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An improved simulation model to predict pre-harvest aflatoxin risk in maize

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ABSTRACT

Aflatoxin is a potent carcinogen produced by *Aspergillus flavus*, which frequently contaminates maize (*Zea mays* L.) in the field between 40° north and 40° south latitudes. A mechanistic model to predict risk of pre-harvest contamination could assist in management of this very harmful mycotoxin. In this study we describe an aflatoxin risk prediction model which is integrated with the Agricultural Production Systems Simulator (APSIM) modelling framework. The model computes a temperature function for *A. flavus* growth and aflatoxin production using a set of three cardinal temperatures determined in the laboratory using culture medium and intact grains. These cardinal temperatures were 11.5 °C as base, 32.5 °C as optimum and 42.5 °C as maximum. The model used a low (≤0.2) crop water supply to demand ratio—an index of drought during the grain filling stage to simulate maize crop's susceptibility to *A. flavus* growth and aflatoxin production. When this low threshold of the index was reached the model converted the temperature function into an aflatoxin risk index (ARI) to represent the risk of aflatoxin contamination. The model was applied to simulate ARI for two commercial maize hybrids, H513 and H614D, grown in five multi-location field trials in Kenya using site specific agronomy, weather and soil parameters. The observed mean aflatoxin contamination in these trials varied from <1 to 7143 ppb. ARI simulated by the model explained 99% of the variation ($p \leq 0.001$) in a linear relationship with the mean observed aflatoxin contamination. The strong relationship between ARI and aflatoxin contamination suggests that the model could be applied to map risk prone areas and to monitor in-season risk for genotypes and soils parameterized for APSIM.

1. Introduction

Maize (*Zea mays* L.) is the third most important cereal used as human food and animal feed worldwide. However, maize is also a favoured host for the aflatoxin producing fungi *Aspergillus flavus* (Bandyopadhyay et al., 2007; Amaike and Keller, 2011). High levels of aflatoxin contamination in maize are quite common in some maize growing regions including those in sub-Saharan Africa (Wagacha and Muthomi, 2008; Hell and Mutegi, 2011; Mutiga et al., 2014). Aflatoxin contamination was particularly serious in eastern Africa where maize was a staple food and fatal aflatoxicosis cases related to the consumption of contaminated maize were frequently reported (Kang'ethe, 2011; Manjula et al., 2009). In Kenya alone around 500 persons have reportedly died due to acute aflatoxicosis since 1980 (Kang'ethe, 2011). Aflatoxin contamination is also a problem in other developing regions of the world (Kensler et al., 2011). While acute poisoning leading to death of humans and livestock represents the most recognizable part of the aflatoxin problem, there are also other more subtle health impacts of this mycotoxin. Chronic exposure to even low doses of aflatoxin increases risk of cancer, and may cause immuno-suppression, poor nutrient absorption, and fetal and infant growth retardation (Henry et al., 1999; Williams et al., 2004; Wild and Gong, 2010; Williams et al., 2010; Kensler et al., 2011).

In many developing countries aflatoxin contamination remains largely undetected due to lack of inexpensive diagnostics tools that can be used in the field, and the prevalence of informal trading of commodities. Given the scope and complexity of the problem, there is a particular need to develop predictive tools that can be

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used for both managing aflatoxin as well as assisting in diagnosing and appropriate handling of risk prone crops. An aflatoxin decision support tool called Afloman, which is based on a peanut aflatoxin model, has assisted in the management of aflatoxin in peanuts in Australia (Chauhan et al., 2010). There is a need to develop and apply similar tools to manage aflatoxin contamination in maize as well.

Aflatoxin contamination in maize can occur during pre-, and post-harvest. Managing pre-harvest contamination should be considered as an obvious target of any intervention as it is an important source of contamination which itself can be significantly above the legal limit of 4 to 20 ppb level for different countries. The residual inoculum could result in further accumulation of aflatoxin during storage if conditions are favourable for aflatoxin production (Hell et al., 2008). On the basis of certain trends in pre-harvest contamination observed in specific agro-ecologies of sub-Saharan Africa, Hell and Mutegi (2011) suggested it should be possible to model pre-harvest aflatoxin contamination. However, this has proved to be a challenging task due to interactions amongst many factors including crop, climate, and soil (Payne et al., 1986). Nevertheless, it is commonly accepted that aflatoxin contamination is a process driven significantly by climatic conditions, with underlying genetic and management components also contributing to susceptibility and risk. In particular, hot and dry conditions during the reproductive phase were recognized to be the key risk factors that pre-dispose the crop to pre-harvest *A. flavus* infection and aflatoxin production (Payne and Widstrom, 1992, Widstrom, 1996; Payne et al., 1986; Luo et al., 2010; Jones et al., 1981; Cotty and Jaime-Garcia, 2007; Cotty et al., 2008). As most modern simulation models are able exploit climate dependencies of various soil and plant processes for different crops to predict their performance in the field, it should also be possible to harness climate dependencies of *A. flavus* and other related species to predict pre-harvest contamination. The APSIM maize model has been recently used to evaluate risk of drought and high temperature to maize grown in the United States (Lobell et al., 2013) and in Australia (Chauhan et al., 2013). Given that several biotic stresses *e.g. Fusarium* cob and charcoal rots are similarly predisposed by climatic conditions, modelling aflatoxin contamination assumes importance. If successful, it should then be possible to model risk posed by other biotic stresses by exploiting their climatic dependencies in a similar way.

A few models for aflatoxin prediction have been proposed that are based on the understanding of interactions that occur amongst the fungus, temperature and water activity (Pitt, 1993; Garcia et al., 2009; Gqaleni et al., 1997; Molina and Giannuzzi, 2002; Abdel-Hadi et al., 2012; Mousa et al., 2011; Astoreca et al., 2012). While some of these models are able to simulate aflatoxin contamination well under *in vitro* conditions (culture media), these have not been extensively applied under field conditions. Probst and Cotty (2012) recently reported a lack of correlation even between the results of *in vitro* and *in vivo* experiments they conducted and hence cautioned on their use for predicting contamination in maize grains.

Only a couple of mechanistic models which exploit climatic dependencies of the *A. flavus* to invade and colonize maize cobs to predict pre-harvest aflatoxin contamination in field grown maize have been proposed in recent years (Chauhan et al., 2008; Battilani et al., 2008, 2013). The more recent version of the model by Battilani et al. (2013) used sporulation, infection, fungal growth and aflatoxin production at different temperatures and water activity as the main components in their modelling approach. They, however, ignored interactions that can occur due to the mismatch of soil moisture and its demand leading to development of drought which seems to be a key driver of pre-harvest contamination. In comparison, the modelling approach of Chauhan et al. (2008) considered sporulation and water activity as non-limiting steps and focused on computing risk of aflatoxin contamination driven by vulnerability of the crop to drought induced by adverse climatic conditions during the grain filling stage. In their model Chauhan et al. (2008) considered that the growth of the fungus and aflatoxin production was driven by temperature and the time spent under drought conditions. The cardinal temperatures used in their prototype model were largely derived from work on peanuts – a sub-terranean crop with similar issues related to aflatoxin contamination (Diener and Davis, 1977) – and has had only limited testing. Also a better indicator was needed to account for temperature-induced changes in vapour pressure deficit that exacerbates drought situation in addition to low soil moisture as risk factors that trigger *A. flavus* invasion and aflatoxin production. The objective of this study, therefore, was to develop maize-specific response parameters of *A. flavus* for the model and evaluate it using contamination data recorded in multi-location trials conducted in Kenya.

2. Materials and methods

2.1. Model description

The maize aflatoxin model was developed as part of the Agricultural Production Systems sIMulator (APSIM) modelling framework. The basic features of APSIM were described by Keating et al. (2003) and that of the prototype aflatoxin model by Chauhan et al. (2008). APSIM simulated maize growth, phenology, yield, and soil water balance using daily input of maximum and minimum temperature, radiation, and rainfall. The APSIM model also simulated the water supply to demand ratio (SDR, unitless) as an indicator of drought which has been used to characterize maize growing environments (Lobell et al., 2013; Chauhan et al., 2013; Harrison et al., 2014). SDR is quite sensitive to temperature because of the latter's relationship with vapour pressure deficit that drives the evapotranspiration demand (Lobell et al., 2013). When the supply matches the evapotranspiration demand then SDR is close to one and as the supply declines or the demand rises either due to high crop growth or increased vapour pressure deficit SDR becomes less than one and represents a degree of drought (Chenu et al., 2013; Lobell et al., 2013).

In the aflatoxin model, first a temperature dependency factor (Aflo_temp_factor) of *A. flavus* was computed using mean ambient temperature (T_{mean_aflo}) and the revised set of new minimum (base) (T_{min_aflo}), optimum (T_{opt_aflo}) and maximum (T_{max_aflo}) cardinal temperatures. The equations that used these three cardinal temperatures to calculate Aflo_temp_factor were: when $T_{mean\_aflo} \geq T_{min\_aflo}$ and $\leq T_{opt\_aflo}$ then

$$\text{Aflo\_temp\_factor} = \frac{T_{mean\_aflo} - T_{min\_aflo}}{T_{opt\_aflo} - T_{min\_aflo}}; \quad (1)$$

and when $T_{mean\_aflo} > T_{opt\_aflo}$ and $< T_{max\_aflo}$ then

$$\text{Aflo\_temp\_factor} = \frac{T_{max\_aflo} - T_{mean\_aflo}}{T_{max\_aflo} - T_{opt\_aflo}}; \quad (2)$$

and when $T_{mean\_aflo} < T_{min\_aflo}$ or $> T_{max\_aflo}$ then

$$\text{Aflo\_temp\_factor} = 0. \quad (3)$$

This temperature dependency factor was then used to compute the aflatoxin risk index (ARI) when SDR was below the threshold value of being ≤ 0.20 during the grain filling stage (stages 8 to 9 in APSIM). This low SDR value was indicative the crop being exposed to severe drought stress (Chauhan et al., 2013) a condition that could favour contamination. The grain filling stage was generally reached within a few days after anthesis. To compute ARI, Aflo_risk was accumulated in a counter so long SDR simulated by the APSIM model remained ≤ 0.2.