TEXAS TECH UNIVERSITY
Libraries™

Evaluation of elite maize inbred lines for reduced Aspergillus flavus infection, aflatoxin accumulation, and agronomic traits

The Texas Tech community has made this publication openly available. Please share how this access benefits you. Your story matter to us.

Citation	Pekar, J.J., Murray, S.C., Isakeit, T.S., Scully, B.T., Guo, B., Knoll, J.E., Ni, X., Abbas, H.K., Williams, P., & Xu, W.. 2019. Evaluation of elite maize inbred lines for reduced Aspergillus flavus infection, aflatoxin accumulation, and agronomic traits. *Crop Science, 59*(6). https://doi.org/10.2135/cropsci2019.04.0206
Citable Link	https://hdl.handle.net/2346/95117
Terms of Use	© 2019 The Author(s). cc-by-nc-nd

Title page template design credit to Harvard DASH.

RESEARCH

Evaluation of Elite Maize Inbred Lines for Reduced *Aspergillus flavus* Infection, Aflatoxin Accumulation, and Agronomic Traits

Jacob J. Pekar,* Seth C. Murray, Thomas S. Isakeit, Brian T. Scully, Baozhu Guo, Joseph E. Knoll, Xinzhi Ni, Hamed K. Abbas, Paul Williams, and Wenwei Xu

ABSTRACT

Mycotoxins produced by the fungus *Aspergillus flavus* are harmful to humans and animals and result in large economic losses. Developing and disseminating resistant germplasm is a critical component to reduce or eliminate the accumulation of pre-harvest aflatoxins in maize (*Zea mays* L.). Ninety-three different inbred lines were evaluated for seven standard agronomic traits during the years of 2012 to 2016 in two locations (nine environments), and inoculated with *A. flavus*. Multiple inbreds were both lower accumulating for aflatoxins and higher yielding than aflatoxin tolerant and high yielding checks. The top seven inbred lines on average had an aflatoxin value of 14.1 ng g^{-1} and an average yield of 3.2 Mg ha^{-1}, while the susceptible checks were 185.9 ng g^{-1} and 2.5 Mg ha^{-1}, respectively. Several inbred lines evaluated in this study have high potential utility in future maize improvement research, such as breeding for resistance, segregating populations for genetic mapping as well as in direct use in hybrids with genetically improved resistance to A. flavus.

J.J. Pekar, S.C. Murray, T.S. Isakeit, Texas A&M Univ., Texas A&M AgriLife Research, College Station, TX 77843-2474; B.T. Scully, USDA-ARS, U.S. Horticulture Research Lab., Ft. Pierce, FL 34945; B. Guo, USDA-ARS, Crop Protection and Management Research Unit, Tifton, GA 31793; J.E. Knoll, X. Ni, USDA-ARS, Crop Genetics and Breeding Research Unit, Tifton, GA 31793; H.K. Abbas, USDA-ARS, Biological Control of Pests Research Unit, Stoneville, MS 38776; P. Williams, USDA-ARS, Corn Host Plant Research Unit, Mississippi State, MS 39762; W. Xu, Texas Tech Univ., Texas A&M Agrilife Research, Lubbock, TX 79403. Received 1 Apr. 2019. Accepted 18 July 2019. *Corresponding author (jacob.pekar@tamu.edu). Assigned to Associate Editor Jode Edwards.

Abbreviations: AF%, Aspergillus flavus percentage; BLUP, best linear unbiased predictor; CS, College Station, Texas; DTA, days to anthesis; DTS, days to silking; EHT, ear height; ex-PVP, expired Plant Variety Protection; EW%, earworm damage percentage; G×E, genotype by environment interaction; PHT, plant height; SERAT, Southeast Regional Aflatoxin Trials; WE, Weslaco, Texas; MS, Mississippi State.

Aflatoxins are a harmful carcinogenic mycotoxin produced by *Aspergillus flavus* Link:FR, which limits the marketability of maize (*Zea mays* L.) grain and reduces the economic value for producers. Actual economic losses are difficult to measure, but are thought to be around $163 million per year in the United States alone regarding maize only, and up to $500 million annually in additional crops such as peanuts (*Arachis hypogaea* L.) and other crops (Wu, 2015). Documented or suspected cases of acute aflatoxin poisoning are numerous throughout the world and result in liver damage, intestinal bleeding, cancer, and even death (Lewis et al., 2005), especially in developing countries which lack infrastructure to test for contamination, and allow contaminated maize to flow freely in local trade. The effects of chronic exposures to aflatoxins are even more challenging to test. More than 100 countries have some type of regulations on aflatoxin concentrations (Wu and Guclu, 2012). In the United States, aflatoxins

Published in Crop Sci. 59:2562–2571 (2019).
doi: 10.2135/cropsci2019.04.0206

© 2019 The Author(s). This is an open access article distributed under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

are regulated for human consumption with an upper limit of 20 (ng g^{-1}), and only maize under 300 (ng g^{-1}) is used for beef, swine, and poultry feed (Stoloff et al., 1991). The limits are set by the federal Department of Agriculture.

It is still unclear why *A. flavus* makes aflatoxins, but preliminary indications are that it might be to prevent insect predation (Drott et al., 2017; Gqaleni et al., 1997). Pre-harvest colonization of maize and the subsequent production of aflatoxins are associated with, and likely are a result of, an increase in physiological stresses of crop production (Klich, 2007). High daytime and nighttime temperatures, along with occurrences of drought and insect pressure, have been shown to increase the occurrences of aflatoxin contamination (Abbas et al., 2002). To reduce pathogen pressure and toxin accumulation, producers decrease stresses through cultural practices and management, when economical. In recent years, atoxigenic strains of *A. flavus* have become an additional management tool to reduce aflatoxins (Abbas et al., 2011); however, while decreasing aflatoxins per se, this typically increases the overall load of *A. flavus*. Heavy infestation of atoxigenic (or toxigenic) *A. flavus* decreases the nutritional value of maize, can contain additional mycotoxins and moldy corn, even if no toxin is present, and is still at least a visual deterrent in food consumption (Lane et al., 2018; Magan et al., 2004). Plant-breeding is an important component of the integrated pest management approach to decrease aflatoxins and *A. flavus* (Brown et al., 2011). Breeding for decreased susceptibility by selecting for heritable segregating traits such as tighter, thicker and closed husk cover, or insect resistance, can reduce toxin accumulation (Widstrom et al., 2003). A major challenge of breeding inbred lines for resistance to aflatoxins, or selecting for increased yield, is that inbred lines appear to be less robust across environments (i.e., experience more genotype × environment interactions, or G×E) in a way that hybrid vigor masks in the hybrids farmers would ultimately grow (Cole et al., 2009; Li et al., 2018; Schnell and Becker, 1986).

To test susceptibility of maize genotypes to *A. flavus* and aflatoxin accumulation uniformly, inoculation must be used. There are two major classes of *A. flavus* inoculation methods, wounding and non-wounding (Tucker Jr. et al., 1986). Wounding inoculation techniques offer consistent infection that mimic insect or mechanical damage, bypassing tolerances from physiological traits such as husk cover or tightness, natural insect tolerance, pericarp thickness, and erect or non-erect (drooping) ears. Non-wounding methods do not bypass other physiological tolerances and evaluate susceptibility in a way that is more relevant to farmers (Williams et al., 2002; Windham et al., 2009). The different types of inoculation methods have various advantages and disadvantages (Williams et al., 2013). In this research, we chose to use a non-wounding technique, ground kernel inoculation (similar to how atoxigenic strains are applied by producers), because it more closely mimics natural inoculation conditions. This technique also reduces the labor needed, allowing the evaluation of a larger number of genotypes and replicates for aflatoxin accumulation.

Despite a number of public breeding efforts, no genotypes have yet been identified that are completely resistant to aflatoxin accumulation, as only quantitative reductions have been found. There have been few collaborative projects to date that investigate diverse public sector materials in common gardens across different environments. Public material bred for decreased susceptibility to aflatoxins in the United States is being developed by the USDA-ARS in Georgia, Mississippi, and North Carolina, and by Texas A&M University (TAMU) at College Station and Texas A&M Agrilife Research at Lubbock (Table S1). The most viable example of joint testing of this material in common gardens has been in the Southeast Regional Aflatoxin Trials (SERAT), which has tested 30 to 40 public breeding sector hybrids for agronomics and aflatoxin accumulation each year since 2003 (Wahl et al., 2017). The SERAT trials have shown that the best public hybrids are more tolerant than current commercial hybrids available to producers; however, many, but not all, public hybrids lack the favorable agronomics and realized yield that producers demand (Wahl et al., 2017). Perhaps more importantly, the SERAT trials demonstrated that a large portion of aflatoxin susceptibility is genetic (22%; for yield this was 19%) and heritable across very diverse but relevant environments. The SERAT trials also demonstrated aflatoxin accumulation is robust to inoculation methods, with a relatively small G×E (13%) but moderate error (39%) components (Wahl et al., 2017). This suggests that relatively few environments are sufficient for identifying superior genetics for *A. flavus* and aflatoxin susceptibility. However, given the large influence of weather on aflatoxin accumulation (Williams et al., 2015), many environments are needed to capture environmental conditions that result in good genotypic separation as observed in SERAT. While SERAT trials evaluated hybrids in common gardens, there have been no similar published trials of inbred evaluations from across diverse public programs' released lines. Inbred line yields and aflatoxin susceptibility are interesting for examining the inheritance of these traits into hybrids (Duvick, 1999). The so-called "Era Studies" demonstrated that the increase in US hybrid yield over decades was correlated to an increase in mid-parent inbred yield and not an increase in heterosis. Yield of inbreds, along with seed size, is directly relevant to the economics of hybrid seed production for farmers. Inbred yield can also be an indicator of fitness or environmental adaptation to the abiotic stresses. Hybrid accumulation of aflatoxins are most relevant to farmers, but understanding which inbreds cause aflatoxin susceptibility is important for scientific understanding and anticipating new hybrid crosses.

14350653, 2019, 6, Downloaded from https://acsess.onlinelibrary.wiley.com/doi/10.2135/cropsci2019.04.0206 by Texas Tech University Libraries, Wiley Online Library on [18/07/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

The purpose of this research was to screen a diverse set of released and pre-released public inbred lines, along with important expired Plant Variety Protection (ex-PVP) commercial lines for low aflatoxins and high yield in Texas environments. Many of the released and to-be-released public breeding lines (Table S1) and all ex-PVP lines have not previously been screened in a common garden for aflatoxin resistance. A total of nine environments, beginning in 2012, were used to screen inbred lines for aflatoxin accumulation and yield in stressful climates of central and southern Texas where high *A. flavus* pressure and aflatoxin production is often prevalent. The specific objectives of this study were to evaluate elite inbred lines from diverse public programs in Texas environments to identify (i) trends in aflatoxin accumulation and agronomic traits; (ii) the most promising inbred germplasm for genetic resistance to *A. flavus* sporulation that results in low aflatoxin; and (iii) differences between this germplasm and the ex-PVP germplasm which presumably are closely related to elite industry lines currently used today.

MATERIALS AND METHODS

Germplasm

A total of 93 inbred lines (Table S1) from across Southern breeding programs and ex-PVPs were evaluated for aflatoxin accumulation in grain across five growing seasons. These inbreds included four southern lines known to be susceptible based on past studies: T173 (West et al., 2001), SC212m, Va35 (Henderson, 1976), and GA209 (Fleming, 1974) and nine lines known for, and most released because of low-aflatoxin accumulations: GT603 (Guo et al., 2011), Mp313E (Scott and Zummo, 1990), Mp420 (Scott and Zummo, 1992), Mp717 (Williams and Windham, 2006), Mp718 and Mp719 (Williams and Windham, 2012), Tx740 (Mayfield et al., 2012), Tx772 (Llorente et al., 2004), and Tx777 (Murray et al., 2019).

Experimental Design and Study Locations

Starting in 2012, two locations were selected for aflatoxin testing of inbreds, College Station (CS) and Weslaco (WE), Texas, where only CS was used in 2016, for a total of nine environments. Limited supplementary irrigation was applied to trials as needed. College Station has an average high temperature of 33.2°C during flowering and an average annual rainfall of 101.8 cm. Weslaco has an average high temperature of 32.2°C during flowering and an average annual rainfall of 63.2 cm. Sowing was delayed about a month longer than optimal in both locations to have higher temperatures during grain fill, which would lead to greater stress and, therefore, a higher potential aflatoxin contamination to differentiate genotypes. In 2013, entries increased from 13 to 38 and increased every subsequent year except 2016. A total of 57 inbred lines were only tested in 1 yr, but in two locations. The remaining 41 were tested in multiple years with multiple locations. The experimental design consisted of a randomized complete block with four replications in 2012 and three replications in subsequent years as the number of inbred lines increased. Each replicate in CS was planted in 1-row plots 6.10-m long with 0.76-m wide alleys; WE had 1-row plots 7.01-m long with 1.01-m alleys.

Aspergillus flavus Inoculation

Inoculum was prepared from stock *A. flavus* isolate NRRL 3357 (Wicklow et al., 1998), which has been commonly used in aflatoxin studies, including many referenced here, on potato dextrose agar. Plates were incubated at 30°C for 5 to 7 d. Once plates had sporulated, they were covered with Parafilm and stored at 4°C. One square cm sample was cut from the source plate and placed in a test tube with 15 mL of sterile water plus 0.01 mL of Tween 20 and vortexed. Additional samples were plated using 0.05 mL from the parent plate and incubated for 5 to 7 d at 30°C. Seven thousand milliliters of whole field corn and 3 L of distilled water were placed into a 46-cm by 38-cm by 13-cm autoclavable plastic tray and covered with aluminum foil and autoclaved for 1 h. Once autoclaved, the tray was transferred into a translucent 140-quart (132 L) plastic bag. Twenty-five to 30 petri dish cultures were blended with 2 L of distilled water and 1 mL of Tween 20. Five hundred milliliters of this mixture was applied to each tray and mixed thoroughly, then placed in a room with a constant temperature of 35°C to 38°C while mixing every 12 h. Sporulation occurred 2 to 3 d after placement. Inoculum was applied to the plots by scattering it on the ground between the rows shortly after the majority of the plots had started silking at a rate of 170 g per 9-linear meters of row. This was usually around 12 d after the initiation of silking.

Traits Measured

Seven agronomic traits were also measured at CS locations, including: 50% of plot flowering as days to anthesis (DTA) and days to silking (DTS), as well as plant height (PHT) from the ground to the tip of the tassel, and ear height (EHT) from the ground to the top ear's node attachment point. Plant height and EHT were not recorded in 2012 at CS, and only PHT was recorded at WE in 2012. Plots were hand harvested at or shortly after maturity, visually rated, shelled and bulked for measurement of plot weight, moisture and test weight using a Dickey-john Mini GAC moisture meter (Dickey-john, Auburn, IL). Bulked grain samples were then ground using a Romer mill (Romer Labs, Union, MO), and aflatoxin analysis was performed using commercially available quantitative enzyme-linked immunosorbent assay (ELISA) kits (Neogen Inc., Lansing, MI). Visual ratings were taken on all harvested ears to evaluate corn earworm (*Helicoverpa zea*) damage, *A. flavus* sporulation, and *Fusarium spp.* sporulation during 2013, 2014, 2015, and 2016 seasons. Corn earworm damage was estimated visually as a percentage of damaged kernels. Fungal sporulation was estimated visually as a percentage of kernel surface area sporulating for *A. flavus*, and 'star bursting' or whitish mycelium on the kernels for *Fusarium spp.*

Statistical Analysis

Statistical analysis was conducted using JMP 12 software (JMP, Version 12. SAS Institute Inc., Cary, NC, 1989–2016). In an attempt to normalize observations, aflatoxin data were transformed using the Box–Cox power transformations formula (Box and Cox, 1964):

$$x'_\lambda = (x^\lambda - 1)/\lambda \quad [1]$$

14350653, 2019, 6, Downloaded from https://acsess.onlinelibrary.wiley.com/doi/10.2135/cropsci2019.04.0206 by Texas Tech University Libraries, Wiley Online Library on [18/07/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

All data were first analyzed jointly to evaluate overall trends and then by individual environments (location × year). In the separate environment analysis, inbred effects were treated as fixed while replications, range, and row effects were random. In the all-years-combined analysis (Eq. [2]), inbreds were fixed while environment, replication, inbred × environment interaction, range, and row effects were random. Here range and row effects are equivalent to row and column effects, but reflect the terminology used in furrow irrigation trials. The model was fit as follows: where m is the grand mean, g_i is the fixed effect of the genotype i, e_k is the random effect of the environment k, $(ge)_{ik}$ is the random effect of the interaction between i and k, $(r/e)_{jk}$ is the random effect of replication j nested within environment k, $(row/e)_{lk}$ is the random effect of row l nested with environment k, $(range/e)_{mk}$ is the random effect of range m nested with environment k, ε_{ijklm} is the random residual error from genotype, environment, replication, row, and range.

$$y_{ijk} = \mu + g_i + e_k + (ge)_{ik} + (r/e)_{jk} + (row/e)_{lk} + (range/e)_{mk} + \varepsilon_{ijklm} \quad [2]$$

All means were compared using Fisher's Protected least significant difference, LSD ($P = 0.05$), and all aflatoxin values were then back transformed and reported as actual ng g^{-1}. Pairwise correlations were analyzed using the multivariate function in JMP software. Repeatability (h^2) was calculated as:

$$h^2 = G / \left(G + \frac{GE}{r} + \frac{\varepsilon}{re}\right) \quad [3]$$

where G, GE, and ε were the variance components of genotype, genotype × environment interaction, and residual error, respectively, with r as number of replications and e as number of environments.

Correlations were compared using Pearson correlation coefficients. Phenotypic correlations were formed on raw data, while genotypic correlations were formed from the best linear unbiased predictor (BLUP) genotypic estimates of each trait.

RESULTS AND DISCUSSION

Exploratory Statistics

Aflatoxin accumulation values on raw data ranged from 0 to 4500 ng g^{-1} with a grand mean of 268 ng g^{-1} across nine environments. Aflatoxin accumulation appeared higher in years where statewide drought was more prevalent (Fig. 1), as expected due to higher stress. Year 2012 had the lowest accumulation means in both WE and CS at 77 ng g^{-1} and 70 ng g^{-1}, respectively. Only 13 entries were used in 2012, and no program lines were significantly better than the tolerant checks (Table S1). The highest mean toxin accumulation occurred during the 2014 season in WE at 542 ng g^{-1}; weather patterns favored hot and dry temperatures with low precipitation during the growing season. In 2016, timely rains during the growing season likely caused the low observed aflatoxin levels (143 ng g^{-1}), but sufficient variation for discrimination of lines was still observed. Visual ear ratings on *A. flavus* sporulation tended to be higher in CS than that in WE, which was unexpected and

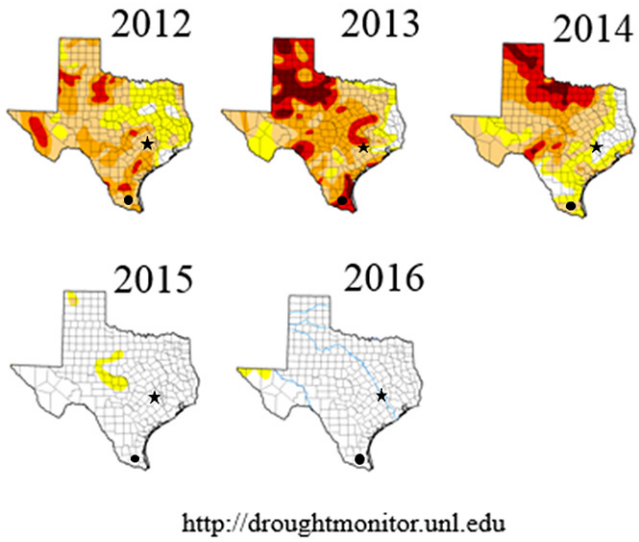

Fig. 1. Drought levels in Texas during flowering time of trials in all years. Darker red colors indicate the most severe drought. College Station is designated as a star and Weslaco is designated as a circle.

possibly associated with irrigation timing during inoculation, which can increase the relative humidity and favor sporulation (Diener et al., 1987; Hesseltine et al., 1966).

Plant height and EHT were recorded in most years, with a PHT range of 76 to 249 cm and an EHT mean of 10 to 112 cm. The Mississippi program material was significantly taller for PHT than all other germplasm at 172 ± 2 and EHT 69 ± 15 cm, respectively. The remaining program materials were not significantly different as groups; however, there were significant differences between entries within each program's material, suggesting that variation and favorable alleles for desired PHT characteristics are present. In Texas and other areas in the southern United States, there have been notable correlations between PHT, EHT, and yield in diverse commercial hybrids (Barrero Farfan et al., 2015; Liu and Wiatrak, 2011; Yin et al., 2011), and in biparental inbred crosses (Chen, 2016). Plant height appears to be an indicator trait of vigor and adaptation to hot and dry southern climates, and might be strongly correlated with yield because certain diverse germplasm can better overcome common stresses associated with southern growing regions (Barrero Farfan et al., 2013). If this hypothesis is correct, then it would suggest that by selecting lines that are taller, they might also be better adapted to this growing region by proxy. Over years, the mean height and days to flowering increased, which can be explained by the increased number of entries from tropical, as opposed to temperate-derived genotypes (Table S1). Tropical derived lines tend to be later in flowering and taller in stature than many of the ex-PVP lines, and to susceptible checks that were first evaluated. Tropical derived germplasm also tend to be less susceptible to aflatoxins as they are usually better adapted to the hotter and dryer climates that favor aflatoxin contamination. The

14350653, 2019, 6, Downloaded from https://acsess.onlinelibrary.wiley.com/doi/10.2135/cropsci2019.04.0206 by Texas Tech University Libraries, Wiley Online Library on [18/07/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Table 1. Variance components and percent total for traits measured in all years and locations combined for transformed aflatoxin value (Aftrans), actual measured aflatoxin accumulation in ng g $^{-1}$ (Afngg), yield (T ha^{-1}), visual fusarium rating (FU), visual *A. flavus* rating (AF), environment (E), visual ear worm rating (EW), days to anthesis (DTA), days to silking (DTS), plant height (PHT), and ear height (EHT).

model	Aftrans	%	Afngg	%	T ha^{-1}	%	FU	%	AF	%
E	3,403***	23	28,161***	10	0.18***	10	44***	9	4***	8
Genotype	4,556***	30	70,263***	26	0.77***	41	206***	42	4*	9
G×E	1,557***	10	42,906***	16	0.37***	20	108***	22	14***	29
Rep[E]	86**	1	NS†	1	0.02*	1	6*	1	NS	0
Range[E]	225***	1	8,259***	3	0.03*	1	NS	1	2**	3
Row[E]	NS		NS	1	0.05***	3	NS	0	NS	0
Residual	5,105	34	115,721	43	0.45	24	125	25	24	51
Total	15,096	100	269,730	100	1.88	100	491	100	47	100
R‡	0.88		0.78		0.98		0.83		0.45	
model	**EW**	**%**	**DTA**	**%**	**DTS**	**%**	**PHT**	**%**	**EHT**	**%**
E	26***	32	62***	75	67***	75	24***	26	11.4***	28
Genotype	14***	17	14***	17	17***	19	36***	39	17***	41
G×E	13***	16	3***	3	2***	3	12***	13	3***	8
Rep[E]	NS	0	0.1**	0	NS	0	NS	0	NS	1
Range[E]	NS	0	NS	0	0.3*	0	2**	2	NS	1
Row[E]	NS	0	NS	0	NS	0	1.1*	1	NS	1
Residual	28	35	3	4	3	3	16.5	18	8.1	20
Total	80	100	82	100	89	100	93	100	41	100
R	0.72		0.93		0.95		0.88		0.92	

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

† NS, not significant; significance tested using Log Likelihood.

‡ R, repeatability.

changes in entries also likely contributed to the decrease of the mean and variance of aflatoxin levels from year to year.

Variance Components and Repeatability: Aflatoxins and Fungal (Ear Rot) Damage

Variance components are useful for evaluating sources of variation in an experiment and allow repeatability to be calculated. Sufficient accumulation of aflatoxins to discriminate inbred lines occurred in all environments. Genotypic variation from the transformed aflatoxin data explained 30% overall and from 26.6% to 68.9% of the total variation within each individual environment. Across the full model, G×E interaction components were lowered by transforming raw aflatoxin accumulation data, which did not fall into residual error, but were distributed to other effects. Residual error variation was decreased using transformed aflatoxin data compared to raw data from 43% to 34%. Residual error was further decreased when using BLUPs compared to the raw data due to the BLUPs accounting for significant field variation (i.e., row and range effects) and replication effects.

Repeatability was used over heritability due to the lack of family structure in the genotypes. Repeatability is typically high for flowering and height, and lower for yield and aflatoxins (Barrero Farfan et al., 2015; Wahl et al., 2017). Across all environments, repeatability for aflatoxin accumulation data was 88% using transformed data, up from 78% on non-transformed raw data (Table 1). Repeatability for aflatoxin accumulation has been moderate or low in previous studies (Wahl et al., 2017; Warburton et al., 2013). Repeatability for aflatoxin accumulation within individual environments was lower than for all other important agronomic traits, but still moderate to high. Logarithmic transformation of aflatoxin accumulation is represented in Table 1 with compared repeatability between trials. Wahl et al. (2017) did not use the Box–Cox transformation method, thus logarithmic transformations were used for comparison. In previous studies, genotypic correlations between visual ratings and wet lab analysis have had *r*-values up to 0.83 and 0.76 for both aflatoxins and fumonisin, respectively (Henry et al., 2009; Horne et al., 2016). In most environments there was very little *A. flavus* sporulation observed. However, *Fusarium* starbursting and sporulation was consistently prevalent. Variance components for genotype were moderate to high for *Fusarium spp.* sporulation (41%), but lower for *A. flavus* sporulation (9.5%) and earworm damage (17.1%). The total percent of variance explained by G×E for *Fusarium spp.* sporulation, *A. flavus* sporulation, and earworm damage were 22.6%, 28.7%, and 15.8%, respectively. Visually rating these phenotypes produced different results across different environments, and would reduce the usefulness of such an observation for prediction of unknown future

14350653, 2019, 6, Downloaded from https://acsess.onlinelibrary.wiley.com/doi/10.2135/cropsci2019.04.0206 by Texas Tech University Libraries, Wiley Online Library on [18/07/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

environments. The environment main effect explained minimal amounts of variation for *Fusarium spp.* and *A. flavus* sporulation. Variation explained by earworm damage from environment was twice the amount than that of G×E, which was explained by an overall increase of pest pressure in certain environments.

Variance Components: Yield and Secondary Agronomic Traits

Environment, genotype, and G×E were all highly significant in the all year model for all traits with replication, range, and row being intermittently significant among traits (Table 1). Genotypic variation for yield (41% overall; Table 1) was less than the variation due to environmental effects and ranged from 47.9% to 91.6% across the nine environments. Many program entries had significantly higher yields than the tolerant and susceptible checks (Table S1). All public programs had at least one entry that yielded better than or equal to the ex-PvPs and all checks. Across all germplasm, the tropical Tuxpan-derived germplasm were among the lowest yielding, while germplasm developed from the two Texas programs containing Argentinian-, Bolivian-, and Cuban-derived germplasm had yields that were significantly higher than that of the Tuxpan germplasm and tolerant checks. Georgia and North Carolina programs both had moderate yield. It is reasonable to expect that some of this was due to the fact the Texas lines were better adapted to the environments being tested. Because each program has diverse lines of origins, the mean performance of the program should not be taken as a benchmark of the program as a whole. Identification of individual superior inbred lines is the primary goal. Within each environment, calculated from the environment × genotype model from Eq. [2], variance components demonstrated the largest sources of variation from genotype, and the least from spatial variation within field (range and row).

When separating environment into location × year, results indicated that PHT had more variation explained by location than by year. Differences between locations of PHTs is normal when planting at different times (WE planted in March and CS in April) and at different latitudes (WE, 26.16° N; CS, 30.63° N). Plants react differently to the oncoming longer days and rapid growing degree days accruements that occur during the growing season (i.e., planting earlier results in shorter maize than later-planted maize). Flowering measurements were the opposite, in that more variation was explained by the year effect than by the location effect. Flowering time (DTS) variation explained within each environment for genotype was consistently over 87% with very little residual error, meaning that flowering measurements were taken with good accuracy and had large repeatable genetic differences. Earlier varieties are favored over later maturing hybrids, thus selecting for earliness in tropical germplasm could aid in favorable hybrid agronomics (Goodman, 2005).

Correlations

The most notable of the many significant genetic correlations across all years were positive correlations between the visual ratings taken on ears for *A. flavus* infection (AF%) and ear worm damage (EW%) during processing and wet lab aflatoxin accumulation ratings (Table 2). *Aspergillus flavus* percentage and EW% were positively significantly correlated to both nontransformed ($r = 0.27$, $r = 0.35$) and transformed aflatoxin data ($r = 0.38$, $r = 0.30$). This suggests that earworm damage was likely a contributor of aflatoxins in these inbred ears, although earworm damage has not often been associated with aflatoxins in previous hybrid studies conducted in Texas (Bibb et al., 2018; Farias et al., 2014; Weaver et al., 2017). However, earworms are known to be an important component to aflatoxins, allowing the opportunistic fungi to access and infect the ear (Jones et al., 1980; Ni et al., 2011). Genotypic correlations for yield and aflatoxin accumulation were nonsignificant for nontransformed data but significant for transformed data ($r = 0.20$★); in contrast, phenotypic correlations for yield and aflatoxins were significant for nontransformed data ($r = -0.12$★★★) but nonsignificant for transformed data (Table 3). Our hypothesis is that there are few or no loci that pleiotropically affect both yield (T ha^{-1}) and aflatoxins (Afngg), however some germplasm bred for resistance were not bred for yield and vice versa. This would be further confirmation that there are genetic linkages between aflatoxins and yield, which could be broken through additional breeding.

Another notable correlation was related to flowering time. Transformed aflatoxin accumulation was significant and highly negatively correlated with DTS (genotypic correlation of −0.40). This increase in aflatoxin accumulation may have resulted from favorable environmental conditions increasing later in the season. A difference in any single day could prove to increase the growth and accumulation to aflatoxin.

Correlations were vastly different for each of the programs' materials and entered checks which suggest that visual ratings used for selection would be highly dependent on varieties and populations; for instance, the Mississippi State (MS) program lines had nonsignificant correlations with AF% and transformed aflatoxin of $r = -0.32$, while the TAMU-CS lines had a correlation of $r = 0.44$ and were significant at $P < 0.10$ in the same analysis (Table 3). The difference in program to program correlations was unexpected but was likely due to segregation of genetic background traits that inhibit the formation of the toxin, but not the producing fungi. This phenomenon has been noted before (Henry et al., 2009). In addition to genetic background, the difference in program to program could be related to the type of inoculation used for testing in the breeding program (nonwounding for TAMU-CS and wounding for MS). It is intriguing to consider that lines with high visual AF% ratings and low actual aflatoxin

14350653, 2019, 6, Downloaded from https://acsess.onlinelibrary.wiley.com/doi/10.2135/cropsci2019.04.0206 by Texas Tech University Libraries, Wiley Online Library on [18/07/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Table 2. Significant Pearson's correlations on all data and all years, across all locations as blest linear unbiased predictors (BLUPs) for days to anthesis (DTA), days to silking (DTS), plant height (PHT), ear height (EHT), yield (T ha^{-1}), visual ratings for ear worm (EW%), visual ratings for aflatoxin sporulation (AF%), visual ratings for fusarium sporulation (FU%), aflatoxins in ng g^{-1} (Afngg), and transformed aflatoxin data (Aftrans).

Variable	by Variable	Phenotypic Correlation	Genotypic Correlation
AF(%)	Afngg	0.18***	0.27***
AF(%)	DTA	−0.23***	−0.21*
AF(%)	DTS	−0.22***	−0.13
AF(%)	EHT	0.07*	0.10
AF(%)	FU(%)	0.2***	0.23***
AF(%)	T ha^{-1}	−0.03	0.12
AF(%)	PHT	0.03	0.12
Afngg	DTA	0.02	−0.19*
Afngg	DTS	0.03	−0.18*
Afngg	EHT	−0.05	−0.03
Afngg	T ha^{-1}	−0.12***	−0.02
Afngg	PHT	−0.07*	0.05
AfTrans	AF(%)	0.22***	0.38***
AfTrans	Afngg	0.71***	0.79***
AfTrans	DTA	0.06	−0.40***
AfTrans	DTS	0.07*	−0.40***
AfTrans	EHT	−0.04	−0.18
AfTrans	EW(%)	0.24***	0.30***
AfTrans	FU(%)	0	0.10
AfTrans	T ha^{-1}	−0.01	0.20*
AfTrans	PHT	−0.01	−0.07
DTS	DTA	0.98***	0.95***
EHT	DTA	0.45***	0.60***
EHT	DTS	0.46***	0.63***
EHT	PHT	0.73***	0.67***
EW(%)	AF(%)	0.27***	0.22***
EW(%)	Afngg	0.24***	0.35***
EW(%)	DTA	−0.36***	−0.13
EW(%)	DTS	−0.34***	−0.10
EW(%)	EHT	0.05	−0.10
EW(%)	FU(%)	0.3***	0.28***
EW(%)	T ha^{-1}	−0.13***	−0.25***
EW(%)	PHT	0.13***	−0.05
FU(%)	Afngg	0.05	0.09
FU(%)	DTA	−0.02	0.04
FU(%)	DTS	−0.05	0.05
FU(%)	EHT	0	0.08
FU(%)	T ha^{-1}	−0.06*	−0.06
FU(%)	PHT	0.12***	0.21*
T ha^{-1}	DTA	−0.06	−0.34***
T ha^{-1}	DTS	−0.11***	−0.45***
T ha^{-1}	EHT	−0.21***	−0.28***
T ha^{-1}	PHT	−0.07*	−0.16
PHT	DTA	0.44***	0.43***
PHT	DTS	0.44***	0.46***

* Significant at the 0.05 probability level.

*** Significant at the 0.001 probability level.

accumulations could have some genetic factor limiting the accrual of toxin while allowing *A. flavus* growth. If confirmed, this alternate form of decreased susceptibility could be used in pyramiding multiple physiological resistances that will decrease overall aflatoxins. The lines of interest that had high AF% but low aflatoxins, thereby deviating from the correlation, included Mp14:179, GT1378, and the only two lines studied with red pericarps. The line (RedEar5-1-4-1-3/RedHybridEar-B-1-1-3)-1-1-B-B-2-B2-B15-B15 has a dark red pericarp, shown to increase phenols (Mahan et al., 2013; Meng et al., 2015) which can act as antioxidants. Antioxidants have been shown to reduce not only *A. flavus* growth but also to inhibit aflatoxin biosynthesis (Nesci et al., 2003). Further supporting this, the line (LH195\X\(RedEar5-1-4-1-3/RedHybridEar-B-1-1-3)-1–1-B-B-2-B2-B15///LH195)-B2////LH195)-1, is a BC_2F_2 red seeded version of LH195; LH195 was a consistently used ex-PVP line in this study which did not deviate from the AF% ratings and aflatoxin level correlation. This finding suggests it could be the red pericarp trait that contributed to overall low aflatoxins, however more investigation is needed to confirm this hypothesis.

Recommendations for Specific Elite Lines

A major goal was to identify individual lines with beyond average responses by programs that appear promising for low aflatoxin accumulations and/or good yield. CY2, GT1307, Mp14:2148, Tx775, Tx777, Tx779, and ANTIGO4 all consistently performed well for both high yield and low aflatoxin accumulation, but most of these were undesirably late in flowering time. Tx777 also performed well when used as a parent in the SERAT trials (Wahl et al., 2017). Several of these inbreds were shared as a parent in the hybrid performance trial of SERAT. Many of these hybrids did exhibit decreased susceptibility to the accrual of aflatoxin which could be inherited from the inbred parent. The earliest maturing of these lines was GT1307, averaging 70 d; this was considerably fewer days (–12) than the tolerant check Mp313E, and similar to that of ex-PVP material. Flowering can be adjusted by selecting different parents to combine and make hybrids. However, the two lines must nic (flower at the same time) to produce sufficient seed, and a split-delay planting can only be used to a point in adjusting inbred nic.

There were four susceptible checks used in testing for reduced aflatoxin accumulation, however, one inbred, CY4, continually accumulated extremely high aflatoxins in multiple years. CY4 accumulated significantly more toxin than the susceptible checks in many locations with accumulations more than 1900 ng g^{-1} more than the next highest inbred. CY4 was evaluated in the South and could be classified as an improved susceptible check while still being relevant to southern growers. CY4 could also be useful to use in a genetic linkage mapping population to better

14350653, 2019, 6, Downloaded from https://acsess.onlinelibrary.wiley.com/doi/10.2135/cropsci2019.04.0206 by Texas Tech University Libraries, Wiley Online Library on [18/07/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Table 3. Pearson's correlations by germplasm group on best linear unbiased predictors (BLUPs) for days to silking (DTS), yield (T ha^{-1}), visual ratings for aflatoxin sporulation (AF%), and transformed aflatoxin data (Aftrans) across all locations and all years.

		GA		LUBB		MS		Susceptible		TAMU	
Variable	by Variable	Correlation	Count	Correlation	Count	Correlation	Count	Correlation	Count	Correlation	Count
T ha^{-1}	DTS	−0.33	20	0.14	15	−0.61***	17	−0.34	5	0.19	24
AF(%)	DTS	−0.23	13	0.18	15	0.29	16	0.68	5	−0.64**	20
AF(%)	T ha^{-1}	0.13	13	0.43	15	−0.22	16	−0.18	5	0.14	20
Aftrans	DTS	−0.03	20	0.27	15	−0.09	17	−0.17	5	−0.43**	24
Aftrans	T ha^{-1}	−0.42*	20	0.42	15	0.50**	17	−0.45	5	−0.11	24
Aftrans	AF(%)	0.54*	13	0.40	15	−0.32	16	−0.62	5	0.44*	20

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

understand the mechanisms for susceptibility; increasing knowledge on how particular inbreds accumulate aflatoxins could yield some insight on decreasing susceptibility, and could be used in breeding to elucidate analysis.

CONCLUSIONS

The most robust inbred lines tested throughout this study have shown substantial reductions in aflatoxin accumulation along with favorable agronomics for hybrid production. While no complete resistance has yet been elucidated, collection of additional alleles towards decreasing susceptibility is essential in developing germplasm that could yield quantitative resistance. Testing across 5 yr and nine environments captured high *A. flavus* infections and showed the high repeatability needed to discriminate germplasm. The common garden test performed in these trials detected trends in how each germplasm directly or indirectly tolerates infection and aflatoxin accumulation, for example, later flowering lines had decreased *A. flavus* infection, likely due, in part, to inoculation avoidance. Using knowledge of these trends can allow effectively selecting parental lines for population development that can be pyramided into future lines; this is currently practiced in multiple public programs across the southern United States. Using the knowledge gained from this study along with many other aflatoxin accumulation studies performed, lines that have better interactions with agronomic, atoxigenic, genetic, and environmental factors can be elucidated, and better germplasm selections can be made in the future.

Conflict of Interest

The authors declare that there is no conflict of interest

Supplemental Material

Supplemental material for this article is available online.

Acknowledgments

The authors would like to thank Steven Anderson, David Rooney, and staff of the Texas A&M AgriLife Research and Extension Center at Weslaco for their help with this research. This research was funded by USDA National Institute of Food and Agriculture Competitive Grant 2014-6804-21836, USDA Hatch funds, USDA-ARS, Texas A&M AgriLife Research, the Aflatoxin Mitigation Center of Excellence, and Texas Corn Producers Board.

References

Abbas, H., R. Zablotowicz, B. Horn, N. Phillips, B. Johnson, X. Jin, and C.A. Abel. 2011. Comparison of major biocontrol strains of non-aflatoxigenic Aspergillus flavus for the reduction of aflatoxins and cyclopiazonic acid in maize. Food Addit. Contam. 28:198–208. doi:10.1080/19440049.2010.544680

Abbas, H.K., W.P. Williams, G.L. Windham, H.C. Pringle, W.P. Xie, and W.T. Shier. 2002. Aflatoxin and fumonisin contamination of commercial corn (Zea mays) hybrids in Mississippi. J. Agric. Food Chem. 50:5246–5254. doi:10.1021/jf020266k

Bibb, J.L., D. Cook, A. Catchot, F. Musser, S.D. Stewart, B.R. Leonard, G.D. Buntin, et al. 2018. Impact of Corn Earworm (Lepidoptera: Noctuidae) on Field Corn (Poales: Poaceae) Yield and Grain Quality. J. Econ. Entomol. 111:1249–1255. doi:10.1093/jee/toy082

Box, G.E., and D.R. Cox. 1964. An analysis of transformations. J. R. Stat. Soc. B 26:211–252.

Brown, R., Z. Chen, M. Warburton, M. Luo, A. Menkir, A. Fakhoury, and D. Bhatnagar. 2011. Proteins associated with aflatoxin-resistance are identified and characterized towards candidacy for breeding markers. Phytopathology 101:S21.

Cole, C.B., D. Bowman, F. Bourland, W. Caldwell, B. Campbell, D. Fraser, and D.B. Weaver. 2009. Impact of heterozygosity and heterogeneity on cotton lint yield stability. Crop Sci. 49:1577–1585. doi:10.2135/cropsci2008.08.0450

Chen, Y. 2016. High-density Linkage Map Construction, Mapping of Agronomic Traits in Tropical Maize (Zea Mays L.) and Validating SNPs Controlling Maize Grain Yield and Plant Height in Southern Hybrid Testcrosses. Ph.D. diss., Texas A&M Univ., College Station, TX.

Diener, U.L., R.J. Cole, T. Sanders, G.A. Payne, L.S. Lee, and M.A. Klich. 1987. Epidemiology of aflatoxin formation by Aspergillus flavus. Annu. Rev. Phytopathol. 25:249–270. doi:10.1146/annurev.py.25.090187.001341

Drott, M.T., B.P. Lazzaro, D.L. Brown, I. Carbone, and M.G. Milgroom. 2017. Balancing selection for aflatoxin in Aspergillus flavus is maintained through interference competition with, and fungivory by insects. Proc. Biol. Sci. 284:20172408. doi:10.1098/rspb.2017.2408

Duvick, D.N. 1999. Heterosis: Feeding people and protecting natural resources. The genetics and exploitation of heterosis in crops: 19–29.

14350653, 2019, 6, Downloaded from https://acsess.onlinelibrary.wiley.com/doi/10.2135/cropsci2019.04.0206 by Texas Tech University Libraries, Wiley Online Library on [18/07/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Barrero Farfan, I.D., G.N. De La Fuente, S.C. Murray, T. Isakeit, P.C. Huang, M. Warburton, P. Williams, et al. 2015. Genome Wide Association Study for Drought, Aflatoxin Resistance, and Important Agronomic Traits of Maize Hybrids in the Sub-Tropics. Plos One 10. doi:10.1371/journal.pone.0117737

Barrero Farfan, I.D., S.C. Murray, S. Labar, and D. Pietsch. 2013. A multi-environment trial analysis shows slight grain yield improvement in Texas commercial maize. Field Crops Res. 149:167–176. doi:10.1016/j.fcr.2013.04.017

Farias, C.A., M.J. Brewer, D.J. Anderson, G.N. Odvody, W. Xu, and M. Sétamou. 2014. Native maize resistance to corn earworm, Helicoverpa zea, and fall armyworm, Spodoptera frugiperda, with notes on aflatoxin content. Southwest Entomol 39:411–426. doi:10.3958/059.039.0303

Fleming, A.A. 1974. Registration of Maize Inbred Ga-209 Germplasm. Crop Sci. 14:911–912. doi:10.2135/cropsci1974.0011183X001400060059x

Goodman, M. 2005. Broadening the US maize germplasm base. Maydica 50:203–214.

Gqaleni, N., J.E. Smith, J. Lacey, and G. Gettinby. 1997. Effects of temperature, water activity, and incubation time on production of aflatoxins and cyclopiazonic acid by an isolate of Aspergillus flavus in surface agar culture. Appl. Environ. Microbiol. 63:1048–1053.

Guo, B.Z., M.D. Krakowsky, X. Ni, B.T. Scully, R.D. Lee, A.E. Coy, and N.W. Widstrom. 2011. Registration of Maize Inbred Line GT603. J. Plant Regist. 5:211–214. doi:10.3198/jpr2010.08.0386crg

Henderson, C.B. 1976. Maize Research and Breeders Manual. Illinois Foundation Seeds, Inc. unspecified.

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. Agron. J. 101:1219–1226. doi:10.2134/agronj2009.0004

Hesseltine, C., O.L. Shotwell, J. Ellis, and R. Stubblefield. 1966. Aflatoxin formation by Aspergillus flavus. Bacteriol. Rev. 30:795.

Horne, D.W., M.S. Eller, and J.B. Holland. 2016. Responses to recurrent index selection for reduced Fusarium ear rot and lodging and for increased yield in maize. Crop Sci. 56:85–94. doi:10.2135/cropsci2015.06.0333

Jones, R., H. Duncan, G. Payne, and K. Leonard. 1980. Factors influencing infection by Aspergillus flavus in silk-inoculated corn. Plant Dis. 64:859. doi:10.1094/PD-64-859

Klich, M.A. 2007. Aspergillus flavus: The major producer of aflatoxin. Mol. Plant Pathol. 8:713–722. doi:10.1111/j.1364-3703.2007.00436.x

Lane, B., S. Sharma, C. Niu, A.W. Maina, J.M. Wagacha, B.H. Bluhm, and C.P. Woloshuk. 2018. Changes in the Fungal Microbiome of Maize During Hermetic Storage in the United States and Kenya. Front. Microbiol. 9:2336. doi:10.3389/fmicb.2018.02336

Lewis, L., M. Onsongo, H. Njapau, H. Schurz-Rogers, G. Luber, S. Kieszak, J. Nyamongo, et al. 2005. Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in eastern and central Kenya. Environ. Health Perspect. 113:1763–1767. doi:10.1289/ehp.7998

Li, Z., L. Coffey, J. Garfin, N.D. Miller, M.R. White, E.P. Spalding, N. de Leon, et al. 2018. Genotype-by-environment interactions affecting heterosis in maize. PLoS One 13:e0191321. [erratum: 14(8): e0219528] doi:10.1371/journal.pone.0191321

Liu, K., and P. Wiatrak. 2011. Corn (Zea mays L.) plant characteristics and grain yield response to N fertilization programs in no-tillage system. Am. J. Agric. Biol. Sci. 6:279–286. doi:10.3844/ajabssp.2011.279.286

Llorente, C.F., F.J. Betran, A. Bockholt, and F. Fojt. 2004. Registration of Tx772 maize. Crop Sci. 44:1036–1037. doi:10.2135/cropsci2004.1036a

Magan, N., V. Sanchis and D. Aldred. 2004. Role of spoilage fungi in seed deterioration. Fungal biotechnology in agricultural, food and environmental applications: 311–323.

Mahan, A., S. Murray, L. Rooney, and K. Crosby. 2013. Combining ability for total phenols and secondary traits in a diverse set of colored (red, blue, and purple) maize. Crop Sci. 53:1248–1255. doi:10.2135/cropsci2012.06.0385

Mayfield, K., F.J. Betran, T. Isakeit, G. Odvody, S.C. Murray, W.L. Rooney, and J.C. Landivar. 2012. Registration of Maize Germplasm Lines Tx736, Tx739, and Tx740 for Reducing Preharvest Aflatoxin Accumulation. J. Plant Regist. 6:88–94. doi:10.3198/jpr2010.12.0675crg

Meng, Q., S.C. Murray, A. Mahan, A. Collison, L. Yang, and J. Awika. 2015. Rapid estimation of phenolic content in colored Maize by near-infrared reflectance spectroscopy and its use in breeding. Crop Sci. 55:2234–2243. doi:10.2135/cropsci2014.11.0767

Murray, S.C., K. Mayfield, J. Pekar, P. Brown, A. Lorenz, T. Isakeit, G. Odvody, et al. 2019. Tx741, Tx777, Tx779, Tx780, and Tx782 Inbred Maize Lines for Yield and Southern United States Stress Adaptation. J. Plant Regist. 13:258–269. doi:10.3198/jpr2017.07.0044crp

Nesci, A., M. Rodriguez, and M. Etcheverry. 2003. Control of Aspergillus growth and aflatoxin production using antioxidants at different conditions of water activity and pH. J. Appl. Microbiol. 95:279–287. doi:10.1046/j.1365-2672.2003.01973.x

Ni, X., J.P. Wilson, G.D. Buntin, B. Guo, M.D. Krakowsky, R.D. Lee, T.E. Cottrell, et al. 2011. Spatial patterns of aflatoxin levels in relation to ear-feeding insect damage in pre-harvest corn. Toxins (Basel) 3:920–931. doi:10.3390/toxins3070920

Schnell, F., and H. Becker. 1986. Yield and yield stability in a balanced system of widely differing population structures in Zea mays L. Plant Breed. 97:30–38. doi:10.1111/j.1439-0523.1986.tb01298.x

Scott, G.E., and N. Zummo. 1990. Registration of Mp313e Parental Line of Maize. Crop Sci. 30:1378. doi:10.2135/cropsci1990.0011183X003000060080x

Scott, G.E., and N. Zummo. 1992. Registration of Mp420 Germplasm Line of Maize. Crop Sci. 32:1296. doi:10.2135/cropsci1992.0011183X003200050054x

Stoloff, L., H. Van Egmond, and D. Park. 1991. Rationales for the establishment of limits and regulations for mycotoxins. Food Addit. Contam. 8:213–221. doi:10.1080/02652039109373971

Tucker, D., Jr., L. Trevathan, S. King, and G. Scott. 1986. Effects of four inoculation techniques on infection and aflatoxin concentration of resistant and susceptible corn hybrids inoculated with Aspergillus flavus. Phytopathology 76:290–293. doi:10.1094/Phyto-76-290

Wahl, N., S.C. Murray, T. Isakeit, M. Krakowsky, G.L. Windham, W.P. Williams, B. Guo, et al. 2017. Identification of Resistance to Aflatoxin Accumulation and Yield Potential in Maize Hybrids in the Southeast Regional Aflatoxin Trials (SERAT). Crop Sci. 57:202–215. doi:10.2135/cropsci2016.06.0519

14350653, 2019, 6, Downloaded from https://acsess.onlinelibrary.wiley.com/doi/10.2135/cropsci2019.04.0206 by Texas Tech University Libraries, Wiley Online Library on [18/07/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

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 Sci. 53:2374–2383. doi:10.2135/cropsci2012.10.0616

Weaver, M.A., H.K. Abbas, M.J. Brewer, L.S. Pruter, and N.S. Little. 2017. Integration of biological control and transgenic insect protection for mitigation of mycotoxins in corn. Crop Prot. 98:108–115. doi:10.1016/j.cropro.2017.03.020

West, D.R., D.R. Kincer, and C.R. Graves. 2001. Registration of T173 parental line of maize. Crop Sci. 41:1375. doi:10.2135/cropsci2001.4141375x

Wicklow, D.T., R.A. Norton and C.E. McAIpin. 1998. β-Carotene inhibition of aflatoxin biosynthesis among Aspergillus flavus genotypes from Illinois corn. Mycoscience 39: 167–172.

Widstrom, N.W., B.Z. Guo, and D.M. Wilson. 2003. Integration of crop management and genetics for control of preharvest aflatoxin contamination of corn. J. Toxicol. Toxin Rev. 22:195–223. doi:10.1081/TXR-120024092

Williams, W., M. Krakowsky, B. Scully, R. Brown, A. Menkir, M. Warburton, and G.L. Windham. 2015. Identifying and developing maize germplasm with resistance to accumulation of aflatoxins. World Mycotoxin J. 8:193–209. doi:10.3920/WMJ2014.1751

Williams, W.P., M.N. Alpe, G.L. Windham, S. Ozkan, and J.E. Mylroie. 2013. Comparison of two inoculation methods for evaluating maize for resistance to Aspergillus flavus infection and aflatoxin accumulation. Int. J. Agron. 2013:972316.

Williams, W.P., P.M. Buckley, and G.L. Windham. 2002. Southwestern corn borer (Lepidoptera: Crambidae) damage and aflatoxin accumulation in maize. J. Econ. Entomol. 95:1049–1053. doi:10.1093/jee/95.5.1049

Williams, W.P., and G.L. Windham. 2006. Registration of maize germplasm line Mp717. Crop Sci. 46:1407–1408. doi:10.2135/cropsci2005.09-0330

Williams, W.P., and G.L. Windham. 2012. Registration of Mp718 and Mp719 Germplasm Lines of Maize. J. Plant Regist. 6:200–202. doi:10.3198/jpr2011.09.0489crg

Windham, G.L., W.P. Williams, L.K. Hawkins, and T.D. Brooks. 2009. Effect of Aspergillus flavus inoculation methods and environmental conditions on aflatoxin accumulation in corn hybrids. Toxin Rev. 28:70–78. doi:10.1080/15569540802450037

Wu, F. 2015. Global impacts of aflatoxin in maize: Trade and human health. World Mycotoxin J. 8:137–142. doi:10.3920/WMJ2014.1737

Wu, F., and H. Guclu. 2012. Aflatoxin regulations in a network of global maize trade. PLoS One 7:e45151. doi:10.1371/journal.pone.0045151

Yin, X., M.A. McClure, N. Jaja, D.D. Tyler, and R.M. Hayes. 2011. In-season prediction of corn yield using plant height under major production systems. Agron. J. 103:923–929. doi:10.2134/agronj2010.0450

14350653, 2019, 6, Downloaded from https://acsess.onlinelibrary.wiley.com/doi/10.2135/cropsci2019.04.0206 by Texas Tech University Libraries, Wiley Online Library on [18/07/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License