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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.

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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 preharvest 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, 2013). ARID might be used to quantify agricultural drought and estimate its effects on crop yields (Woli et al., 2012, 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.