Inheritance of Resistance to Aspergillus Ear Rot and Aflatoxin in Corn Genotypes

K. W. Campbell and D. G. White

Graduate research assistant and professor, respectively, Department of Plant Pathology, University of Illinois, Urbana 61801.
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ABSTRACT

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The inheritance of resistance to Aspergillus ear rot of corn (*Zea mays*) caused by *Aspergillus flavus* was studied in progeny derived from crosses between resistant (LB31, L317, CI2, N6, 75-R001, B37Ht2, OH513, Tex6, and H103) and susceptible (B73 and/or Mo17) inbreds following inoculation. In 1992 and 1993 the parental, F_1, F_2, F_3, and both backcross generations of 11 crosses were tested. The number of generations evaluated for each cross was dependent on the year. Parental and F_1 generations, as well as five F_2 and three F_3 populations, were evaluated for aflatoxin content in 1992 and 1993. Generation mean analysis indicated additive and dominance gene action were of primary importance in resistance to Aspergillus ear rot. Dominance genetic effects estimates ranged from 0.0 to 87.3% of the variation between generation means. Inbreds Tex6, LB31, CI2, and OH513 consistently had the highest levels of resistance. Frequency distributions of aflatoxin content of ears on F_2 plants and ears on F_3 families (lines) of the Mo17 × Tex6 and B73 × LB31 populations were highly skewed toward the resistant parent, indicative of genic dominance. The F_2 and F_3 generations indicated various levels of transgressive segregation for resistance to Aspergillus ear rot and to aflatoxin production.

Additional keywords: maize, mycotoxin.

Preharvest infection of corn kernels by *Aspergillus flavus* Link:Fr. causes ear and kernel rot, resulting in reduced grain quality and, potentially, aflatoxin contamination of grain. Since the early 1970's researchers have been evaluating corn germ plasm for resistance and determining the inheritance of the resistance (4,8,9,12,16,20,25,27,29,32–34). Studies on the inheritance of resistance are important to determine the gene action controlling resistance in order to develop appropriate breeding procedures. In previous research using diallel mating designs to study the genetics of resistance to Aspergillus ear rot and aflatoxin production, results have been confounded by environment, inoculation technique, and kernel sampling procedure. Most studies have shown additive genetic effects are more important than dominance effects for determination of resistance to aflatoxin production in previously identified sources of resistance. Zuber et al. (35) found general combining ability (GCA) was significant, but specific combining ability (SCA) was nonsignificant in a diallel mating design among eight inbreds. A pinboard inoculation technique was used, and whole ears were sampled for aflatoxin content. Gardner et al. (9) evaluated seven of the inbreds used in the study by Zuber et al. (35) in a diallel mating design. In their study both GCA and SCA were significant, but SCA accounted for two-thirds of the total genetic variation indicating dominance genetic effects were of primary importance for resistance. A pinboard inoculation technique also was used, but only injured kernels were sampled for aflatoxin content. In contrast, Darrah et al. (8) found GCA was highly significant and accounted for most of the genetic variation when they evaluated the same seven inbreds used in the Zuber et al. (35) and Gardner et al. (9) studies in a diallel mating design across five environments. A "modified natural inoculation" technique was used, in which silks were sprayed with a conidial suspension of *A. flavus* 5 days after midsilk and ears were covered with plastic and paper bags, and whole ears were sampled for aflatoxin content.

Widstrom et al. (33) found significant GCA effects, but nonsignificant SCA effects in two sets of corn inbreds (nine dent inbreds and eight sweet corn inbreds) in separate studies using diallel mating designs. Plants were inoculated by injecting a liquid conidial suspension under the ear husk with a hypodermic syringe. Entire ears were shelled and aflatoxin assays were completed on the bulked grain. Gorman et al. (12) found GCA was nonsignificant for aflatoxin content, and SCA was significant only for aflatoxin G_2 content in a diallel mating design evaluated in three environments. The lack of significance was attributed to relatively low levels of aflatoxin produced among the F_1 crosses evaluated. The diallel was composed of seven corn inbreds with the leafy (*Lfy*) gene backcrossed into each respective inbred. Ears were slash inoculated (a knife was dipped into a conidial suspension of *Aspergillus parasiticus* Speare and slashed through husks, thereby injuring one row of kernels), and uninjured kernels on both sides of the inoculated kernels were assayed for aflatoxin content.

Limited amounts of germ plasm have been evaluated for resistance to *A. flavus* and aflatoxin production. Much of the germ plasm used in previous genetic studies matures too late for use in the midwestern United States (25,26,32) and is not genetically related to commercially used inbreds or hybrids. Many commercial hybrids in the midwestern United States are produced using derivatives of inbreds B73 and/or Mo17. Therefore, genetic studies with these inbreds would be useful to identify sources of resistance with alleles for resistance to incorporate into commercial inbreds. The objectives of this study were: (i) to determine the

Corresponding author: D. G. White; Fax: 217/244-1230

types and magnitudes of gene action for resistance to Aspergillus ear rot from previously identified resistant inbreds (4) in 11 crosses containing B73 and/or Mo17 as a susceptible parent in a generation mean analysis mating design; (ii) to examine parental and F_1 generations for resistance to aflatoxin production; and (iii) to examine the relationship between Aspergillus ear rot and aflatoxin production in five F_2 and three F_3 corn populations.

MATERIALS AND METHODS

Aspergillus ear rot evaluations. In 1992, nine corn inbred lines previously identified as conferring resistance to Aspergillus ear rot in F_1 crosses (4) were evaluated at the Agronomy-Plant Pathology South Farm, Urbana, IL. Inbred lines LB31, L317, CI2, N6, and 75-R001 were crossed with the susceptible inbred B73, and LB31, B37Ht2, OH513, Tex6, H103 and 75-R001 were crossed with the susceptible inbred Mo17. Genetic analyses were based on P_1 (the susceptible parent, either B73 or Mo17), P_2 (the resistant parent), F_1, F_2, BCP_1 of all 11 crosses, and the F_3 generation for crosses involving LB31, L317, 75-R001, B37Ht2, and H103. The F_2, F_3, and BCP_1 generations were produced by selfing the F_1 and F_2 generations and crossing F_1 plants with the P_1 generation, respectively. The individual F_3 families (lines) were produced from bulked F_2 seed (20 to 30 F_1 plants were selfed, harvested, and seed was bulked). Plants were grown in single-row plots 5.34 m long, 0.76 m apart, with 12 to 18 plants per row. The experimental design was a randomized complete block with three replications of the P_1, P_2, and F_1 generations and two replications of the F_2, BCP_1, and F_3 generations. Each replication included two plots for the P_1, P_2, and F_1 generations; five plots for the F_2 generation; eight plots for the BCP_1 generation; and 80 to 100 plots (individual F_3 families) for the F_3 generations. The parental and F_1 generations for each cross were grouped adjacently within replications.

In 1993, the study was expanded to include the BCP_2 generation for the LB31, N6, 75-R001, B37Ht2, Tex6, and H103 crosses and F_3 generations for the N6 and Tex6 crosses. The experimental design was a randomized complete block with five replications of the P_1, P_2, F_1, F_2, BCP_1, and BCP_2 generations and two replications of each F_3 generation. Plots were the same as in 1992, and each replication included two plots of the P_1, P_2, and F_1 generations; five plots of the F_2 generation; seven plots of the BCP_1 and BCP_2 generations; and 39 to 98 plots of the F_3 generations. Plots of the parental and F_1 generations were grouped adjacently as in 1992. Experiments were planted 6 May 1992 and 7 May 1993.

Inoculation and rating. Inoculum was an equal mixture of conidia of four isolates of *A. flavus* (NRRL isolates 6536, 6539, 6540, and an isolate obtained from corn grain in Illinois in 1988). The selected isolates produced the highest levels of ear rot and aflatoxin production in a 1990 isolate virulence study (D. G. White, *unpublished*). Inoculum was produced on potato-dextrose agar in petri dishes incubated at 28°C with 12 h of light for 12 to 16 days. Cultures were blended with water and filtered through a double layer of cheesecloth. The resulting spore suspension was adjusted to 2×10^6 spores per milliliter by dilution with distilled water, and two drops of Tween 20 per 100 ml were added. Inoculum was prepared immediately prior to inoculation. Five milliliters of the spore suspension was injected under the husk leaves of the primary ear of each plant 20 to 24 days after midsilk with a pinboard inoculator (3). Forty to fifty days after inoculation, inoculated ears were husked and a visual rating of 1 to 10 (1 = 10% to 10 = 100% ear rot throughout the inoculated area) was determined for each ear.

Evaluation of parental and F_1 generations for aflatoxin production. Resistance to aflatoxin production in the parental and F_1 generations of the 11 crosses was examined in separate inbred and F_1 experiments in 1992 and 1993 at Urbana, IL (2). Plants were grown in two-row plots 5.34 m long, 0.76 m apart, with 12 to 18 plants per row. Plots were arranged in a randomized complete block design with three replications in 1992 and six replications in 1993. Inoculum preparation and inoculations were the same as previously described (3). Plots were harvested 60 to 70 days after midsilk, dried in a forced-air dryer for 3 days, and ears were machine shelled and bulked for aflatoxin analysis.

Evaluation of F_2 and F_3 populations for Aspergillus ear rot and aflatoxin production. In 1992, individual F_2 plants were evaluated for Aspergillus ear rot and aflatoxin production for the following crosses (number in parenthesis indicates the number of F_2 plants evaluated): Mo17 × Tex6 (44), Mo17 × 75-R001 (37), Mo17 × OH513 (39), B73 × N6 (54), and B73 × CI2 (49). Plants were grown in single-row plots 5.34 m long, 0.76 m apart, with 12 to 15 plants per row. Primary ears on F_2 plants were self-pollinated to produce F_3 seed. Twenty days after pollination, pollination bags were removed, ears were inoculated, and pollination bags replaced over the ear. Fifty days after inoculation, ears were husked and rated for ear rot. At maturity, ears were dried in a forced-air dryer for 3 days and shelled. A random 50-g sample (obtained using a Borno divider) of kernels from individual ears on F_2 plants was assayed for aflatoxin content.

In 1993, 44, 98, and 94 F_3 families of the Mo17 × Tex6, B73 × LB31, and B73 × 75-R001 crosses, respectively, that were evaluated for ear rot also were evaluated for aflatoxin production. Harvest and shelling procedures were the same as the F_2 plant evaluations, except plants were open-pollinated and aflatoxin analyses were done on grain samples from bulked F_3 family ears (consisting of 10 to 17 ears).

Quantification of aflatoxin content in 1992 and 1993. Samples were ground with a Romer (model 2A) grinding/subsampling mill (Romer Labs, Inc., Union, MO) to pass through a 1-mm screen. The 1992 aflatoxin assays were completed by E. H. Gendloff (University of Wisconsin—Madison). An indirect competitive enzyme-linked immunosorbent assay (ELISA) was used as follows. Ground grain (0.5 g) was weighed into disposable plastic cups. The samples were extracted with 70% methanol and stored at 0°C. Wells of Nunc maxisorp immunoplates (Nunc Intermed, Roskilde, Denmark) were coated with 100 µl of a 2,000-fold dilution of aflatoxin B_1-BSA (bovine serum albumin) conjugate (Sigma Chemical Co., St. Louis) in carbonate-bicarbonate coating buffer (7) and incubated at 4°C overnight or longer. All subsequent incubations were at 37°C for 30 min. The plates were washed, and each well was blocked with 100 µl of 0.1% BSA (Sigma fraction V) in phosphate-buffer solution (PBS) (7). After incubating and washing, 50 µl of sample or standard (diluted in PBS) was added, followed by 50 µl of anti-rabbit immunoglobulin G (aflatoxin B_1-KLH [keyhole limpet hemocyanin], Sigma) which had been previously diluted 2,000-fold in the blocking buffer. After incubating and washing, 50 µl of goat anti-rabbit peroxidase (Sigma) diluted 2,000-fold in blocking buffer was added. Substrate (phosphate-citrate with sodium perborate diluted 100-fold) was added after another incubation and wash. The reaction was stopped with HCl, and optical densities were determined as previously described (7). The analysis of the data to quantify aflatoxin content in the samples was done as described by Gendloff et al. (10). The ELISA routinely detected aflatoxin content in the sample greater than 2 ng/ml. Aflatoxin contents of 2 ng/ml or less were recorded as zero.

In 1993, an indirect competitive ELISA technique was again used. Ground grain (0.5 g) was weighed into disposable plastic cups. The samples were extracted with 70% methanol and stored at 0°C. Wells of Dynatech immulon 4 plates (Dynatech Laboratories, Inc., Chantilly, VA) were coated with 100 µl of a 1,000-fold dilution of aflatoxin B_1-BSA conjugate (A-6655, Sigma) in carbonate-bicarbonate coating buffer and incubated at 30°C for 30 min. All subsequent incubations were at 30°C for 15 min. The plates were washed five times, and 50 µl of a 1,000-fold dilution (dilutions were in PBS) of sample or standard was added, fol-

lowed by 50 µl of a 6,000-fold dilution of anti-rabbit immunoglobulin G (8679, Sigma) and placed on a shaker for 15 min. Plates were washed five times, and 100 µl of a 1,000-fold dilution of goat anti-rabbit peroxidase (Sigma) was added and incubated at 30°C for 15 min. Substrate (phosphate-citrate with sodium perborate diluted 100-fold) was added after another wash. The reaction was stopped with HCl, and optical densities and analyses of data for quantification of aflatoxin content were determined as in 1992.

Data analysis. Significance of additive, dominance, and the three digenic epistasis effects was determined on the unweighted means of ear rot ratings by generation mean analysis, using the method of Hayman (13). Increasingly complex models were fitted by least squares regression to the generation means of each population. These models were:

$$\bar{Y}_k = m + e_k$$
$$\bar{Y}_k = m + \alpha_k d + e_k$$
$$\bar{Y}_k = m + \alpha_k d + \beta_k h + e_k$$
$$\bar{Y}_k = m + \alpha_k d + \beta_k h + \alpha_k^2 i + 2\alpha_k\beta_{kj} j + \beta_k^2 l + e_k$$

In these models $\bar{Y}_k$ = the mean of the kth generation, m = a constant, α_k and β_k are coefficients determined by the degree of genetic relationship of the kth generation, d = pooled additive effects, h = pooled dominance effects, i = pooled additive × additive effects, j = pooled additive × dominance effects, l = pooled dominance × dominance effects, and e = experimental error. The coefficients appropriate to the generations of this study are given by Hayman (13). Residual variation (deviations) among generation means after fitting the additive-dominance model was attributed to epistasis.

Broad-sense heritability estimates (calculated as the ratio of the genotype variance over the phenotypic variance) of the F_2 generation were estimated by $h^2 = (s_p^2 - s_e^2)/s_p^2$, in which s_p^2 is the phenotypic variance of individuals in the F_2 generation and s_e^2 is the environmental variance among individuals of the same genotypes, estimated by pooling the within-plot sums of squares and degrees of freedom of the inbred parents (21). Estimates of the minimum number of effective factors (n) were estimated using the formula of Wright as cited by Burton (1): $n = 0.25(0.75 - h + h^2)D^2/(s_{F2}^2 - s_{F1}^2)$; $h = (\bar{x}_{F1} - \bar{x}_{P2})/(\bar{x}_{P1} - \bar{x}_{P2})$; $D = \bar{x}_{P1} - \bar{x}_{P2}$.

Aflatoxin values from individual F_2 plants and F_3 families (mean of two replicates) from each of the eight populations were separated into classes as follows: 0 to 20, 21 to 50, 51 to 100, 101 to 150, 151 to 200, 201 to 300, 301 to 400, 401 to 500, 501 to 1,000, and greater than 1,000 ng/g. The frequency distributions were examined. Pearson's and Spearman's rank correlation coefficients were calculated between ear rot ratings and aflatoxin values and rank of individual F_2 plants or F_3 families for ear rot and aflatoxin values within years, respectively, for the eight populations. Since the generation mean data was unbalanced, the general linear model (GLM) procedure was used for analyses (SAS Institute Inc., Cary, NC). In those crosses when a significant generation × year (G × Y) interaction was detected, the G × Y mean square was used to test genetic effects.

RESULTS

Ear rot resistance. Ear rot ratings of the resistant parents ranged from 1.3 to 4.4 for Tex6 and L317, respectively (Table 1). The F_1 generation ear rot ratings ranged from 1.9 in the Tex6 cross to 4.6 in the B37Ht2 cross. The susceptible parent ear rot ratings ranged from 3.3 to 4.4 in Mo17 and B73, respectively. Although different inbreds were crossed with B73 and Mo17 (inbreds LB31 and 75-R001 were crossed with both B73 and Mo17), frequency distributions of ear rot ratings of various generations with B73 as the susceptible parent (Figs. 1A, B, E, and F and 2A to F) were generally not as skewed toward lower ear rot

TABLE 1. Mean Aspergillus ear rot ratings and aflatoxin values of parental, F_1, F_2, F_3, and backcross generations and deviations of the F_1 mean from the midparent value[a] in each of 11 corn crosses studied in 1992 and 1993 at Urbana, IL

		Ear rot[b]								Aflatoxin (ng/g)[c]			
Cross	Year	P_1[d]	BCP_1	F_1	F_2	F_3	BCP_2	P_2[e]	F_1-MP[a]	P_1	P_2	F_1	F_1-MP
B73 × LB31	1992	4.1	4.1	2.5	3.6	3.4	…[f]	2.2	−0.6	475	84	35	−344**[g]
	1993	4.4	3.9	3.1	3.6	3.6	3.2	2.5	−0.4	674	97	68	−318**
B73 × L317	1992	4.1	3.5	3.4	3.4	4.2	…	3.2	−0.1	475	29	320	68
	1993	4.4	3.8	2.5	3.5	…	…	4.4	−1.9**	674	259	62	−423**
B73 × CI2	1992	4.1	2.9	3.0	3.2	…	…	2.2	−0.6	475	24	317	68
	1993	4.4	3.5	3.7	2.8	…	…	1.7	0.6	674	72	148	−225*
B73 × N6	1992	4.1	3.3	2.9	3.6	…	…	2.1	−0.2	475	113	253	−41
	1993	4.4	3.2	3.9	3.5	3.5	4.2	3.3	0.0	674	106	105	−285*
B73 × 75-R001	1992	4.1	3.7	1.9	3.3	4.0	…	2.4	−0.5**	475	773	29	−595*
	1993	4.4	4.1	3.2	3.3	3.6	4.0	3.8	−0.9	674	938	104	−702**
Mo17 × LB31	1992	3.3	3.0	1.9	2.6	3.2	…	2.2	−0.5	321	84	124	−79
	1993	3.6	3.7	2.0	3.2	3.2	3.4	2.5	−1.1*	167	97	44	−88
Mo17 × B37Ht2	1992	3.3	3.1	3.7	3.9	4.4	…	3.4	…	321	…	56	…
	1993	3.6	3.6	4.6	3.8	3.8	4.5	3.4	1.1**	167	462	104	−211*
Mo17 × OH513	1992	3.3	3.0	3.0	3.3	…	…	3.2	−0.9	321	19	16	−154**
	1993	3.6	2.9	2.7	3.0	…	…	3.2	−0.7*	167	168	46	−122*
Mo17 × Tex6	1992	3.3	2.8	1.9	2.7	…	…	1.3	0.0	321	0	4	−157**
	1993	3.6	2.8	2.0	2.0	2.1	1.6	1.5	−0.6	167	65	4	−112**
Mo17 × 75-R001	1992	3.3	…	2.5	3.0	…	…	3.7	−0.8*	321	773	30	−517*
	1993	3.6	…	3.2	3.3	…	3.5	3.8	−0.5	167	938	96	−457*
Mo17 × H103	1992	3.3	3.2	2.7	3.5	3.6	…	2.9	−0.3	321	448	42	−343*
	1993	3.6	3.2	3.4	3.2	3.5	4.2	3.1	0.0	167	280	212	−12

[a] Midparent value = MP = $(P_1 + P_2)/2$.

[b] Ear rot ratings were based on a 1–10 scale where: 1 = 10% to 10 = 100% of the inoculated area rotted. The P_1, P_2, and F_1 generations had three replications, and the F_2, BCP_1, and F_3 generations had two replications in 1992. In 1993, the P_1, P_2, F_1, F_2, BCP_1, and BCP_2 generations had five replications and the F_3 generations were replicated twice.

[c] Measured by indirect competitive enzyme-linked immunosorbent assay. Samples were run in triplicate. Aflatoxin values are a mean of three replications and six replications in 1992 and 1993, respectively.

[d] P_1 was the susceptible parent in the cross (either B73 or Mo17).

[e] P_2 was the resistant parent in the cross.

[f] … Indicates missing generation due to unavailability of seed.

[g] Asterisks (*,**) indicate difference is statistically significant at $P = 0.05$ and $P = 0.01$, respectively.

ratings as were crosses using Mo17 as the susceptible parent (Figs. 1C and D and 3A to F). The mean ear rot rating of the F_1 generation was significantly less than the midparent value in the B73 × 75-R001 and Mo17 × 75-R001 crosses in 1992, and the B73 × L317, Mo17 × LB31, Mo17 × B37Ht2, and Mo17 × OH513 crosses in 1993, indicating genic dominance.

Crosses with the inbreds Tex6 (Figs. 1C and D), CI2 (Figs. 1E and F), and LB31 (Figs. 1A and B and 3A and B) consistently had the highest levels of resistance to Aspergillus ear rot. The F_1, F_2, BCP_1, BCP_2, and F_3 generations of Mo17 × Tex6 (Figs. 1C and D) were highly skewed toward resistance, indicative of genic dominance. Generations of the LB31 crosses (Figs. 1A and B and 3A and B) also were skewed toward LB31, particularly in the Mo17 × LB31 cross (Figs. 3A and B). Lines OH513 (Figs. 3E and F) and B37Ht2 (Figs. 3C and D) had moderate levels of ear rot resistance, although generations were not as highly skewed toward the resistant parent as were the generations of the LB31, Tex6, and CI2 lines. Inbred lines L317, N6, and 75-R001 were as susceptible to Aspergillus ear rot as B73 when parental generations were examined (Figs. 2A, C, and E, respectively). However, F_2 and F_3 generations in the N6 and 75-R001 crosses (Figs. 2D and F) show segregation toward moderate to high levels of ear rot resistance.

Least squares estimates of genetic effects in the Mo17 × 75-R001 and Mo17 × H103 crosses were nonsignificant (Table 2) and were therefore excluded from subsequent tables and figures. In the statistical analyses of the remaining crosses with Mo17, LB31 and Tex6 had highly significant additive and dominance genetic effects, whereas the B37Ht2 and OH513 analyses had significant dominance genetic effects. With the exception of L317, additive genetic effects were significant in all of the crosses with B73, and dominance genetic effects were significant in the B73 ×

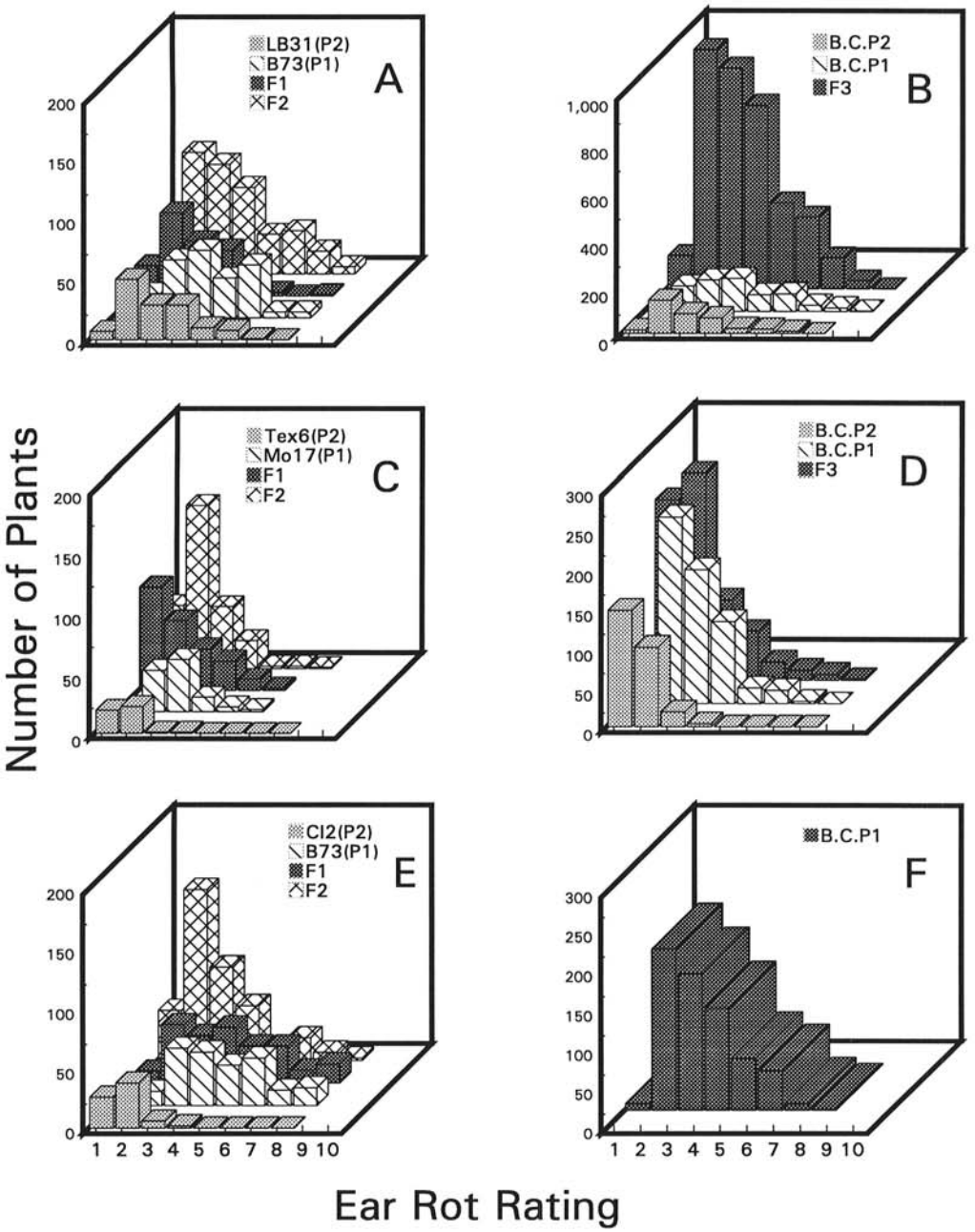

Fig. 1. Frequency distributions of ear rot ratings of three maize populations evaluated in 1992 and 1993. **A,** Parental (B73 and LB31), F_1, and F_2 generations of the B73 × LB31 cross. **B,** BCP_1, BCP_2, and F_3 generations of the B73 × LB31 cross. **C,** Parental (Mo17 and Tex6), F_1, and F_2 generations of the Mo17 × Tex6 cross. **D,** BCP_1, BCP_2, and F_3 generations of the Mo17 × Tex6 cross. **E,** Parental (B73 and CI2), F_1, and F_2 generations of the B73 × CI2 cross. **F,** BCP_1 generation of the B73 × CI2 cross.

L317 and B73 × 75-R001 crosses (Table 2). Additive and dominance genetic effects accounted for 5.7 to 90.0% and 0.0 to 87.3% of the total variation, respectively, among generation means in the nine crosses with significant additive or dominance effects (Table 3). Significant deviations from the additive dominance genetic model were detected in the B73 × CI2, B73 × N6, and Mo17 × LB31 crosses (Table 2) and accounted for 14.7, 57.3, and 49.5% of the variation, respectively (Table 3). In the B73 × CI2 cross, the magnitude of the deviation was small compared to the additive effect. The B73 × LB31 and B73 × L317 crosses had significant generation × year (G × Y) interactions (Table 2). The ear rot rating of the B73 × LB31 F_1 generation increased 19% from 1992 to 1993, which may have accounted for the G × Y interaction. With the B73 × L317 cross the resistant parent and F_1 generation ear rot means increased 27 and 26%, respectively, between 1992 and 1993. Year mean squares were significant in the B73 × LB31, B73 × N6, B73 × 75-R001, and Mo17 × 75-R001 crosses.

Estimates of broad-sense heritabilities were low to moderately high and ranged from 12 to 68% (Table 3). The Mo17 × B37Ht2 cross had a negative number of effective factors, indicating that the F_1 variance (s_{F1}^2) was higher than the F_2 variance (s_{F2}^2).

Resistance to aflatoxin production. Aflatoxin values of the resistant parents ranged from 0 to 938 ng/g (Table 1). Inbred lines LB31, CI2, and Tex6 had aflatoxin values less than 100 ng/g in both 1992 and 1993. The F_1 generation of Tex6 × Mo17 had an aflatoxin value less than 20 ng/g in both years. Several inbreds (75-R001, B37Ht2, and H103) had aflatoxin values as high or higher than the aflatoxin values of the susceptible parents, yet the F_1 generation had aflatoxin values lower than the resistant parent. Aflatoxin values of the F_1 generation were significantly less than the midparent value in the B73 × LB31, B73 × 75-R001, Mo17 ×

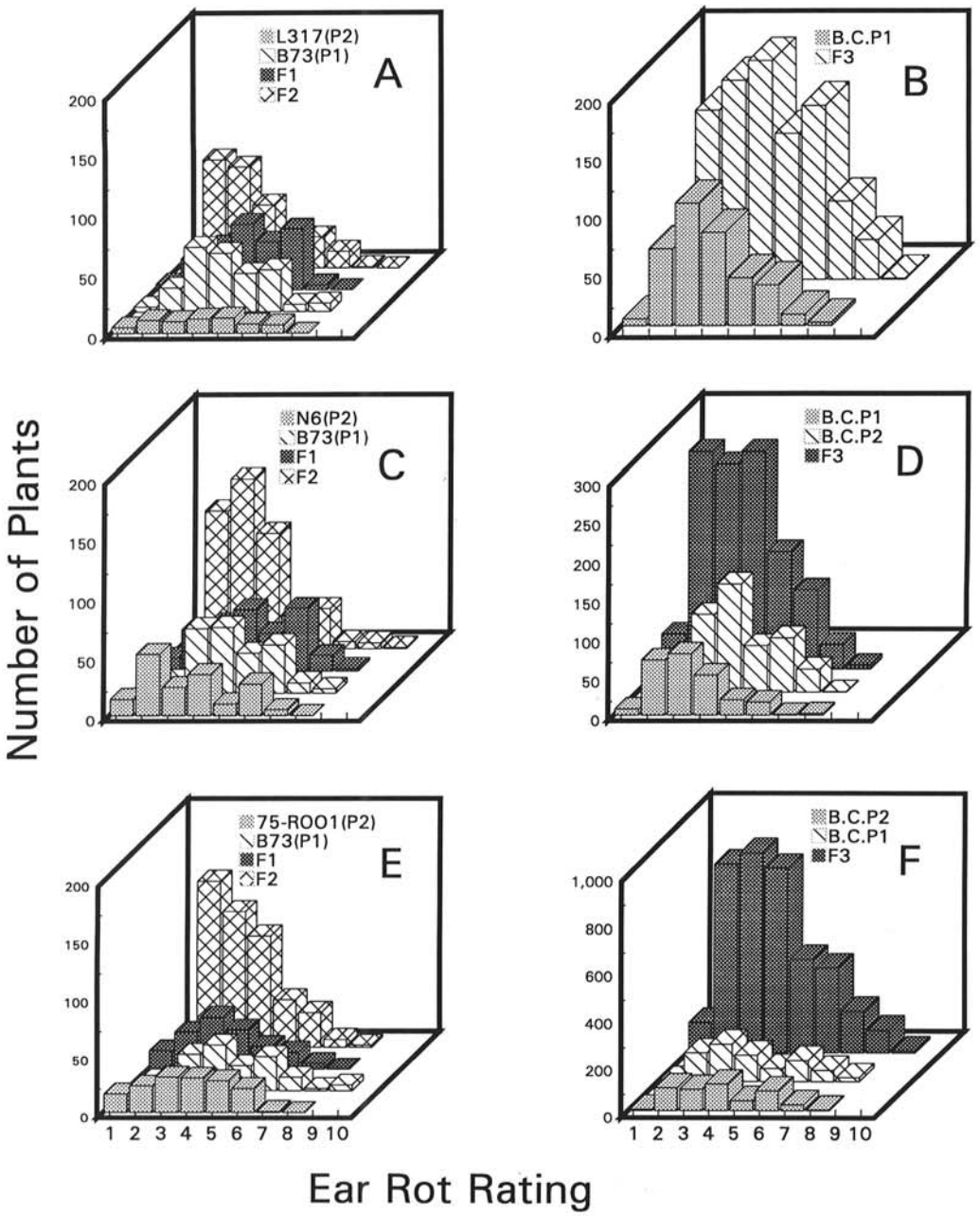

Fig. 2. Frequency distributions of ear rot ratings of three maize populations evaluated in 1992 and 1993. **A,** Parental (B73 and L317), F_1, and F_2 generations of the B73 × L317 cross. **B,** BCP_1 and F_3 generations of the B73 × L317 cross. **C,** Parental (B73 and N6), F_1, and F_2 generations of the B73 × N6 cross. **D,** BCP_1, BCP_2, and F_3 generations of the B73 × N6 cross. **E,** Parental (B73 and 75-R001), F_1, and F_2 generations of the B73 × 75-R001 cross. **F,** BCP_1, BCP_2, and F_3 generations of the B73 × 75-R001 cross.

OH513, Mo17 × Tex6, Mo17 × 75-R001, and Mo17 × H103 crosses in 1992. In 1993, mean aflatoxin values of the F_1 generation were significantly less than the midparent value in every cross except the Mo17 × LB31 and Mo17 × H103 crosses.

The frequency distributions of aflatoxin content for ears on F_2 plants in the Mo17 × Tex6 F_2 population were skewed toward the resistant parent Tex6, with 91% of ears on F_2 plants in the 0 to 20 ng/g class (Fig. 4A). Many of these ears had less than 2 ng/g aflatoxin content. The ears on F_3 families of Mo17 × Tex6 also were skewed toward Tex6, with 23% of the families in the 0 to 20 ng/g class, and 61% of the families with aflatoxin content below 100 ng/g (Fig. 5A). Frequency distributions of aflatoxin content of ears on F_2 plants of the B73 × CI2, Mo17 × OH513, and Mo17 × 75-R001 populations all had more than 60% of ears on F_2 plants in the 0 to 20 ng/g class (Fig. 4B–D). B73 × N6 had the lowest frequency (55%) of ears on F_2 plants in the 0 to 20 ng/g class (Fig. 4E). The frequency distributions of aflatoxin content of ears on F_2 plants from the Mo17 × 75-R001 and B73 × N6 populations had the highest percentage of ears on F_2 plants in the greater than 1,000 ng/g class with 14 and 17%, respectively. The frequency distributions of aflatoxin content of ears on F_3 families from the B73 × LB31 population (Fig. 5B) contained 41% of the ears on F_3 families in the first three classes, but was not as skewed toward the lower aflatoxin classes as F_3 families of the Mo17 × Tex6 population (Fig. 5A). The inbred 75-R001 had aflatoxin values higher than either B73 or Mo17 (Figs. 4D and 5C). Yet, ears on F_2 and F_3 generations with either B73 or Mo17 had moderate (Fig. 5C) to low (Fig. 4D) levels of aflatoxin.

The frequency distributions of aflatoxin content of ears on individual F_2 plants and ears on F_3 families indicated transgressive segregation for susceptibility in all eight populations. Also, several F_2 populations (B73 × CI2, Mo17 × 75-R001, and B73 × N6) and all of the F_3 populations demonstrated various levels of transgressive segregation for resistance. This was particularly true with

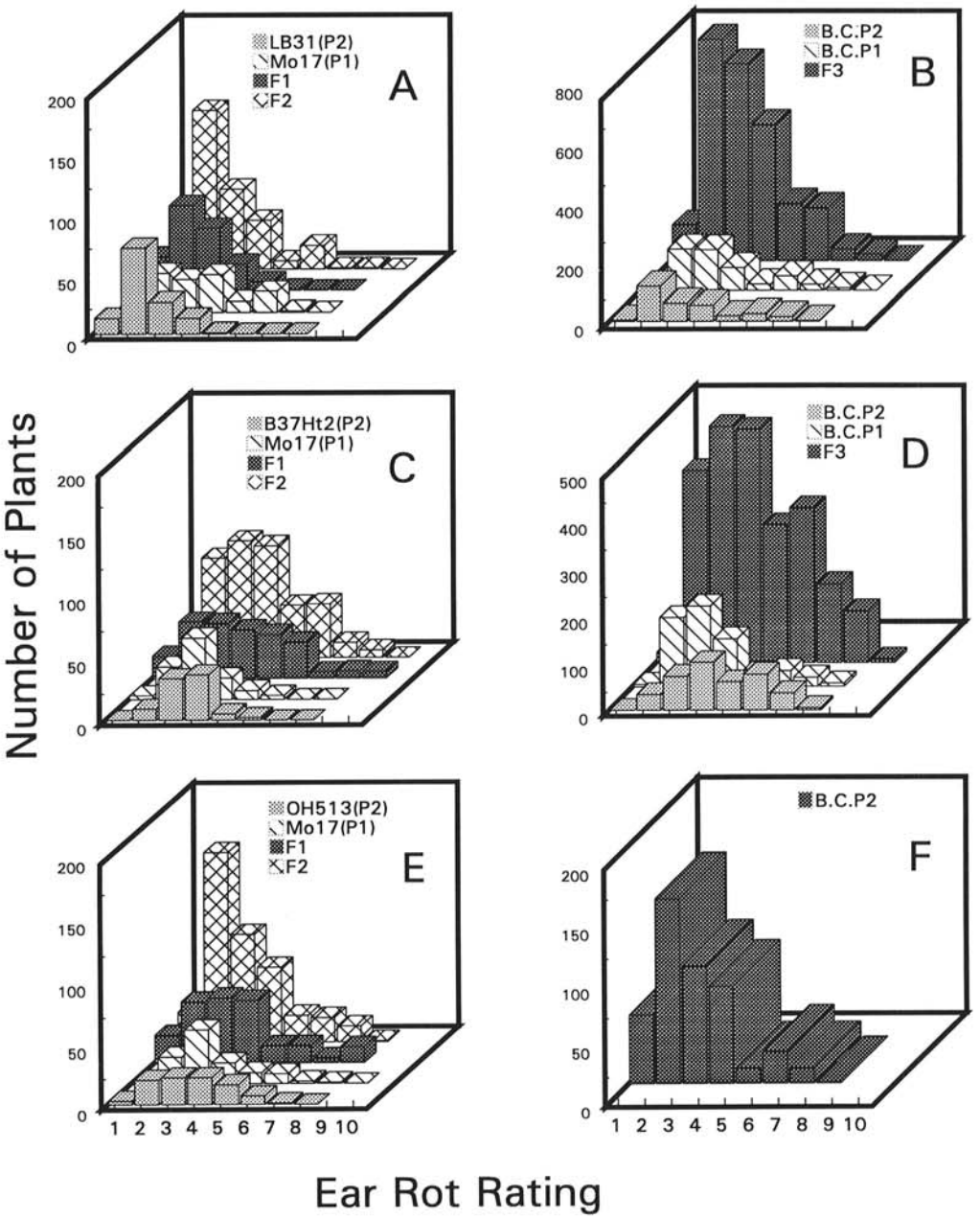

Fig. 3. Frequency distributions of ear rot ratings of three maize populations evaluated in 1992 and 1993. **A,** Parental (Mo17 and LB31), F_1, and F_2 generations of the Mo17 × LB31 cross. **B,** BCP_1, BCP_2, and F_3 generations of the Mo17 × LB31 cross. **C,** Parental (Mo17 and B37Ht2), F_1, and F_2 generations of the Mo17 × B37Ht2 cross. **D,** BCP_1, BCP_2, and F_3 generations of the Mo17 × B37Ht2 cross. **E,** Parental (Mo17 and OH513), F_1, and F_2 generations of the Mo17 × OH513 cross. **F,** BCP_2 generation of the Mo17 × OH513 cross.

the Mo17 × 75-R001 cross, in which 80% of ears on F_2 plants and 39% of ears on F_3 families had lower levels of aflatoxin content than either parent. Additionally, 40% of the Mo17 × Tex6 F_3 families had aflatoxin values lower than Tex6.

Relationship between ear rot ratings and aflatoxin production. Pearson's correlation coefficients and Spearman's rank correlation coefficients between ear rot ratings and aflatoxin content ranged from 0.35 to 0.49 and 0.22 to 0.50, respectively (Table 4). Pearson's correlation coefficients were nonsignificant or relatively low due to random variation between the ear rot ratings and aflatoxin content (Figs. 6 and 7). For example, many of the ears on F_2 plants had ear rot ratings greater than or equal to five, but 0 ng/g aflatoxin content (Fig. 6). There also were ears on F_2 plants with ear rot ratings less than or equal to four, but aflatoxin values greater than 1,000 ng/g. This also was true with the ears on F_3 families, particularly the F_3 population of B73 × 75-R001 (Fig. 7C) in which ears on five F_3 families had mean ear rot ratings less than four, but aflatoxin content greater than 500 ng/g. In general, visual ratings of ears were not a particularly good measure of the entire range of aflatoxin values. However, ears on F_2 plants and F_3 families with low levels of ear rot (i.e., less than or equal to 4.0) typically had less than 300 ng/g aflatoxin content. The exception to this was the B73 × 75-R001 cross in which 15% of the F_3 families had ear rot ratings less than or equal to 4.0, but aflatoxin content greater than 300 ng/g. Spearman's rank correlation coefficients for the F_3 crosses were low ($r_s = 0.22$) in the B73 × 75-R001 cross to moderate in the Mo17 × Tex6 and B73 × LB31 crosses ($r_s = 0.50$ and $r_s = 0.49$, respectively).

DISCUSSION

Based on significant genetic additive and dominance effects in the generation mean analysis, moderate to high heritabilities, and low estimates of effective factors, selection for resistance to Aspergillus ear rot should be effective when appropriate sources of resistance are used. Although estimates of the number of effective factors are imprecise, they are reasonable estimates of the relative magnitude of gene number (22). The frequency distributions of ear rot ratings and aflatoxin production of the Mo17 × Tex6 and B73 × LB31 crosses indicate that F_3 family selection for resistance to aflatoxin production may be effective for an inbred line development program. To lower the cost of aflatoxin assays, line development programs from these two crosses could be as follows: self, inoculate, and select F_2 plants based on low ear rot

TABLE 2. Analyses of variance of mean Aspergillus ear rot ratings of parental, F_1, F_2, F_3, and backcross generations in each of 11 inbreds crossed with Mo17 and/or B73 studied in 1992 and 1993

	Inbred crosses with Mo17											
	LB31		B37Ht2		OH513		Tex6		75-R001		H103	
	df[a]	m.s.[b]	df	m.s.	df	m.s.	df	m.s.	df	m.s.	df	m.s.
Block (years)	6	0.21	6	0.17	6	0.32	6	0.14	6	0.18	6	1.09**
Year (Y)	1	1.63	1	0.95	1	0.73	1	0.40	1	3.23*	1	0.02
Generations (G)	6		6		4		6		4		6	
Additive	1	4.60**[c]	1	0.46	1	0.98	1	18.43**	1	0.0	1	0.23
Dominance	1	1.51**	1	3.38*	1	3.42*	1	1.73**	1	0.94	1	0.02
Deviations	4	1.50*	4	1.05	2	0.48	4	0.10	2	0.27	4	0.40
G × Y	5	0.13	5	0.31	4	0.24	4	0.18	3	0.2	5	0.22
Error	31	0.47	31	0.59	24	0.40	29	0.29	22	0.63	31	0.35
	Inbred crosses with B73									
	LB31		L317		CI2		N6		75-R001	
	df[a]	m.s.[b]	df	m.s.	df	m.s.	df	m.s.	df	m.s.
Block (years)	6	0.34	6	0.57	6	1.03	6	0.32	6	0.29
Year (Y)	1	1.16**[c]	1	0.39	1	0.03	1	4.07**	1	5.13**
Generations (G)	6		5		4		6		6	
Additive	1	22.52**	1	1.57	1	33.77**	1	6.3**	1	5.97**
Dominance	1	2.55	1	12.89*	1	0.16	1	0.49	1	6.87**
Deviations	4	0.57	3	0.10	2	2.89*	4	2.28*	4	0.96
G × Y	5	0.56**	4	1.21*	4	0.47	4	0.42	5	0.81
Error	31	0.16	26	0.44	23	0.48	29	0.37	31	0.44

[a] df = degrees of freedom.
[b] m.s. = mean squares.
[c] Asterisks (*,**) indicate significance at $P = 0.05$ and $P = 0.01$, respectively.

TABLE 3. Percentage of variation in Aspergillus ear rot severity among generation means of nine corn crosses accounted for by additive, dominance, and epistatic genetic effects and estimates of broad-sense heritabilities (h^2) and number of effective factors (n) involved in resistance[a]

	Genetic effect			Estimate	
Pedigree	Additive	Dominance	Epistasis	Broad-sense heritability % (h^2)[b]	Effective factors (n)[c]
B73 × LB31	82.3	9.3	8.4	57	0.70
B73 × L317	10.6	87.3	2.1	30	1.32
B73 × CI2	85.3	0.0	14.7	50	7.72
B73 × N6	39.6	3.1	57.3	32	1.02
B73 × 75-R001	35.8	41.2	23.0	12	1.15
Mo17 × LB31	38.0	12.5	49.5	56	0.24
Mo17 × B37Ht2	5.7	42.0	52.3	68	−1.16
Mo17 × OH513	18.3	64.0	17.7	48	0.50
Mo17 × Tex6	90.0	8.0	2.0	47	3.26

[a] Percentage of variation and estimates were based on Aspergillus ear rot data pooled over 2 years.
[b] Broad-sense heritabilities (h^2) estimated by the formula (21): $h^2 = [(s_{F2}^2 - s_e^2)/s_{F2}^2] \times 100$.
[c] Numbers of effective factors (n) estimated by the formula (1): $n = 0.25(0.75 - h + h^2)d^2/(s_{F2}^2 - s_{F1}^2)$; where $h = (\bar{x}_{F1} - \bar{x}_{P2})/(\bar{x}_{P1} - \bar{x}_{P2})$ and $d = \bar{x}_{P1} - \bar{x}_{P2}$.

ratings (i.e., less than or equal to 4.0). Selections from subsequent generations would then be based on both low ear rot and aflatoxin values. Due to the inherent variability of aflatoxin production (17), selection of F_3 families and subsequent generations should be based on data from replicated trials. Additive and dominance genetic effects appear to be of primary importance in resistance to Aspergillus ear rot. The magnitude of the genetic additive or dominance effect was dependent on the cross studied and on the year of study. Generation mean analysis has been used to detect quantitatively inherited traits including disease resistance (6,14,23,28). Limitations associated with generation mean analysis lie in the assumptions made. Apart from the usual assumptions concerning the analysis of variance, additional assumptions include the isodirectional distribution of genes between the two parental lines, the absence of linkage between interacting loci, and the absence of selection favoring certain gametes. A further limitation is the lack of sensitivity of generation mean analysis to detect types of gene action when individual gene effects of differing direction cancel each other and are not detected by means (22).

Additive genetic effects were of primary importance in B73 × LB31, B73 × CI2, and Mo17 × Tex6 crosses, whereas genetic dominance was of primary importance in B73 × L317, Mo17 × OH513, and B73 × 75-R001 crosses. Genic dominance may be of greater importance than indicated by generation mean analysis. The "susceptible" line Mo17 appears to be moderately resistant to Aspergillus ear rot (Table 1) and contributes favorable alleles in crosses. This violates the assumption of isodirectional distribution of alleles, resulting in significant deviations from the simple additive dominance genetic model. Consequently, partitioning the

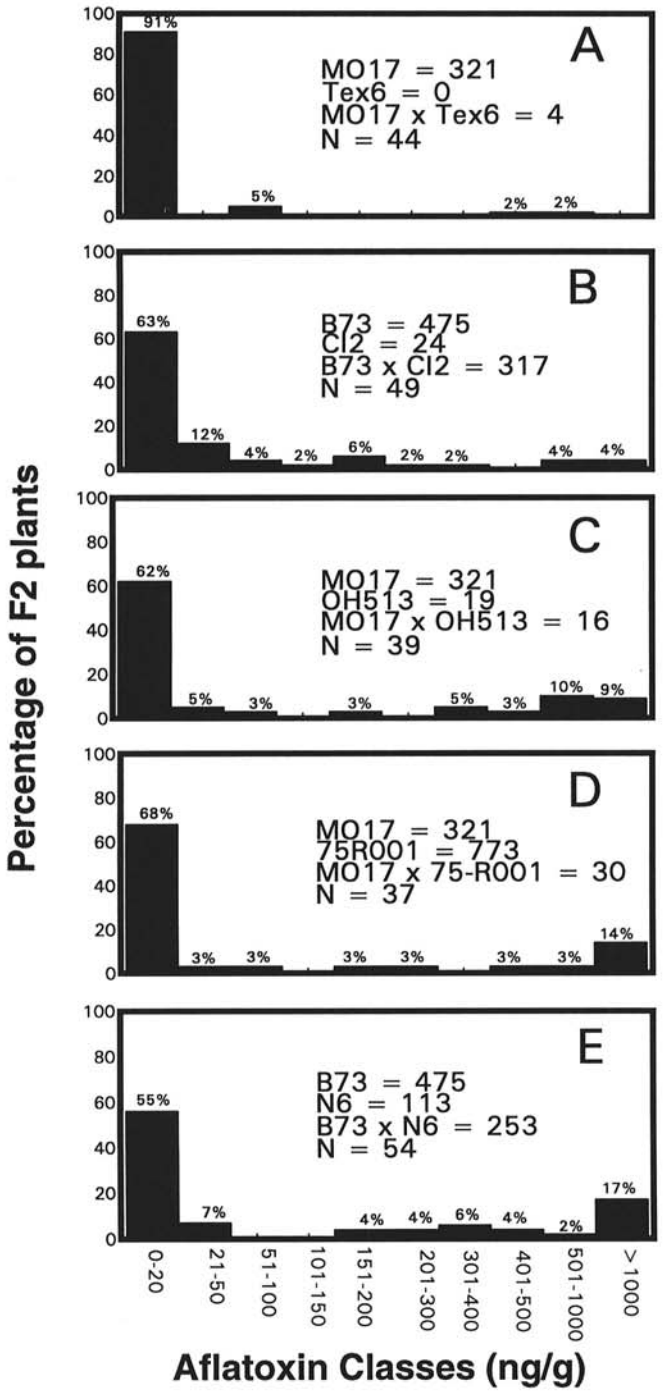

Fig. 4. Frequency distributions of aflatoxin on F_2 plants in five maize populations evaluated in 1992. **A,** Mo17 × Tex6. **B,** B73 × CI2. **C,** Mo17 × OH513. **D,** Mo17 × 75-R001. **E,** B73 × N6. Parental and F_1 generation aflatoxin values are listed in their respective frequency distribution. N represents the number of F_2 plants evaluated for each population.

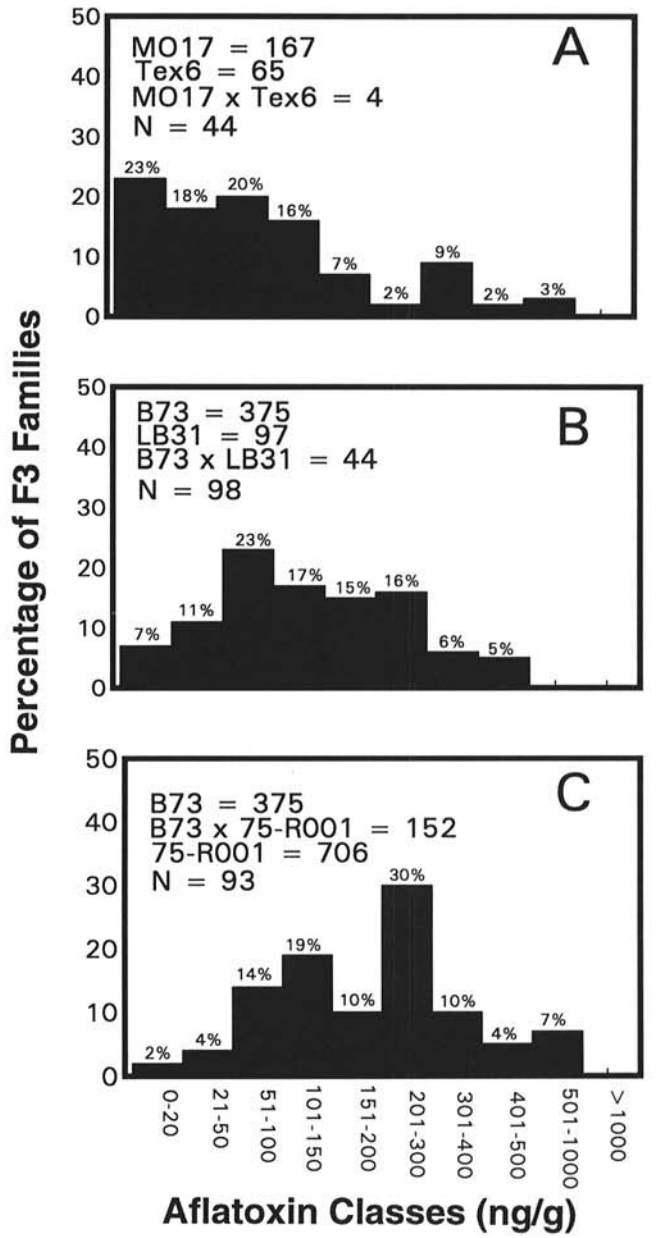

Fig. 5. Frequency distributions of aflatoxin content of ears on F_3 families (averaged over two replications) in three corn populations evaluated in 1993. **A,** Mo17 × Tex6. **B,** LB31 × B73. **C,** B73 × 75-R001. Parental and F_1 generation aflatoxin values are listed in their respective frequency distribution. N represents the number of F_3 families evaluated for each population.

TABLE 4. Relationship between Aspergillus ear rot and aflatoxin production in eight corn populations at Urbana, IL, in 1992 and 1993

Cross	Number of F_2 plants or F_3 families	Population	Year	Correlation coefficient	
				r_p[a]	r_s[b]
Mo17 × Tex6	30	F_2[c]	1992	N.S.	N.S.
B73 × CI2	48	F_2	1992	0.35*[d]	0.45**
Mo17 × OH513	38	F_2	1992	0.45**	0.37*
Mo17 × 75-R001	36	F_2	1992	0.39*	0.45**
B73 × N6	46	F_2	1992	N.S.	0.34*
Mo17 × Tex6	44	F_3[e]	1993	0.45**	0.50**
B73 × LB31	98	F_3	1993	0.49**	0.49**
B73 × 75-R001	93	F_3	1993	N.S.	0.22*

[a] Pearson's correlation coefficients.
[b] Spearman's rank correlation coefficients.
[c] Ear rot ratings and aflatoxin values (aflatoxin assays were completed on grain subsamples of individual F_2 ears) from individual F_2 plants were used in the calculations.
[d] Asterisks (*,**) indicate significance at $P = 0.05$ and $P = 0.01$, respectively.
[e] Mean F_3 family ear rot ratings (mean of all F_3 plants in a row × two replications) and aflatoxin values (aflatoxin assays were completed on grain subsamples from F_3 family ear bulks × two replications) were used in the calculations.

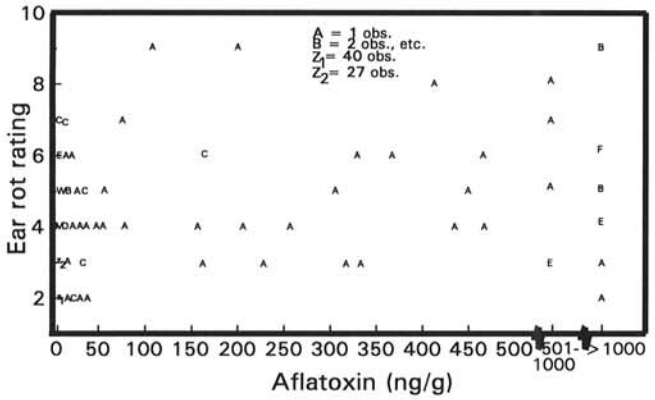

Fig. 6. Relationship between *Aspergillus flavus* ear rot ratings and aflatoxin values of 198 individual F_2 plants for crosses (number in parenthesis indicates the number of F_2 plants evaluated): Mo17 × Tex6 (44), B73 × CI2 (49), Mo17 × OH513 (39), Mo17 × 75-R001 (37), and B73 × N6 (54) evaluated in 1992 at Urbana, IL.

percentage of variation among generation means due to various genic effects of segregating generations into additive or dominance variance components within the Mo17 crosses was biased. For example, additive genetic effects accounted for 90.0% of variation in the Mo17 × Tex6 ear rot means (Table 2), yet ear rot means of F_1 and subsequent generations (Figs. 1C and D) are all skewed toward Tex6, indicative of genic dominance. In addition, environment is very important in the development of Aspergillus ear rot and aflatoxin production (11,15,18,19,24). In 1993, environmental conditions were unusually wet, which may have had a limiting effect on growth of *A. flavus*, resulting in a lack of uniform disease development. Coefficients of variation calculated from the analyses of variance of plot mean ratings varied from 7.3 to 20.0% in 1992 compared with 13.0 to 27.3% in 1993. The inflated error mean squares may well have prevented detection of real but small effects due to dominance and epistasis in the generation mean analysis. Although significant deviations from the additive dominance genetic model were detected in three of the crosses studied, they were of major importance in only the B73 × N6 and Mo17 × LB31 crosses in which they accounted for 57.3 and 49.5%, respectively, of the total variation among generation means (Table 3). Deviations could have been due to an inadequate scale to measure Aspergillus ear rot, variability of inoculation technique between individuals (i.e., some ears were wounded more severely), nonallelic interaction of genes, or the failure to meet assumptions made in generation mean analysis. Significant generation × year (G × Y) interaction in the analysis of variance (Table 2) can be interpreted as genotype × environment interactions. This effect was of a higher magnitude (G × Y sums of squares were much higher than the other crosses) in the B73 × L317 cross. Much of this interaction was probably due to the failure of the mean ear rot ratings of L317 to be similar in both 1992 and 1993 (Table 1). In 1993, L317 had a ear rot mean of 4.4, which was equal to that of B73.

The analyses were based on unweighted least squares analyses. Mather et al. (22) suggested using weighted least squares if the assumptions about a common variance of generation means are not met. Moll et al. (23) compared weighted and unweighted analyses of a population having nearly a fivefold range among variances of generation means. The R^2 values for both analyses were quite similar. Variances of generation means reported here were very similar; consequently, unweighted analyses were considered appropriate.

The highest levels of resistance to aflatoxin production were in the Mo17 × Tex6 (Figs. 4A and 5A) and B73 × LB31 (Fig. 5B) crosses. Crosses with CI2 (Fig. 4B) and OH513 (Fig. 4C) also appear to have moderately high levels of resistance to aflatoxin production. The mean F_1 aflatoxin values were significantly less than the midparent values in 1992 and 1993 in the B73 × LB31, Mo17 × OH513, and Mo17 × Tex6 crosses, indicative of genic dominance (Table 1). Inbred 75-R001 had extremely high aflatoxin values, but in crosses with B73 or Mo17 contributed alleles for resistance. This was indicated in the aflatoxin values of the F_1 generation, which were significantly less than midparent values in crosses with both B73 and Mo17. Although these sources of resistance may have aflatoxin contents beyond an acceptable level in tests using our inoculation technique, with natural infection this level of resistance may be sufficient.

Transgressive segregation in the F_2 and F_3 populations evaluated for aflatoxin production indicates that crosses of two lines with intermediate resistance could produce progenies with higher levels of resistance than either parent. Furthermore, resistance in inbreds such as Tex6, LB31, OH513, and CI2 that demonstrate genic dominance for resistance can be incorporated into widely used inbreds (i.e., derivatives of B73 and/or Mo17) through backcross breeding programs for improvement of midwest hybrids.

Other studies (15,20,29) have reported a moderate to high correlation between kernel infection and/or ear rot ratings and aflatoxin content. In our study, Pearson's correlation coefficients between ear rot ratings and aflatoxin values were nonsignificant or relatively low. The selected crosses used in the study all had low levels of Aspergillus ear rot (4), and there were no "susceptible" crosses included in the study. Consequently, there was a narrow range of response in the Aspergillus ear rot values. Most of the F_2 plants and F_3 families (Figs. 6 and 7, respectively)

had ear rot values in the 2.0 to 6.0 range. Although the aflatoxin values had a wider range, the F_2 populations and the Tex6 × Mo17 F_3 population were highly skewed toward the low aflatoxin values.

Another possible explanation for the nonsignificant correlation coefficients was that different mechanisms of plant resistance may be responsible for resistance to the ear rot and aflatoxin production phases of the disease, and these mechanisms may differ with genotypes. Zuber (34) stated, "Mycotoxin levels in corn could be controlled by inherited differences in the ability to 1) resist invasion of the fungus into the kernel, 2) minimize amount of fungal growth within a kernel or 3) inhibit mycotoxin synthesis." Although mechanical wounding of kernels simulates insect damage, wounding circumvents aleurone and pericarp resistance to infection. Since the pathogen is allowed direct access into the kernel (via wound sites), the plant has only two resistance mechanisms: minimize the amount of fungal growth within a kernel and/or inhibit mycotoxin synthesis.

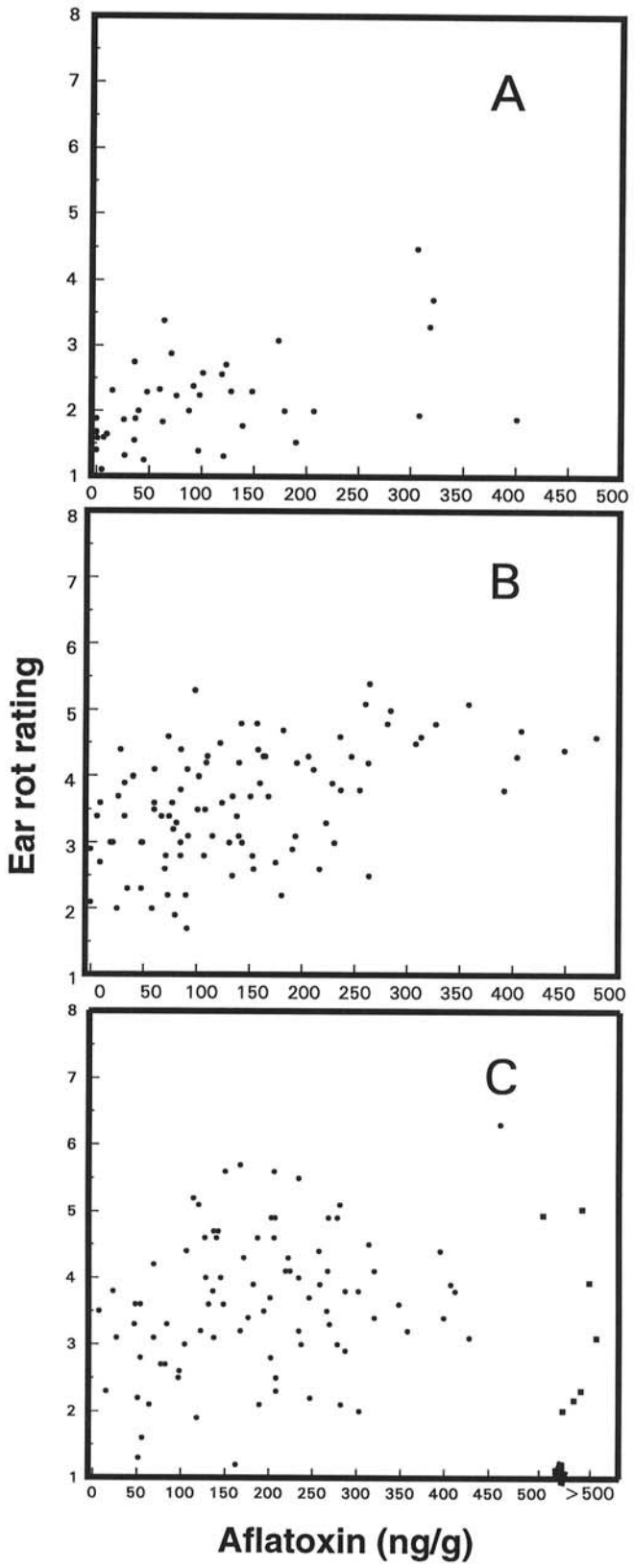

Fig. 7. Relationship between mean *Aspergillus flavus* ear rot ratings and aflatoxin values of F_3 families in three maize populations evaluated in 1992. **A,** Mo17 × Tex6. **B,** B73 × LB31. **C,** B73 × 75-R001.

Several factors are known to influence infection and aflatoxin production by *A. flavus* in corn (12,24), but the biochemical basis of these effects is unknown. No plant compound associated with decreased production of aflatoxin in feed crops has been identified, thus plant breeders do not have a convenient marker for resistance (24). Compounds that are induced by pathogen attack have been called pathogenesis-related (PR) proteins (5,30). These induced proteins include chitinases, β-1,3 glucanases, proteinase inhibitors, and ribosome-inactivating proteins. Also, a compound in corn seeds known as zeamatin has been reported as inhibitory to various fungi (31). Genotypes with low Aspergillus ear rot ratings and high aflatoxin content may contain gene(s) which inhibit or minimize fungal growth within kernels, but not the prerequisite gene(s) for resistance to aflatoxin production. The opposite would be true of genotypes with high levels of ear rot and low levels of aflatoxin content. Inbreds Tex6, LB31, CI2, and OH513 with low levels of Aspergillus ear rot and aflatoxin content may have compounds that inhibit both kernel colonization and aflatoxin production.

A line development program was initiated in 1992 to develop inbred lines with high levels of resistance to Aspergillus ear rot and aflatoxin production for public release. Currently, we have F_5 lines from the B73 × LB31 cross. When grown in replicated trials these genotypes have low levels of Aspergillus ear rot and aflatoxin content. In 1994, several of these F_5 lines were crossed with two widely used inbred lines (derivatives of Mo17) and evaluated for Aspergillus ear rot. In 1995, selections from these populations will be evaluated in separate experiments for yield and resistance to Aspergillus ear rot and aflatoxin production. We also are developing resistant inbreds from the Mo17 × Tex6 F_3 families that can be substituted for Mo17 derivatives.

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