Evaluation of Corn Genotypes for Resistance to Aspergillus Ear Rot, Kernel Infection, and Aflatoxin Production

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ABSTRACT

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Resistance of corn (*Zea mays* L.) hybrids and inbreds to ear rot, kernel infection, and aflatoxin contamination caused by *Aspergillus flavus* was evaluated following inoculation. Thirty-five F_1 hybrids from crosses of selected inbreds with the susceptible inbreds B73 and/or Mo17 were evaluated for 3 years in Illinois. Fifteen of the F_1 hybrids also were evaluated in Mississippi. Resistant and susceptible inbreds per se also were evaluated for 2 years in Illinois. Inbreds Tex6, Y7, and Mp420, and the F_1 hybrids with these inbreds, consistently had the greatest resistance to Aspergillus ear rot, kernel infection, and aflatoxin production in the Illinois environment. Grain of the F_1 hybrids with these inbreds also had less aflatoxin in Mississippi. Pearson and Spearman rank correlation coefficients indicated that Aspergillus ear rot ratings provided a more accurate estimate of aflatoxin contamination of grain than did kernel infection. From evaluations of the F_1 hybrids and the inbred parents per se, inbreds were identified that may contain alleles for resistance not found in B73 and/or Mo17.

Additional keyword: mycotoxin

Aspergillus ear and kernel rot of corn (*Zea mays* L.) is caused by *Aspergillus flavus* Link:Fr. The disease and the associated production of aflatoxin in grain are prevalent in the midwestern U.S. during years with drought conditions. Aflatoxin, a secondary metabolite produced by the fungus in kernels either before or after harvest, is carcinogenic to a number of animal species, and can seriously affect marketing of corn grain. Corn grain with more than 20 ng/g (ppb) aflatoxin cannot be sold through interstate commerce, and some countries will not buy grain with contamination greater than 10 ng/g (17).

The most effective control of Aspergillus ear and kernel rot and the possible aflatoxin contamination of corn grain is the use of genetically resistant hybrids. Natural outbreaks of the disease usually are sporadic, therefore identification of resistant genotypes from natural infection is unreliable (7,32). Consequently, inoculation techniques that produce uniformly high levels of ear rot and aflatoxin must be used. Kernel wounding has produced levels of ear rot and aflatoxin sufficient to differentiate genotypes (1,2,12,13,19).

Differences in Aspergillus ear rot, kernel infection, and aflatoxin production have been observed in commercial dent corn hybrids (2,11,12,14,15), in experimental F_1 hybrids (3,9,12,23,28–31), in inbreds (20,27), in open-pollinated varieties (32), in sweetcorn (29), and in popcorn (16). Although genotypic resistance to Aspergillus ear rot and aflatoxin production is known, high levels of resistance have not been incorporated into commercially valuable hybrids. Additionally, since much of the previously identified resistant germ plasm matures too late for use in the midwestern U.S. (21,28), it is of limited use in breeding commercial inbreds and hybrids for resistance in that area. Many commercial hybrids in the midwestern U.S. are produced using derivatives of inbreds B73 and/or Mo17. The objective of this study was to identify inbreds useful for improving the resistance of commercial inbreds related to B73 and/or Mo17.

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MATERIALS AND METHODS

1991 to 1993 Illinois experiments. In the summer of 1990, inbreds from the University of Illinois Plant Pathology corn collection were crossed with the susceptible inbreds Mo17 and/or B73, to produce F_1 hybrids that were evaluated for resistance to Aspergillus ear rot in 1991. The inbreds from the collection included inbreds released by agricultural experiment stations in the U.S.; inbreds from Canada, Europe, Mexico, Northern China, India, and South Africa; and lines previously identified in the southern U.S. as resistant to *A. flavus* (22). Most of the lines are relatively adapted to the midwestern U.S.

In separate experiments in 1991, 1,189 F_1 hybrids with Mo17 (Mo17 experiment) and 978 F_1 hybrids with B73 (B73 experiment) were pinboard inoculated (2) and evaluated for resistance to Aspergillus ear rot. Two commercial hybrids, Com19 and Com79, were included for comparison as susceptible (2). Hybrids were planted 1 May 1991 in single-row plots 5.34 m long with 12 to 18 plants per row. Plots were spaced 0.76 m apart and arranged in a randomized complete block design with two replications. Eighteen F_1 hybrids from the B73 experiment and 17 F_1 hybrids from the Mo17 experiment were selected for further study (Table 1) (3) based on low ear rot ratings.

In 1992 and in 1993, the selected F_1 hybrids (Table 2) were reevaluated and parental inbreds (Table 3) also were evaluated for Aspergillus ear rot, kernel infection, and aflatoxin production. Susceptible inbreds B73, Mo17, and CO158 were included for comparison in the inbred experiment in both years. In 1993, MAS: pw,nf, (susceptible), MAS:gk (resistant) (28), and MP420 (resistant) (21) also were included for comparison. Two susceptible F_1 hybrids, CO158 × B73 and CO158 × Mo17 were included in the F_1 hybrid experiment in both years. The resistant hybrid MP420 × Tx601 was included in 1993. Experimental units were two-row plots, with rows 5.34 m long, spaced 0.76 m apart, with 12 to 18 plants per row. Plots were arranged in a randomized complete block design with three replicates in 1992 and six replicates in 1993. Experiments were planted 6 May 1992 and 7 May 1993 at the University of Illinois Agronomy/Plant Pathology South Farm, Urbana.

Inoculum, inoculation technique, and kernel plating for Illinois experiments. Inoculum was an equal mixture of conidia from four isolates of *A. flavus* (NRRL isolates 6536, 6539, 6540, and an isolate from Illinois collected in 1988). Isolates were selected on the basis of high virulence (D. G. White, *unpublished*). *Aspergillus flavus* was grown in petri dishes on potato-dextrose agar for 12 to 16 days at 28°C with 12-h light. Cultures, including media, were blended with distilled water and filtered through double layered cheesecloth. Conidial concentrations were estimated with a hemacytometer and adjusted with distilled water. Two drops of Tween 20 per 100 ml were added. Concentrations of 1×10^5 conidia/ml were used in 1991 and 1×10^6 conidia/ml in 1993. Conidial suspensions were prepared immediately prior to use. Primary ears of

Table 1. Mean ear rot and aflatoxin values of F_1 hybrids pinboard inoculated with *Aspergillus flavus* in 1991 at Urbana, Ill.

Pedigree	Ear rot[a]	Aflatoxin ng/g	
		Wounded[b]	Non-wounded[c]
B37Ht2 × Mo17	2.60	58,104[d]	2,751
B40 × Mo17	2.80	19,930[d]	1,603[d]
B9 × Mo17	3.60	68,185	1,938
CH66-17 × B73	4.00	98,715	3,714
CI2 × B73	2.20	46,630	1,235
CO158 × B73	2.50	103,777	4,963
CO158 × Mo17	4.40	–[e]	–
FR809 × Mo17	4.10	43,915[d]	–
F486 × B73	5.10	12,332	12,209
H103 × Mo17	2.00	16,646[d]	1,450
KYS × B73	3.60	73,130	1,668
KY58 × B73	3.00	71,682	1,479
LB31 × B73	4.40	22,026	1,446
LB31 × Mo17	2.30	62,943[e]	1,494
L317 × B73	2.80	10,300	2,696
MS214 × Mo17	1.50	8,185	1,702[d]
MI82 × B73	3.50	56,953	2,207
NC232 × B73	2.10	49,020	1,107
ND363 × Mo17	2.50	12,707	2,100[d]
N6 × B73	3.30	67,507	2,464
N8 × Mo17	4.60	16,646	1,719
OH513 × Mo17	1.60	4,023	398[d]
OH516 × B73	1.50	29,143	1,844
SDPO31 × B73	3.50	11,613	12,964
SDP262 × Mo17	3.50	24,342	2,207
SD18 × B73	3.50	20,130	2,835
SP292 × Mo17	2.20	–	1,260
Tex6 × Mo17	3.30	8,433	1,063
TR213 × B73	2.70	26,107	2,696
T115 × Mo17	2.10	63,576	2,207
Y7 × Mo17	3.50	11,158[d]	1,900
33-16 × B73	3.20	53,103	2,252
75-R001 × B73	2.10	33,522	14,617
75-R001 × Mo17	4.30	–	–
75-R012 × B73	3.20	21,806	1,386
Com19	6.90	31,887[d]	1,199[d]
Com79	7.38	9,135[d]	1,001[d]
Mean	5.47[f] (5.45)[g]	37,275	2,934
LSD/LSR (0.05)[h]	3.86[f] (4.51)[g]	7	NS[i]
CV (%)[i]	24[f] (25)[g]	9	10

[a] Ratings on 1 to 10 scale: 1 = 10% to 10 = 100% of inoculated area rotted. Values are a mean of two replicates.

[b] Wounded kernels were removed from within inoculated area and assayed for aflatoxin production. Values are geometric means (antilogarithm of logarithmic means) of two replicates. High-pressure liquid chromatography used for all aflatoxin analyses.

[c] Two rows of kernels surrounding inoculated area were removed and assayed for aflatoxin. Values are geometric means (antilogarithm of logarithmic means) of two replicates.

[d] Kernels from only one replicate were analyzed for aflatoxin.

[e] – indicates missing data due to lost samples.

[f] From experiment in which 1,189 Mo17 F_1 hybrids were evaluated for Aspergillus ear rot (3).

[g] From experiment in which 978 B73 F_1 hybrids were evaluated for Aspergillus ear rot (3).

[h] Least significant difference (LSD) and least significant ratio (LSR) statistics used for ear rot and aflatoxin values, respectively, to compare between treatment means. LSR is antilog of LSD of logarithmic data, appropriate for comparison between geometric means. Means whose ratios exceed LSR values are significantly different ($P = 0.05$).

[i] NS, not significant; CV, coefficient of variation.

each plant were inoculated 20 to 24 days following the midsilk growth stage (50% of ears with emerged silks) using a pinboard inoculator (2). The pinboard inoculator consisted of seven rows of 23 pins mounted in an aluminum bar, with 0.8 cm of the point ends exposed. Located in the center of the pins was a larger needle through which the conidial suspension was injected under the ear husk. The inoculator was mounted at the end of a spray gun that was attached to a backpack sprayer. The inoculator was aligned with the ear axis, the pins forced through the husk into kernels, and 5 ml of the suspension injected under the husk. Forty to fifty days after inoculation, ears were husked, and a visual rating of 1 to 10 (1 = 10% to 10 = 100% of the inoculated area rotted) was determined for each ear and averaged for each plot.

In 1991, ears were harvested 60 to 70 days after midsilk and dried in a forced air dryer for 3 days. Wounded kernels within the inoculated area and undamaged kernels from the surrounding two rows were removed separately from each ear and bulked for each plot. Kernels were ground with a Romer Mill (Model 2A) Grinding/Subsampling Mill (Romer Labs, Inc., Union, Mo.) to pass through a 1-mm screen and arbitrarily selected subsamples were analyzed for aflatoxin.

Table 2. Mean ear rot and aflatoxin values, and percentage of corn kernels infected by *Aspergillus flavus* of F_1 hybrids pinboard inoculated in 1992 and 1993 at Urbana, Ill.

Pedigree	1992[a]			1993[a]		
	Ear rot[b]	Aflatoxin[c] (ng/g)	Kernel infection (%)[d]	Ear rot	Aflatoxin (ng/g)	Kernel infection (%)
B37Ht2 × Mo17	3.76	19	27	4.85	85	43
B40 × Mo17	2.49	23	19	3.82	232	23
B9 × Mo17	4.78	795	14	5.26	133	42
CH66-17 × B73	4.62	99	12	5.93	232	38
CI2 × B73	4.85	244	25	4.05	144	34
CO158 × B73	4.17	292	14	5.84	149	46
CO158 × Mo17	5.16	160	7	–[e]	–	–
FR809 × Mo17	3.10	136	25	2.77	36	38
F486 × B73	4.67	220	40	4.31	139	35
H103 × Mo17	2.75	21	10	3.61	166	24
KYS × B73	2.86	111	32	3.34	7	19
KY58 × B73	4.84	445	19	4.78	125	36
LB31 × B73	2.49	19	11	3.35	38	25
LB31 × Mo17	2.23	29	16	2.88	31	22
L317 × B73	3.36	87	19	2.74	37	23
MS214 × Mo17	3.67	36	18	5.59	268	33
MI82 × B73	6.40	77	30	4.41	68	31
Mp420 × Tx601[f]	–	–	–	2.90	2	27
NC232 × B73	2.87	139	34	4.33	98	41
ND363 × Mo17	2.85	71	7	5.15	147	42
N6 × B73	2.90	150	9	4.07	54	70
N8 × Mo17	1.80	12	4	4.04	64	19
OH513 × Mo17	3.03	15	20	3.19	31	32
OH516 × B73	–	–	–	2.71	22	25
SDPO31 × B73	2.35	133	19	5.56	137	30
SDP262 × Mo17	4.88	943	18	5.14	171	42
SD18 × B73	2.43	188	23	5.45	267	46
SP292 × Mo17	–	–	–	4.39	107	28
Tex6 × Mo17	1.85	4	14	2.14	3	33
TR213 × B73	5.31	249	28	3.73	104	32
T115 × Mo17	2.59	7	7	2.06	18	42
Y7 × Mo17	2.65	21	12	2.31	10	27
33-16 × B73	4.84	333	17	3.44	39	56
75-R001 × B73	1.82	29	7	3.69	58	36
75-R001 × Mo17	2.48	8	18	3.18	44	32
75-R012 × B73	3.37	89	44	3.53	116	33
Mean	3.46	158	19	4.03	99	34
LSD/LSR (0.05)[g]	2.28	13	21	1.22	4	21
CV (%)[h]	40	36	70	27	27	54

[a] Values are a mean of three replicates in 1992 and six replicates in 1993.

[b] Ratings on a 1 to 10 scale: 1 = 10% to 10 = 100% of the inoculated area rotted.

[c] Values are geometric means (antilogarithm of logarithmic means). Measured by indirect competitive enzyme-linked immunosorbent assay. Samples were run in triplicate.

[d] Kernels were plated on malt salt agar and incubated for 7 to 10 days. One hundred kernels and fifty kernels randomly selected, were plated for each entry in 1992 and 1993, respectively.

[e] Indicates missing data due to unavailability of seed.

[f] Only three replicates were evaluated in 1993.

[g] Least significant difference (LSD) and least significant ratio (LSR) statistics used to compare between treatment means: LSD values applicable for ear rot and kernel infection means and LSR values applicable for aflatoxin values. LSR is antilog of LSD of logarithmic data, appropriate for comparison between geometric means. Means whose ratios exceed LSR values are significantly different ($P = 0.05$).

[h] Coefficient of variation.

In 1992 and 1993, plots were harvested 60 to 70 days after midsilk and dried in a forced air dryer for 3 days. Due to the extremely high aflatoxin concentrations from kernels within the inoculated area in 1991, kernels were shelled from entire ears (15 to 25 ears/plot) and bulked.

Subsamples for kernel plating were obtained using a Borno divider. One hundred whole kernels from each plot in 1992 and fifty whole kernels from each plot in 1993 were arbitrarily selected, surface sterilized in a 1.6% sodium hypochlorite (30% commercial bleach) solution for one minute, and rinsed in sterile water. Kernels were plated on malt salt agar (10 kernels/plate). After incubation for 7 to 10 days, the percentage of kernels from which *A. flavus* grew was recorded. The remainder of the bulked corn grain was ground for aflatoxin analyses.

Quantification of aflatoxin. In 1991, samples were extracted (10) and analyzed for aflatoxin B_1 using high pressure liquid chromatography (25) at the Veterinary Medicine Center at the University of Illinois, Urbana.

In 1992, aflatoxin B_1 assays were done by E. H. Gendloff at the University of Wisconsin-Madison. An indirect competitive enzyme-linked immunosorbent assay (ELISA) was used as follows: 0.5 g of ground grain was weighed into disposable plastic cups, and the samples were extracted with 70% methanol and stored at 0°C. Wells of Nunc Maxisorp Immunoplates (Nunc Intermed, Rosklide, Denmark) were coated with 100 µl of a 2,000-fold dilution of aflatoxin B_1–bovine serum albumin (BSA) conjugate (Sigma Chemical Co., St. Louis, Mo.) in carbonate-bicarbonate coating buffer (5) 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-buffered saline (PBS) (5). After incubating and washing, 50 µl of sample or standard (diluted in PBS) was added, followed by 50 µl of anti-rabbit (aflatoxin B_1–keyhole limpet hemocyanin [KLH], Sigma), which had been previously diluted 2,000-fold in the above 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 (5). All samples were run in triplicate. Data were analyzed to quantify aflatoxin in the samples as described by Gendloff (8). The ELISA procedure routinely detected aflatoxin in the sample down to 2 ng/ml.

In 1993, aflatoxin B_1 assays were done at the University of Illinois. An indirect competitive ELISA technique was used again. One-half gram of ground grain 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, 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 PBST [PBS Tween: 0.01 mol/liter sodium phosphate buffer containing 0.15 mol/liter of NaCl, ph 7.4, with 0.05%, vol/vol, Tween 20]) of sample or standard were added, followed by 50 µl of a 6,000-fold dilution of anti-rabbit (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 reac-

Table 3. Mean ear rot and aflatoxin values, and percentage of corn kernels infected by *Aspergillus flavus* of inbreds pinboard inoculated in 1992 and 1993 at Urbana, Ill.

	1992[a]			1993[a]		
Inbred	Ear rot[b]	Aflatoxin[c] (ng/g)	Kernel infection (%)[d]	Ear rot	Aflatoxin (ng/g)	Kernel infection (%)
B37Ht2	–[e]	–	–	3.76	283	20
B40	2.63	1,052	19	4.23	90	29
B73	4.12	430	26	4.76	492	31
B9	5.18	442	29	4.68	261	31
CH66-17	5.75	723	19	4.20	108	45
CI2	2.20	14	13	1.89	33	16
CO158	6.25	1,259	31	5.71	186	62
FR809	3.80	260	20	4.33	343	35
F486	2.61	2,971	25	3.86	259	50
H103	2.93	300	4	3.57	178	45
KYS	4.67	10	–	2.88	310	20
KY58	3.31	39	11	2.72	208	36
LB31	2.18	52	16	2.71	44	41
L317	2.76	29	45	4.79	232	53
MAS:gk[f]	–	–	–	4.28	74	46
MAS:pw,nf[f]	–	–	–	4.35	189	29
Mo17	3.25	318	8	3.28	79	60
MP420	–	–	–	2.68	47	24
MS214	3.26	57	34	3.07	56	33
MI82	4.54	232	13	4.08	225	20
NC232	3.41	53	17	2.47	44	52
ND363	3.84	95	24	5.59	607	26
N6	2.14	35	21	3.50	63	51
N8	3.16	35	163	3.61	69	33
OH513	3.20	19	–	3.37	99	37
OH516	4.25	7	33	2.15	23	20
SDP031	4.05	174	11	4.64	357	47
SDP262	3.38	177	20	3.50	339	43
SD18	3.19	314	24	4.66	533	52
SP292	4.16	291	22	4.67	619	43
Tex6	1.61	2	9	1.73	9	11
TR213	3.28	428	21	4.12	44	43
T115	3.75	77	14	2.33	39	12
Y7	1.90	26	13	2.95	54	28
33-16	3.23	18	27	5.28	827	37
75-R001	2.60	114	17	4.06	795	44
75-R012	3.40	566	30	3.95	375	40
Mean	3.50	322	20	3.69	238	36
LSD/LSR (0.05)[g]	1.31	22	20	1.16	5	20
CV (%)[h]	23	40	59	28	27	47

[a] Values are a mean of three replicates in 1992 and six replicates in 1993.
[b] Ratings on a 1 to 10 scale: 1 = 10% to 10 = 100% of the inoculated are rotted.
[c] Values are geometric means (antilogarithm of logarithmic means). Measured by indirect competitive enzyme-linked immunosorbent assay. Samples were run in triplicate.
[d] Kernels were plated on malt salt agar, and incubated for 7 to 10 days. One hundred kernels and fifty kernels were plated for each entry in 1992 and 1993, respectively.
[e] Indicates missing data due to unavailability of seed.
[f] Only three replicates were evaluated in 1993.
[g] Least significant difference (LSD) and least significant ratio (LSR) statistics were used to compare between treatment means; LSD values were applicable for the ear rot and kernel infection means and LSR values were applicable for the aflatoxin values. LSR is antilog of LSD of logarithmic data and is appropriate for comparison between geometric means. Means whose ratios exceed the LSR values are significantly different ($P = 0.05$).
[h] Coefficient of variation.

tion was stopped with HCL. Samples were run in triplicate. Optical densities and analyses of data for quantification of aflatoxin were determined as in 1992.

Table 4. Mean ear rot and aflatoxin values of F_1 hybrids pinboard inoculated with *Aspergillus flavus* in 1993 at Starkville, Miss.[a]

Pedigree	Ear rot[b]	Aflatoxin (ng/g)[c]
CI2 × B73	3.48	4,889
FR1064 × LH123	3.82	1,743
H103 × Mo17	3.82	2,840
KYS × B73	3.54	3,658
LB31 × B73	4.00	4,067
LB31 × Mo17	4.76	6,135
L317 × B73	4.44	3,031
MS214 × Mo17	2.71	2,718
N6 × B73	3.54	1,430
N8 × Mo17	4.83	1,465
OH513 × Mo17	5.34	2,806
OH516 × B73	3.38	1,312
Tex6 × Mo17	2.85	128
Y7 × Mo17	2.92	991
75-R001 × Mo17	3.79	4,998
75-R001 × B73	3.56	5,202
P3362	3.66	1,053
MAS:gk	3.78	2,275
MAS:pw,nf	6.50	6,117
Mp420 × Tx601	3.39	21
Mean	3.91	2,844
LSD/LSR (0.05)[d]	0.82	5
CV (%)[e]	15	15

[a] Values were a mean of four replicates.
[b] Ratings on a 1 to 10 scale: 1 = 10% to 10 = 100% of the inoculated area rotted.
[c] Values are geometric means (antilogarithm of logarithmic means). Measured by indirect competitive enzyme-linked immunosorbent assay. Samples were run in triplicate.
[d] Least significant difference (LSD) and least significant ratio (LSR) statistics were used to compare between treatment means for the ear rot and aflatoxin values, respectively. LSR is antilog of LSD of logarithmic data and is appropriate for comparison between geometric means. Means whose ratios exceed LSR values are significantly different ($P = 0.05$).
[e] Coefficient of variation.

1993 Mississippi experiment. To further evaluate F_1 hybrids from the 1991 and 1992 Illinois experiments in a southern environment, 15 F_1 hybrids and five resistant or susceptible hybrids or populations for comparison were evaluated for Aspergillus ear rot and aflatoxin production at the Plant Science Farm, Mississippi State, Miss. (Table 4). The 15 F_1 hybrids were selected from the 1991 and 1992 Illinois F_1 hybrid experiments based on low Aspergillus ear rot ratings and aflatoxin values. The resistant population MAS:gk, the resistant hybrid Mp420 × Tx601, the susceptible population MAS:pw,nf, the susceptible hybrids Com62 (2), and FR1064 × LH123 (a midwestern commercial hybrid) were included for comparison. Experimental plots were planted 7 April 1993 in single-row plots, 5 m long with 20 plants per row. Plots were 0.76 m apart in a randomized complete block experimental design with four replicates.

Inoculum, inoculation technique, and aflatoxin assay for the Mississippi experiment. *Aspergillus flavus* (NRRL 3357) was grown on corn cob grits in 500-ml Erlenmeyer flasks, each containing 50 g of grits and 100 ml of H_2O. After 12 to 14 days, conidia were washed from the surface of the grits with sterile, distilled water containing 2 drops of Tween 20 per 100 ml. Conidia concentrations were estimated with a hemacytometer and adjusted to 1×10^6 conidia/ml with sterile distilled water. Five milliliters of the suspension was injected under the husk leaves of the primary ear 17 to 20 days after midsilk as previously described. Forty days after inoculation, ears were husked, and a visual rating of 1 to 10 was determined for each ear and averaged for each plot. Ears were harvested, dried at 42°C for 7 days, machine shelled, and kernels bulked. Aflatoxin determinations were completed at the University of Illinois using an indirect competitive ELISA technique as previously described.

Statistical analysis. Data were analyzed using SAS software (SAS Institute, Cary, N.C.). Because the aflatoxin data had a wide range of values, including some with nondetectable values (i.e., less than 2 ng/g), the data were transformed to ln(ng/g) to stabilize variances (24). Samples with nondetectable aflatoxin values were recorded as 2 ng/g, and antilogs of the logarithmic means produced geometric means. Analyses of variance were computed for tests of significance for the transformed aflatoxin, ear rot, and kernel infection values between genotypes, years, locations, and their respective interactions. A restricted least significant difference (LSD) test (4) was used to determine differences among ear rot and kernel infection entry means and the least significant ratio (LSR), which is the antilog of the LSD, was computed for comparisons between the aflatoxin geometric means (24). Interaction mean squares were tested by using the appropriate pooled error term. Entries and locations were assumed to be fixed, but years were considered random variables. The year × genotype interaction was significant for the combined Illinois F_1 hybrid and the Illinois inbred experiments; therefore, the LSD, LSR, and coefficient of variation (CV) values are based on error terms from individual years (Tables 1–3). Pearson and Spearman rank correlation coefficients were calculated between the dependent variables at each location within years.

RESULTS

Illinois F_1 hybrid evaluations. In 1991, the F_1 hybrids differed significantly for ear rot ratings in both the Mo17 and B73 experiments. The selected F_1 hybrids differed significantly for aflatoxin content in the wounded kernels, but were nonsignificant in the nonwounded kernels. Ear rot ratings ranged from 1.50 in the MS214 × Mo17

Table 5. Relationship between Aspergillus ear rot percentage, aflatoxin content, and kernel infection in inbred and F_1 hybrid evaluations at Urbana, Ill.

	Correlation coefficient (r)									
					F_1 hybrid evaluations					
	Inbred evaluations[a]				Illinois[b]				Mississippi[c]	
	1992		1993		1992		1993		1993	
Pairs of dependent variables	r_p[d]	r_s[e]	r_p	r_s	r_p	r_s	r_p	r_s	r_p	r_s
Ear rot (%)[f] × aflatoxin (ng/g)	NS[g]	NS	0.58**[h]	0.68**	0.52**	0.73**	0.80**	0.81**	0.59**	0.45*
Ear rot (%) × kernels infected (%)[i]	NS	NS	0.46**	0.39*	NS	NS	NS	0.43**	–[j]	–
Kernels infected (%) × aflatoxin (ng/g)	NS	NS	NS	NS	NS	NS	NS	NS	–	–

[a] Correlation coefficient of the 35 inbreds based on means of three replicates in 1992 and six replicates in 1993.
[b] Correlation coefficient of the 35 F_1 hybrids based on means of three replicates in 1992 and six replications in 1993.
[c] Correlation coefficient of the 20 F_1 hybrids based on means of four replicates.
[d] Indicates Pearson correlation coefficients.
[e] Indicates Spearman correlation coefficients.
[f] Ratings on a 1 to 10 scale: 1 = 10% to 10 = 100% of the inoculated area rotted.
[g] Nonsignificant correlation coefficient.
[h] (*,**) = significant at the 5% and 1% levels, respectively.
[i] Kernels were plated on malt salt agar and incubated for 7 to 10 days. One hundred kernels and fifty kernels were plated for each entry in 1992 and 1993, respectively.
[j] Indicates no kernel-plating assays were done.

and OH516 × B73 F_1 hybrids to 7.38 in the susceptible Com79 (Table 1). The aflatoxin experimental means for the 37 entries were 37,275 ng/g (range of 4,023 to 103,777 ng/g) for the wounded and 2,934 ng/g (range of 398 to 14,617 ng/g) for the nonwounded kernels. Environmental conditions in 1991 were favorable for Aspergillus ear rot and aflatoxin production, because higher than average temperatures and lower than average rainfall occurred. Entries L317 × B73, MS214 × Mo17, OH513 × Mo17, Tex6 × Mo17 and Y7 × Mo17 had low (relative to the experimental mean) aflatoxin values in both wounded and nonwounded kernels. Entry CO158 × B73 had a relatively low (2.50) ear rot rating, but extremely high aflatoxin values (103,600 ng/g for wounded and 5,250 ng/g for nonwounded kernels). Pearson and Spearman rank correlation coefficients were nonsignificant between ear rot and aflatoxin (Table 5).

In the 1992 and 1993 F_1 hybrid experiments, hybrids differed significantly for ear rot, kernel infection, and aflatoxin. Ear rot ratings were relatively consistent between experiments in 1991, 1992, and 1993. The experimental mean of percent kernel infection was higher in 1993 than in 1992. Aflatoxin values were much lower in the 1992 and 1993 F_1 hybrid experiments than in 1991, probably due to kernel sampling from entire ears rather than just from the inoculated area. Mean aflatoxin values were 158 ng/g (range of 4 to 943 ng/g) in 1992, and 99 ng/g (range of 3 to 268 ng/g) in 1993 (Table 2). Environmental conditions were extremely cool and wet during 1993, which may have had a detrimental effect on aflatoxin production. Several of the F_1 hybrids (B9 × Mo17, KY58 × B73, SDP262 × Mo17, and 33-16 × B73) had aflatoxin values higher than the susceptible CO158 × B73 and CO158 × Mo17. In 1992, Tex6 × Mo17 had the lowest aflatoxin value (4 ng/g), but was not significantly different (aflatoxin LSR) from crosses of B40, H103, LB31, MS214, N8, OH513, T115, Y7, and 75-R001 with Mo17 or crosses of LB31 and 75-R001 with B73. In 1993, the Tex6 × Mo17 aflatoxin value was not significantly different from the Y7 × Mo17, KYS × B73, and Mp420 × Tx601 values.

Pearson and Spearman rank correlation coefficients were significant between ear rot and aflatoxin values in the 1992 and 1993 F_1 hybrid experiments (Table 5). The other significant correlation coefficient in the F_1 hybrid experiments was the Spearman rank correlation coefficient between ear rot rating and kernel infection.

Illinois inbred evaluations. Inbreds differed significantly for ear rot, kernel infection, and aflatoxin. Ear rot ratings were consistent between 1992 and 1993, although the kernel infection increased 80% from 1992 to 1993. Mean aflatoxin values were 567 ng/g (range of 10 to 3,983 ng/g) in 1992 and 363 ng/g (range of 50 to 1,799 ng/g) in 1993.

Inbred Tex6 had the lowest ear rot rating and aflatoxin value in both years. Inbreds 33-16, Y7, OH516, OH513, N8, N6, L317, KY58, KYS, and CI2 did not differ significantly from Tex6 for aflatoxin in 1992. In 1993, Tex6 did not differ significantly from inbreds T115, TR213, OH516, NC232, Mp420, LB31, and CI2 for aflatoxin. Inbreds SD18, SDP262, F486, FR809, and 75-R001 consistently had the highest aflatoxin values.

Pearson and Spearman rank correlation coefficients were nonsignificant in the 1992 inbred experiment (Table 5). In the 1993 inbred experiment, Pearson and Spearman rank correlation coefficients were significant for ear rot and aflatoxin, significant for ear rot and kernel infection, and nonsignificant for aflatoxin and kernel infection.

Mississippi F_1 hybrid evaluations. Differences among F_1 hybrids were significant for both ear rot ratings and aflatoxin. The ear rot ratings had a mean of 3.91, with a range of 2.71 in the MS214 × Mo17 F_1 hybrid to 6.50 in the susceptible population MAS:pw,nf (Table 4). The Mississippi location had the highest aflatoxin values, with a mean of 2,844 ng/g (range of 21 to 6,135 ng/g). The drought conditions that existed in Mississippi in 1993 were likely a contributing factor to the higher aflatoxin. Mp420 × Tx601 had the lowest aflatoxin value (21 ng/g), but was poorly inoculated (i.e., very few wounded kernels) due to extremely thick husk coverage. Tex6 × Mo17 and Y7 × Mo17 had the lowest ear rot and aflatoxin values of the F_1 hybrids identified as resistant in experiments in Illinois. Tex6 × Mo17 had the lowest aflatoxin value (128 ng/g) of those genotypes that were wounded, and was significantly different from both Y7 × Mo17 and the resistant MAS:gk. Pearson and Spearman rank correlation coefficients were significant between the ear rot ratings and aflatoxin values (Table 5).

DISCUSSION

A report of a portion of these results has already been presented (3). This paper expands on that report. including results on F_1 hybrids from several years and two locations, and evaluations of the inbreds per se. Inbreds Tex6, Y7, and Mp420 consistently had the highest levels of resistance to *A. flavus* infection (both ear rot and kernel infection) and to aflatoxin production in the Illinois environment when evaluated as inbreds per se or in hybrid combination. The F_1 hybrids of Tex6 and Y7 crossed with the susceptible inbred Mo17 compared quite favorably with Mp420 × Tx601, which is a "resistant × resistant" cross (22), and MAS:gk, a southern adapted, narrow-based population selected by N. W. Widstrom for resistance to aflatoxin and kernel infection (28). Inbreds LB31, CI2, OH513, T115, OH516, N6, and N8 also had moderate to high levels of resistance to Aspergillus ear rot and aflatoxin production in experiments in Illinois, but had high aflatoxin values when evaluated in hybrid combination in Mississippi. With the exceptions of Tex6 and Y7, the inbreds used in the F_1 hybrids evaluated in Mississippi were relatively adapted to the midwest (i.e., short to moderate relative maturities). Inbred Tex6 was selfed from a southern corn variety (Whitemaster, PI401763) from Texas. The derivation of the late-maturing inbred Y7 is unknown. Evaluation of genotypes adapted for the midwestern U.S. may be too severe in a southern environment where environmental conditions are more conducive to Aspergillus ear rot and aflatoxin production following severe wounding by the pinboard inoculator. For example, LB31 × B73 had 19 ng/g and 38 ng/g aflatoxin in 1992 and 1993, respectively, in the Illinois environment (Table 2). When LB31 × B73 was evaluated in Mississippi in 1993 using the same inoculation technique, it had 4,067 ng/g aflatoxin (Table 4). Tex6 × Mo17 had the lowest aflatoxin value of any of the resistant × susceptible entries in Mississippi with 128 ng/g, and consistently had low aflatoxin values as an inbred per se and in crosses with Mo17 in Illinois.

Genotypes with low ear rot ratings generally had lower aflatoxin values. Pearson correlation coefficients were moderately high (r = 0.52 in 1992 and r = 0.80 in 1993) between ear rot ratings and aflatoxin values in the Illinois F_1 hybrid evaluations, and moderately high in the 1993 Illinois inbred (r = 0.58) and Mississippi F_1 hybrid (r = 0.59) experiments. Tucker et al (26) also reported a moderately high Pearson correlation coefficient of r = 0.56 ($P \leq$ 0.01) between Aspergillus ear rot ratings and aflatoxin, in a study of two resistant and two susceptible single cross hybrids. The nonsignificant Pearson correlation coefficient between the ear rot and aflatoxin values in the 1991 F_1 hybrid and 1992 inbred experiments may have been due to the genotypes with low ear rot values and high aflatoxin values. For example, inbreds B40, 75-R012, SD18, and F486 had lower ear rot ratings, but higher aflatoxin values, than the experimental mean. The number of replications also was increased (three in 1992 vs. six in 1993), which may have given a better approximation of the Aspergillus ear rot and aflatoxin values for each genotype, and, consequently, significant correlation coefficients. Generally, Spearman rank correlation coefficients indicated a consistent relationship between ear rot rankings and aflatoxin rankings. In the Illinois F_1 hybrid experiments, Spearman rank correlation coefficients were r_s = 0.73 in 1992 and r_s = 0.81 in 1993 between ear rot and aflatoxin. Genotypes with low rankings for ear rot

(i.e., low ear rot ratings) also had low rankings for aflatoxin (i.e., low aflatoxin values).

Pearson correlation coefficients between kernel infection and aflatoxin were not significant for the 1992 and 1993 Illinois F_1 hybrid and inbred experiments. Other studies have reported moderate to high correlations between kernel infection and aflatoxin. Tucker et al. (26) reported a Pearson correlation coefficient of $r = 0.88$ ($P \leq 0.01$) between kernel infection and aflatoxin when 260 kernels were plated from each of four genotypes. Payne et al. (18) reported a Pearson correlation coefficient of $r = 0.79$ ($P \leq 0.01$) between kernel infection and aflatoxin, when 200 kernels were plated from the commercial hybrid Pioneer 3147. In this study, 100 and 50 kernels were plated for each plot in 1992 and 1993, respectively. This small sample size was possibly a contributing factor to higher error mean squares (22), and may have contributed to the nonsignificant Pearson correlation coefficients between kernel infection and aflatoxin.

Aspergillus flavus is weakly pathogenic and Illinois environments often are less than optimal for disease development. Kernel wounding and introduction of inoculum throughout the wounded area is necessary to develop high levels of infection to classify genotypes accurately in the Illinois environment. The pinboard technique allows screening large numbers of genotypes with sufficient kernel wounding and inoculum introduction throughout the wounded area. To identify resistant genotypes, a large number can be screened for reaction to ear rot, and those with low ratings can be selected for aflatoxin assays, thus reducing the time and expense of aflatoxin assays.

Although mechanical wounding of kernels simulates insect damage, wounding circumvents the silk channel and destroys the kernel aleurone and pericarp. Since the pathogen is allowed direct access into the kernel (via wound sites), two resistance mechanisms are possible: (i) minimize the amount of fungal growth within and between kernels; and/or (ii) inhibit mycotoxin synthesis by biochemical means. Since the pinboard method only identifies resistance that functions in the kernel after wounding, this may cause some genotypes to be incorrectly rated as susceptible when the resistance mechanism is expressed through characteristics of silks, pericarp, or aleurone layer (1,6). Environment, kernel sampling techniques, and the inherent variability of aflatoxin production are all factors that may bias results. Genotypes frequently change ranks for ear rot, kernel infection, and aflatoxin values across replications, locations, and years. Genotype × environment, genotype × year, and genotype × environment × year interactions make accurate and repeatable genotype classification difficult. Because of this variability, genotypes should be evaluated in different environments over years to generate sufficient data to accurately classify genotypes as resistant, moderately resistant, etc.

The objective of our study was to identify sources of resistance with alleles not found in B73 or Mo17. By evaluating both the F_1 hybrids with the susceptible inbreds B73 and/or Mo17, and the inbreds per se, we were better able to identify genotypes that consistently had the highest levels of resistance to Aspergillus ear rot and aflatoxin production. With inoculations, these inbreds and/or F_1 hybrids may have higher than acceptable aflatoxin contamination; however, this level of resistance may be sufficient with natural infection. The efficacy of the inoculation technique will be validated when inbreds developed from breeding programs using inoculation for evaluation and selection are found to be resistant in commercial hybrids under natural conditions favorable for Aspergillus ear rot and aflatoxin production.

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