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# Minimum Temperature, Rainfall, and Agronomic Management Impacts on Corn Grain Aflatoxin Contamination

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## ABSTRACT

Aflatoxins are a group of toxins produced by fungi found on corn (*Zea mays* L.). Aflatoxin contamination can make it unmarketable. Fortunately, management practices that reduce stress during critical growth stages lessen contamination. A study was conducted at Fairhope, AL (2010–2014), and Prattville, AL (2013–2014), to evaluate the effect of planting dates, plant densities, and in-season weather conditions on preharvest aflatoxin contamination. The experiment had a split-split-plot design replicated six times, with inoculation method assigned to the main plots, planting date to the subplots, and planting density to the sub-subplots. Results showed that delaying planting from mid-March to mid-April reduced aflatoxin levels and increasing the planting density from 44,480 to 74,130 plants $ha^{-1}$ did not impact toxin accumulation. Multiple linear regression indicated that minimum air temperature and rainfall models could explain from 50 to 76% of the observed aflatoxin variability. However, the effect of both variables on aflatoxin contamination levels changed during the period presilking (14 d prior) to physiological maturity. Minimum temperature alone had the strongest positive influence on aflatoxin over the 2 wk after mid-silk. A reduction in rainfall during 2 wk prior mid-silk and from Day 43 after mid-silk to physiological maturity resulted on high aflatoxin contamination levels. In conclusion, a better understanding of the influence of weather variables on corn contamination may lead to better crop management and development of more accurate prediction systems.

## Core Ideas

- Evaluation of the influence of planting date on corn aflatoxin.
- Evaluation on how in-season weather conditions influence corn aflatoxin.
- Evaluation of the influence of planting density on corn aflatoxin.
- Identification of the growing season periods when the aflatoxin risk is highest.
- Evaluation of the relationship between yield and corn aflatoxin.

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Corn (*Zea mays* L.) is a major worldwide crop that contributes significantly to the economy, because it is used in crop rotations, as animal feed, in the fermentation industry, and for direct human consumption (Abbas et al., 2002, 2017; Battilani et al., 2016). In 2016, the corn harvested area in the Americas, Asia, Africa, and Europe equaled 70, 63, 37, and 18 million ha, respectively (FAO STAT, http://www.fao.org/faostat/en/#data/RM). Despite its importance, corn grain infection by *Aspergillus flavus* and subsequent aflatoxin contamination is a worldwide concern (Battilani et al., 2016; Henry et al., 2009; Warburton et al., 2013). Naturally contaminated grain has been reported in several countries other than the United States (e.g., Australia, France, Hong Kong, Italy, Kenya, Mozambique, Philippines, Thailand, and Uganda) (Battilani et al., 2016; Blaney et al., 2008; Chauhan et al., 2008; Lewis et al., 2005; Shephard, 2008; Shotwell, 1977). Consequently, corn grain quantity and quality is highly impacted by aflatoxin contamination. As a result of its high toxicity, aflatoxins are associated with the greatest losses and management costs among mycotoxins; therefore, the United States Food and Drug Administration agency (US FDA) has set 20 $\mu g\ kg^{-1}$ as the upper limit for corn contamination with aflatoxins that can be used for human consumption or feed of young animals (Fountain et al., 2014; Robens and Cardwell, 2003; U.S. Food and Drug Administration, 2000). These toxins are deleterious to both humans and animals, because of their immunosuppressant, mutagenic, teratogenic, and carcinogenic effects, and as such, also have a significant impact on the corn grain industry (Hernández-Martínez and Navarro-Blasco, 2010; Payne, 1992; Wahl et al., 2017). Balancing the economic importance of corn with the public health risk associated with contaminated food consumption is crucial.

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**Abbreviations:** APDA, acidified potato dextrose agar; ELISA, enzyme-linked immunosorbent assay; HPLC, high-performance liquid chromatography; PD1, mid-March planting; PD2, mid-April planting; U.S. FDA, United States Food and Drug Administration agency.

Weather and climate fluctuations influence corn infection by *Aspergillus flavus* and have an impact on the extent of contamination (Cotty and Jaime-Garcia, 2007). Infection by *A. flavus* and subsequent kernel contamination is more severe in tropical and subtropical areas; however, contamination might occur in temperate areas as well (Battilani et al., 2016; Payne, 1992; Windham et al., 2009). In the southern United States, preharvest aflatoxin contamination is a constant concern, especially during growing seasons characterized by high temperature, low humidity, and drought (Abbas et al., 2012, 2006, 2002; Damianidis et al., 2015; Kebede et al., 2012; Scully et al., 2016). Contamination is commonly exacerbated in seasons characterized by above normal temperatures and below average rainfall during the reproductive and grain-filling growth stages (Hawkins et al., 2008; Wahl et al., 2017; Widstrom et al., 1990; Windham et al., 2009). Among others, heat and drought stresses are considered to diminish corn resistance and, thus, predispose crops to *A. flavus* infection and subsequent in-field contamination (Bruns, 2003; Damianidis et al., 2015; Fountain et al., 2014; Hawkins et al., 2008; Wahl et al., 2017; Windham et al., 2009). However, Abbas et al. (2007) reported a negative correlation between heat stress and aflatoxin contamination detected in preharvest corn, thus demonstrating the complex relationships and interactions between biotic and abiotic factors contributing to the phenomenon.

The high variability in aflatoxin contamination among seasons and locations might be partially explained by the complexity of host resistance to *A. flavus* and genotype × environment interactions that influence the expression of this trait (Fountain et al., 2014; Okoth et al., 2017). These observations are supported by studies that have shown strong correlations between aflatoxin contamination and plant stress (Fountain et al., 2014; Hawkins et al., 2008; Jones et al., 1981; Pechanova et al., 2011; Rodriguez-del-Bosque, 1996; Widstrom et al., 1990; Windham et al., 2009).

Agronomic management practices that reduce abiotic stress can reduce aflatoxin contamination in corn (Payne, 1992). Practices that have been tested include plant density, planting date, nutrition, and insect control (Abbas et al., 2007; Bruns, 2003; Bruns and Abbas, 2005; Jones and Duncan, 1981; Jones et al., 1981; Payne, 1992; Rodriguez-del-Bosque, 1996). Hansen et al. (2013) reported that water stress results in an up regulation of genes associated with drought and down regulation of genes linked to nutrient uptake and disease resistance. These drought-linked processes could predispose corn to aflatoxin contamination. However, two studies failed to show correlation between plant density and aflatoxin contamination (Abbas et al., 2012; Rodriguez-del-Bosque, 1996). Optimum plant density depends on the genotype × environment × management interaction, including factors such as relative maturity groups, soil class, soil plant-available water, management practices, and location (Assefa et al., 2016; Bruns and Abbas, 2005; Mastrodomenico et al., 2018). In recent years, elite corn hybrids are grown at plant populations equal to or greater than 79,000 plants $ha^{-1}$; a 50,000 plants $ha^{-1}$ increase compared with the pre-hybrid era (Mastrodomenico et al., 2018; Gonzalez et al., 2018). These changes suggest the need to develop new site-specific strategies to mitigate corn aflatoxin contamination. Additionally, altering planting dates can impact plant exposure to heat and drought stress during the reproductive stages, and thus, facilitate aflatoxin concentration in corn grain (Abbas et al., 2007; Bruns and Abbas, 2006; Rodriguez-del-Bosque, 1996). For example, Lillehoj et al. (1980) reported that corn planted in May in Georgia and Florida had greater aflatoxin contamination than corn planted in April and June. Therefore, by altering the aforementioned management practices, and thus, exposing the crop to less stress during the critical growth stages, could reduce high aflatoxin contamination levels.

Because environmental conditions are highly variable between locations, local field studies are needed to elucidate how management practices and environmental conditions interact to influence toxin biosynthesis and accumulation. The hypothesis is that agronomic management practices reducing corn plant stress lower preharvest aflatoxin contamination levels. Our objectives were to: (i) assess the effect of planting date and plant density on preharvest aflatoxin contamination in rainfed corn grown in the Coastal Plains of south and central Alabama, (ii) identify which weather variables influence aflatoxin contamination of corn, (iii) determine the relative weight of significant weather variables on contamination of corn for the Coastal Plains of Alabama, and (iv) determine time periods during the growing season when weather variables are associated with aflatoxin contamination of corn.

## MATERIALS AND METHODS

### Experimental Sites and Cultivation Practices

Field experiments were conducted at the Gulf Coast Research and Extension Center, in Fairhope, AL (30°32′ N, 87°52′ W) from 2010 to 2014, and at the Prattville Agricultural Research Unit, near Prattville, AL, USA (32°25′ N, 86°26′ W) from 2013 to 2014. The soil series at Fairhope and Prattville were a Malbis fine sandy loam (fine-loamy, siliceous, subactive, thermic Plinthic Paleudults) and a Lucedale sandy loam (fine-loamy, siliceous, subactive, thermic Rhodic Paleudults), respectively. Cultivation practices varied over season and location depending on equipment availability, but did not differ from the recommended agronomic practices in the state of Alabama (Mask and Mitchell, 1988). Briefly, 1 to 2 wk before planting a field area (approximately 900–1000 $m^2$) was strip-tilled at Fairhope each season, while at Prattville in 2013 and 2014 a conventional tillage (disk harrowed, chisel plowed, and smoothed with Lely Roterra) and paratill (in-row subsoiling with a KMC Paratill) (Kelley Manufacturing Co, Tifton, GA) were applied, correspondingly. Generally, corn was planted following soybean [*Glycine max* (L.) Merr.] or cotton (*Gossypium* spp.). During the winter (November–March) the land was either left fallow (Prattville, 2013–2014), or planted to a wheat (*Triticum aestivum* L.), rye (*Secale cereale* L.), or wheat and rye mixture cover crop prior to corn cultivation (Fairhope 2010–2014). At Fairhope the crop residues at or after planting were rye, wheat, or wheat and rye mixture in 2012, 2013, and 2014, respectively. Soybean residue was prevalent at Prattville in 2014. Fertilization and soil pH adjustments followed the recommendations of Auburn University Soil Test Laboratory (Auburn University, 2012) (Table 1). In most cases, N fertilization occurred twice during the growing season with one-third of the total N applied at planting and two-thirds at the V6 growth stage (Table 1). In each growing season, the corn hybrid Pioneer 31P42 (HX1, LL,

Table 1. Planting, mid-silk, physiological maturity, harvest dates, and fertilization for each location and growing season.

| Year | Treatment† | Planting date | Mid-silk‡ | Physiological maturity§ | Harvest¶ | N fertilization date and rate# | P and K fertilization date and rate |
|---|---|---|---|---|---|---|---|
| | | | | | | kg ha$^{-1}$ | |
| Fairhope | | | | | | | |
| 2010 | PD1 | 19 Mar. | 29 May (71) | 11 July (43) | 10 Aug. | 15 Mar. (22)†† | 15 Mar. (45 of P and K)†† |
| | PD2 | 15 Apr. | 10 June (56) | 22 July (42) | 10 Aug. | 29 Apr. (145)‡‡ | |
| 2011 | PD1 | 17 Mar. | 29 May (73) | 11 July (43) | 8 Aug. | 16 Mar. (22) | 16 Mar. (67 of P and 45 of K) |
| | PD2 | 12 Apr. | 13 June (62) | 26 July (43) | 23 Aug. | 26 Apr. (145) | |
| 2012 | PD1 | 20 Mar. | 25 May (66) | 20 July (56) | 7 Aug. (74) | 19 Mar. (22) | 19 Mar. (45 of P and K) |
| | PD2 | 13 Apr. | 15 June (63) | 2 Aug. (48) | 25 Aug. (71) | 26 Apr. (145) | |
| 2013 | PD1 | 14 Mar. | 1 June (79) | 23 July (52) | 13 Aug. (73) | 4 Mar. (20), 14 Mar. (50), 30 Apr. (100)§§ | 4 Mar. (45 of P and K) |
| | PD2 | 18 Apr. | 21 June (64) | 5 Aug. (45) | 24 Aug. (64) | 4 Mar. (20), 18 Apr. (50), 23 May (100) | |
| 2014 | PD1 | 21 Mar. | 7 June (78) | 28 July (51) | 11 Aug. (65) | 12 Mar. (20)¶¶ | 12 Mar. (45 of P and K) |
| | PD2 | 14 Apr. | 19 June (66) | 6 Aug. (48) | 11 Aug. (53) | 7 May (140) | |
| Prattville | | | | | | | |
| 2013 | PD1 | 15 Mar. | 9 June (86) | 29 July (50) | 22 Aug. (74) | 15 Mar. (56), 3 May (112)## | 28 Jan. (67 of P) |
| | PD2 | 16 Apr. | 21 June (66) | 7 Aug. (47) | 3 Sept. (74) | 4 Apr. (56), 29 May (112) | |
| 2014 | PD1 | 22 Mar. | 8 June (78) | 29 July (51) | 15 Aug. (68) | 24 Mar. (56), 2 May (112) | |
| | PD2 | 21 Apr. | 22 June (62) | 8 Aug. (47) | 4 Sept. (74) | 21 Apr. (56), 21 May (112) | |

† PD1 and PD2 are standard planting date (mid-March) and late planting date (mid-April), respectively.

‡ Numbers in parenthesis are days from planting date to mid-silk.

§ Numbers in parenthesis are days from mid-silk to physiological maturity.

¶ Numbers in parenthesis are days from mid-silk to hand harvest (harvest maturity).

# Numbers in parenthesis correspond to N rate.

†† At Fairhope in all years, the first application corresponded to a blended fertilizer (NPK). The values on the PD1 row corresponded to N, P, and K rates broadcasted over the entire study area.

‡‡ At Fairhope from 2010 to 2012, the rate on the PD2 row was the second N application over the study area. The N form was urea–ammonium nitrate as 28–0–0–5 solution.

§§ From 2013 to 2014, the second and third N rates were applications of ammonium nitrate as 33–0–0.

¶¶ In 2014, the indicated rate was applied over the entire study area.

## At Prattville in 2013 and 2014, 34% N urea–ammonium nitrate was used on the first and second N applications.

RR2; 121 d relative maturity group) was planted in four row plots 9.1 m long. The row spacing within each experimental unit (plot) was 96 and 90 cm in Fairhope and Prattville, respectively.

The experimental design at both locations was a split-split-plot arrangement in a randomized complete block design with six blocks. The inoculation treatment was assigned to whole plots, whereas planting date and plant density factors were allocated to subplots and sub-subplots, respectively. Inoculation treatments were: (i) non-inoculation (control), to resemble natural infection and subsequent contamination; and (ii) artificial inoculation, to ensure uniform kernel exposure to *A. flavus* conidia, allowing for potential aflatoxin accumulation at concentrations sufficient to reveal possible effects of planting date and/or plant density on aflatoxin synthesis regardless of environmental conditions (Abbas et al., 2002). Experimental plots were inoculated approximately 7 d after R1 growth stage. There were two planting dates: (i) a standard planting date (approximately mid-March) and (ii) a late planting date (approximately mid-April) (Table 1). Corn was seeded at rates designed to produce standing populations of 44,480, 54,360, 64,250, and 74,130 plants ha$^{-1}$ (densities D1, D2, D3, and D4, respectively). In the 2010 growing season, only the first three densities (D1, D2, and D3) were included in the study.

## Inoculum Preparation and Inoculation Technique

### Seasons 2010–2012

Between 2010 and 2012 growing seasons, cracked corn was infested with *A. flavus* and was used as the inoculant carrier. Briefly, 7.5 kg of cracked corn were soaked overnight with 2390 mL of distilled water, bagged, and autoclaved for 55 min at 121°C. Fifty mL of chlorotetracycline (1%) plus streptomycin (1%), then 20 mL of 2,5-dichloro-4-nitroaniline (Botran) (0.3 g mixed into 900 mL acetone) were added and mixed to 7.5 kg autoclaved corn. Cultures of field-collected toxigenic *A. flavus* isolates were grown on acidified potato dextrose agar (APDA) on 9-cm Petri dishes at room temperature (approximately 25°C) for 5 to 7 d. The APDA was prepared by mixing 39 g of potato dextrose agar (IBI Scientific, Peosta, IA) into 1 L of distilled water. The solution was autoclaved and cooled to 55°C, and then 1 mL of lactic acid was added. Conidia were rinsed and collected from the plates with sterile water containing 0.01% Tween 20. Prepped corn (456 g) was placed in small plastic bags, inoculated with 20 mL of spore suspension, homogenized by manual agitation every 24 h, and incubated for 3 d at room temperature (approximately 25°C). Fungus-infested corn was scattered by hand in the middle two rows onto the soil at a rate of 1057 kg ha$^{-1}$ a week prior mid-silk (R1).

### Seasons 2013–2014

Data analysis from the first three seasons indicated that spreading *A. flavus*–infested cracked corn on the soil did not effectively promote infection and subsequent contamination. Therefore, side needle wounding, using an Idico tree-marking gun fitted with a 14-gauge hypodermic needle (Idico Products Co., New York), was used (Abbas et al., 2002; Windham et al., 2009; Zummo and Scott, 1989). Inoculum preparation

Table 2. Significance of factor effects and aflatoxin mean concentrations by factor at Fairhope and Prattville, AL, during the study period between 2010 and 2014.

| | Fairhope | | | | | Prattville | |
|---|---|---|---|---|---|---|---|
| | Year | | | | | Year | |
| Effect† | 2010 | 2011 | 2012 | 2013 | 2014 | 2013 | 2014 |
| | $P > F$ | | | | | | |
| IN | 0.6134 | 0.0654 | 0.5133 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| PD | 0.6749 | 0.0009 | 0.4428 | <0.0001 | 0.0209 | 0.4502 | 0.4623 |
| D | 0.9794 | 0.1589 | 0.2619 | 0.5972 | 0.1390 | 0.6661 | 0.3274 |
| IN × PD | 0.807 | 0.238 | 0.6976 | <0.0001 | 0.9825 | 0.0956 | 0.1821 |
| PD × D | 0.9984 | 0.8578 | 0.9114 | 0.0411 | 0.054 | 0.5661 | 0.3104 |
| IN × D | 0.7603 | 0.0888 | 0.8036 | 0.9367 | 0.3586 | 0.5340 | 0.9725 |
| I × PD × D | 0.0145 | 0.8772 | 0.8274 | 0.7961 | 0.9689 | 0.0460 | 0.9274 |
| | Aflatoxin least square mean estimates (μg kg$^{-1}$)‡ | | | | | | |
| IN | | | | | | | |
| I§ | 5.1 | 88.6 | 18.6 | 141.2a | 202.9a | 673.2a | 147.0a |
| No-I | 7.0 | 47.5 | 37.2 | 3.3b | 0.4b | 3.4b | 3.9b |
| PD | | | | | | | |
| PD1¶ | 6.5 | 115.4a | 28.0 | 49.5a | 19.8a | 57.9 | 28.3 |
| PD2 | 5.4 | 36.3b | 24.9 | 11.2b | 12.4b | 49.2 | 24.0 |
| IN × PD | | | | | | | |
| I × PD1 | 5.3 | 191.4 | 14.3 | 553.6a | 253.6 | 878.0 | 185.2 |
| I × PD2 | 4.9 | 40.7 | 24.2 | 35.4b | 162.3 | 516.2 | 116.6 |
| No-I × PD1 | 8.0 | 69.5 | 54.0 | 3.6c | 0.7 | 2.9 | 3.6 |
| No-I × PD2 | 6.0 | 32.4 | 25.6 | 3.1c | 0.1 | 3.9 | 4.3 |
| PD × D | | | | | | | |
| PD1 × D1# | 6.2 | 106.0 | 81.5 | 40.5ab | 34.0 | 56.9 | 29.9 |
| PD1 × D2 | 6.7 | 67.2 | 15.3 | 84.3a | 17.3 | 69.6 | 28.8 |
| PD1 × D3 | 6.7 | 216.8 | 34.6 | 42.4ab | 20.6 | 43.5 | 30.1 |
| PD1 × D4 | | 114.7 | 13.7 | 41.4ab | 12.5 | 65.2 | 24.9 |
| PD2 × D1 | 5.0 | 24.6 | 32.7 | 15.5bc | 9.4 | 57.9 | 15.1 |
| PD2 × D2 | 5.7 | 24.0 | 33.5 | 8.2c | 15.6 | 45.1 | 25.0 |
| PD2 × D3 | 5.6 | 53.5 | 20.5 | 8.5c | 16.2 | 47.2 | 35.0 |
| PD2 × D4 | | 54.6 | 16.9 | 14.5bc | 9.9 | 47.7 | 24.7 |

† IN = inoculation method, PD = planting date, D = plant density.

‡ Least squares means in the same column followed by different letter are significantly different at $P \leq 0.05$ (Tukey test).

§ I and No-I refer to artificial inoculation and natural infection methods, respectively.

¶ PD1 and PD2 represent planting date at mid-March and mid-April, respectively.

# D1, D2, D3, and D4 correspond to plant densities of 44,480, 54,360, 64,250, and 74,130 plants ha$^{-1}$, respectively.

was carried on as described in detail by Abbas et al. (2002) and Windham et al. (2009). Briefly, 50 g of sterile corn cob grits (size 2040, Grit-O-Cobs, Maumee, OH) were soaked with 100 mL of sterile distilled water and placed in 500-mL flasks. Inoculum of toxigenic *A. flavous* isolates was incubated at 28°C for 3 wk. Conidia of *A. flavus* grown in each flask were washed with a 500 mL solution of distilled water with 0.1% Tween 20. To ensure adequate infection, modifications included: (i) the use of four native Alabama aflatoxigenic *A. flavus* isolates (E316.1, PV11027, SM310.3, and TV203.1) collected, isolated, and preserved by K.L. Bowen (Plant Pathology Laboratory, Auburn University) (Campbell and White, 1994; Walker and White, 2001); and (ii) four inoculum suspensions standardized to $9.0 \times 10^6$ conidia mL$^{-1}$ (Zummo and Scott, 1989) prepared from each toxigenic isolate and mixed at equal volumes (v/v/v/v) to produce the final inoculum suspension. Inoculations were made on the top ear of each plant in the middle two rows (30 plants per row) approximately 7 d after R1 by injecting around 2 mL of suspension into one or two corn kernels.

## Harvest and Aflatoxin Contamination Assessment

Ten upper-most corn ears were hand-harvested at harvest maturity from the center rows of each plot. Ears were machine-shelled. Grain originating from the same experimental unit were manually mixed and a subsample (250 g) was obtained for aflatoxin assessment (Jaime-Garcia and Cotty, 2003). The remaining corn in plots was harvested with a small plot combine. Yield per plot was added to hand-harvested to arrive at whole plot weight and reported at 15.5% moisture content. Important growth stage days per growing season and location for different planting dates are provided in Table 1.

Kernels (250 g) from sampled ears were ground to <2 mm with a Thomas-Wiley Laboratory Mill, model 4 (Swedesboro, NJ). Total aflatoxin quantitative assessment was done on 10 g of ground corn per treatment (Veratox test, Neogen Corp., Lansing, MI) following manufacturer instructions. Veratox is a competitive direct enzyme-linked immunosorbent assay (ELISA) test with detection limit and quantitation range equal to 1.4 and 5 to 50 μg kg$^{-1}$, respectively. All assays exceeding the upper quantitation threshold (50 μg kg$^{-1}$) were diluted as needed and reassayed

(Bowen et al., 2014). Duplicates were run for more than 10% of the samples to verify aflatoxin concentration. Samples from the 2014 season were analyzed using high-performance liquid chromatography (HPLC) at the Biological Control of Pests Research Unit, USDA-ARS, Stoneville, MS, as described by Abbas et al. (2015). Briefly, 20 g of ground corn (<0.02 mm) was extracted in 100 mL of 70% methanol. Samples were diluted one-half with acetonitrile, and 800 μL of the mixture was applied to a 1.5 mL extract-clean reservoir mini column packed with aluminum oxide. The eluate (20 μL) was injected onto a Waters HPLC system described by Abbas et al. (2015) at a column temperature of 30°C. An aflatoxin standard mixture (no. A9441; Sigma-Aldrich, St. Louis) and additional standard dilutions were studied using methanol/water/acetic acid (310:190:0.5, vol/vol/vol) elutant. The same solution was used as blank. The detection limit was 0.1 ng $g^{-1}$. The HPLC and ELISA methods were found comparable (H.K. Abbas, personal communication, 2015).

## Weather Parameters

Air temperature and rainfall were measured at both experimental sites for the 2013 and 2014 seasons with a HOBO Pendant Temperature/Alarm Data Logger (model 64k-UA-001-64, Onset Computer Corp., Bourne, MA), and a tipping bucket rain gauge (RainWise Inc., Trenton, ME). Sensors were mounted on a PVC pipe and installed at each field at the beginning of the growing season. Between 2010 and 2012, maximum temperature, minimum temperature, and rainfall data were collected by the weather stations of the Alabama Mesonet Weather Data network (AWIS, http://www.awis.com/mesonet/index.html) located at each research unit. Monthly weather summaries are provided in Supplemental Table S1.

A total of 15 weather variables were evaluated (3 weather variables × 5 time windows) for their association with end-of-season aflatoxin contamination. Maximum and minimum daily air temperatures were averaged, while cumulative rainfall was considered, as well, for consecutive time periods or windows beginning 2 wk before R1 and extending up to physiological maturity (R6). The five time windows considered were: (i) 14 d before (-I), and (ii) 14 d after R1 (I), (iii) from Day 15 to Day 28 after R1 (II), (iv) from Day 29 to Day 42 following R1 (III), and (v) a variable day-length window starting on Day 43 after R1 and extending up to physiological maturity day (ranging from 0 to 14 d, depending on planting date and growing season) (IV). Mid-silk was designated as the reference day for two reasons: (i) to remove part of the variability intrinsically related to different locations, planting dates, and growing seasons; and (ii) previous work indicating that the critical time for corn infection and contamination occurs around and beyond R1 (Betrán and Isakeit, 2004; Hawkins et al., 2008; Payne et al., 1988; Windham et al., 2009).

## Statistical Analysis

Aflatoxin concentrations (μg $kg^{-1}$) were log-transformed [$\log_{10}$ (μg $kg^{-1}$+1)] to stabilize the variance, and all the analyses thereafter were done on transformed data. Aflatoxin values reported are the geometric means (antilogarithm of the logarithmic mean). Yield data met normality assumptions and therefore no transformation was needed. Generalized linear mixed model analyses in SAS version 9.3 using the PROC GLIMMIX procedure (SAS Institute, 2010) were performed. Fixed effects means were compared at level of significance $\alpha = 0.05$ with least-square means differences adjusted with Tukey's test to control for experiment wise error. In a preliminary analysis, aflatoxin data were pooled (e.g., over each location and seasons 2013 and 2014), with year and location considered fixed effects ($P \leq 0.05$). This analysis (not shown) revealed the effects as being significant. Therefore, the subsequent analyses for each response variable were performed separately by year and location with block, block × inoculation, and block × inoculation × planting dates considered as random effects. Because the inoculation method from 2010 to 2012 was different from the last 2 yr of the study, the analysis of the main effects on aflatoxin included only the last 2 site-years with the same inoculation method.

Table 3. Effects for aflatoxin contamination and yield when pooled over locations and seasons 2013-2014.

| Effect† | Aflatoxin | Yield |
|---|---|---|
| | $P > F$‡ | |
| Year | 0.0003 | 0.1096 |
| Location | <0.0001 | 0.0001 |
| Year × Location | 0.2182 | 0.4834 |
| Inoculation§ | <0.0001 | 0.0125 |
| Year × Inoculation | 0.8148 | 0.6501 |
| Location × Inoculation | 0.8852 | 0.6930 |
| Planting date | <0.0001 | 0.0001 |
| Year × Planting date | 0.0319 | <0.0001 |
| Location × Planting date | 0.0013 | <0.0001 |
| Inoculation × Planting date | <0.0001 | 0.0728 |
| Planting density | 0.6834 | <0.0001 |
| Year × Planting density | 0.0625 | 0.0055 |
| Location × Planting density | 0.5959 | <0.0001 |
| Inoculation × Planting density | 0.8197 | 0.5364 |
| Planting density × Planting date | 0.2427 | 0.0020 |

† Up to two-way fixed effects interactions presented herein.

‡ Effects are significant at $\alpha = 0.05$.

§ Inoculated plots corresponded only to the side-needle inoculation treatment.

Multiple linear regression analyses were conducted to investigate potential relationships between aflatoxin contamination due to natural infection and the calculated weather variables for five time intervals before and after R1 growth stage. In all models, aflatoxin concentration was averaged over six treatment replicates. In an initial step, a group of five rainfall or five temperature-derived variables were considered separately as explanatory variates in the regression models. Analysis with raw maximum temperature data indicated that the residuals were not normally distributed; attempts to transform the data did not fix the normality issue. Therefore, results of the regression analysis with maximum temperature variables as independent factors could be misleading, and thus, those results are not presented. In a second step, five rainfall and five minimum air temperature variables were entered together into the linear model and tested as explanatory factors. Significant independent variables for all models were determined by employing stepwise selection (PROC REG, SAS version 9.3) with entry and exit criteria levels equal to 0.1. Regression analysis between aflatoxin contamination and grain yield were conducted with data from the Fairhope site. Linear relationships were evaluated by running univariate linear regression analyses with PROC REG procedure (SAS version 9.3). For the 2010 and 2010–2012 regression analyses, aflatoxin

contamination data [$\log_{10}(\mu g\ kg^{-1}+1)$] and grain yield were used as response and explanatory variables, respectively, after being averaged by treatments (inoculation × planting date × density). When the analysis included 2010–2014 data, only observations from the non-inoculated treatment were included.

## RESULTS AND DISCUSSION

### Weather Conditions

Season-long climatic conditions in Fairhope in 2010 and 2011 were warmer and drier than the season-long historical average (Supplemental Table S1). At Fairhope in 2012, monthly cumulative rainfall from March to April was 173 mm below the historical average, but from May to July, a period that coincides with the grain filling time, rainfall was exceeded by 95 mm compared with the historical average values. In the 2013 and 2014 growing seasons, the rainfall was above and temperature below the historical average. In 2013, June and July rainfall at Fairhope was above average. In 2014, not only rainfall in July but also August were above average values. At Prattville, the 2013 growing season was cooler and drier than the historical average values. Season-long mean maximum and mean minimum temperatures were 1.2 and 0.2°C lower than the historical average, whereas the area received 81.6 mm less rainfall compared with the historical average (Supplemental Table S1). In 2014, the growing season was wetter than the historical average.

### Treatment Effects on Aflatoxin Contamination

#### Inoculation Effect on Aflatoxin Contamination

At Fairhope, spreading cracked corn previously inoculated with *A. flavus* during the 2010 to 2012 seasons did not increase aflatoxin levels ($P > 0.05$) compared with natural infection treatment (Table 2). This inoculation method was effective only under the extremely dry conditions encountered in 2011 (Supplemental Table S1), when the mean aflatoxin concentrations exceeded the US FDA action limit of 20 μg of aflatoxin per kg of grain (Table 2). Olanya et al. (1997) suggested that *A. flavus* conidia production and dispersal are influenced by weather conditions. A rapid increase in the soil-borne population of *A. flavus* during hot, dry years may influence aflatoxin development by increasing the available inoculum for corn silk infection (McGee et al., 1996). Thus, it is likely that conidial dispersal from the inoculum source to silks was favored by the prevailing weather conditions between R1 and R4 during 2011, but not in 2010 or 2012. Additionally, plants with reduced drought stress, as during the 2010 and 2012 seasons, were expected to be more resistant to infection and toxin accumulation.

In contrast, the side needle inoculation treatment used in 2013 and 2014 induced aflatoxin contamination at significantly greater concentrations ($P < 0.05$) compared with the non-inoculated treatment at both study locations (Table 2). Wounding inoculation methods introduce conidia directly to kernels, bypassing potential plant resistance mechanisms to infection, and they are likely less sensitive to environmental influences than natural infection and non-wounding inoculation methods (Windham et al., 2003, 2009). At Prattville in 2013, the use of the side needle inoculation treatment resulted in three- to five-fold greater toxin concentrations than the toxin concentrations observed in 2014 and 2013 for the same treatment in Fairhope, respectively. These results might be explained by: (i) dry conditions in May and June and (ii) below historical average rainfall and higher day–night temperatures in June, which coincided with the R1 growth stage (Supplemental Table S1). Additionally, rainfall in July and August was above the historical averages (Supplemental Table S1), which increased corn grain moisture and delayed harvest, resulting in a prolonged time exposure window for aflatoxin synthesis (Jaime-Garcia and Cotty 2003; Jones et al., 1981).

Natural infection did not result in high contamination levels. On average, natural infection rates resulted in aflatoxin concentrations that ranged from 0.4 up to 47.5 $\mu g\ kg^{-1}$ across all years and locations (Table 2). At Fairhope, only in 2011 and 2012, the natural infection treatment resulted in contamination concentrations greater than the action level (20 $\mu g\ kg^{-1}$) (Table 2). At Prattville, the concentration resulting from natural infection was below the action limit (3.4–3.9 $\mu g\ kg^{-1}$). These results agree with Windham et al. (2009), who reported that aflatoxins contamination due to natural infection is more influenced by maximum temperature and rainfall parameters around silking–pollination than contamination imposed by side needle technique. Soil-borne populations of *A. flavus* have been considered the main source of inoculum in the corn agroecosystem in Iowa (Olanya et al., 1997) and tends to decline when conditions are not favorable (e.g., during wet and cool seasons) (McGee et al., 1996; Shearer et al., 1992). This may explain the reduction in aflatoxin concentrations observed for the non-inoculated treatment for years following the 2011 growing season in Fairhope (McGee et al., 1996; Shearer et al., 1992). A less stressed plant tends to develop a more robust canopy, which may act as a physical barrier to keep air-borne inoculum from silks (Jones et al., 1981). Water stress could reduce the plant's ability to respond to wound recovery and pest resistance (Hansen et al., 2013), and thus, promote insect feeding (Parsons and Munkvold, 2010) and subsequent infection by *A. flavus* (Bowen et al., 2014).

In this study natural infection treatment and the inoculated plots with infested cracked corn resulted in aflatoxin accumulation greater than 20 $\mu g\ kg^{-1}$ only for Fairhope 2011, an extremely dry year (Supplemental Table S1 and Table 2). If year and location were considered fixed, analysis over pooled data for 2013 and 2014, showed a significant inoculation effect ($P < 0.0001$) (Table 3).

#### Planting Date Effect on Aflatoxin Contamination

Planting date effect on aflatoxin contamination varied among years (Table 2). Mid-March planting (PD1) generally resulted in greater aflatoxin contamination at the end of the season compared with mid-April (PD2) planting, but this difference was only significant ($P < 0.05$) at Fairhope in 2011, 2013, and 2014. Analysis of pooled data for both locations and seasons 2013–2014 when the same inoculation method was used, indicated significant planting date effects ($P < 0.0001$) (Table 3), with least square means estimates for aflatoxin contamination being 35.8 and 20.8 $\mu g\ kg^{-1}$ for mid-March and mid-April plantings, respectively. At Fairhope in 2011, an extremely dry year (Supplemental Table S1), mid-March planted corn was exposed to 3.4°C higher minimum temperatures and received less cumulative rainfall (7.6 mm less) than the mid-April corn at the end of the grain-filling period (IV window) (Supplemental Table S2), which may explain the different

aflatoxin contamination concentrations observed. At Fairhope in 2013, the corn planted in mid-March received 9.1 mm of rain during the 2-wk period before R1 (-I), whereas for the same period the mid-April planted corn received 103.6 mm of rain (Supplemental Table S2). Additionally, the mid-March planted corn was exposed to 1°C higher minimum temperatures and received 207.3 mm less rainfall than mid-April corn from Day 15 to Day 28 after R1 (II) (Supplemental Table S2). It appears that drought and heat stress just before R1 (-I) and at the II time period after R1, respectively, might predispose mid-March planted corn to greater *A. flavus* infection and subsequent contamination than the mid-April planted corn. This could partially explain the greater aflatoxin contamination concentrations observed for corn seeded in mid-March than mid-April (Table 2). At Fairhope in 2014, average minimum temperatures for mid-March planted corn were 1 and 2°C higher during the II and IV time periods, respectively, compared with mid-April planted corn (Supplemental Table S2). It appears that mid-March corn was exposed to greater night heat stress than mid-April corn during the aforementioned time periods, which might explain the greater contamination concentrations observed for mid-March planted corn compared with mid-April corn. Lillehoj et al. (1980), reported that planting dates affected aflatoxin synthesis and concentration in naturally infected corn. Corn grown in 1977 in Georgia and Florida, had significantly lower contamination concentrations when planted on 1 April than 1 May. Furthermore, late planting in the spring season in Mexico, exposed plants to highest minimum temperatures between V6 and VT and resulted in the greatest aflatoxin concentrations than early spring planting (Rodriguez-del-Bosque, 1996). Under the environmental conditions prevailing during our study, planting in mid-March resulted in significantly different (3 site-years) or numerically greater aflatoxin contamination levels (4 site-years), 4.3 $\mu g\ kg^{-1}$ on average, than did planting in mid-April (Table 2). In contrast, a 3-yr study in Arkansas revealed lower aflatoxin contamination on corn planted in mid-April, early, compared with planting in early-May (Abbas et al., 2007). Bruns and Abbas (2006) found nonsignificant grain contamination between planting dates (early vs. late April) in a study conducted in Mississippi between 2002 and 2004.

Literature and our findings confirm the variability and the location-specific response of corn to planting dates across the United States (Bruns, 2003) due to the influence of climatic conditions on plant growing season lengths. The influence of these factors suggests that additional local studies are needed. This knowledge may allow mitigating grain toxin contamination more effectively in the vicinity of interest. The spatio-temporal aflatoxin levels differences observed in this study suggest the impact that weather factors have on infection and contamination.

### Plant Density Effect on Aflatoxin Contamination

Plant density effect on aflatoxin concentration was nonsignificant ($P > 0.05$) when individual years per location were considered (Table 2). A similar trend was observed for pooled data analysis ($P = 0.6834$, Table 3). No interactions that included plant density were significant except for the inoculation × planting date × planting density interaction at Prattville in 2013, and the planting date × plant density interaction at Fairhope in 2013 (Table 2). These results confirm the findings of Rodriguez-del-Bosque (1996), Abbas et al. (2012), and Bruns and Abbas (2005), which also reported that plant density was nonsignificant. Contrasting to the lack of plant density effect on aflatoxin contamination on our study, corn yield increased as plant population increased (Supplemental Table S3). Modern hybrids, which possess an erect up-right leaf architecture, can more efficiently withstand the plant-to-plant competition for limited resources than older hybrids (Bruns, 2003; Mastrodomenico et al., 2018; Van Roekel and Coulter, 2011). As a result, under prevailing environmental conditions, it is possible the herein tested plant densities failed to differentiate stress levels over a hypothetical threshold necessary to induce a significant effect on aflatoxin synthesis in the grain. This agrees with Clay et al. (2009), who reported that under crowded conditions (74,500 vs. 149,000 plants $h^{-1}$), the photosynthetic capacity of corn was downregulated, but a change in direct competition for plant-available water was not detected.

## Relationship of Weather Conditions with Aflatoxin Contamination

### Rainfall Model

As the rainfall model suggests, the influence of cumulative rainfall per se on pre-harvest aflatoxin contamination due to natural infection changes over the season (Table 4). More specifically, cumulative rainfall several days before physiological maturity (IV) could explain 25% of aflatoxin concentration at harvest. Also, total rainfall 2 wk prior to R1 (-I) and over 2 wk period starting the third week after R1 (II) explained 19 and 6% of aflatoxin contamination, respectively (Table 4).

The rainfall model indicated that rainfall variables were negatively associated with aflatoxin contamination (Table 4). Thus, reduced rain for the 2-wk period prior to R1 increased toxin concentrations in the grain. Interestingly, the rainfall 2-weeks before R1 (-I) accounted for more than 37% (partial $R^2 = 0.19$) of the aflatoxin variability explained by the rainfall model (Table 4). Similarly, Windham et al. (2009) showed that cumulative rainfall 21 to 42 d prior to inoculation (7 d after R1) was negatively correlated with aflatoxin contamination in corn grown in a loam but not in silty clay loam. Thus, they suggested that moisture stress prior to R1 may increase aflatoxin synthesis.

The rainfall model also indicated that cumulative rainfall at the end of the season (IV) could explain approximately 20% more of the aflatoxin variability observed at the end of the season than the total rainfall at time period II (Table 4). Similar to our study, Windham et al. (2009) observed that cumulative rainfall was negatively correlated with natural aflatoxin contamination in corn 21 to 35 d after R1. Widstrom et al. (1990) in a 5-yr study at Tifton, GA, did not find significant correlation between average rainfall over 20- to 40- and 40- to 60-d time periods following R1 and aflatoxin concentration in corn grown on loamy sand. Among others, differences in weather patterns with the subsequent stresses imposed on the host and the fungi, and different soil types, insects, and microorganismal communities between the regions studied, may have contributed to the aforementioned discrepancies.

### Minimum Temperature Model

In this study, minimum temperature was closely associated with aflatoxin contamination observed in the non-inoculated

Table 4. Stepwise multiple linear regression models on the effect of weather variables on aflatoxin contamination (μg kg$^{-1}$) due to natural infection for combined data for Fairhope (2010–2014) and Prattville (2013–2014), AL.

| Independent variable† | Estimated coefficient | SE | $R^2$ | Adjusted- $R^2$ | Root mean squared error | CV | $Pr > \|t\|$ | Variance inflation factor |
|---|---|---|---|---|---|---|---|---|
| Rainfall model | | | | | | | | |
| Model | | | 0.5005 | 0.4687 | 0.3736 | 52.68 | <0.0001 | |
| Intercept | 1.4035 | 0.1189 | | | | | <0.0001 | 0.00 |
| Rain (-I) | –0.0048 | 0.0008 | 0.1886 | | | | <0.0001 | 1.13 |
| Rain (II) | –0.0011 | 0.0006 | 0.0596 | | | | 0.0646 | 1.02 |
| Rain (IV) | –0.0090 | 0.0019 | 0.2524 | | | | <0.0001 | 1.14 |
| Minimum temperature model | | | | | | | | |
| Model | | | 0.6024 | 0.5770 | 0.2701 | 37.18 | <0.0001 | |
| Intercept | –3.7375 | 1.0060 | | | | | 0.0005 | 0.00 |
| Temp min (-I) | –0.1528 | 0.0351 | 0.1385 | | | | <0.0001 | 2.11 |
| Temp min (I) | 0.1611 | 0.0660 | 0.3099 | | | | 0.0185 | 2.56 |
| Temp min (II) | 0.1810 | 0.0424 | 0.1540 | | | | <0.0001 | 1.51 |
| Overall model | | | | | | | | |
| Model | | | 0.7596 | 0.7443 | 0.2592 | 36.55 | <0.0001 | |
| Intercept | –6.2577 | 0.7083 | | | | | <0.0001 | 0.00 |
| Rain (-I) | –0.0053 | 0.0006 | 0.1886 | | | | <0.0001 | 1.16 |
| Rain (IV) | –0.0027 | 0.0016 | 0.2739 | | | | 0.0922 | 1.66 |
| Temp min (IV) | 0.3177 | 0.0311 | 0.2971 | | | | <0.0001 | 1.66 |

† Rainfall and minimum air temperatures were averaged over 14-d intervals around mid-silk; (-I) = 14 d prior mid-silk, (I) = 14 d after mid-silk, (II) = Days 15–28 after mid-silk, (III) = Days 29–42 after mid-silk, (IV) = remaining days up to physiological maturity. Dependent variable was aflatoxin content $y = \log_{10}$(μg kg$^{-1}$+1) averaged for six replicates for different environments specified as: Location × Non– Inoculated × Planting dates × Densities.

treatments. The regression analysis indicated that preharvest aflatoxin contamination increased as minimum temperature increased over the 2 wk after R1 (I) (partial $R^2$ = 31.0%, Table 4). Additionally, minimum temperature corresponding to the 14-d period prior to R1 (-I) and the 15- to 28-d period after R1 (II) were also significant ($P < 0.1$).

Elevated minimum temperatures during the 14-d interval following R1 (I) explained more than half of the total aflatoxin variability accounted for by the model (Table 4). This interval coincides with the timespan when silks are green–yellow (freshly pollinated) and yellow–brown. Previous work has reported that colonization of silks and kernel infection was significantly greater when inoculum was applied at yellow–brown rather than brown silks (Marsh and Payne, 1984). Silk colonization rate depends on the physiological stage of the tissue, temperature, humidity, and moisture, with higher day–night temperatures promoting colonization of senescing silks and the downward growth of the fungi through the silk channel (Diener et al., 1987; Marsh and Payne, 1984).

Minimum temperature over 14 d starting the third weeks after mid-silk (II) was also positively associated with preharvest aflatoxin contamination concentrations (Table 4). This interval commonly includes the R3 (milk) growth stage. Marsh and Payne (1984) showed that *A. flavus* was readily isolated through the season from the silks with the higher presence of *A. flavus* observed at milk (R3), whereas internal grain infection was observed at dent stage (R5). It is likely that our findings reflect colonization and infection processes for the aforementioned timespan. Furthermore, since the temperature range for rapid production and toxin accumulation is between 20 and 35°C (Schroeder and Hein, 1967), this period may be additionally indicative of increased aflatoxin synthesis resulting from higher minimum temperature at night.

The minimum temperature model also indicated that a reduction of night (minimum) temperature over 14 d prior to R1 might increase aflatoxin contamination (Table 4). A drop in minimum temperature may result in a significant increase in diurnal temperature variation, and thus, dew formation (FAO, 2012). Fungus growth, development and successive conidia production could be promoted under wet conditions, resulting in an increase of primary and/or secondary inoculum in the soil, plant debris, and/or plant canopy. A higher minimum temperature just before flowering should have the opposite effect (drier conditions) leading to less air-borne *A. flavus* inoculum available for silk colonization, infection, and subsequent aflatoxin contamination later in the season.

### Overall Model

Evaluating average minimum air temperatures and cumulative rainfall variables together as response variates indicated that aflatoxin contamination due to natural infection was influenced by cumulative rainfall and average minimum temperature for the timespan from Day 43 following R1 through physiological maturity (IV) (Table 4). Rainfall, for the 14 d prior to R1 (-I), was also significant at $\alpha = 0.1$. Rainfall and minimum temperature between Day 43 after R1 up to physiological maturity (IV) are the primary variables influencing aflatoxin contamination in the Coastal Plains of Southern and Central Alabama as indicated by partial $R^2$ of 27.4 and 29.7%, respectively (overall model, Table 4). Reduced rainfall and high temperature are the usual climatic factors associated with aflatoxin contamination problems in several crops including corn (Jaime-Garcia and Cotty, 2003). In our study, the overall model indicated that reduced rainfall for the 14 d prior to R1 (-I) and from Day 43 through physiological maturity (IV)

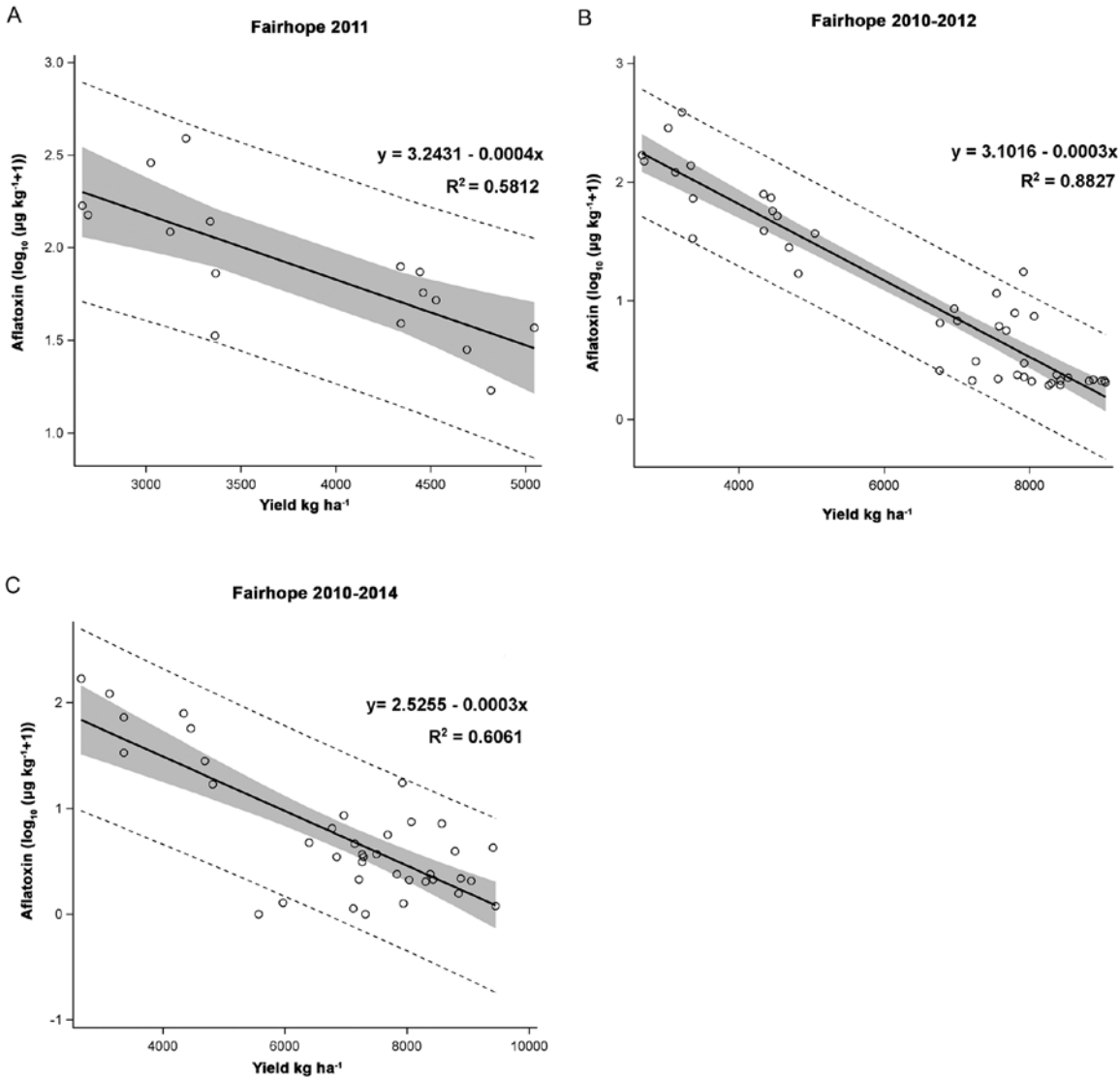


Fig. 1. Relationship between aflatoxin contamination ($log_{10}$ (µg $kg^{-1}$+1)) and grain yield (kg $ha^{-1}$) at Fairhope. Each marker on parts A and B corresponds to average values from all treatments (inoculation, planting date, plant density). Part C excluded inoculated observations. Shaded area and area enclosed by the upper and lower dotted lines represent 95% confidence and prediction limits, respectively.

exacerbated aflatoxin contamination in corn grain. Similarly, an increase in minimum temperature at the end of the grain-filling period (from Day 43 after R1 up to physiological maturity, [IV]) increased contamination concentrations. Plant resistance to fungal infection and toxin synthesis is a quantitative trait involving numerous genes and is highly influenced by genotype × environment interactions (Fountain et al., 2014). On the one hand, reactive oxidative species are produced as plant response to biotic (i.e., pathogen attack) and abiotic stresses (i.e., heat and drought) (Torres et al., 2006; Vellosillo et al., 2010; You and Chan, 2015). On the other hand, exposure of *A. flavus* to oxidative stress may be a prerequisite for aflatoxin biosynthesis (Fountain et al., 2014).

This study revealed short time periods (mainly 14-d intervals) during the growing season when a particular weather variable had a significant effect on toxin synthesis. It also showed that the degree to which rainfall and minimum temperature influenced aflatoxin contamination in corn changed over the growing season.

### Relationship between Aflatoxin Contamination and Yield

A strong negative relationship between aflatoxin concentration and yield was observed at Fairhope in 2011 (model $P$ = 0.0006, RMSE = 0.2517, coefficient of variation = 13.37) (Fig. 1A). Regression analysis between aflatoxin concentration and yield showed a strong negative linear relationship for Fairhope 2010– 2012 with $R^2$ = 0.8827 as well (model $P$ < 0.0001, RMSE = 0.2528, coefficient of variation = 24.69) (Fig. 1B). If only natural infection data were included in the analysis, the fitted linear model for Fairhope 2010–2014 could explain 60.6% of the variability (model $P$ < 0.0001, RMSE = 0.3913, coefficient of variation = 53.05) (Fig. 1C). Those findings are in agreement with several other studies (Betrán and Isakeit, 2004; Bowen et al., 2014; Jones et al., 1981; Rodriguez-del-Bosque, 1996). Evidently, as indicated by seasonal yield (Supplemental Table S3) and weather data summaries (Supplemental Table S1), corn crops should have experienced less stress in Fairhope for the 2010, 2012, 2013, and 2014 seasons than in 2011. The same conclusion could be drawn for corn grown at Prattville in both seasons when compared with

the extremely dry 2011 year in Fairhope. A better managed crop grown under reduced weather extremes, and thus, less stressed, will commonly produce greater yield and would be expected to have lower concentrations of aflatoxin contamination (Bowen et al., 2014; Jones et al., 1981; Rodriguez-del-Bosque, 1996). As noted by Bowen et al. (2014), the negative linear relationship between yield and aflatoxin contamination does not reveal a causal relationship; rather, a third factor, such as inadequate water, could limit corn yield, while at the same time drought might promote aflatoxin concentration.

## CONCLUSIONS

Results from this study showed a relationship between in-season weather conditions and aflatoxin contamination. Changes in minimum temperature and rainfall explained between 50 and 76% of the observed aflatoxin variability. However, high aflatoxin levels could be driven by an increase in minimum temperature during the 28 d after R1 growth stage, but mainly the two subsequent weeks to R1. Lack of rainfall, especially during the 2 wk prior to R1, partially the R5 growth stage, and the R6 growth stage will increase aflatoxin levels.

This study also showed that under the environmental conditions of south and central Alabama, agronomic practices could have an influence on corn yield and aflatoxin contamination. The effect of planting date on aflatoxin contamination and yield was significant but variable across environments. Delaying planting from mid-March to mid-April reduced aflatoxin levels in most site-years. Planting densities did not influence aflatoxin accumulation in corn, but did impact yield in 6 of the 7 site-years. When data from individual seasons were considered alone, a significant negative linear relationship was illustrated between aflatoxin and yield only for the extremely dry 2011 season in Fairhope.

Those findings emphasize the importance of weather conditions (minimum temperature and rainfall) and their interaction with agronomic management on corn aflatoxin contamination. Moreover, they further elucidate how local weather may influence *A. flavus* growth, development, corn infection, and subsequent aflatoxin contamination, which may lead to better crop management and development of more accurate prediction systems.

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## SUPPLEMENTAL MATERIAL

Table S1. Monthly weather conditions and historical average values for Fairhope and Prattville, AL, during the study period.

Table S2. Average maximum air temperature, average minimum air temperature, and cumulative rainfall for five time windows corresponding with the two planting dates tested at the two study locations.

Table S3. Analysis of variance for corn grain yield growing at different planting dates and planting densities in Fairhope, AL (2010–2014), and Prattville, AL (2013–2014).

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