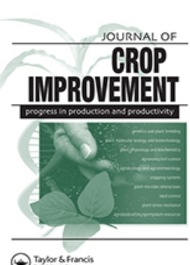

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Field evaluation of resistance to aflatoxin accumulation in maize inbred lines in Kenya and South Africa

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ABSTRACT

Aflatoxin, a carcinogenic toxin, is produced mainly by Aspergillus flavus and Aspergillus parasiticus. Contamination of maize (Zea mays L.) grain by these fungi occurs before harvest, and the easiest strategy to prevent this is to develop/use maize varieties resistant to Aspergillus spp. and aflatoxin accumulation. The objective of this investigation was to identify potential sources of resistance among 23 maize inbred lines (13 obtained from the MAIZE Competitive Grants Initiative, International Maize and Wheat Improvement Centre and 10 from Agricultural Research Council, South Africa). The inbred lines were planted in a randomized complete-block design at two locations each in Kenya and South Africa. Maize ears were inoculated at silking with three toxigenic strains of A. flavus. The inoculated ears in each plot were harvested at 12–18% moisture, dried, and visually assessed for Aspergillus ear rot (AER). Aflatoxin concentration in the kernels was determined using liquid chromatography–tandem mass spectrometry. Significant variation for both AER and aflatoxin concentration existed among the inbred lines at both locations in Kenya and one location in South Africa. Combined analysis revealed a significant ($p < 0.001$) lines × locations interaction for both AER and aflatoxin concentration. Higher incidences of AER (0–86.0%) and aflatoxin concentration (0.21–6.51 µg/kg) were recorded at Kiboko in Kenya than at the other three locations. A stronger genetic correlation ($r_G = 0.936$, $p < 0.0001$) between the AER and aflatoxin concentration was recorded in Potchefstroom than at the other three locations. Repeatability of aflatoxin concentration was high at Kiboko (0.87) and Potchefstroom in South Africa (0.74). Three inbred lines, CML247, CML444, and CML495, emerged as potentially useful sources of resistance to AER and aflatoxin accumulation as they showed low levels of aflatoxin contamination in both localities in Kenya and in South Africa.

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Introduction

Maize (*Zea mays* L.) is the major staple food crop in Kenya grown at various altitudes, from the coastal strip to >1600 m above sea level in the highlands, with an annual production of 4 million metric tons of grain (Gov. of Kenya, 2016; Snipes and Kamau 2013). Maize is grown mainly by small-scale farmers, located in the Rift Valley and Western regions, who produce >75% of the total maize in Kenya. About 95% of the maize produced in the country serves the subsistence needs, with per capita consumption of 98 kg per year and is sold in domestic markets (Gov. of Kenya (Government of Kenya) 2016; Snipes and Kamau 2013). Maize also serves as a staple food in several other eastern and southern African countries, of which South Africa is the main producer of maize, with an annual production of 10–12 million metric tons (BFAP, 2011; DAFF (Department of Agriculture, Forestry and Fisheries, South Africa) 2016). Commercial growers, however, produce most of the South African maize. Maize production in East Africa is often adversely affected by two fungi, *Aspergillus flavus* and *Aspergillus parasiticus*, which cause *Aspergillus* ear rot (AER) and liberate toxic secondary metabolites known as aflatoxins.

Following the consumption of maize contaminated with *A. flavus* and aflatoxins, several cases of aflatoxicosis were reported from Kenya. In 1981, an outbreak of aflatoxicosis occurred following a severe drought followed by heavy rains during the harvest (Ngindu et al. 1982). The worst outbreak of the disease in Kenya was in 2004 when 317 cases were reported and 125 people died in the Makueni and Kitui districts in eastern Kenya (Probst, Njapau, and Cotty 2007). Since then, cases of aflatoxicosis among people have been reported every year because of high levels of aflatoxins in their predominantly maize-based diet. Chronic exposure to aflatoxins is more serious than acute aflatoxicosis (Lauren et al. 2005; Okoth et al. 2012a; Okoth and Kola, 2012b). Aflatoxin B1 (AFB_1) is the most potent naturally occurring carcinogen known (Liu and Wu 2010; Squire 1989).

The production of aflatoxins starts in the field soon after maize kernels are colonized by *A. flavus* and/or *A. parasiticus*. Aflatoxin continues to accumulate during kernel maturation. The Kenya Bureau of Standards has set 10 μg/kg as the acceptable limit of total aflatoxins in maize for both human and animal consumption (Brown et al. 2013b; KBS (Kenya Bureau of Standards) 2013), which is lower than the limit of 20 μg/kg set by the United States Food and Drug Administration. Testing of food and feed quality in Kenya, however, has not been effective because of the high cost of mycotoxin analyses and poor infrastructure to evaluate food quality. Most foodstuffs and feeds sold in local markets do not go through any quality control measures. While Kenya records regular occurrence of aflatoxicosis, high fumonisin levels predominate in South Africa (Ncube et al. 2011).

Management of aflatoxins in the maize value chain includes prevention of fungal infection in the field and growth of the fungus in maize kernels, inhibition of aflatoxin production, and the degradation of aflatoxins in the grains (Bandyopadhyay et al. 2016; Maina et al. 2016). In Kenya, proper and affordable storage facilities and the use of non-toxigenic *A. flavus* strains (ACDI-VOCA 2015; Bandyopadhyay et al. 2016; Ehrlich 2014) are currently being evaluated for aflatoxin management. Host–plant resistance is considered a desirable and cost-effective approach (Williams 2006; Williams et al. 2014), and inbred lines with pre-harvest resistance to *A. flavus* have been identified by several researchers (Menkir et al. 2008; Scott and Zummo 1988; Widstrom 1996; Widstrom, McMillian, and Wilson 1987; Zuber et al. 1978).

The application of new tools of proteomics and genomics in identification of resistance mechanisms is a positive step toward the pursuit of significant resistance in maize (Henry, Gary, and Michael 2012; Brown et al. 2013a; 2013b; Williams et al. 2014; Williams and Windham 2015). However, no commercial maize varieties with resistance to aflatoxin accumulation have been released because some of the resistant parental maize inbred lines currently available generally do not possess acceptable agronomic attributes. The search therefore continues to identify new sources of resistance to aflatoxin contamination in adapted maize germplasm. Warburton and William (2014) provide a comprehensive analysis of the progress made in the pursuit for aflatoxin resistance. Resistance in most of the lines that had been earlier identified as resistant and used in breeding was highly dependent on the environment (Scott and Zummo 1988; Widstrom, Wilson, and McMillian 1984, 1986; Zuber et al. 1983). Some of the newer germplasms evaluated for resistance to aflatoxin showed more or less repeatable resistance under varying environments (Gorman et al. 1991; Guo et al. 2001; McMillian, Widstrom, and Wilson 1993; Walker and White 2001; Williams and Windham 2001) and possessed favorable agronomic traits (Betran, Isakeit, and Odvody 2002; Li et al. 2002; Williams and Windham 2006). However, transferring the resistance genes from these lines into elite cultivars has been less than effective because of the large number of genes involved (Cary et al. 2011). Some of these genes reportedly had only a small effect; and resistance imparted by some others was environment-dependent, which hampered progress from breeding for resistance to aflatoxin contamination of maize grain. Identification of quantitative trait loci (QTL) conditioning resistance to aflatoxin accumulation could aid in developing aflatoxin-resistant maize using marker-assisted selection (Alwala et al. 2008; Kelley et al. 2012; Warburton et al. 2013; Willcox et al. 2013; Zhang, Kang, and Magari 1997). A pool of lines showing resistance could not only increase breeder's options for germplasm improvement but also provide material for use in additional QTL mapping experiments.

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Most maize inbred lines developed in Africa possess desirable traits, such as yield, good husk cover, and stress tolerance but with no consideration of susceptibility to aflatoxin accumulation (Brown et al. 2016). The objective of this study was to screen selected, adapted maize inbred lines in Kenya and South Africa to identify those with resistance to *Aspergillus* infection and aflatoxin accumulation. Such material should be useful for establishing breeding populations and developing *A. flavus*-resistant germplasm/cultivars.

Materials and methods

Germplasm

Twenty-three maize inbred lines of diverse origin (mid-altitude Africa and lowland/sub-tropical adaptation) were used in this study (Table 1). These included 13 maize inbred lines from the MAIZE Competitive Grants Initiative, International Maize and Wheat Improvement Centre (CIMMYT) Kenya and 10 inbred lines from the Agricultural Research Council's Grain Crops Institute (ARC-GCI) in Potchefstroom, South Africa. The lines from Kenya had been observed to exhibit low AER severity under field conditions (Dr. D. Makumbi, personal communication), and they were also characterized for resistance to *Fusarium* ear rot (FER) and fumonisins (Rose et al. 2017). The lines from South Africa were characterized as resistant to FER and fumonisins (Rose et al. 2016, 2017; Small et al. 2012).

Field locations, experimental design, and management

The trials were conducted in Kenya at the Kenya Agricultural and Livestock Research Organization stations at Katumani (1°35'S, 37°14′E, 1600 m asl) and Kiboko (37°75′ E, 2°15′ S; 975 m asl) in 2013. These stations are situated in Machakos County in the semi-arid eastern Kenya, which is considered an aflatoxin hot spot (Lauren et al. 2005). The region has bimodal rainfall, with long rains falling in March–May and short rains from October to December/January. The long rainy season is characterized by prolonged heavy rainfall compared with the short rains that occur for a few weeks in the season. At Kiboko, the average rainfall from December to May was 75.2 mm, and maximum and minimum temperatures were 31.5°C and 18.3°C, respectively. The average rainfall, and maximum and minimum temperatures measured at Katumani were lower (2.5 mm, 26.4°C, and 15.0°C, respectively) during the same time period.

Field trials in South Africa were conducted at Potchefstroom (26°73'S, 27°07′E; 1349 m asl) in the North West province and at Vaalharts (27° 95'S, 24°83′E; 1180 m asl) in the Northern Cape province during the 2012/2013 maize-growing season. At Potchefstroom, the average rainfall,

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and maximum and minimum temperatures from December to May were 97.3 mm, 27.5°C, and 12.5°C, respectively. The average rainfall measured at Vaalharts was lower (75.3 mm), whereas the mean maximum temperature was higher (30.6°C) during the same period. The experiments were conducted using a randomized complete-block design with three replications at all four locations. Each entry was planted in a single 10-m-long plot. The inter-row spacing was 0.90 m, whereas intra-row spacing was 0.30 m. Four border rows of a commercial maize hybrid were planted around each field at each location. Standard agronomic practices, including application of irrigation water and fertilizer, were followed as recommended for each location.

Table 1. List of maize inbred lines, their origin, and characteristics.

Line	Name/pedigree	Origin†	Key characteristics
1	CB-222	ARC	Mid-altitude (MA) adaptation, tolerant to Fusarium ear rot (FER), white grain
2	CB-248	ARC	MA adaptation, tolerant to FER, white grain
3	CKL05003	CIMMYT	MA adaptation, turcicum leaf blight (TLB), and grey leaf spot (GLS) tolerant, semi-dent, white grain
4	CKL05015	CIMMYT	MA adaptation, TLB, and maize streak virus (MSV) tolerant, semi-flint
5	CKL05019	CIMMYT	MA adaptation, intermediate maturity, TLB, and GLS tolerant, flint, white
6	CKL05022	CIMMYT	MA adaptation, TLB, and GLS tolerant, flint, white
7	CML182	ARC	Subtropical adaptation, quality protein maize line, dent, white
8	CML247	CIMMYT	Lowland tropical (LT) adaptation, GLS tolerant, semi-dent, white
9	CML264	CIMMYT	LT adaptation, flint, white
10	CML390	ARC	MA adaptation, MSV tolerant, flint, white
11	CML442	CIMMYT	MA adaptation, drought tolerant, dent, white, susceptible to ear rot
12	CML444	CIMMYT	MA adaptation, TLB and GLS tolerant, drought tolerant, semi-dent, white
13	CML495	CIMMYT	LT adaptation, flint, white
14	I137tnW	ARC	MA adaptation, susceptible to FER, white grain
15	La Posta Seq C7-F103-2-1-1-1xMIRTC5 Bco F80-4-2-1-1-1-3-1-B-B-B	CIMMYT	LT adaptation, drought-tolerant background
16	MIRTC5 Bco F78-2-2-1-1-1xDERRc2 15-3-7-1-1-B-B-B-B	CIMMYT	LT adaptation, semi-flint, white
17	P502c2-185-3-4-2-3-B-2-B-B-B-B-B-B	CIMMYT	LT adaptation, semi-dent, white
18	R119W	ARC	MA adaptation, susceptible to FER, white grain
19	R2565Y	ARC	MA adaptation, susceptible to FER, white grain
20	RO549W	ARC	MA adaptation, tolerant to FER, white grain
21	US2540W	ARC	MA adaptation, tolerant to FER, white grain
22	VL06688	CIMMYT	MA adaptation, semi-dent, white susceptible to ear rot
23	VO617y-2	ARC	MA adaptation, tolerant to FER, white grain

†ARC = Agricultural Research Center, South Africa; CIMMYT = International Maize and Wheat Improvement Center.

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Inoculum preparation

Three *A. flavus* isolates (V201365, V100130, and V100095) from the Makueni and Nandi districts (Okoth et al. 2012b) were used as inoculum for the field trials conducted in Kenya. For inoculation of the field trials in South Africa, three toxigenic strains of *A. flavus* (MRC 3951, MRC 3952, and MRC 3954) were obtained from the Medical Research Council's Programme on Mycotoxins and Experimental Carcinogenesis unit (MRC-PROMEC, Tygerberg, South Africa).

The inoculum for field trials was prepared by growing the *A. flavus* isolates on sterile maize kernels. The kernels (50 g) were first soaked in 25-mL sterile distilled water in 250-mL conical flasks for 6 hr or overnight, after which they were autoclaved at 120°C for 40 min. The isolates were transferred onto the kernels in the conical flasks, mixed well, and incubated at 30°C. To prevent clumping, the conical flasks were shaken once daily. After 7–14 days, fungal conidia were washed from the kernels with a soap solution consisting of 40 µL Tween20 added to 20-mL H_2O. The suspension was thereafter sieved using sterile double cheesecloth, and the conidia were collected in a beaker. The concentration of the conidia was then determined with a hemocytometer, and for inoculation purposes, a concentration of 1×10^6 conidia per mL was prepared using sterile distilled water. Inoculum of each of the three isolates was raised separately and only mixed a few minutes before inoculating maize ears. Equal quantities of the three *A. flavus* isolates per country were mixed thoroughly before field inoculations were conducted. Before use, the conidial suspension was refrigerated at 4°C for a maximum of 72 hr, and the capped plastic bottles were submerged in ice during use in the field.

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Field inoculation

The primary maize ear on each plant was inoculated using the silk-channel method (Zummo and Scott 1989). Inoculation was done after at least 50% of the individual plants in a plot had emerged silks and when the silk length was at least 2.5 cm. The ears were inoculated once. Two milliliters of the well-mixed conidial suspension was slowly injected into each maize ear through the silk channel using a 10-mL syringe and sterile needle (gauge 18). Because the inbred lines were of diverse maturity, inoculation of different lines was staggered accordingly. Two inbred lines (CML442 and VL06688), characterized as susceptible to AER and aflatoxin accumulation under natural infection were used as positive controls. All inoculated ears were labeled for identification at harvest.

Assessment of AER Rating

Inoculated ears in each plot were harvested by hand at 12–18% moisture, determined using a field moisture meter (Superpro®, Supertech Agroline,

Bogense, Denmark). The AER was visually estimated using a method adapted from Henry et al. (2009). Per ear percentage ear rot (the area of a rotten ear) was determined using the following scale: 0% = no ear rot symptoms, 25% = rot covering a quarter of the ear, 50% = rot covering half of the ear, 75% = rot covering three quarters of the ear, and 100% = rot covering the entire ear. The average AER per plot was then calculated. The ears from each plot were bulked and hand shelled. The grains were then thoroughly mixed and a 250-g sample was collected, milled, and stored at 4°C until aflatoxin extraction was performed.

Aflatoxin quantification

Aflatoxin content in maize inbred lines was determined by the dilute-and-shoot method using liquid chromatographic tandem mass spectrometry (LC-MS/MS). Aflatoxins were extracted from subsamples of 5 g each according to Rose et al. (2016) and submitted to the Central Analytical Facility at Stellenbosch University, South Africa, for the quantification of AFB_1 and AFB_2. A mixture of aflatoxins B and G, guaranteed 95% pure, was purchased from Sigma Aldrich (St. Louis, Missouri, USA) to serve as standards. Ten percent of the total number of samples was evaluated in triplicate (three samples per plot) to determine the sample variation for aflatoxin content. Additionally, 10% of the samples in each LC-MS/MS assay was analyzed in triplicate to determine the within-assay variation. A dilution series, ranging from 0.15×10^{-4} to 0.38 µg/kg for AFB_1 and AFG_1 and 0.44×10^{-5} to 0.11 µg/kg for AFB_2, and 0.83×10^{-5} and 0.08 µg/kg for AFG_2, was analyzed with field trial samples. Each standard and sample (5 µL) was injected into the LC-MS/MS system, and samples with results above the calibration curve limit were diluted with 70% methanol and reanalyzed.

Data analysis

The data on AER and aflatoxin content were log-transformed [$\ln(y + 1)$] to normalize data before conducting analyses of variance (ANOVA). Individual location and across locations ANOVA were conducted using PROC GLM of SAS (SAS Institute 2011). Transformed means were back-transformed to original values for reporting. Means were separated using Fisher's Protected LSD (Lentner and Bishop 1986). Repeatability (R) was calculated as the proportion of genetic variance divided by the total phenotypic variance and estimated for each individual trial as

$$R = \sigma_g^2 / \left[\sigma_g^2 + (\sigma_e^2 / r)\right]$$

where σ_g^2 is the genotypic variance, σ_e^2 is the residual variance, and r is the number of replications. Repeatability for AER and aflatoxin content and the

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genotypic correlation between the two traits were estimated using META-R software (Alvarado et al., 2016).

Results

AER Infection

ANOVA revealed significant differences among the inbred lines for AER at three of the four locations (Table 2). Lines × locations interaction for AER was significant ($p < 0.001$). AER expression was higher at the Kenyan locations compared with the South African locations (Table 3). The AER severity was higher in the majority of lines at Kiboko compared with Katumani in Kenya; whereas at Potchefstroom and Vaalharts, the lines expressed almost similar AER severity. Lines that showed the least AER in both countries were CML390, CKL05022, CML247, and P502c2-185-3-4–2-3-B-2-B*5 (P502c2). Lines that showed some of the highest AER severity at different locations in both countries were CB248 RO549W, CML264, VL06688, CML442, and CKL05003. Lines CML182, R119W, RO549W, and VO617y-2 had higher AER than others in Potchefstroom. Repeatability for AER ranged from 0.41 at Vaalharts to 0.87 at Katumani.

Response of maize inbred lines to A. flavus and genotypic correlation between traits

The mean aflatoxin content in maize inbred lines varied significantly ($p < 0.001$) at three locations and across countries (Table 2). Locations and lines × locations interaction were significant ($p < 0.001$) for aflatoxin content. Aflatoxin content was higher in the inbred lines at Kiboko compared with Katumani and Potchefstroom (Table 4). Inbred lines with significantly higher

Table 2. Mean squares from analysis of variance for *Aspergillus* ear rot rating (AER) and aflatoxin content at four locations and across locations in Kenya and South Africa in 2013.

Source of variation	*df*	Katumani	Kiboko	Potchefstroom	Vaalharts	*df*	Across locations
				AER rating (%)			
Loc						3	29.209***
Rep(Loc)						8	0.332ns†
Line	22	1.680***	4.979***	0.191*	0.212ns	22	3.269***
Line × Loc						62	1.333***
Error	44	0.222	0.873	0.081	0.125	168	0.336
				Aflatoxin (AFB_1 and AFB_2) content (µg/kg)			
Loc						3	11.229***
Rep(Loc)						8	0.003ns
Line	22	0.001**	0.805***	2.018E-04***	0.006ns	22	0.200***
Line × Loc						62	0.217***
Error	44	3.45E-04	0.104	5.296E-05	0.003	168	0.028

*. **, ***Significant at 0.05, 0.01, and < 0.001 levels, respectively. †ns = not significant.

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Table 3. *Aspergillus* ear rot (AER) rating in maize inbred lines evaluated in different localities in Kenya and South Africa in 2013.

	Aspergillus ear rot rating (%)†			
	Kenya		South Africa	
Inbred line	Katumani	Kiboko	Potchefstroom	Vaalharts
CML390	1.47 ± 0.39	1.00 ± 0.54	1.06 ± 0.17	1.14 ± 0.23
CML444	1.00 ± 0.27	3.65 ± 1.97	1.26 ± 0.21	1.58 ± 0.32
CML182	1.68 ± 0.46	3.88 ± 2.09	2.02 ± 0.33	1.10 ± 0.22
VO617y-2	1.75 ± 0.48	2.91 ± 1.57	1.68 ± 0.28	1.12 ± 0.23
RO549W	6.46 ± 1.75	10.07 ± 9.47	1.87 ± 0.31	2.54 ± 0.52
US2540W	1.00 ± 0.27	17.56 ± 3.63	1.25 ± 0.21	2.33 ± 0.48
R119W	1.00 ± 0.27	6.74 ± 3.63	1.89 ± 0.31	1.04 ± 0.21
CB248	5.50 ± 1.49	66.46 ± 35.85	1.90 ± 0.31	1.00 ± 0.20
CB222	5.97 ± 1.62	2.28 ± 1.23	1.08 ± 0.18	1.37 ± 0.28
I137tnW	1.00 ± 0.27	11.78 ± 6.35	1.52 ± 0.25	1.19 ± 0.24
R2565y	2.52 ± 0.68	2.73 ± 1.47	1.63 ± 0.27	1.71 ± 0.35
CKL05003	5.85 ± 1.59	14.16 ± 7.64	1.05 ± 0.17	1.66 ± 0.34
CKL05015	1.82 ± 0.49	7.79 ± 4.20	1.05 ± 0.17	1.45 ± 0.30
CKL05019	1.00 ± 0.27	16.33 ± 8.81	1.00 ± 0.16	1.12 ± 0.23
CKL05022	1.21 ± 0.33	1.00 ± 0.54	1.00 ± 0.16	1.21 ± 0.25
CML247	2.43 ± 0.66	1.00 ± 0.54	1.07 ± 0.18	1.02 ± 0.21
CML495	1.13 ± 0.31	4.66 ± 2.51	1.01 ± 0.17	1.30 ± 0.26
CML264	1.04 ± 0.28	33.07 ± 17.84	1.07 ± 0.18	1.18 ± 0.24
MIRTC5	1.69 ± 0.46	4.08 ± 2.20	1.00 ± 0.16	1.43 ± 0.29
P502c2	1.60 ± 0.44	1.00 ± 0.54	1.25 ± 0.21	1.00 ± 0.20
LaPosta	1.17 ± 0.32	3.32 ± 1.79	1.25 ± 0.21	1.00 ± 0.20
CML442	9.30 ± 2.53	16.77 ± 9.05	NP‡	NP
VL06688	6.00 ± 1.63	86.99 ± 46.92	NP	NP
Mean	2.05	13.88	1.29	1.31
$LSD_{(0.05)}$	2.17	4.65	1.60	1.79
Repeatability	0.87	0.82	0.57	0.41

†Values are mean ± SE.
‡Not planted.

aflatoxin levels than the positive control inbred lines (VL06688 and CML442) at Kiboko were P502c2, CKL05015, CKL05003, and I137tnW (Table 4). Lines CB222, CML247, CML444, CML495, LaPostaSeqC7-F103-2-1-1-1xMIRTC5 BcoF80-4-2-1-1-1-3-1-B*5 (LaPosta), and CML390 accumulated less aflatoxin than the positive controls. Repeatability for aflatoxin content ranged from 0.39 at Vaalharts to 0.87 at Kiboko. A significant ($p < 0.001$) genotypic correlation between AER severity and aflatoxin content was detected at Katumani and Potchefstroom but not at the other two locations (Table 5). A stronger genotypic correlation between the two traits ($r_G = 0.936$, $p < 0.0001$) was found at Potchefstroom than at the other three locations.

Discussion

Screening germplasm for resistance to aflatoxin accumulation under field conditions provides an opportunity to select superior germplasm. Field screening for resistance to aflatoxin accumulation, however, is difficult

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Table 4. Aflatoxin accumulation in inoculated ears of maize inbred lines evaluated in different localities in Kenya and South Africa in 2013.

	Aflatoxin (AFB_1 and AFB_2) content (µg/kg)[†]			
	Kenya		South Africa	
Inbred line	Katumani	Kiboko	Potchefstroom	Vaalharts
CML390	1.01	1.56 ± 0.29	1.00	1.00 ± 0.00
CML444	1.00	1.47 ± 0.28	1.00	1.00 ± 0.03
CML182	1.03	3.40 ± 0.63	1.00	1.03 ± 0.03
VO617y-2	1.00	2.16 ± 0.40	1.02	1.00 ± 0.03
RO549W	1.02	3.10 ± 0.58	1.00	1.10 ± 0.04
US2540W	1.02	4.07 ± 0.76	1.00	1.04 ± 0.03
R119W	1.00	1.79 ± 0.33	1.02	1.00 ± 0.03
CB248	1.06	1.63 ± 0.30	1.03	1.12 ± 0.04
CB222	1.06	1.21 ± 0.23	1.00	1.00 ± 0.03
I137tnW	1.00	5.88 ± 1.10	1.01	1.01 ± 0.03
R2565y	1.00	1.60 ± 0.30	1.01	1.08 ± 0.04
CKL05003	1.00	5.51 ± 1.03	1.00	1.18 ± 0.04
CKL05015	1.00	6.26 ± 1.17	1.00	1.00 ± 0.03
CKL05019	1.00	1.98 ± 0.37	1.00	1.06 ± 0.04
CKL05022	1.00	1.56 ± 0.29	1.00	1.03 ± 0.03
CML247	1.00	1.28 ± 0.24	1.00	1.07 ± 0.04
CML495	1.00	1.46 ± 0.27	1.00	1.06 ± 0.04
CML264	1.00	1.63 ± 0.30	1.00	1.00 ± 0.03
MIRTC5	1.00	2.04 ± 0.38	1.01	1.02 ± 0.03
P502c2	1.00	4.72 ± 0.88	1.00	1.05 ± 0.04
LaPosta	1.00	1.50 ± 0.28	1.00	1.02 ± 0.03
CML442	1.00	2.85 ± 0.53	NP[‡]	NP
VL06688	1.00	2.63 ± 0.49	NP	NP
SE	0.01	–	0.01	–
Mean	1.01	2.32	1.01	1.04
$LSD_{(0.05)}$	1.03	1.70	1.01	1.10
Repeatability	0.62	0.87	0.74	0.39

[†]Values are mean ± SE.
[‡]Not planted.

Table 5. Genotypic correlations (r_G) between AER rating and aflatoxin (AFB_1 and AFB_2) content in different locations in Kenya and South Africa.

Country	Location	Genetic correlation
Kenya	Katumani	$r_G = 0.544$, $p = 0.007$
Kenya	Kiboko	$r_G = 0.300$, $p = 0.163$
South Africa	Potchefstroom	$r_G = 0.936$, $p < 0.0001$
South Africa	Vaalharts	$r_G = 0.080$, $p = 0.730$

because *Aspergillus* is not uniformly distributed, but this difficulty is overcome by use of artificial inoculation. Significant variation among inbred lines in this study indicated the presence of sufficient genetic variation for both AER and aflatoxin accumulation, which meant that this genetic variability can be exploited for developing cultivars resistant to AER and aflatoxin accumulation in the maize grain. The consistently high repeatability for the two traits at the Kenyan locations suggested

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that it would be possible to make selection progress for these two traits. The repeatability for aflatoxin accumulation recorded in this study was within the range reported by Betrán et al. (2006) in a study involving quality protein maize (QPM) inbred lines and testcrosses but lower than that reported by Chiuraise et al. (2016) in a study involving mid-altitude maize in South Africa. The range of repeatability for AER recorded in this study was similar to that reported by Chiuraise et al. (2016).

Inbred lines with resistance to *A. flavus* infection and subsequent accumulation of aflatoxin were identified in this study. Six inbred lines (CB222, CML247, CML444, CML495, LaPosta, and CML390) were relatively resistant to *A. flavus* infection at both Katumani and Kiboko. Kiboko recorded higher rainfall and temperature during the season, which promoted significantly more AER development and aflatoxin accumulation when compared with Katumani in Kenya. These results should be viewed with caution because disease development was not uniform at all locations. Resistance or susceptibility of all the inbred lines included in the study should be validated in further field tests and perhaps with the use of two different inoculation techniques. The side-needle inoculation technique might be a good choice as it has been reported to be effective in germplasm evaluation (Windham and Williams 2002). Additionally, the kernel screening procedure developed by Li and Kang (2005) could be utilized to validate genotype reaction before declaring lines as resistant to *A. flavus* in breeding programs targeting both fungal infection and aflatoxin accumulation. However, it is encouraging to note that inbred line CML247, with low aflatoxin accumulation and low AER in this study, was also reported as resistant in a different study (Williams et al. 2014). This indicates that this line certainly holds promise as a source of resistance to aflatoxin accumulation. CML247 would be an important addition to African-adapted germplasm reported to be resistant to aflatoxin accumulation (Brown et al. 2016; Chiuraise et al. 2016; Menkir et al. 2008). It is also interesting to note that in another study (Rose et al. 2016), two lines (CML444 and CML390) possessing resistance to aflatoxin accumulation were found to be resistant to FER and fumonisin accumulation, whereas line CB222 was found to be tolerant to fumonisin accumulation. Therefore, these lines, if confirmed as resistant, could be a good resource for improving resistance to aflatoxin accumulation. Robertson-Hoyt et al. (2007) reported that genes responsible for resistance to ear rots are genetically linked to those responsible for resistance to mycotoxin contamination.

In the present study, there was a significant lines × locations interaction for both AER and aflatoxin accumulation. This suggested that the inbred lines responded differently to inoculation with *A. flavus* at the different locations and that resistant lines would need to be identified for each location. The presence of significant genotypes × locations interaction is common for aflatoxin accumulation (Betrán et al. 2006; Giorni, Bertuzzi, and Battilani 2016; Williams et al.

2011; Williams, Windham, and Buckley 2008). Differences in genotypic response across locations may be a result of different climatic/environmental conditions experienced at the different locations. A dry and hot climate favors *A. flavus* infection and aflatoxin accumulation (Chauhan, Wright, and Rachaputi 2008; Cotty and Jaime-Garcia 2007; Fountain et al. 2015, 2014; Windham and Williams 2002). Widstrom et al. (1990) noted that aflatoxin concentration was highly correlated with minimum and maximum daily temperatures and daily evapotranspiration after silking. It is also possible that inoculation carried out on separate days might have caused some variation because of differing environmental conditions experienced by lines after inoculation (Williams et al. 2011). The climatic conditions at the two South Africa locations seemed unfavorable for *A. flavus* growth and aflatoxin production. However, locations with favorable climate for AER development and aflatoxin accumulation have been reported in South Africa (Chiuraise et al. 2016). The low disease severity in South Africa could also be attributable to differences in virulence of the different strains used in the two countries. The significant lines × locations interaction might also indicate the need to develop region-specific maize germplasm that is resistant to aflatoxin accumulation. In general, genotype variability across locations would make breeding for reduced aflatoxin accumulation a challenge. There was a moderately to strongly significant genotypic correlation between AER and aflatoxin accumulation at two locations in this study (Katumani $r_G = 0.544$, $p = 0.007$; Potchefstroom $r_G = 0.936$, $p < 0.0001$). The strong genotypic correlation suggested that selection of lines for reduced AER should result in reduced aflatoxin accumulation among the lines. This result supported the findings of Robertson-Hoyt et al. (2007) in a study with the temperate maize germplasm. In studies with maize inbred lines (Henry et al. 2009) and segregating families (Chiuraise et al. 2016), a significant correlation between AER and aflatoxin accumulation has also been reported.

In conclusion, several tropical maize inbred lines with the potential resistance to AER development and aflatoxin accumulation were identified. A number of lines potentially resistant to aflatoxin accumulation were also reported as being resistant to fumonisin accumulation, which enhances their potential as an important breeding resource. Resistance of these lines to aflatoxin accumulation should be validated using different inoculation techniques across locations. This information will be essential to African maize breeding programs targeting development of aflatoxin-resistant germplasm and cultivars.

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Declaration of interest

The authors declare no conflict of interest.

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Author Contributions

Authors S.O., A.V. and B.C.F. conceived and designed the experiments. Authors B.C.F. and D.M. provided germplasm. H. S, L.J.R., I.B., M.M., S.O. and A.O. conducted all field trials and generated the data; D.M., and L.J.R. analyzed the data; S.O and D.M. wrote the manuscript.

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References

ACDI-VOCA, 2015. Storage and Drying for Aflatoxin Prevention Project (AflaSTOP). http://acdivoca.org/sites/default/files/attach/legacy/site/Lookup/Kenya-flaSTOP/$file/Kenya-AflaSTOP.pdf.

Alvarado, G., M. López, M. Vargas, A. Pacheco, F. Rodríguez, J. Burgueño, and J. Crossa, 2016. META-R (Multi Environment Trail Analysis with R for Windows) Version 6.01. International Maize and Wheat Improvement Center. http://hdl.handle.net/11529/10201.

Alwala, S., C. A. Kimbeng, W. P. Williams, and M. S. Kang. 2008. Molecular markers associated with resistance to *Aspergillus flavus* in maize grain: QTL and discriminant analyses. *Journal of New Seeds* 9 (1):1–18.

Bandyopadhyay, R., A. Ortega-Beltran, A. Akande, C. Mutegi, J. Atehnkeng, L. Kaptoge, A. L. Senghor, B. N. Adhikari, and P. J. Cotty. 2016. Biological control of aflatoxins in Africa: Current status and potential challenges in the face of climate change. *World Mycotoxin Journal* 9 (5):771–89.

Betrán, F. J., S. Bhatnagar, T. Isakeit, G. Odyody, and K. Mayfield. 2006. Aflatoxin accumulation and associated traits in QPM maize inbreds and their testcrosses. *Euphytica* 152:247–57.

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Betran, F. J., T. Isakeit, and G. Odvody. 2002. Aflatoxin accumulation of white and yellow maize inbreds in diallel crosses. *Crop Science* 42 (6):1894–901. BFAP (Bureau for food and agricultural policy), 2011. The South African Agricultural Baseline, pp 90 http://www.bfap.co.za/documents/baselines/BFAP_Baseline_2011.PDF.

Brown, R. L., M. Abebe, C. Zhi-Yuan, B. Deepak, Y. Jiujiang, Y. Haibo, and E. C. Thomas, 2013b. COMESA and EAC Regional Maize Trade Policy Paper, 2003. http://www.ifpri.org/sites/default/files/p6regional_maize.pdf.

Brown, R. L., A. Menkir, Z. Y. Chen, D. Bhatnagar, J. Yu, H. Yao, and T. E. Cleveland. 2013a. Breeding aflatoxin-resistant maize lines using recent advances in technologies–A review. *Food Addit & Contam* 8:1382–91.

Brown, R. L., W. P. Williams, G. L. Windham, A. Menkir, and Z.-Y. Chen. 2016. Evaluation of African-bred maize germplasm lines for resistance to aflatoxin accumulation. *Agronomy* 6 (2):24. doi:10.3390/agronomy6020024.

Cary, J. W., K. Rajasekaran, R. L. Brown, M. Luo, Z. Y. Chen, and D. Bhatnagar. 2011. Developing resistance to aflatoxin in maize and cottonseed. *Toxins* 3 (6):678–96.

Chauhan, Y. S., G. C. Wright, and N. C. Rachaputi. 2008. Modelling climatic risks of aflatoxin contamination in maize. *Australian Journal of Experimental Agriculture* 48:358–66.

Chiuraise, N., J. Derera, K. S. Yobo, C. Magorokosho, A. Nunkumar, and N. F. P. Qwabe. 2016. Progress in stacking aflatoxin and fumonisin contamination resistance genes in maize hybrids. *Euphytica* 207:49–67.

Cotty, P. J., and R. Jaime-Garcia. 2007. Influences of climate on aflatoxin producing fungi and aflatoxin contamination. *International Journal of Food Microbiology* 119:109–15.

DAFF (Department of Agriculture, Forestry and Fisheries, South Africa), 2016. Abstract of agricultural statistics 2016, pp 7. http://www.daff.gov.za/Daffweb3/Portals/0/Statistics%20and%20Economic%20Analysis/Statistical%20Information/Abstract%202016%20.pdf.

Ehrlich, K. C. 2014. Non-aflatoxigenic *Aspergillus flavus* to prevent aflatoxin contamination in crops: Advantages and limitations. *Frontiers in Microbiology* 5:50. doi:10.3389/fmicb.2014.00050.

Fountain, J. C., P. Khera, L. Yang, S. N. Nayak, B. T. Scully, R. D. Leef, Z.-Y. Cheng, R. C. Kemerait, R. K. Varshney, and B. Guo. 2015. Resistance to *Aspergillus flavus* in maize and peanut: Molecular biology, breeding, environmental stress and future perspectives. *The Crop Journal* 3:229–37.

Fountain, J. C., B. T. Scully, X. Ni, R. C. Kemerait, R. D. Lee, Z. Y. Chen, and B. Guo. 2014. Environmental influences on maize-*Aspergillus flavus* interactions and aflatoxin production. *Frontiers in Microbiology* 5:40. doi:10.3389/fmicb.2014.00040.

Giorni, P., T. Bertuzzi, and P. Battilani. 2016. Aflatoxin in maize, a multifaceted answer of *Aspergillus flavus* governed by weather, host-plant and competitor fungi. *Journal of Cereal Sciences* 70:256–62.

Gorman, D. P., M. S. Kang, T. Cleveland, and R. L. Hutchinson. 1991. Field aflatoxin production by *Aspergillus flavus* and *A. parasiticus* on maize kernels. *Euphytica* 61 (3):187–91.

Gov. of Kenya (Government of Kenya), 2016. Food and Agricultural data network. CountrySTAT. http://www.countrystat.org/home.aspx?c=KEN.

Guo, B. Z., R. G. Li, N. W. Widstrom, R. E. Lynch, and T. E. Cleveland. 2001. Genetic variation within maize population GT-MAS: Gk and the relationship with resistance to Aspergillus flavus and aflatoxin production. *TAG Theoretical and Applied Genetics* 103 (4):533–39.

Henry, W. B., L. W. Gary, and H. B. Michael. 2012. Evaluation of maize germplasm for resistance to aflatoxin accumulation. *Agronomy Journal* 1:28–39.

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Henry, W. B., W. P. Williams, G. L. Windham, and L. K. Hawkins. 2009. Evaluation of maize inbred lines for resistance to *Aspergillus* and Fusarium ear rot and mycotoxin accumulation. *Agronomy Journal* 101:1219–26.

Institute, S. A. S. 2011. *SAS/STAT 9.3 User's guide*. Cary, NC: SAS Institute Inc.

KBS (Kenya Bureau of Standards), 2013. The Benchmark. The official magazine of the Kenya Bureau of Standards, Issue 19, pp 39.

Kelley, R. Y., W. P. Williams, J. E. Mylroie, D. L. Boykin, J. W. Harper, G. L. Windham, et al. 2012. Identification of maize genes associated with host plant resistance or susceptibility to *Aspergillus flavus* infection and aflatoxin accumulation. *PLoS ONE* 7 (5):e36892. doi:10.1371/journal.pone.0036892.

Lauren, L., M. Onsongo, H. Njapau, H. Schurz-Rogers, G. Luber, S. Kieszak, J. Nyamongo, L. Backer, M. Dahiye, A. Misore, K. DeCock, and C. Rubin; the Kenya Aflatoxicosis Investigation Group. 2005. Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in Eastern and Central Kenya. *Environmental Health Perspectives* 113:12.

Lentner, M., and T. Bishop. 1986. *Experimental design and analysis*. Blacksburg, VA: Valley Book Company.

Li, R., and M. S. Kang. 2005. Genetics of Resistance to Field Kernel Infection by *Aspergillus flavus* in Maize. *Journal of Crop Improvement* 15:11–32.

Li, R., M. S. Kang, O. J. Moreno, and L. M. Pollak. 2002. *Field resistance to Aspergillus flavus from exotic maize (Zea mays L.) germplasm*, 11–15. Plant Genetic Resources Newsletter (FAO).

Liu, Y., and F. Wu. 2010. Global burden of aflatoxin-induced hepatocellular carcinoma: A risk assessment. *Environmental Health Perspectives* 118:818–24.

Maina, A. W., J. M. Wagacha, F. B. Mwaura, J. W. Muthomi, and C. P. Woloshuk. 2016. Postharvest practices of maize farmers in Kaiti District, Kenya and the impact of hermetic storage on populations of *Aspergillus* spp. and aflatoxin contamination. *Journal of Food Research* 5 (6):53.

McMillian, W. W., N. W. Widstrom, and D. M. Wilson. 1993. Registration of GT-MAS: Gk maize germplasm. *Crop Science* 33 (4):882–882.

Menkir, A., R. L. Brown, R. Bandyopadhyay, and T. E. Cleveland. 2008. Registration of six tropical maize germplasm lines with resistance to aflatoxin contamination. *Journal of Plant Registrations* 2:246–50.

Ncube, E., B. C. Flett, C. Waalwijk, and A. Viljoen. 2011. *Fusarium* spp. and levels of fumonisins in maize produced by subsistence farmers in South Africa. *South African Journal of Science* 107:1–7. Accessed October 19, 2016. http://www.sajs.co.za 10.4102/sajs.v107i1/2.367.

Ngindu, A., P. R. Kenya, D. M. Ochieng, T. M. Omondi, W. Ngare, and D. Gatei. 1982. Outbreak of acute hepatitis caused by aflatoxin poisoning in Kenya. *Lancet* 1:1346–48.

Okoth, S., B. Nyongesa, V. Ayugi, E. Kang'ethe, H. Korhonen, and V. Joutsjoki. 2012b. Toxigenic potential of Aspergillus species occurring on maize kernels from two agro-ecological zones in Kenya. *Toxins* 4:991–1007.

Okoth, S. A., and M. Kola. 2012a. Market samples as a source of chronic aflatoxin exposure in Kenya. *African Journal of Health Sciences* 20:56–61.

Okoth, S. A., Kola, M. A. 2012b. Market samples as a source of chronic aflatoxin exposure in Kenya. *Afr. J. Health Sci* 20 (1-2), 56–61.

Probst, C., H. Njapau, and P. J. Cotty. 2007. Outbreak of an acute aflatoxicosis in Kenya in 2004: Identification of the causal agent. *Applied and Environmental Microbiology* 73:2762–64.

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Robertson-Hoyt, L. A., J. Betran, G. A. Payne, D. G. White, T. Isakeit, C. M. Maragos, T. L. Molnar, and J. B. Holland. 2007. Relationships among resistances to Fusarium and Aspergillus ear rots and contamination by fumonisin and aflatoxin in maize. *Phytopathology* 97:311–17.

Rose, L. J., M. Mouton, I. Beukes, B. C. Flett, C. Van Der Vyver, and A. Viljoen. 2016. Multi-environment evaluation of maize inbred lines for resistance to Fusarium ear rot and fumonisins. *Plant Diseases* 100:2134–44.

Rose, L. J., S. Okoth, I. Beukes, A. Ouko, M. Mouton, B. C. Flett, D. Makumbi, and A. Viljoen. 2017. Determining resistance to *Fusarium verticillioides* and fumonisin accumulation in maize inbred lines resistant to *Aspergillus flavus* and aflatoxins. *Euphytica* 213:93. doi:10.1007/s10681-017-1883-7.

Scott, G. E., and N. Zummo. 1988. Sources of resistance in maize to kernel infection by *Aspergillus flavus* in the field. *Crop Science* 28 (3):504–07.

Small, I. M., B. C. Flett, W. F. Marasas, A. McLeod, M. A. Stander, and A. Viljoen. 2012. Resistance in maize inbred lines to *Fusarium verticillioides* and fumonisin accumulation in South Africa. *Plant Disease* 96 (6):881–88.

Snipes, K., and C. N. Kamau. 2013. Corn update report. Grain and feed. USDA Foreign Agricultural Service. *Global Agricultural Information Network* 18 (4):181–85. http://gain.fas.usda.gov/Recent%20GAIN%20Publications/Corn%20Update%20Report%20_Nairobi_Kenya_1-14-2013.pdf.

The South African Agricultural Baseline. http://www.bfap.co.za/documents/baselines/BFAP_Baseline_2011.PDF.

Squire, R. A. 1989. Ranking animal carcinogens: A proposed regulatory approach. *Science* 214:887–91.

Walker, R. D., and D. G. White. 2001. Inheritance of resistance to Aspergillus ear rot and aflatoxin production of corn from CI2. *Plant Disease* 85 (3):322–27.

Warburton, M. L., and W. P. Williams. 2014. Aflatoxin resistance in maize: What have we learned lately? *Advances in Botany 2014* Article ID 352831:10. doi:10.1155/2014/352831.

Warburton, M. L., W. P. Williams, G. L. Windham, S. C. Murray, W. Xu, L. K. Hawkins, and J. F. Duran. 2013. Phenotypic and genetic characterization of a maize association mapping panel developed for the identification of new sources of resistance to and aflatoxin accumulation. *Crop Science* 53 (6):2374–83.

Widstrom, N. W. 1996. The aflatoxin problem with corn grain. *Advances in Agronomy* 56:219–80.

Widstrom, N. W., W. W. McMillian, R. W. Beaver, and D. M. Wilson. 1990. Weather associated changes in aflatoxin contamination of preharvest maize. *Journal of Production Agriculture* 3:196–99.

Widstrom, N. W., W. W. McMillian, and D. M. Wilson. 1987. Segregation for resistance to aflatoxin contamination among seeds on an ear of hybrid maize. *Crop Science* 27 (5):961–63.

Widstrom, N. W., D. M. Wilson, and W. W. McMillian. 1984. Ear resistance of maize inbreds to field aflatoxin contamination. *Crop Science* 24 (6):1155–57.

Widstrom, N. W., D. M. Wilson, and W. W. McMillian. 1986. Differentiation of maize genotypes for aflatoxin concentration in developing kernels. *Crop Science* 26 (5):935–37.

Willcox, M. C., G. L. Davis, M. L. Warburton, G. L. Windham, H. K. Abbas, J. Betrán, J. B. Holland, and W. P. Williams. 2013. Confirming quantitative trait loci for aflatoxin resistance from Mp313E in different genetic backgrounds. *Molecular Breeding* 32 (1):15–26.

Williams, W. P. 2006. Breeding for resistance to aflatoxin accumulation in maize. *Mycotoxin Research* 22:27–32.

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Williams, W. P., M. D. Krakowsky, B. T. Scully, R. L. Brown, A. Menkir, M. L. Warburton, and G. L. Windham. 2014. Identifying and developing maize germplasm with resistance to accumulation of aflatoxins. *World Mycotoxin Journal* 8 (2):193–209.

Williams, W. P., S. Ozkan, A. Ankala, and G. L. Windham. 2011. Ear rot, aflatoxin accumulation, and fungal biomass in maize after inoculation with *Aspergillus flavus*. *Field Crops Research* 120:196–200.

Williams, W. P., and G. L. Windham. 2001. Registration of maize germplasm line Mp715. *Crop Science* 41 (4):1374–1374.

Williams, W. P., and G. L. Windham. 2006. Registration of maize germplasm line Mp717. *Crop Science* 46 (3):1407–09.

Williams, W. P., and G. L. Windham. 2015. Aflatoxin accumulation in a maize diallel cross. *Agriculture* 5:344–52.

Williams, W. P., G. L. Windham, and P. M. Buckley. 2008. Diallel analysis of aflatoxin accumulation in maize. *Crop Science* 48:134–38.

Windham, G. L., and W. P. Williams. 2002. Evaluation of corn inbreds and advanced breeding lines for resistance to aflatoxin contamination in the fields. *Plant Disease* 86:232–34.

Zhang, Y., M. S. Kang, and R. Magari. 1997. Genetics of resistance to kernel infection by *Aspergillus flavus* in maize. *Plant Breeding* 116:146–52.

Zuber, M. S., O. H. Calvert, W. F. Kwolek, E. B. Lillehoj, and M. S. Kang. 1978. Aflatoxin B1 Production in an Eight-Line Diallel of *Zea mays* infected with *Aspergillus flavus*. *Phytopathology* 68:1346–49.

Zuber, M. S., L. L. Darrah, E. B. Lillehoj, L. M. Josephson, A. Manwiller, G. E. Scott, R. T. Gudauskas, E. S. Horner, N. W. Widstrom, D. L. Thompson, and A. J. Bockholt. 1983. Comparison of open-pollinated maize varieties and hybrids for preharvest aflatoxin contamination in the southern United States. *Plant Disease* 67 (2):185–87.

Zummo, N., and G. E. Scott. 1989. Evaluation of field inoculation techniques for screening maize genotypes against kernel infection by *Aspergillus flavus* in Mississippi. *Plant Diseases* 73:313–16.

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