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Evaluating a generic drought index as a predictive tool for aflatoxin contamination of corn: from plot to regional level

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ABSTRACT

Corn (*Zea mays* L.) kernel infection by *Aspergillus flavus* and subsequent aflatoxin accumulation in grain can have a deleterious effect on both humans and animals that consume contaminated grain. Predicting the aflatoxin risk is challenging due to complex interactions of biotic and abiotic stress factors that govern and exacerbate the phenomenon. The goal of this study was to determine whether a drought index could be used to predict the risk for pre-harvest aflatoxin contamination in corn. Risk assessment was approached at: 1) field (plot) level with data obtained from an in-field controlled experiment (Mississippi study), and 2) state level, where corn fields were sampled at a county level (Georgia study). The data used for this study consisted of historical records on aflatoxin contamination collected over thirteen growing seasons from 2000 to 2011, 2013, and 2014 at Mississippi State, Mississippi (1), and from random corn fields in 53 counties across Georgia between 1977 and 2004 (2). A controlled experiment was conducted at Mississippi with two soil types (a Leeper silty clay loam and a Myatt loam), and three commercial hybrids characterized by different susceptibility levels to aflatoxin contamination. The Agricultural Reference Index for Drought (ARID), a generic drought index for calculating drought on daily basis was evaluated as an aflatoxin risk prediction tool. Mid-silk day was selected to split each growing season into two time periods, which were further divided into positive and negative weeks representing weeks after and before mid-silk, respectively. Weekly ARID factors were calculated for all periods to evaluate the in-season alterations in aflatoxin risk. In both studies, multiple logistic regression models were used to predict aflatoxin risk as a function of the weekly ARID values. In Mississippi, risk level changes were additionally tested according to soil type and corn hybrid aflatoxin susceptibility. The United States Food and Drug Administration restricts corn grain consumption by humans and young animals if the contamination level is above 20 μg/kg; thus, this threshold (20 μg/kg) was selected to develop a binary dependent variable for the logistic model from the raw aflatoxin data. The results revealed that ARID might be used as a predictive tool to assess aflatoxin risk, soil type and hybrid susceptibility to aflatoxin contamination were statistically significant independent factors, and there are critical week windows during the growing season when changes in drought conditions affect the likelihood for aflatoxin contamination. These findings can be used to minimize risk by adapting site-specific management strategies such as triggering irrigation during critical risk weeks, selecting the most appropriate hybrid for a given site/location based on soil type, and determining optimum harvest date.

Keywords: *Aspergillus flavus*; infection; logistic regression; modeling; risk assessment; maize

1. Introduction

Aflatoxin contamination in corn is a worldwide issue since the toxins have adverse health effects on humans and domestic animals (Damianidis et al., 2015; Robens and Cardwell, 2003). Moreover, aflatoxin contamination raises food-safety concerns and impacts the trade of corn grain and its byproducts, and thus, results in significant economic losses (Abbas et al., 2012; Blandino et al., 2008; CAST, 2003). Aflatoxins are difuro-cumarins biosynthesized secondary metabolites through a polyketide pathway (Fountain et al., 2014; Mishra and Das, 2003; Probst and Cotty, 2012) produced by several fungul species belonging to *Aspergillus* section *Flavi* (CAST, 2003) with *A. flavus* and *A. parasiticus* being the most common and of major concern (CAST, 2003; Diener et al., 1987; Klich, 2007). The most prevalent naturally occurring forms of aflatoxins include the toxins B_1, B_2, G_1, and G_2, with types B and G being usually synthesized by *A. parasiticus* and *A. nomius*, while *A. flavus* mainly produces B_1 and B_2 aflatoxins (Klich, 2007).

Despite aflatoxins discovery followed an outbreak of Turkey "X" disease in England in 1960 (Austwick and Ayerst, 1963; Bayman and Cotty, 1990; Blount, 1961; Richard, 2008; Sargeant et al., 1961; Spensley, 1963), in many countries, the extent of aflatoxin contamination is not well known since there is a reluctance to report the problem (Payne and Widstrom, 1992). Aflatoxins are considered carcinogenic, mutagenic, teratogenic, and hepatotoxic compounds for both humans and animals (Blandino et al., 2008; Blaney et al., 2008; CAST, 2003; Fountain et al., 2014; Molina and Giannuzzi, 2002). Therefore, 48 countries have established regulatory actions and are monitoring aflatoxin contamination in food, with 21 countries establishing tolerance levels in feedstuffs (Dohlman, 2003; Hawkins et al., 2008; Mishra and Das, 2003). The United States Food and Drug Administration (U.S. FDA) restricts consumption of corn grain by humans and young animals if contamination levels exceed 20 μg of aflatoxin/kg of grain (U.S. Food and Drug Administration, 2000).

Aflatoxin synthesis is more likely to occur in areas with tropical and subtropical climates (Streit et al., 2012). In recent decades, severe aflatoxicosis outbreaks have been reported in Kenya, India, and Malaysia (Lewis et al., 2005; Shephard, 2008). Recently, significant pre-harvest corn contamination was reported in Northern Italy (Battilani et al., 2008a; Giorni et al., 2007; Piva et al., 2006) and in Australia (Blaney et al., 2008). In the United States, corn infection and subsequent contamination is a chronic economic and health concern in the South (Davis et al., 1986; Diener et al., 1987; Payne and Widstrom, 1992). Given favorable weather patterns, in-field contamination may also occur in Midwest as well (Payne and Widstrom, 1992; Wallin and Minor, 1986; Zuber and Lillehoj, 1979).

Aflatoxin contamination occurs both pre-harvest and post-harvest. One tactic to mitigate contamination problems is to reduce the risk of infection prior to harvest. (Chauhan et al., 2015). This should reduce residual inoculum in harvested corn grain which is a source of further contamination under poor storage conditions. The in-field contamination is highly variable both within a field and among geographic areas and seasons (Battilani et al., 2008a; Hawkins et al., 2008), reflecting the effect weather conditions have on *A. flavus* incidence (Cotty and Jaime-Garcia, 2007) and plant predisposition to infection/contamination (Fountain et al., 2014).

Aflatoxin contamination is exacerbated in seasons characterized by higher temperatures and lower than normal rainfall that may expose corn plants to drought stress from silking and through grain fill (Diener et al., 1987; Payne and Widstrom, 1992; Windham et al., 2009). Agricultural drought occurs when plant available water in the soil does not meet the atmospheric demand for evapotranspiration (Woli et al., 2012). Critical time windows when the risk for corn aflatoxin contamination changes were identified in numerous studies (Battilani et al., 2008a; Damianidis et al., 2015; Hawkins et al., 2008; Widstrom et al., 1990; Windham et al., 2009). This includes: 1) a window extending between days 65 and 85 following planting when heat stress may result in increased contamination (Hawkins et al., 2008), and 2) the decadal intervals from late June to late August when drought, as quantified by an aridity index, were significantly correlated with aflatoxin contamination (Battilani et al., 2008a). Conclusively, drought stress around silking and during kernel development are the key risk factor for elevated *Aspergillus* infection and aflatoxin contamination in corn at the end of the season (Damianidis et al., 2015; Diener et al., 1987; Luo et al., 2010; Payne et al., 1986; Windham et al., 2009).

Models have been used to answer questions related to research, crop management, policymaking, and to assess the risk associated with human and animal health (Garcia et al., 2009; Prandini et al., 2009). If aflatoxin risk could be predicted, then human/animal health concerns, and the subsequent economic losses, could be minimized. Numerous *in vitro* studies had reported modeling efforts to predict aflatoxin contamination based on variables such as temperature, water activity, and pH (Abdel-Hadi et al., 2012; Garcia et al., 2013; Molina and Giannuzzi, 2002; Pitt, 1993). Although those models could predict contamination, they have not been evaluated under field conditions (Chauhan et al., 2008; 2015).

Several attempts to predict the in-field aflatoxin corn contamination based on environmental conditions have been recently reported by using empirical or mechanistic models (Battilani et al., 2008a; 2013; Chauhan et al., 2008; 2015). However, development of mechanistic models might require data or assumptions based on data coming from *in vitro* studies (e.g. sporulation, dispersal, germination, infection, fungal growth, and toxin production rates) that may not be always readily available. Additionally, aflatoxin production is strain and media specific (Luchese and Harrigan, 1993; Sweeney and Dobson, 1998), making for challenging model development and application. Moreover, contamination levels from *in vitro* studies do not always correlate well with *in vivo* observations (Probst and Cotty, 2012). Therefore, models developed with data generated from artificial media (*in vitro*) should be used with caution for in-field corn aflatoxin contamination assessment (Chauhan et al., 2015).

Ideally, an early predictive system should be simple in its approach, easy to implement, and should provide satisfactory predictive accuracies. Logistic regression is a multivariate technique that satisfies those criteria and has been used in human (Fei et al., 2017; Tu et al., 1994) and plant epidemiology to assess risk and guide disease management decisions (Battilani et al., 2008a; Paul and Munkvold, 2004). It has been used previously to assess the in-field risk of 1) gray leaf spot of corn, caused by *Cercospora zeae-maydis* (Paul and Munkvold, 2004), and 2) fumonisin contamination in corn (Battilani et al., 2008b). Battilani et al. (2008a) extended this approach to predict aflatoxin contamination in corn in Northern Italy by using as independent variable an aridity index. However, in their approach Battilani et al. (2008a) did not take into consideration the relationship between soil plant available water and the evapotranspiration demand during the growing season which may lead to drought; a prerequisite for aflatoxin contamination in corn (Diener et al., 1987; Payne and Widstrom, 1992). The Agricultural Reference Index for Drought (ARID), a generic and simple to use drought index, takes into account plant available water and daily evapotranspiration (Woli et al., 2012; Woli et al., 2013). ARID might be used to quantify agricultural drought and estimate its effects on crop yields (Woli et al., 2012; Woli et al., 2013). However, assessing in-season aflatoxin contamination in corn with a generic drought index in the Southeastern United States has yet to be done. The hypothesis driving this study was that changes in spatial and in-season drought lead to changes in the risk for aflatoxin contamination of corn. Therefore, the objectives of this study were to: 1) determine whether a drought index could be used to predict the risk for aflatoxin contamination in corn, 2) assess in-season risk differences among soil types and among hybrids, and 3) explore the applicability to predict the risk at regional level when minimum data are available.

2. Materials and Methods

Two aflatoxin datasets were used in the study including aflatoxin contamination data collected from field experiments in Mississippi State, Mississippi (MS), USA; and data on aflatoxin contamination from corn samples collected from randomly surveyed fields across 53 counties in South Georgia (GA), USA.

2.1 Mississippi dataset

Field experiments were conducted from 2000 to 2011, 2013, and 2014 at the R. R. Foil Plant Science Research Center located at Mississippi State, Mississippi (Windham et al., 2009). The experimental design was a split plot design with corn hybrids assigned to the main plots, while inoculation methods (natural infection, side needle, and spray silks) were allotted to sub-plots (Windham et al., 2009). Hybrids were selected and classified into three categories based on their susceptibility to infection by *Aspergillus flavus* and subsequent aflatoxin contamination. Two of the cultivars were characterized as moderately susceptible (indicated hereafter as hybrid 1 and hybrid 2) and a third (hybrid 3) as highly susceptible to aflatoxin contamination. Starting in 2000 and up to 2005 the experiment was conducted for two soil types, a Leeper silty clay loam (Fine, smectitic, nonacid, thermic Vertic Epiaquepts) and a Myatt loam (Fine-loamy, siliceous, active, thermic Typic Endoaquults). The water-holding capacity at field capacity of the Leeper silty clay loam and the Myatt loam was 28% and 21%, respectively. From 2006 and after, the study was conducted only for the heavier soil type (Leeper silty clay loam). Corn ear samples were harvested from each plot, processed, and analyzed for aflatoxin contamination (μg/kg) as described by Windham et al. (2009). Contamination data related to natural infection by *A. flavus* were only considered for the analysis herein. This comprehensive database contained 240 aflatoxin contamination observations and was divided into a model development dataset and a model evaluation dataset. Twenty percent of the data were randomly selected to create an evaluation dataset, while the rest of the data (80%) were used for model development.

2.2 Georgia dataset

A total of 818 corn samples were collected from random farm fields located in 53 counties in Georgia from 1977 to 2004. Up to the late 1990's, aflatoxin contamination and identification was determined by Thin Layer Chromatography (Brown et al., 1993; Guo et al., 1995), and thereafter (from 2000 to 2004), the VICAM AflaTest® (VICAM, Watertown, Massachusetts) analytical method was used. The database was unbalanced, since fields were not sampled from all counties every season. Samples were assigned to Georgia counties where the sampled corn field was located. The comprehensive survey database was randomly separated into a model development dataset (80% of the data) and evaluation (20% of the data) dataset.

2.3 Quantifying seasonal drought

The Agricultural Reference Index for Drought (ARID) is a simple drought index used to monitor, predict, and estimate the effect of drought timing and degree on crop yields (Woli et al., 2012). ARID reflects seasonal in-field drought conditions, requires a minimal number of site specific weather parameters, and is calculated on a daily basis. ARID values range from 0 to 1; 0 indicates no water deficit, and 1 signifies maximum water deficit.

Ideally, weather parameters required to calculate ARID include daily maximum, minimum, and dew point temperatures along with precipitation, wind speed, potential evapotranspiration (ET_o), and solar radiation. However, ARID calculations could be completed even when weather parameters are missing (e.g. daily maximum temperature, minimum temperature, and rainfall will suffice) (Woli et al., 2012). For the Mississippi data, ARID calculations were based on weather data provided by the Mississippi Agricultural and Forestry Experimental Station, while meteorological data for Georgia analysis were retrieved from: 1) DAYMET database covering the timespan from 1980 – 2004 (Thornton et al., 2014), and 2) CRONOS database for seasons 1977 to 1979 (State Climate Office of North Carolina, 2016). For Mississippi data, ET_o was estimated by FAO Penman-Monteith method (Allen et al., 2006); for Georgia, where wind speed and dew temperature data were not available for the seasons studied, ET_o was alternatively estimated by Hargreaves equation as described by Allen et al. (2006). Whenever the Hargreaves equation is used for ET_o estimation in a region, comparison with ET_o estimates by the FAO Penman-Monteith model is suggested (Allen et al., 2006). Univariate regression analysis indicated that for the study area in Georgia the two methods were comparable (R^2 ranged from 0.9360 to 0.9807; for the eight locations tested).

2.4 Logistic regression – concepts

Logistic regression requires the dependent variable to be formulated as a binary factor, and it can be used to estimate the probabilities for an event to occur based on preselected independent predictors. The logistic regression model can be described by the equation:

$$P(Event) = \frac{e^{\beta_o+\beta_1\chi_1+\beta_2\chi_2+\cdots+\beta_n}}{1+e^{\beta_o+\beta_1\chi_1+\beta_2\chi_2+\cdots+\beta_n}} \qquad (1)$$

where *e* is the exponential constant, β_o, β_1, β_2, and β_n are the estimated coefficients, and x_1, x_2, and x_n are the independent variables. P(Event) is the probability for an event to occur; in this study to have a contaminated sample.

In binary response models (e.g. logistic models), model assessment can be accomplished by generating the Receiver Operating Characteristic (ROC) curve and calculating the area under the ROC curve (AUC) (Damianidis et al., 2015; SAS, 2015). The AUCs of the developed and the evaluated models are then compared for equality at a preselected level of significance with the ROC curve of a model predicting by chance (model with only intercept).

The AUC provides a graphical summary to assess the predictive power of a binary model (Allison, 2012). It does not depend on an arbitrary cutpoint value needed for the construction of a classification table, which inherently has an influence on the classification of test results as events or non-events (Allison, 2012). The area under the ROC curve takes values from 0 to 1; larger values correspond to stronger associations between predicted and observed values. A value of 0.5000 corresponds to a model with an intercept only, and thus, with no predictive power. The more the ROC curve departs from the forty five degree line the more accurate the model predicts.

2.5 Database development for both studies

Prior to conducting the logistic analysis the aflatoxin contamination data were transformed to a binary variable. The created binary variable was assigned a value of 1 (contaminated) and 0 (not contaminated) when the aflatoxin levels of the corn samples were greater and smaller than

20 μg/kg, respectively; the limit set by U.S. FDA to restrict corn consumption by humans and young animals.

Because the risk of aflatoxin contamination will be predicted based on in-season changes in drought conditions, weekly ARID values were calculated for two time intervals surrounding mid-silk day. Thus, each season was divided into positive weeks and negative weeks indicating time periods before and after mid-silk, respectively. The calculated weekly ARID values were used as independent predictors in the logistic models to assess in-season risk changes in aflatoxin contamination. Mid-silk was selected as a reference day for two reasons: 1) to remove the portion of the variability related to the different growing seasons, since plant growth and development depends greatly on weather conditions that are particular for each year and do not coincide with calendar days, and 2) as indicated in the literature, the likelihood for infection and contamination is greater around corn silking (Hawkins et al., 2008; Windham et al., 2009).

2.6 Predicting aflatoxin risk with logistic regression at field (plot) level – Mississippi study

For the current analysis, ARID was evaluated as a predictive tool for pre-harvest aflatoxin contamination. Multiple logistic regression was used to predict aflatoxin risk as a function of weekly ARID values, soil type, and hybrid susceptibility to infection and contamination. Additionally, risk level changes were studied in their association to soil type and corn hybrids. Statistical analyses were carried on with PROC logistic procedure in SAS version 9.3.

Inclusion of all the weekly ARID values as predictive variables in the model resulted in high multicollinearity. Multicollinearity makes the estimated coefficients more unstable, and one way to mitigate the issue is by dropping collinear variables (Allison, 2012). Thus, all potential predictors (weekly ARID values) of aflatoxin risk were initially tested at the univariable level for significance (p-value = 0.05). The Variance Inflation Factor (VIF) of the retained predictors was < 5, indicating that multicollinearity was alleviated and more robust estimates could be obtained. Logistic model development followed by including only significant weekly ARID independent variables as identified in the first step, and their association to the outcome at the multivariable level was also tested. The logistic model used for the Mississippi data set analysis is given by the following equation:

$$P(Aflatoxin) = \frac{e^{\beta_0+\beta_1 Soil+\beta_2 Hybrid+\beta_{x1} Week_{x1}+\cdots+\beta_{xn} Week_{xn}}}{1+e^{\beta_0+\beta_1 Soil+\beta_2 Hybrid+\beta_{x1} Week_{x1}+\cdots+\beta_{xn} Week_{xn}}} \quad (2)$$

where P(Aflatoxin) is the probability to have aflatoxin contamination above the selected threshold (20 μg/kg), e is the base of natural logarithm, β_o, β_1, β_2, β_{x1},...β_{xn} are the estimated coefficients, and Soil, Hybrid, $Week_{x1}$,…$Week_{xn}$ are the independent variables that were entered into the model. Stepwise selection with entry and exit criteria levels equal to 0.10 and 0.20, respectively, was employed to define significant independent predictors during the model development phase.

The predictive power of the developed model was assessed by external evaluation using the independent evaluation dataset (SAS, 2015). The estimated AUC for the developed model along with the ROC curve computed when the fitted model was applied to the independent dataset (external evaluation) were compared for equality at level of significance α = 0.05 with the uninformative model (a model predicting by chance; AUC = 0.5000).

2.7 Predicting aflatoxin risk with logistic regression at regional level – Georgia study

The data from Georgia were utilized to determine if ARID can be used as a tool to predict aflatoxin risk at a regional scale. Briefly, the comprehensive survey database was randomly separated into a model development and evaluation datasets, and a binary response variable was constructed from the original aflatoxin contamination data as previously described.

Missing data of planting dates and mid-silk day from the Georgia dataset forced several assumptions in order to retrieve that information from other sources. Planting dates from the state variety trials conducted by the University of Georgia at the experimental field research units in the Coastal Plain Region of Georgia in Tifton, Plains, and Midville, and the Wiregrass Research and Extension Center in Headland, Alabama were available from 1977 to 2004 (Alabama Cooperative Extension System et al., 2016; The University of Georgia CAES, 2016). For each season, the four planting dates retrieved from the aforementioned corn trial studies, were averaged, and thus, a potential planting date for each year was calculated. In a given year, all the Georgia counties with aflatoxin contamination data were assigned the calculated averaged planting date as the actual planting date. Starting from this calculated planting date, mid-silk days were estimated based on growth degree units (GDU), calculated as [(daily maximum temperature + daily minimum temperature/2) - 10^{o}C]. Estimated mid-silk stage, which occurred when 1250 – 1300 GDU were accumulated (Lee, 2016, personal communication) was used to split the growing season into weekly intervals following (positive) or preceding (negative) that day and weekly ARID values were calculated.

Multicollinearity as indicated by VIF < 5 for independent model parameters was not an issue in this analysis. Thus, weekly ARID values starting at week nine before mid-silk and up to week nine after mid-silk were used as predictor variables for model development. Model development and model evaluation were done as described in the previous section. The logistic model was represented by:

$$P(Aflatoxin) = \frac{e^{\beta_0+\beta_{x1}Week_{x1}+\cdots+\beta_{xn}Week_{xn}}}{1+e^{\beta_0+\beta_{x1}Week_{x1}+\cdots+\beta_{xn}Week_{xn}}} \tag{3}$$

where P(Aflatoxin) is the probability for aflatoxin contamination above the selected threshold (20 μg/kg), e is the base of natural logarithm, β_o, β_{x1},...β_{xn} are the estimated coefficients, $Week_{x1}$,…$Week_{xn}$ are the independent weekly ARID values entered into the model. Significant independent predictors were identified by stepwise selection having entry and exit criteria levels equal to 0.05.

3. Results and Discussion

3.1 Predicting the risk at a field (plot) level

Significant predictors (p-value < 0.10) for aflatoxin contamination risk in corn in Mississippi were soil type, hybrid, and drought levels represented by weekly-ARID values before and after mid-silk. Odds ratio estimates indicated an increased aflatoxin risk for the highly susceptible hybrid (hybrid 3) when compared to hybrids 1 and 2, which were characterized as moderately susceptible (Table 1). Additionally, corn grown in the heavier soil type (Leeper silty clay loam) showed a lower likelihood for aflatoxin contamination above the selected threshold of 20 μg/kg than corn grown in the Myatt loam. This agrees with observations from other studies indicating higher pre-harvest contamination levels for corn grown in coarse sandy soils than corn grown in finer textured soils (Davis et al., 1986; Jones et al., 1981). Due to the lower water holding capacities of the coarser textured soils, potentially, the plants are more prone to water stress through the growing season than when grown in heavier soil types.

Table 1: Odds ratio estimates and profile-likelihood confidence intervals for statistically significant independent variables for Mississippi data analysis as determined from the logistic regression model via stepwise selection with entry and exit values set to α = 0.10 and α = 0.20, respectively.

Effect	Unit	Odds Ratio Estimates	90% Confidence Limits	
Moderately resistant 1 vs susceptible	1	0.127	0.057	0.285
Moderately resistant 2 vs susceptible	1	0.195	0.091	0.418
Silty clay loam vs loam	1	0.198	0.095	0.411
Week 4 before mid-silk	0.1	1.226	1.044	1.440
Week 1 before mid-silk	0.1	1.325	1.151	1.525
Week 4 after mid-silk	0.1	1.224	1.079	1.390
Week 8 after mid-silk	0.1	0.853	0.758	0.960

Moderately resistant 1 & Moderately resistant 2 are moderately susceptible hybrids.
Susceptible is highly susceptible hybrid.
Silty clay loam = Leeper silt clay loam.
Loam = Myatt loam

The critical growing season periods when changes in in-field drought conditions influence the risk for aflatoxin contamination, included weeks four and one before mid-silk and weeks four and eight after mid-silk day (Table 1). Moreover, a 0.1 increase in in-field drought, as quantified by ARID, during weeks four and one before mid-silk and week four after mid-silk, revealed that the predicted odds for contamination to be above the preselected threshold of 20 μg/kg was 22.6, 32.5, and 22.4% higher than the odds of not having contamination, respectively. Battilani et al. (2008a) had shown that drought had an influence on aflatoxin contamination in corn in Northern Italy, and defined as critical for contamination the timespan starting the last decade (10 days) of June and the first and last decade of August. Additionally, aflatoxin occurrence have usually been associated with higher than normal temperatures and low rainfalls around silking/pollination and grain filling period (20 – 60 days after flowering), both conditions that may increase drought stress (Diener et al., 1987; Payne and Widstrom, 1992; Widstrom et al., 1990; Windham et al., 2009).

Interestingly, in this study the predicted odds to have an event (contaminated sample) were 14.7% smaller than the odds to not have contamination for every 0.1 drought increase, as indicated by ARID index, during week eight after mid-silk (a near to harvest window) (Table 1). Rewetting events late in the season coinciding with the timespan just prior or during harvest delayed corn drying, favor unremitting aflatoxin synthesis, and thus, may increase toxin accumulation, particularly in years conducive for infection and contamination (Cotty and Jaime-Garcia, 2007; Jaime-Garcia and Cotty, 2003; Jones et al., 1981).

The relative risk for corn aflatoxin contamination above the threshold of 20 μg/kg was higher for all three hybrids when cultivated in the lighter soil type (Myatt loam) compared to the heavier soil type (Leeper silty clay loam) (Figure 1 and Figure 2). Increase in drought conditions during week four following mid-silk, other things equal, resulted in an increased contamination risk, as well, for all the scenarios shown in Figure 1.

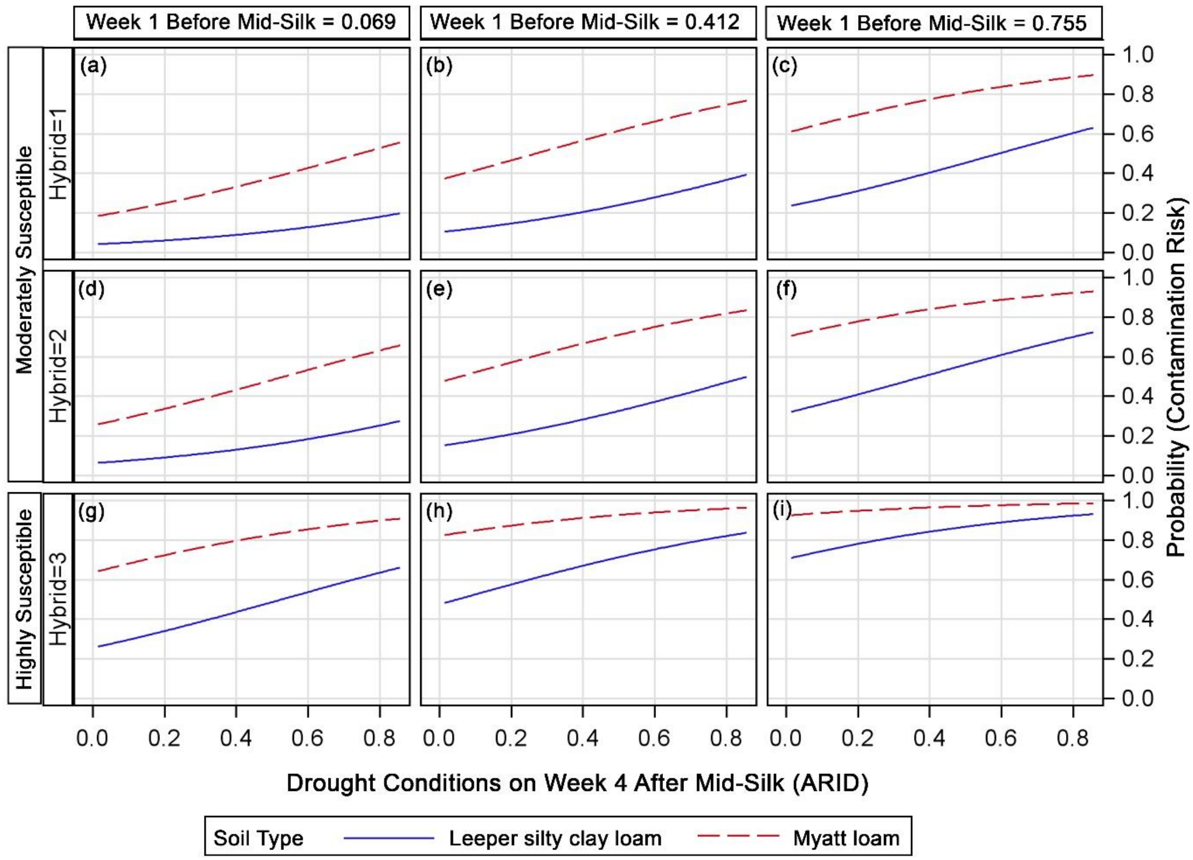

Figure 1: Predicted probabilities (Mississippi analysis) for having aflatoxin contamination above the threshold of 20 μg/kg with changes in drought conditions on the fourth week after mid-silk, given that moderately (Hybrids 1 & 2) and highly (Hybrid 3) susceptible hybrids are cultivated under Leeper silty clay loam (solid line) and Myatt loam (dashed line). Three scenarios (columns) are presented herein for each hybrid (rows): 1) low (ARID=0.069), 2) medium (ARID=0.412) and 3) severe (ARID=0.755) drought conditions for the week prior mid-silk. Week four before mid-silk and week eight after mid-silk were set fixed to their respective mean values.

For example, we found that the predicted probabilities for contamination above the threshold of 20 μg/kg were greater than 50% (y-axis), when a highly susceptible hybrid (hybrid 3) was exposed to moderate (ARID = 0.412) or extreme drought (ARID = 0.755) during the week prior to mid-silk, even when no or low drought (e.g. ARID < 0.200, x-axis) occurred on week four after mid-silk, regardless of soil type (Figure 1h and Figure 1i). In contrast, if the moderately susceptible hybrids (hybrid 1 and 2) were cultivated in the Leeper silty clay loam, the likelihood for contamination above the legal limit (20 µg/kg) was less than 50% (y-axis) even when the crops were exposed to extreme drought conditions (ARID > 0.800, x-axis) on week four following mid-silk (Figure 1a-b, Figure 1d-e). This holds when the crop cultivated in the Leeper silty clay loam was exposed to low stress (ARID = 0.069, Figure 1a and Figure 1d) or moderate stress (ARID = 0.412, Figure 1b and Figure 1e) the week preceding mid-silk.

Our study indicates that drought conditions close to corn harvest in Mississippi reduce aflatoxin contamination levels when compared with the earlier vegetative and reproductive crop stages. The impact of reduced drought late in the season on aflatoxin accumulation in corn grain was indicated in all scenarios presented in Figure 2.

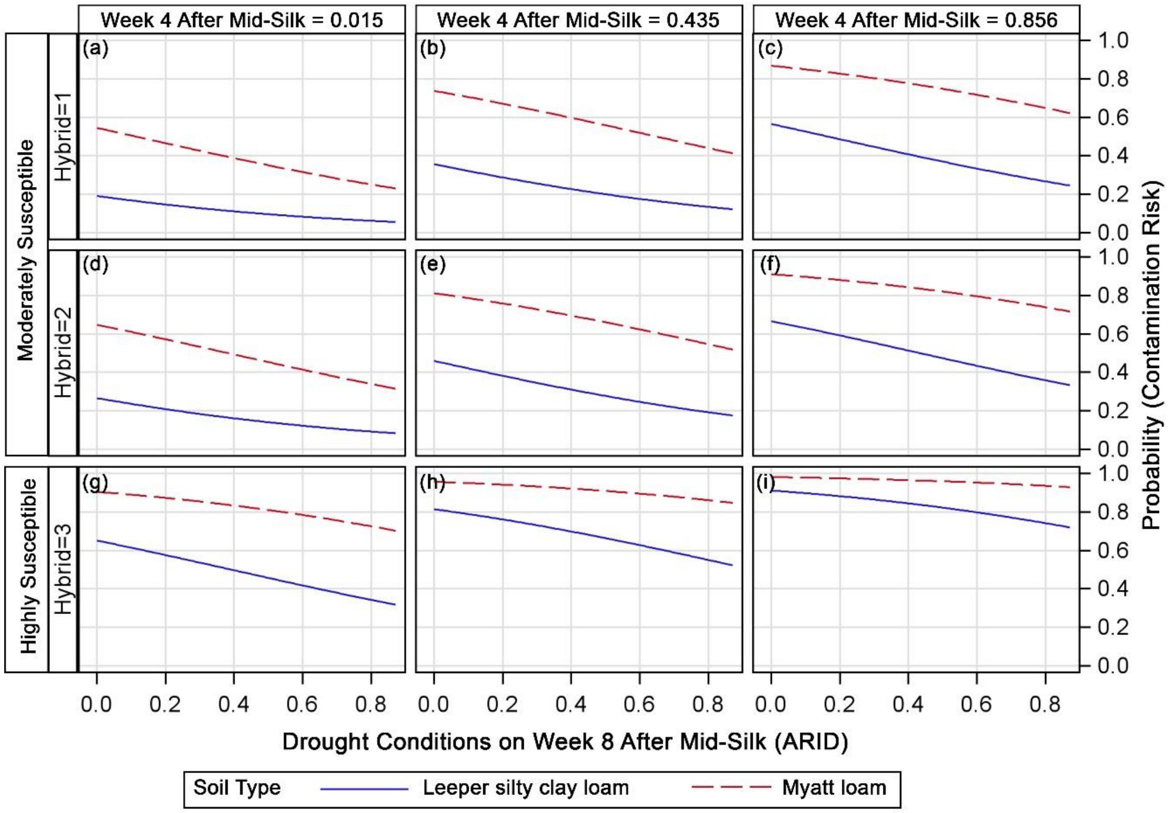

Figure 2: Predicted probabilities (Mississippi analysis) for having aflatoxin contamination above the threshold of 20 μg/kg with changes in drought conditions on the eighth week after mid-silk, given that moderately (Hybrids 1 & 2) and highly (Hybrid 3) susceptible hybrids are cultivated under Leeper silty clay loam (solid line) and Myatt loam (dashed line). Three scenarios (columns) are presented herein for each hybrid (rows): 1) low (ARID=0.015), 2) medium (ARID=0.435) and 3) severe (ARID=0.856) drought conditions for the week four after mid-silk. Week four and one before mid-silk were set fixed to their respective mean values.

For all the scenarios studied, late in-season drought (week eight after mid-silk) decreased the relative risk for grain contamination for both soil types, regardless of hybrid type (Figure 2). Drought is commonly associated with higher than normal surface temperatures, prolonged periods of no or minimal precipitation (McNab and Karl, 1991), and drier air than usual (Baldwin, 1957; McNab and Karl, 1991; Potter, 1958). Thus, our results are in agreement with Cotty (2001) who suggested that aflatoxin contamination of a mature cotton seed is promoted by warm temperature, high relative humidity (above 85%) or wetting events at or after ball opening. Similarly, our findings agree with Jaime-Garcia and Cotty (2003) who showed that increased rainfall late in the season was associated with increased seed contamination levels in cotton in Texas. A drought increase late in the season might be associated with drier air than usual, and thus, less than normal dew formation, which could explain the reduced aflatoxin contamination risk with drought increase on week eight after mid-silk. This agrees with McMillian et al. (1985) who found that when corn ears were water sprayed from the third to sixth week after full silk they had higher levels of contamination than non-sprayed ears. It was suggested that heavy morning dews may promote preharvest corn contamination in the Southeast US (McMillian et al., 1985). Likewise, an increase in drought is usually associated with higher air temperatures. August average minimum temperature was negatively associated with aflatoxin incidence and severity in corn studies over nine locations in USA (Sisson, 1986). The eighth week after mid-silk in our study corresponds to a week before harvest, and depending on the season, ranged from the first calendar week of August to the first calendar week of September. It was suggested by Sisson (1986) that higher night temperatures could hasten corn maturation and reduce incidence of dew formation, a factor that could be related to fungal development and aflatoxin synthesis, and could explain the findings of our work on week eight after mid-silk. Battilani et al. (2008a), showed that in Northern Italy, aflatoxin contamination risk was higher when drought increased on the last ten day window of June, and the first and last ten day windows of August. In the Mississippi study, the contamination probability was increased by increasing ARID values on weeks four and one before mid-silk, and on week four after mid-silk that correspond to late vegetative and early reproductive corn stages, respectively. However, our data showed that the risk for aflatoxin contamination was reduced by increasing drought on week eight after mid-silk (a week prior to harvest).

The variability in drought conditions during the periods found in this study impact the extent of preharvest aflatoxin contamination in corn. Timing and the degree of drought, along with soil type and hybrid resistance on infection and subsequent toxin accumulation can significantly change the likelihood for aflatoxin contamination. Seasonal fluctuations drive the dynamic relationships in the micro-organismal community, change the fungal community structure along with the quantity of both aflatoxigenic producers and the available primary and/or secondary inoculum in the field (Cotty and Jaime-Garcia, 2007). Plant exposure to different drought stress levels when crops are grown in different soil types with variable soil plant available water may predispose corn to *A. flavus* infection and subsequent aflatoxin contamination. Different genotypes respond to environmental stresses in different ways. For example, in most of the genotypes tested greater levels of contamination were observed for corn crops indicating the highest physiological responses to drought and heat stresses, thus revealing a relationship between aflatoxin accumulation and those stresses (Kebede et al., 2012). In the same study, an aflatoxin resistant genotype had the lowest contamination levels despite being one of the most stressed crops; this suggested that the resistance mechanism for this genotype might be more complex. The findings indicate that soil type and genotype are likely to influence the final toxin accumulation in the grain, and may explain contradictory reports among different studies.

3.2 Model evaluation – Mississippi analysis

Binary response models, including logistic regression models, can be assessed by calculating the AUC. The AUC for the developed model was equal to 0.8233, and was forecasting significantly better than a model predicting by chance (AUC = 0.5000; p-value < 0.0001) (Figure

3).

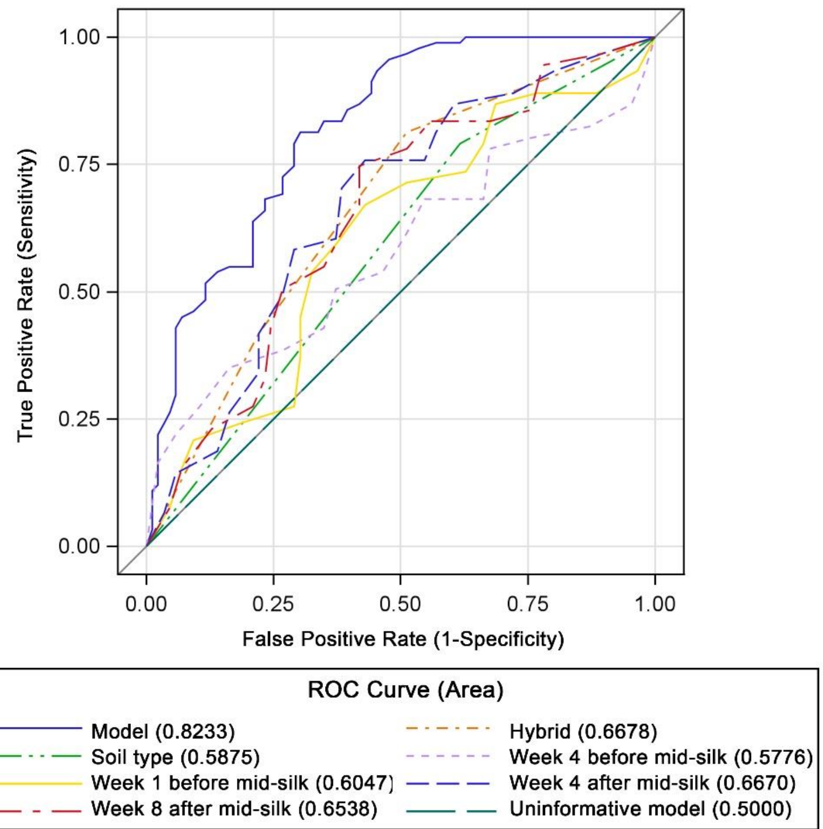

Figure 3: Receiver Operating Characteristic curves for the fitted model (Model), and for each significant variable as identified in the logistic model, indicating the relative weight of each predictor variable on the studied association (Aflatoxin predicted probabilities vs. predictor variates). Uninformative is the model with no predictive power (predicting by chance). Values in parentheses correspond to area under the curve (AUC) calculated for each particular model tested; Mississippi analysis.

Applying the fitted model to the evaluation dataset resulted in a negligible decrease in the predictive power (AUC = 0.8092) (Figure 4). A significant contrast test (p-value < 0.0001) indicated that the developed model was better than the uninformative model (AUC = 0.5000) when applied to the evaluation dataset. Therefore, the proposed predictive model could correctly predict the risk in nearly 82% of the cases. Thus, ROC curve analysis showed that the developed model proposed herein could identify true positives and minimize false negatives at acceptable levels of accuracy.

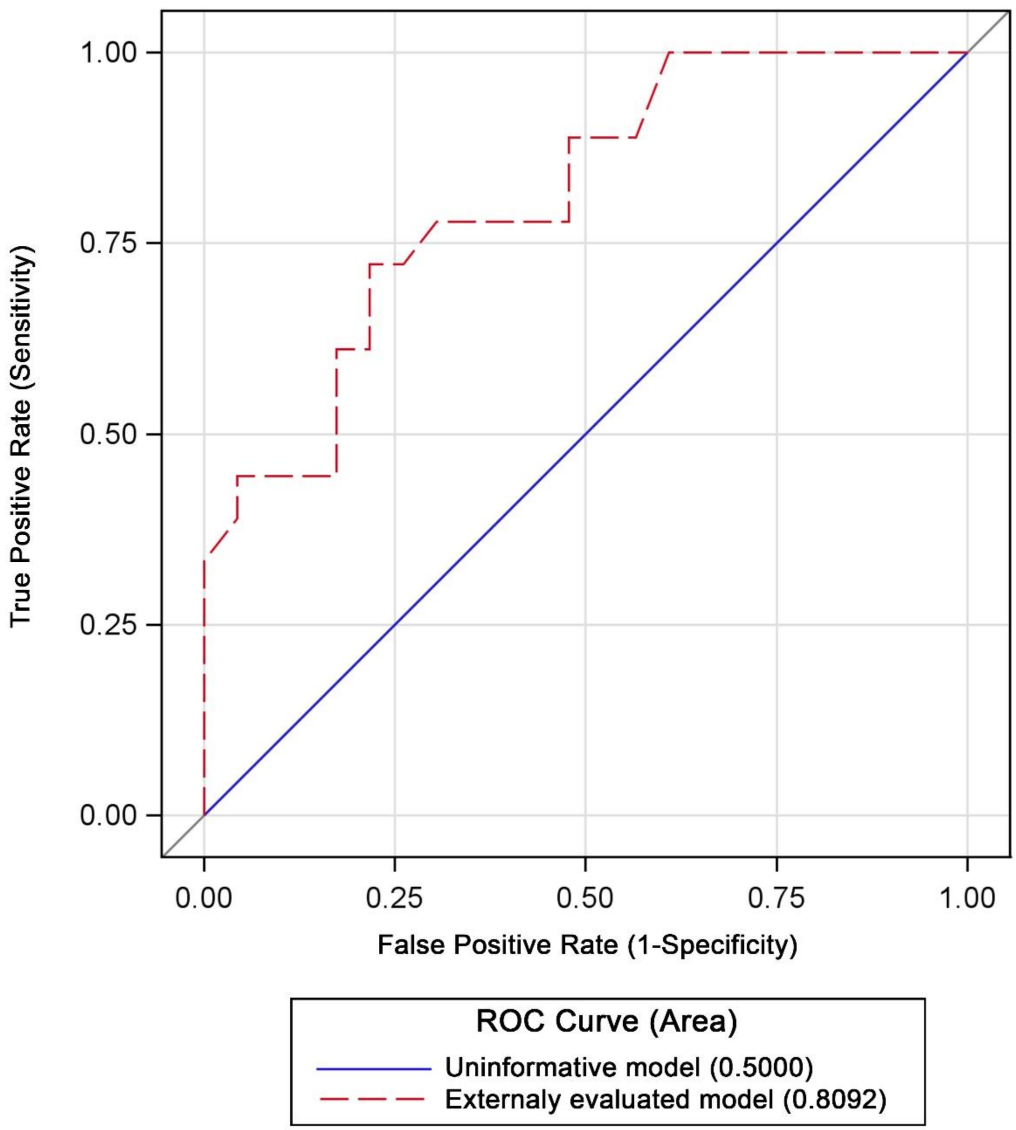

Figure 4: Receiver Operating Characteristic curve from applying the fitted model (Externally evaluated model) to the evaluation dataset, compared to the AUC (Area Under the Curve) of the uninformative model (Model). Values in parentheses correspond to calculated AUC; Mississippi analysis.

As indicated by their respective ROC curves, when weekly ARID values four and eight after mid-silk along with hybrid resistance to infection and subsequent contamination were considered as individual predictors, they had the highest relative impact on the measured associations (Figure 3). In contrast, sub-models with only soil type or week four before mid-silk as independent factors alone had the lowest AUC's equal to 0.5841 and 0.5673, respectively (Figure 3). All the single independent variable sub-models (Figure 3) had a significantly reduced predictive power when compared to the overall model (p-value < 0.0001). However, when the sub-models were compared to the uninformative model (AUC = 0.5000), their discriminative power between events and non-events was statistically significant (p-value < 0.0151) for all but the sub-model having week four before mid-silk alone as an independent variable (p-value = 0.0732).

3.3 Predicting the risk at a regional level

The logistic regression model showed that significant predictors (p-value ≤ 0.05) for aflatoxin contamination in Georgia were the calculated drought levels during weeks eight, seven, and three before mid-silk, along with weeks two, four, and nine following mid-silk (Table 2). The effect of drought on the likelihood of corn aflatoxin contamination changed both in magnitude and direction (positive and negative) through the season. For example, a 0.1 increase in drought conditions during week four and nine after mid-silk was estimated to increase the odds of having contamination above the 20 μg/kg legal limit by 71.8 and 77.0%, respectively. Interestingly, if the average weekly ARID value for weeks eight and three before mid-silk was increased by a value of 0.1, then the predicted odds for contamination were 3.4 and 1.3 lower than the odds of non-events (contamination below 20 μg/kg). Similarly, the model showed that a reduced drought on week two after mid-silk resulted in an increased risk. In contrast, an increased early drought stress (week 7 before mid-silk) significantly increased the likelihood for aflatoxin accumulation above the action limit (20 μg/kg) in the grain at the end of the season.

Table 2: Odds ratio estimates and profile-likelihood confidence intervals for statistically significant independent variables for Georgia data analysis as determined from the logistic regression model via stepwise selection with entry and exit values set to α = 0.05.

Effect	**Unit**	**Odds Ratio Estimate**	**95% Confidence Limits**	
Week 8 before mid-silk	0.1	0.295	0.182	0.430
Week 7 before mid-silk	0.1	3.167	1.978	5.884
Week 3 before mid-silk	0.1	0.749	0.576	0.955
Week 2 after mid-silk	0.1	0.729	0.574	0.907
Week 4 after mid-silk	0.1	1.718	1.360	2.245
Week 9 after mid-silk	0.1	1.77	1.403	2.308

This study showed that the risk for aflatoxin accumulation changes over the growing season. Drought conditions at particular weekly intervals relative to mid-silk, had influenced the likelihood for contamination above the legal limit (20μg/kg) set by U.S. FDA. Moreover, the estimated probability for aflatoxin contamination to exceed 20 μg/kg was defined by the level of dry/wet cycles in the preceding critical timespans. For example, if the plant was exposed to low to moderate drought on week seven (ARID < 0.492) before mid-silk, then the risk remained well below 20%, even if extreme drought occurred on week four after mid-silk (ARID = 1.000) (Figure 5a-c). However, if the ARID value on week four following mid-silk was ≥ 0.6, reflecting a moderate in-field drought situation, then the probability for contamination above the legal limit exceeded 40% (Figure 5e).

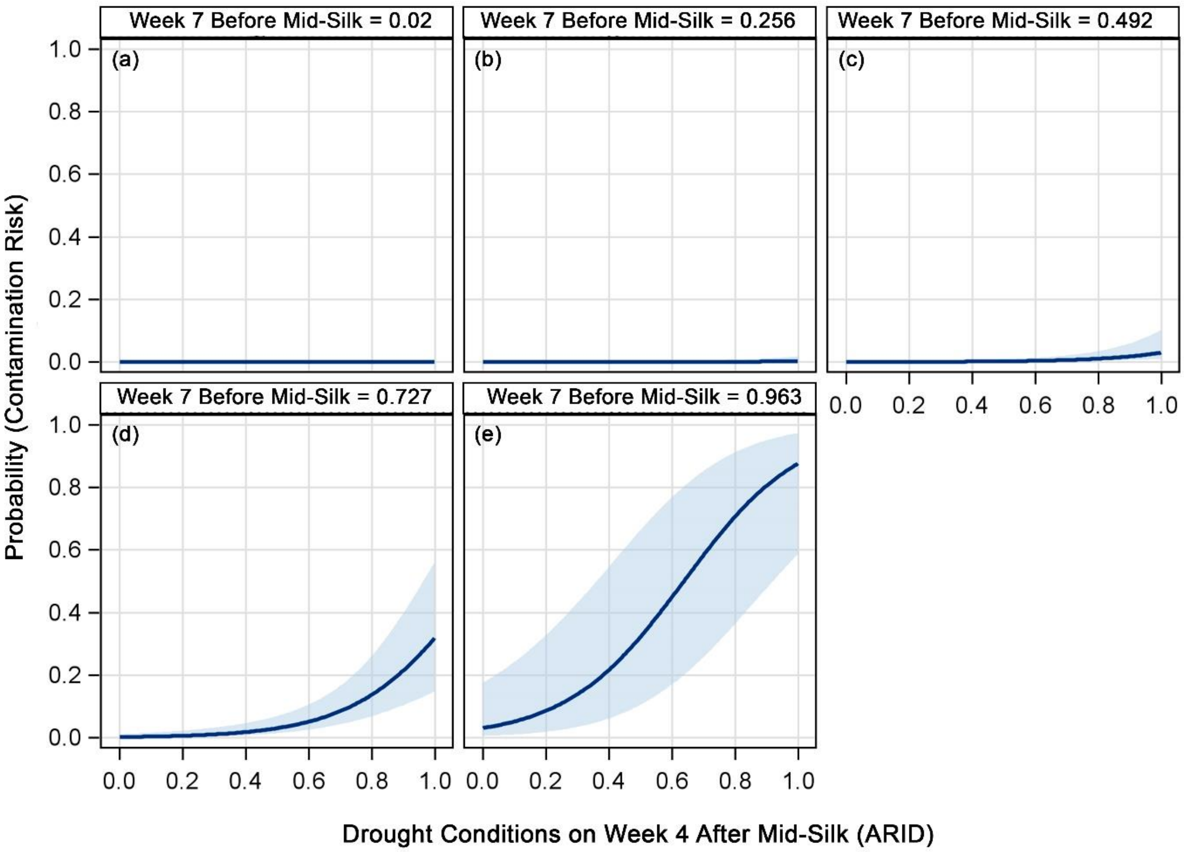

Figure 5: Predicted probabilities (Georgia analysis) plot panel indicating aflatoxin risk change when drought conditions are changing on week four after mid-silk for five different aridity scenarios early in the seasons (week 7 before mid-silk). ARID values for the rest of the weeks were set equal to their mean. Shaded band represent confidence limits at $\alpha = 0.1$.

Under extreme drought conditions on week nine (ARID > 0.800) and week four (ARID = 0.998) after mid-silk, other things being equal, the likelihood for aflatoxin contamination above the action level (20 μg/kg) approached 40% (Figure 6e). Those observations may reflect not only the complex interactions that take place between biotic and abiotic factors impacting fungal growth, sporulation, inoculum dissemination, infection, toxin synthesis, and accumulation, they also indicate the interconnectivity and the interdependence of process, that constantly change during the growing season and occur at the host plant level, fungal level, and each particular environment.

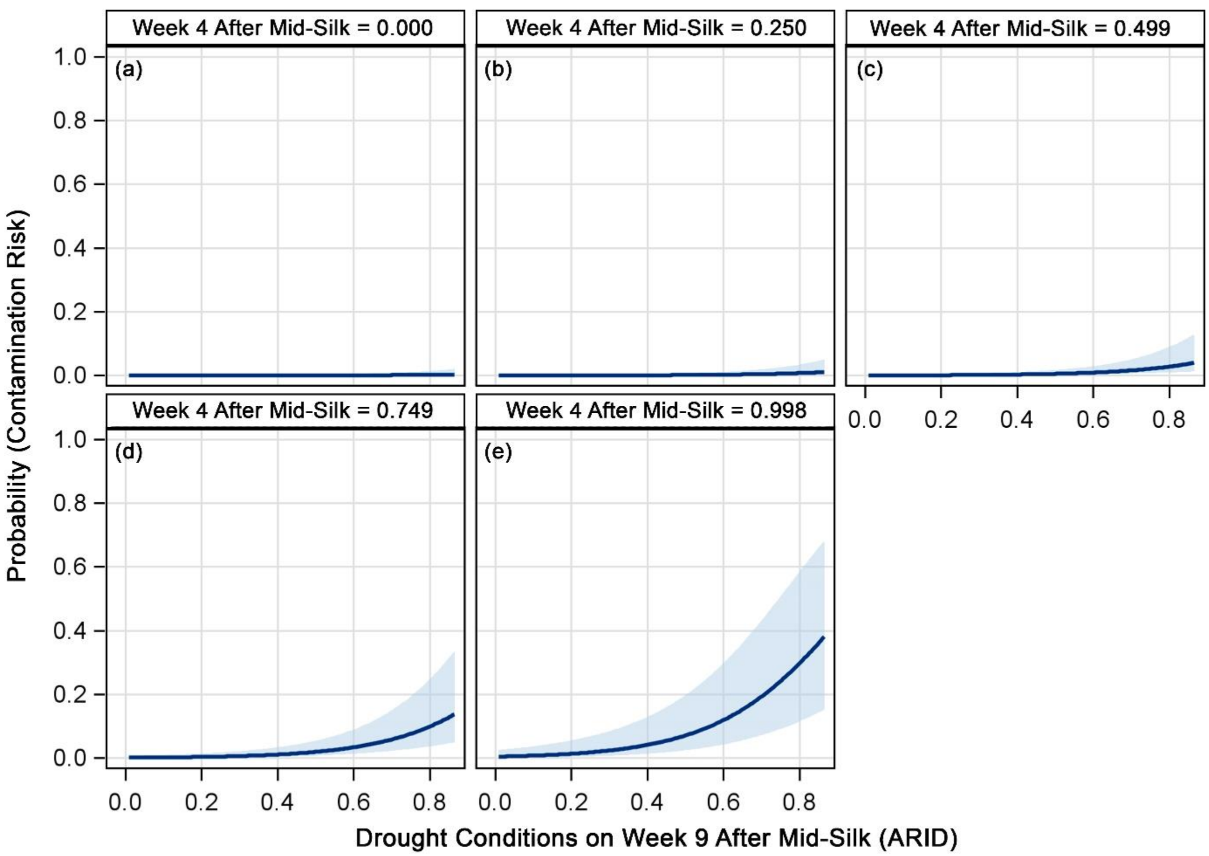

Figure 6: Predicted probabilities (Georgia analysis) plot panel indicating aflatoxin risk change when drought conditions are changing on week nine after mid-silk for five different aridity scenarios for week four following mid-silk. ARID values for the rest of the weeks were set equal to their mean. Shaded band represent confidence limits at $\alpha = 0.1$.

As shown in this study, predicting aflatoxin risk at field (plot) level based on soil type, hybrid, and drought conditions is promising. Predicting the likelihood of contamination at a regional level might be more challenging due to multiple soil types, the very different weather conditions encountered across a region and their effect on the fungus, the host, and their interactions. Consequently, questions have been raised about the feasibility of the methodology proposed herein. Lack of data (e.g. planting date, crop growth stage, soil types, hybrids) added to the overall uncertainty, and required informative assumptions (i.e. determination of potential planting dates for a given area and season) and estimations (e.g. forecasting mid-silk day by calculating GDU). In regional studies, due to data limitations, meteorology might be the only driving factor available to assess risk, and thus, a simple predictive system might be more desirable and applicable. For all these reasons, our approach to assess the likelihood of corn contamination above the legal limit at county level was based only on minimum weather data (maximum temperature, minimum temperature, and rainfall).

3.4 Model evaluation – Georgia analysis

Contaminated and non-contaminated samples used for model development equaled to 620 (93.37%) and 44 (6.63%) samples, correspondingly. The evaluation dataset contained 154 samples, 91.56 and 8.44% were classified as non-events (had aflatoxin contamination below the 20 μg/kg threshold) and events, respectively. The AUC for the developed model was 0.9744 (p-value < 0.0001) and was predicting significantly better than the model predicting by chance (AUC = 0.5000) (Figure 7).

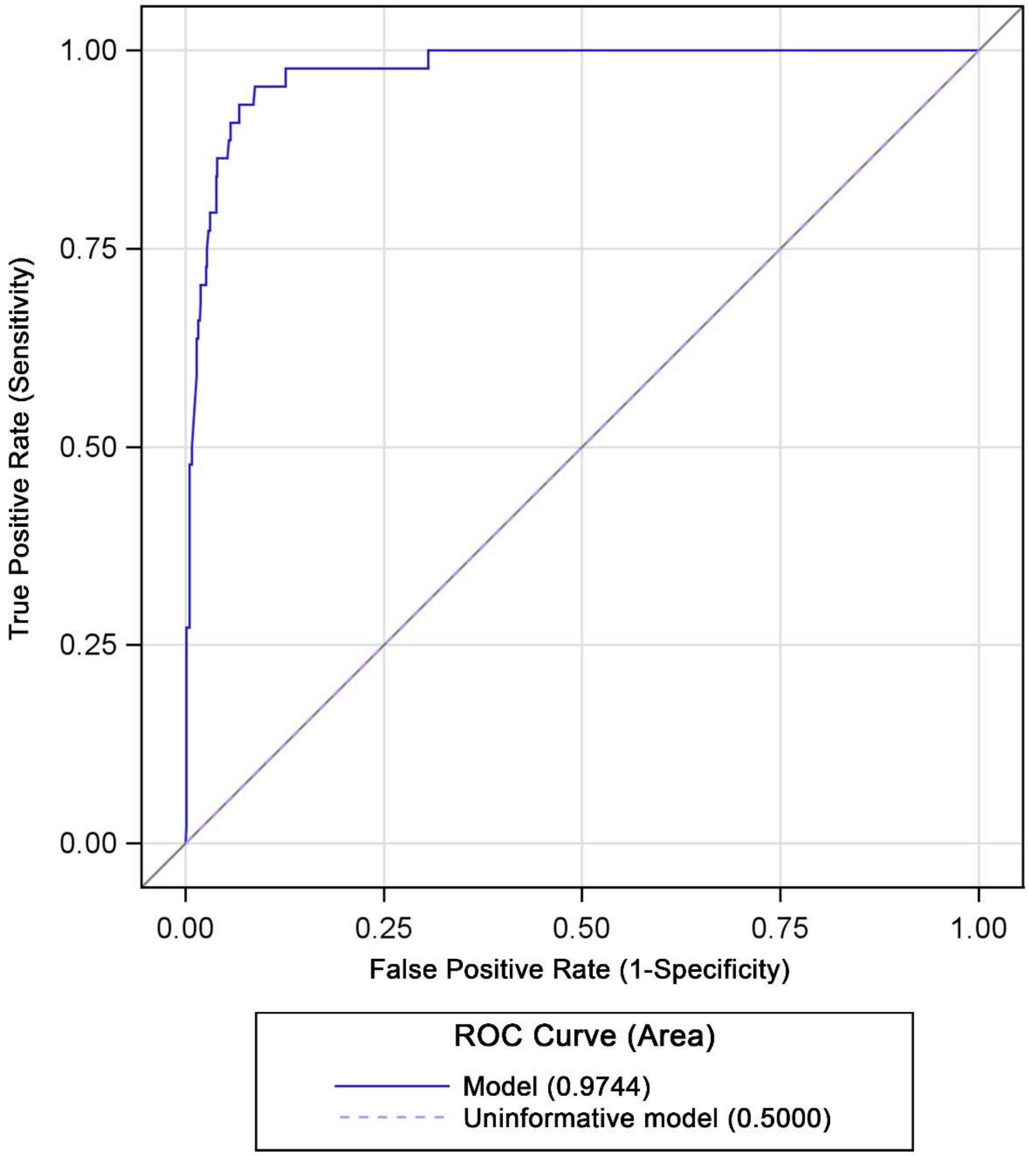

Figure 7: Receiver Operating Characteristic curve for the fitted model (Model). Uninformative is the model with no predictive power (predicting by chance). Values in parentheses correspond to calculated area under the curve (AUC); Georgia analysis.

When the developed model was applied to the evaluation dataset, the ROC curve dropped to 0.9177 (Figure 8). Despite that, the ROC contrast test was significant (p-value < 0.0001), indicating that the developed model was more accurate in predicting contaminated from non-contaminated samples when compared to the uninformative model (AUC = 0.5000).

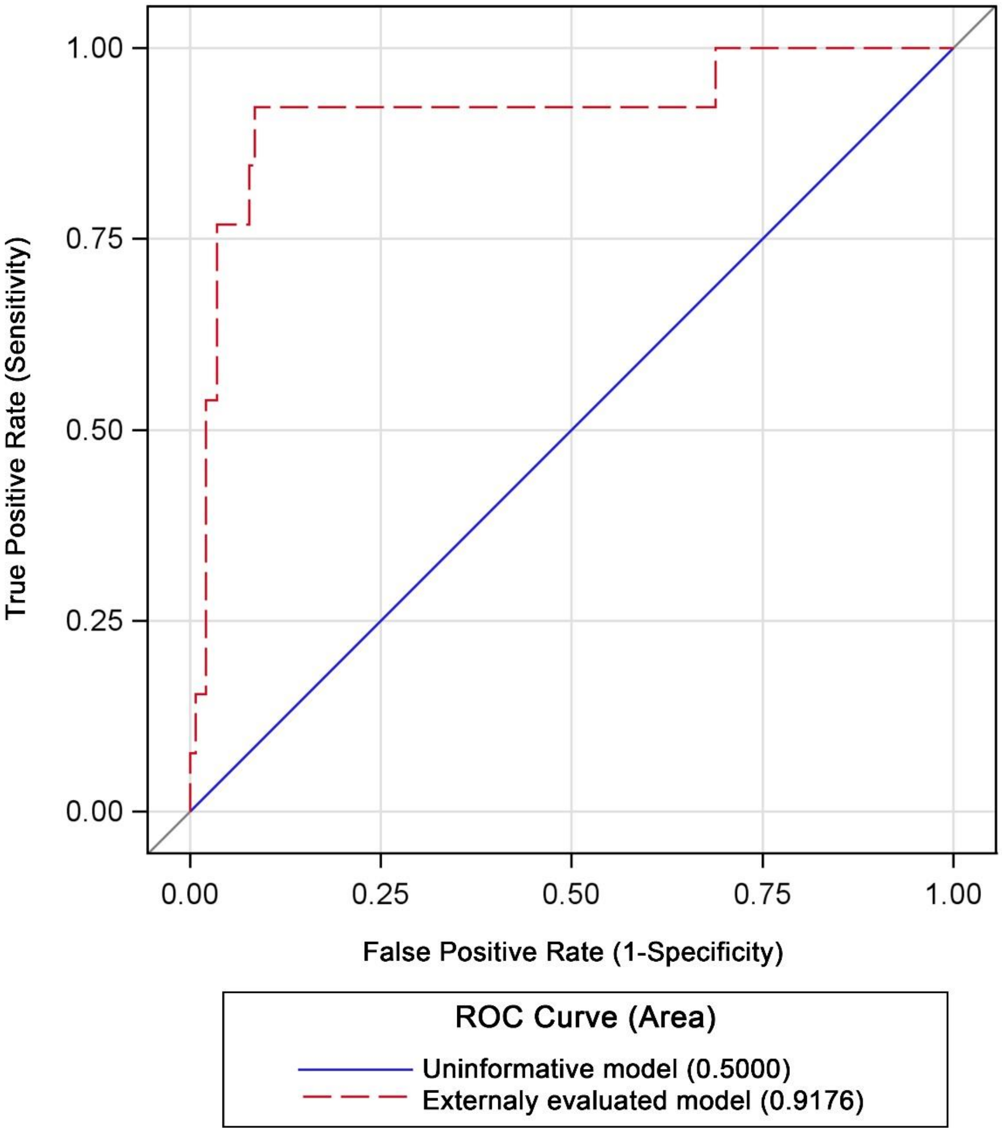

Figure 8: Receiver Operating Characteristic curve from applying the fitted model (Externally evaluated model) to the evaluation dataset, compared to the AUC of the uninformative model (Model). Values in parentheses correspond to calculated AUC; Georgia analysis.

3.5 Comparing the results – field (plot) versus regional

Analyses using both Mississippi dataset and Georgia dataset indicated that drought, as quantified by weekly ARID values, is a significant driving factor that influences the risk for contamination. However, the timespans (weeks) indicated as significant varied between the Mississippi and Georgia data sets (Table 1 and Table 2). Only week four after mid-silk was identified as significant by both models. Additionally, both studies are reflective of what has been observed by other researchers (Hawkins et al., 2008; Windham et al., 2009), as well; infection and subsequent aflatoxin contamination are likely influenced by environmental stresses (e.g. drought, temperature, and moisture stresses) occurring prior to silking. Perhaps more attention and studies under controlled environments (greenhouse) considering corn vegetative growth stages might be needed to determine if those early stresses provoke physiological responses/processes at the plant and/or fungal level on the variability in toxin accumulation observed at the end of the season.

The differences on the results from both studies may be related to the assumptions made on the Georgia data. The planting dates and mid-silk stages were estimated, and corn samples were collected from fields within a county but there is not information on the specific location. These issues may have reduced the power of the model especially because weekly ARID calculations rely on that information. From experience, it is known that weather, and particularly rainfall amount and distribution, can be highly variable even over relatively short distances. Therefore, interpretation of odds estimates from the regional model has to be approached with extreme caution. For example, the odds estimates for week three prior to mid-silk and week two after mid-silk derived from the Georgia analysis suggest that increase in drought results in lower risk (Table 2). This contradicts other studies that have shown that extended aflatoxin levels in corn are commonly encountered in seasons or at field locations associated with drought stress (Abbas et al., 2002; 2004; Davis et al., 1986; Diener et al., 1987; Windham et al., 1999). Abbas et al. (2004) had shown that the incidence of *A. flavus* propagules recovered from corn grains was greater in a field site that had received supplemental water compared to other field sites. The opposite trend was observed for aflatoxin contamination levels; moreover, no association between aflatoxin contamination and colonization levels was detected. Thus we may conclude that the odds ratios for week three prior to mid-silk and week two following mid-silk, as suggested by the Georgia model, are likely erroneous.

We consider the model from the Mississippi study more robust, since the data obtained were derived from a controlled in-field experiment. Hence, potential strategies to mitigate aflatoxin contamination should rely more on the information derived from the controlled (Mississippi) experiment which is in agreement with the principle knowledge of the phenomenon. Therefore, the Georgia results should be rather considered as a preliminary work illuminating the potential of the proposed methodology to assess the risk over a larger regional area; however, a more detailed georeferenced database will be necessary to address the limitations and contradictions observed herein. Additionally, agronomic information such as hybrid type, growth stages (at least planting dates and/or mid-silk) may add to the robustness of a future regional model.

3.6 Potential strategies to mitigate pre-harvest contamination

Site-specific management strategies could be adapted at the beginning or through the season to minimize host plant stress, which may reduce aflatoxin risk. Use of irrigation during critical risk weeks may reduce the risk (Fortnum and Manwiller, 1985; Jones et al., 1981; Payne et al., 1986); but irrigation amount and timing should be based on crop needs, atmospheric conditions, and soil water holding capacity. Planting date adjustment, within-field planting density and/or selection of hybrids with suitable relative maturities to reduce plant exposure to drought stress during critical growth timespans should be considered as well (Abbas et al., 2007; Bruns and Abbas, 2006; Alvarado-Carrillo, et al.; 2010; Payne and Widstrom, 1992). A grower could consider selecting of the most appropriate hybrid for a given site/region based on soil type and drought risk assessment in a particular locality. Separation of the field into management zones based on risk stratification criteria (e.g. soil texture, plant available water holding capacity, electrical conductivity, and/or soil organic matter content) could be considered as well. Separation of the field into management zones will allow to: 1) plant appropriate hybrid type per zone, 2) segregate harvest if necessary, and 3) apply variable rate irrigation/fertigation at different zones as needed. Determination of best harvest timing may be based on the predicted contamination risk for a particular season and location (Battilani et al., 2013). Therefore, decisions for early harvest, subsequent grain drying, and proper grain storage aiming to reduce/cease further infection and toxin accumulation, might be an option (Hell and Mutegi, 2011); but the additional associated cost needs to be accounted for. Grain storage segregation based on risk prediction for different harvested lots is an option (Ni, et al., 2011).

In addition, risk maps (Battilani et al., 2016) by county or by regions within a single state or regional risk maps could be generated using ARID data. Those regional aflatoxin risk maps might be useful for adaption or implementation of risk mitigation strategies including: 1) selection of drought tolerant corn hybrids in high risk areas, 2) cultivating cover crops for soil moisture conservation on high risk areas, 3) shifting in planting dates, and 4) applying variable rate irrigation and seed to minimize plant water stress. Moreover, the logistic model used herein to predict aflatoxin risk in corn, could be incorporated into decision support systems (Chauhan et al., 2015) and develop on-line tools to predict the risk earlier in season based on changes in drought conditions during corn growth and development. This may allow for more informative, effective, and efficient crop management decisions by the producers and the agri-business sectors (Battilani et al., 2013; Chauhan et al., 2015; Cotty and Jaime-Garcia, 2007).

4. Conclusions

Results from the control experiment (Mississippi) analysis indicated that ARID could be used as a predictive tool for aflatoxin risk assessment. Hybrid susceptibility to infection/contamination, along with soil type contributed significant to predicting aflatoxin occurrence. Additionally, this work identified significant weeks during the growing season when changes in drought had an influence on the likelihood of aflatoxin contamination. This study illuminated that the critical timespan for infection and subsequent contamination extend both prior and beyond mid-silk. Time windows, as indicated by the Mississippi study when changes in drought have the greatest influence on aflatoxin risk, included weeks four prior and after mid-silk, among others. Additionally, the highly susceptible hybrid grown in lighter soil showed a higher risk for aflatoxin contamination with changes in drought conditions during critical week windows compared to the moderately susceptible hybrids grown in the heavier soil. The proposed methodology was extended from field (plot) level to a regional scale (Georgia study), and the results are presented here in as well. Both predictive models were externally assessed on independent datasets and showed high accuracy in classifying samples as contaminated above or below the preselected threshold (20 μg/kg). Identifying critical weeks influencing the risk for contamination early in the season may allow farmers, researchers, and extension specialists to monitor changes of aflatoxin risk with in-season drought changes, and thus, make more informative management decision in an effort to mitigate the problem (Battilani et al., 2013; Chauhan et al., 2015; Cotty and Jaime-Garcia, 2007). This is true particularly during years characterized by conducive to toxin accumulation conditions. Finally, this work emphasizes the effect drought timing and drought severity has on pre-harvest corn aflatoxin risk alterations during the season and further illuminates the impact drought has on contamination levels under different environments.

Acknowledgments

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