ordaz, J., Vazquez, B. I., Franco, C. M., and Cepeda, A. 2001. New additive for culture media for rapid identification of aflatoxin-producing *Aspergillus* strains. *Appl. Environ. Microbiol.* 67:4858-4862.
17. Gardner, C. A. C., Darrah, L. L., Zuber, M. S., and Wallin, J. R. 1987. Genetic control of aflatoxin production in maize. *Plant Dis.* 71:426-429.
18. Gorman, D. P., and Kang, M. S. 1991. Preharvest aflatoxin contamination in maize: Resistance and genetics. *Plant Breed. (Z. Pflanzenzucht.)* 107(1):1-10.
19. Gorman, D. P., Kang, M. S., Cleveland, T., and Hutchinson, R. L. 1992. Combining ability for resistance to field aflatoxin accumulation in maize grain. *Plant Breed.* 109:296-303.
20. Hallauer, A. R., and Miranda, J. B. 1988. *Quantitative Genetics in Maize Breeding*. Iowa State University Press, Ames.
21. Hamblin, A. M., and White, D. G. 2000. Inheritance of resistance to *Aspergillus* ear rot and aflatoxin production of corn from Tex6. *Phytopathology* 90:292-296.
22. Horn, B. W., Greene, R. L., Sobolev, V. S., Dorner, J. W., Powell, J. H., and Layton, R. C. 1996. Association of morphology and mycotoxin production with vegetative compatibility groups in *Aspergillus flavus*, *A. parasiticus*, and *A. tamarii*. *Mycologia* 88:574-587.
23. Jones, R. K., Duncan, H. E., and Hamilton, P. B. 1981. Planting date, harvest date and irrigation effects on infection and aflatoxin production by *Aspergillus flavus* in field corn. *Phytopathology* 71:810-816.
24. Jones, R. K., Duncan, H. E., Payne, G. A., and Leonard, K. J. 1980. Factors influencing infection by *Aspergillus flavus* in silk-inoculated corn. *Plant Dis.* 64:859-863.
25. Kwolek, W. F., and Shotwell, O. L. 1979. Aflatoxin in white corn under loan. V. Aflatoxin prediction from weight percent of bright greenish-yellow fluorescent particles. *Cereal Chem.* 56:342-345.
26. Lillehoj, E. B., Kwolek, W. F., Horner, E. S., Widstrom, N. W., Josephson, L. M., Franz, A. O., and Catalano, E. A. 1980. Aflatoxin contamination of preharvest corn: Role of *Aspergillus flavus* inoculum and insect damage. *Cereal Chem.* 57:255-257.
27. Lillehoj, E. B., Kwolek, W. F., Manwiller, A., DuRant, J. A., LaPrade, J. C., Horner, E. S., Reid, J., and Zuber, M. S. 1976. Aflatoxin production in several corn hybrids grown in South Carolina and Florida. *Crop Sci.* 16:483-485.
28. Lillehoj, E. B., Kwolek, W. F., Peterson, R. E., Shotwell, O. L., and Hesselstine, C. W. 1976. Aflatoxin contamination, fluorescence, and insect damage in corn infected with *Aspergillus flavus* before harvest. *Cereal Chem.* 53:505-512.
29. Lillehoj, E. B., Kwolek, W. F., Zuber, M. S., Bockholt, A. J., Calvert, O. H., Findley, W. R., Guthrie, W. D., Horner, E. S., Josephson, L. M., King, S., Manwiller, A., Sauer, D. B., Thompson, D., Turner, M., and Widstrom, N. W. 1980. Aflatoxin in corn before harvest: Interaction of hybrids and locations. *Crop Sci.* 20:731-734.
30. Lillehoj, E. B., Manwiller, A., Whitaker, T. B., and Zuber, M. S. 1982. South Carolina corn yield trial samples as probes for the natural occurrence of aflatoxin in preharvest kernels. *Cereal Chem.* 59:136-138.
31. Lillehoj, E. B., Manwiller, A., Whitaker, T. B., and Zuber, M. S. 1983. Hybrid differences in estimation of preharvest occurrence of bright greenish-yellow fluorescence and aflatoxin in corn. *J. Environ. Qual.* 12:216-219.
32. Marsh, P. B., Simpson, M. E., Ferretti, R. J., Merola, G. V., Donoso, J., Craig, G. O., Trucksess, M. W., and Work, P. S. 1969. Mechanism of formation of a fluorescence in cotton fiber associated with aflatoxin in the seeds at harvest. *Agric. Food Chem.* 17:468-472.
33. Mather, K., and Jinks, J. L. 1982. *Biometrical Genetics*. 3rd ed. Chapman and Hall, London.
34. ORA. 1995. Adulteration with aflatoxin, CPG 7120.26. Page 268 in: FDA/ORA Compliance Policy Guides Manual, Section 555.400. Issued 1980. U.S. Food and Drug Administration, Office of Regulatory Affairs, Washington, DC.
35. Papa, K. E. 1986. Heterokaryon incompatibility in *Aspergillus flavus*. *Mycologia* 78:98-101.
36. Payne, G. A. 1992. Aflatoxin in maize. *Crit. Rev. Plant Sci.* 10:423-440.
37. SAS Institute. 2000. *SAS User's Guide. Statistical Analysis System Institute, Inc., Cary, NC.*
38. Scott, G. E. 1996. Association of bright greenish yellow fluorescence and aflatoxin in grain of maize inbreds. *Maydica* 41:43-48.
39. Shotwell, O. T., Goulden, M. L., and Hesselstine, C. W. 1972. Aflatoxin contamination: Association with foreign material and characteristic fluorescence in damaged corn kernels. *Cereal Chem.* 49:459-465.
40. Shotwell, O. L., Goulden, M. L., Jepson, A. M., Kwolek, W. F., and Hesselstine, C. W. 1975. Aflatoxin occurrence in some white corn under loan, 1971. III. Association with bright greenish-yellow fluorescence in corn. *Cereal Chem.* 52:670-677.
41. Smith, M. S., and Riley, T. J. 1992. Direct and interactive effects of planting date, irrigation, and corn earworm (Lepidoptera: Noctuidae) damage on aflatoxin production in preharvest field corn. *J. Econ. Entomol.* 85:998-1106.
42. Troyer, A. F. 1999. Background of U.S. hybrid corn. *Crop Sci.* 39:601-626.
43. Walker, R. D., and White, D. G. 2001. Inheritance of resistance to *Aspergillus* ear rot and aflatoxin production of corn from CI2. *Plant Dis.* 85:322-327.
44. Wicklow, D. T. 1999. Influence of *Aspergillus flavus* strains on aflatoxin and bright greenish yellow fluorescence of corn kernels. *Plant Dis.* 83:1146-1148.
45. Widstrom, N. W. 1996. The aflatoxin problem with corn grain. *Adv. Agron.* 56:219-280.
46. Widstrom, N. W., McMillian, W. W., Beaver, R. W., and Wilson, D. M. 1990. Weather-associated changes in aflatoxin contamination of preharvest maize. *J. Prod. Agric.* 3:196-199.
47. Widstrom, N. W., Wilson, D. M., and McMillian, W. W. 1984. Ear resistance of maize inbreds to field aflatoxin contamination. *Crop Sci.* 24:1155-1157.
48. Zuber, M. S., Calvert, O. H., Kwolek, W. F., Lillehoj, E. B., and Kang, M. S. 1978. Aflatoxin B₁ production in an eight-line diallel of *Zea mays* infected with *Aspergillus flavus*. *Phytopathology* 68:1346-1349.
49. Zuber, M. S., Darrah, L. L., Lillehoj, E. B., Josephson, L. M., Manwiller, A., Scott, G. E., Gudauskas, R. T., Horner, E. S., Widstrom, N. W., Thompson, D. L., Bockholt, A. J., and Brewbaker, J. L. 1983. Comparison of open-pollinated maize varieties and hybrids for preharvest aflatoxin contamination in the southern United States. *Plant Dis.* 67:185-187.
50. Zummo, N., and Scott, G. E. 1994. Pathogenicity of *Aspergillus flavus* group isolates in inoculated maize ears in Mississippi. *Bio-deterioration Res.* 4:217-224.