Field Crops Research 178 (2015) 91–99

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

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

Yashvir Chauhan[a,*], Jeff Tatnell[a], Stephen Krosch[a], James Karanja[b], Benoit Gnonlonfin[c], Immaculate Wanjuki[c], James Wainaina[c], Jagger Harvey[c]

[a] Department of Agriculture and Fisheries, Kingaroy 4610, QLD, Australia
[b] Kenya Agricultural and Livestock Research Organization (KALRO), Box 340-90100, Katumani, Machacos, Kenya
[c] Biosciences Eastern and Central Africa-International Livestock Research Institute (BecA-ILRI) Hub, PO Box 30709, Nairobi, Kenya

ARTICLE INFO

Article history:
Received 19 August 2014
Received in revised form 30 March 2015
Accepted 30 March 2015
Available online 21 April 2015

Keywords:
APSIM
Aspergillus flavus
Drought
Mycotoxin
Temperature
Zea mays L.

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

* Corresponding author. Tel.: +61 7 41600759.
E-mail addresses: yash.chauhan@daf.qld.gov.au, yschauhan@hotmail.com (Y. Chauhan).

http://dx.doi.org/10.1016/j.fcr.2015.03.024

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.

When the SDR ≤ 0.20 and maize growth stage ≥8

$$\sum \text{Aflo\_risk} = \text{Aflo\_risk} + (1 \times \text{Aflo\_temp\_factor}) \quad (4)$$

$$\text{ARI} = \sum \text{Aflo\_risk} \times 10 \quad (5)$$

The multiplier '10' in equation 5 was to bring ARI in the 0 to 100 range. A different multiplier could also be used to directly convert Aflo_risk into aflatoxin content. However, this would require strain specific calibration. The upper limit of ARI was kept as 100 which equated to a very high level of contamination.

The model assumed that *A. flavus* fungal inoculum was always available for infection and hence two steps—sporulation and spore germination were considered non-limiting and were not included in the model. Only the exposure of maize to drought (SDR ≤ 0.2) during any part of the grain filling stage was treated as a trigger point for infection and proliferation of the fungus and aflatoxin production in the grains. The model also assumed that water activity for spore germination was non-limiting as moisture content of maize grains before physiological maturity was always > 24% at which water activity of 1 was achieved (Payne et al., 1998).

2.2. Determination of cardinal temperatures for aflatoxin production

Three laboratory experiments were conducted to determine cardinal temperatures for *A. flavus* growth and aflatoxin production. *A. flavus* was grown in culture media in experiments 1 and 2, and on intact grains in experiment 3. In all the three experiments, incubation at different temperatures was done in dark. All inoculations were done in a biological safety cabinet (II).

In experiment 1, toxigenic strain #5307 of *A. flavus* collected from the soil from the Coalstoun Lakes region of Queensland, Australia, was grown on Czapak yeast agar (CYA) and corn meal agar (CMA, MP Biomedicals, LLC, Germany) media in 90 mm diameter covered plastic Petri plates. The strain was applied with a pin head in the centre of each Petri plate. There were seven temperature treatments (10, 15, 20, 25, 30, 35, and 40 °C) maintained in separate incubators. There were four replications for each treatment and all replications for each temperature treatment were kept within the same incubator. A Tinytag data logger (Hastings Data Loggers) was kept in each incubator to monitor temperature. The experiment was terminated after five days (120 h). The colony diameter was measured without opening the lids of Petri plates. Immediately afterwards, the Petri plates were kept in the freezer maintained at <−20 °C prior to determination of aflatoxin.

In experiment 2, two strains of *A. flavus*, #5307, and #5316 also from the soil of the Coalstoun Lakes region of Queensland, Australia, were cultured on CYA medium in 90 mm diameter plastic Petri plates as in experiment 1. In addition, these Petri plates were kept in 2 l polyethylene boxes. The atmosphere inside the boxes was saturated by water vapour by adding 100 ml deionized water to the base of each box. This saturation was considered necessary due to a sudden drop in aflatoxin production observed at >30 °C in experiment 1, which was suspected to have occurred due to lower relative humidity achieved in the Petri dishes at higher temperatures. The level of Petri plates was raised sufficiently to prevent this water entering in them. There were seven temperature treatments (10, 15, 20, 25, 30, 35, and 40 °C) maintained in separate incubators. There were four replications for each treatment. Experiment 2 was conducted as per experiment 1 from this point onwards. For both experiments 1 and 2, CYA medium was prepared by dissolving 1 g K_2HPO_4, 30 g sucrose, 5 g yeast extract, 15 g agar, 10 ml of Czapek concentrate, 1 ml Smith's trace metal solution in 1 l distilled water. The Czapek concentrate consisted of 25 g KCl, 150 g $NaNO_3$, 25 g $MgSO_4 \cdot 7H_2O$, and 0.5 g $FeSO_4 \cdot 7H_2O$ dissolved in 500 ml distilled water. The trace metal solution consisted of 0.5% $CuSO_4$, and 1% Zn SO_4 in distilled water.

In experiment 3, *A. flavus* strain #5307 was grown on intact grains of Pioneer hybrid 34N43 only to measure aflatoxin production as colony growth was difficult to measure on grains. For this experiment 40 g of maize kernels autoclaved for 15 min at 121 °C was placed in 90 mm sterile plastic Petri plates. Eight millilitres of deionized water containing 10^6 spores/ml was then poured over the kernels. This spore suspension by itself did not contain any detectable amount of aflatoxin. This spore suspension in addition to serving as a source of inoculum increased the grain moisture content from 9% to 33%, which is the moisture maize kernels normally have close to physiological maturity. The Petri plates were covered with their lids and then placed in 2 l covered plastic boxes containing 100 ml deionized water at the base to saturate the box atmosphere as described above. These boxes were placed in incubators maintained at seven different temperatures (10, 15, 20, 25, 30, 35, and 40 °C). There were a few extra Petri-plates for the 25 and 30 °C treatments to periodically determine when to terminate the experiment, which occurred at 72 h after the incubation commenced.

The aflatoxin content in all three experiments was determined using the Vicam procedure (Vicam Science and Technology, 1999). Samples were processed in 80% methanol and measurements made on a VICAM Fluorometer, Series 4 (BBI Source Scientific, USA) according to manufacturer's instructions.

2.3. Evaluation of the model

To generate aflatoxin contamination data for model evaluation, five field trials were conducted at four Kenya Agriculture and Livestock Research Organisation (KALRO) field station locations in Kenya including two sowings at Kiboko in 2011 and 2012, and one trial each at Katumani, Perkerra and Mtwapa in 2012 (Table 1). The trial sites had either maize or other crops prior to planting. These trials were planted at 75 cm row-to-row and 25 cm plant-to-plant distances resulting in a population of 5.3 plants/m^2. The maize crop received 60 kg nitrogen/ha the timing of which is shown in Table 2. The trials were irrigated at different times (Table 2), except at Mtwapa where the crop was raised entirely as rainfed. While a number of hybrids were included in the trials in both seasons we selected only tropical Hybrids 513 (H513) and 614 D (H614) for which we had similar hybrids (H511 and H614) parameterized in APSIM (Keating et al., 2003). H513 flowered and matured up to 10 days earlier compared to H614. These hybrids were grown in three replicated rows (biological replicates) at Kiboko in 2011 and seven replicated rows in all 2012 trials. Ten ears per replicate were artificially inoculated. This inoculation was done to make sure that *A. flavus* was present as screening different genotypes for susceptibility to the fungus was also one of the objectives of the trials. Inoculation was done to ensure that all genotypes had an equal chance of being infected with the same inoculum strain. Each entry was inoculated by injecting 1 ml of 10^8 conidia/ml into the silk channel when the entry had reached 50% silking, using a local toxigenic *A. flavus* strain. At harvest these inoculated cobs within a replication were bulked to constitute a sample.

The manager module of the user interface of the APSIM model was configured to incorporate equations described in Section 2.1. Subsequently, the model's capability to simulate seasonal (at Kiboko) and locational variation (all locations) in pre-harvest aflatoxin contamination in different environments was evaluated.

The manager module of the user interface was also configured to incorporate agronomic details of the trials and to include two commercial hybrids H511 (as a proxy of H513, which has a similar phenological development pattern and is recommended for cultivation in similar environments), and H614 in separate simulations.

Table 1
Details of field trials conducted in Kenya.

Location (village)	District	Latitude	Longitude	Altitude (m)	Dates of		
					Planting	Inoculation[a]	Harvest
Kiboko	Kibwezi	2°1′S	37°4′E	892	22/08/11	4/11/11 10/11/11	12/1/12
Kiboko	Kibwezi	2°1′S	37°4′E	892	26/06/12	14/9/12 27/9/12	08/11/12
Katumani	Machakos	1°3′S	37°1′E	1100	30/05/12	15/9/12 24/9/2012	29/10/12
Mtwapa	Kilifi	3°6′S	39°4′E	15	26/05/12	27/7/12 4/8/12	05/10/12
Perkerra (Marigat)	Baringo	0°3′N	36°1′E	1067	23/06/12	5/9/12 17/9/12	24/10/12

[a] The first inoculation date is for H513 and the second for H614.

Table 2
Soil depth, total plant available water-holding capacity (PAWC) when full profile, nitrogen and irrigation amounts and days after sowing (DAS) of their application.

Location	Soil depth (cm)	PAWC (mm)	Nitrogen (kg/ha)(DAS)	Irrigation (mm)(DAS)
Kiboko 2011	150	147	30(0), 30(49)	40 (0)
Kiboko 2012	150	147	30(0), 30(35)	12(43), 35(49).35(55), 28(60), 20(65), 19(73), 32(81)
Katumani	170	218	60(0)	25(0), 25(7), 25(14), 25(21),25(26)
Mtwapa	100	43	30(0), 30(42)	Nil
Perkerra	120	174	60(0)	40(0), 40(60)

The input of irrigation and their timings as shown in Table 2 were incorporated into the module to simulate aflatoxin risk index.

Daily climatic data of these trials were collected using Decagon (USA) automatic weather stations installed at each trial site. Soil water holding capacity of the experimental sites described in different publications was used for modelling purpose (Table 2). The soil at Kiboko holds about 147 mm plant available water to a 1.5 m depth and Katumani 218 mm to a 1.7 m depth (Keating et al., 1991; Probert et al., 2001). The soil at Perkerra holds about 170 mm plant available water to 1.2 m depth (Mwangi, 1983; Kipkorir et al., 2002), and at Mtwapa 43 mm to 1.0 m depth (Nandwa and Chege, 1996). Changes in the U' parameter of the soils of two sites including Kiboko and Mtwapa, which controls the second stage evaporation, were made to ensure that the simulated maturity of the later maturing hybrid H614 coincided with its harvest date. In Kiboko soil the 'U' parameter of evaporation was reduced from 9 originally present to 4 and for Mtwapa this parameter was increased from 4 originally present to 5.5. The same soil parameters were used for both hybrids, and for two seasons at Kiboko. The starting available soil water at all locations was considered to be 100% at the time of sowing at each location as sowings were taken up after a significant rainfall event or irrigated soon after sowing to initiate germination.

2.4. Relative susceptibility of maize types to aflatoxin contamination

The maize aflatoxin model was developed to assess aflatoxin risk rather than absolute level of contamination in diverse environments. Nevertheless to be of practical value, the risk predicted by the model was expected to have some relationship with observed levels of contamination. The slope and intercept of this relationship should ideally be similar irrespective of environments or seasons. However, if there were genetic differences in susceptibility of maize this may not happen. Also the strains' ability to produce aflatoxin could differ (Niles et al., 1985; Probst et al., 2007; Probst and Cotty, 2012). In the laboratory experiment 4 we therefore examined if the method we used to obtain the temperature response of *A. flavus* could also detect interaction between maize and strain types to assist interpretation of results.

In this experiment we compared a yellow maize hybrid to represent the yellow maize, which is commonly grown in Australia, and a white maize hybrid to represent the white maize, which is grown in Kenya. Seeds of Australian yellow maize hybrid 34N43, and white maize hybrid KHCIM12 with parents derived from Kenya were collected from the 2012–2013 harvest at Kingaroy, Queensland Australia. The kernel moisture content of both hybrids was 13.8% at harvest.

Twenty four gram sub-samples of seed (20 g on the dry weight basis) were placed in 90 mm diameter glass Petri plates and autoclaved at 121 °C for 15 min. After cooling the Petri plates to room temperature, 3.3 ml spore suspension of strains #5307 and #5316 containing approximately 10^6 spores/ml was added to each plate using a self-measuring pipette. There was also an un-inoculated control in which 3.3 ml deionized water was added. The experiment had four replicated Petri plates for each treatment. These were kept in 2 l plastic boxes filled with 100 ml de-ionized water as described above, with one of the boxes also having a Tinytag logger to record temperature and humidity fluctuations inside the box. These covered boxes were placed in an incubator maintained at 32.5 °C. The experiment was terminated after 72 h by removing the samples from the incubator and placing them in a deep freezer having <20 °C temperature. Aflatoxin content in the grains was determined using the Vicam procedure for maize in 0 to 300 ppb range (Vicam Science and Technology, 1999).

2.5. Statistical analysis

Data of laboratory experiments were statistically analysed using the GENSTAT program (VSN International, 2011). In the laboratory experiments, zero values of aflatoxin were replaced by 1 as

the minimum detection limit. All aflatoxin values in the laboratory experiments were then transformed as their natural log +1 as the range of differences amongst treatments was too large. APSIM simulated ARI for field trials were regressed against the geometric mean values of aflatoxin contamination of each hybrid grown at each site. Thus, there were 10 observations. The performance of the model was evaluated by comparing R^2 and the significance of the relationship of the level of contamination with the independent variable that is ARI.

3. Results

3.1. Cardinal temperatures for aflatoxin production

The coefficient of variation for colony growth and aflatoxin production in experiment 1 and 2 was less than 6% and for aflatoxin production and in experiment 3 with intact grains was 15.9%.

In experiment 1, the temperature, culture medium and temperatures × culture medium interaction effects were highly significant for the colony growth rate (Fig. 1a). Colony growth in the mid temperature range was significantly less on CMA medium as compared with CYA medium. However, the response patterns on both media, which could be described by 3rd order polynomial equations, were nearly identical with similar minimum (base), optimum and maximum (ceiling) temperatures.

In experiment 2 (Fig. 1b), which included strains #5307 and #5316 grown only on CYA medium, the cardinal temperatures for growth were similar to those observed in experiment. The strain × temperature interaction for the colony growth rate was highly significant with the growth rate of strain #5307 being greater compared to that of strain #5316 between 25 and 35 °C. The response of both strains to temperature could be described by 3rd order polynomial equations.

By establishing separate relationships between the rising and decreasing colony growth rate regions given in Fig. 1, the base temperature (T_{min_aflo}) was found to be 11.5 °C, optimum (T_{opt_aflo}) 32.5 °C and maximum temperature (T_{max_aflo}) 42.5 °C.

The optimum temperature for aflatoxin production on CYA medium in both experiments 1 and 2 was similar (Fig. 2), but less compared to that for colony growth in them (Fig. 1). In experiment 1, no aflatoxin could be detected on CMA medium. In experiment 2 the strain × temperature interaction was highly significant ($p \leq 0.01$) for aflatoxin production. In this experiment the overall amount of aflatoxin produced by strain #5316 at 18 to 30 °C temperature was significantly more ($p \leq 0.01$) compared to by strain #5307 (Fig. 2a).

Although growth rates of the two strains were not significantly different, there were visible differences in colony appearance in Petri plates at different temperatures with intensity of dark colour #5316 being appreciably more than that of strain #5307 (Fig. 3). This may be indicative of greater conidia production by #5316 which was consistent with its higher aflatoxin production potential. The gradation of darker colour of the two strains, however, did not exactly match with the aflatoxin content observed at different temperatures as there was very little aflatoxin produced at 35 °C by strain #5316 although that treatment had the highest intensity of dark colour.

In experiment 3 with whole maize grains the effect of temperature on aflatoxin production was highly significant ($p \leq 0.01$). The optimum temperature for aflatoxin production on maize grains was different compared to that found on CYA medium in both

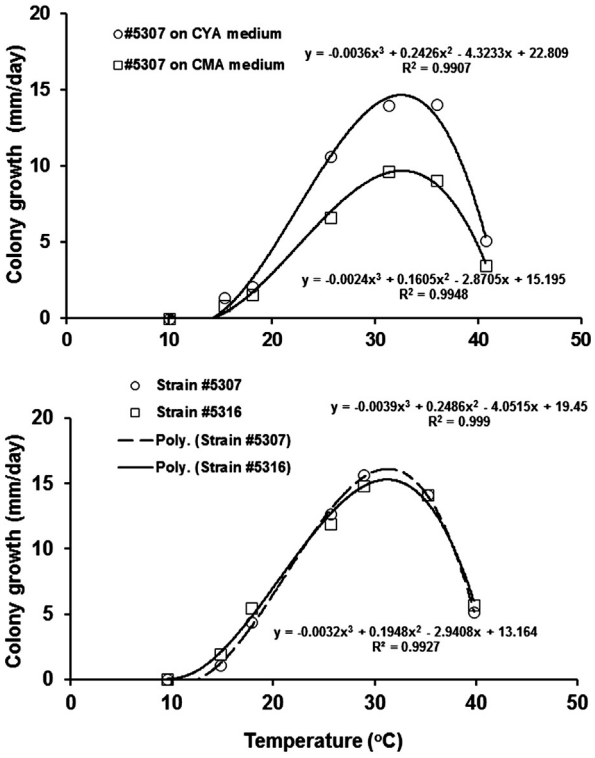

Fig. 1. Growth response of strain #5307 on CYA and CMA media (a) and of strains #5307 and #5316 *A. flavus* on CYA media at different incubation temperatures (b). The standard error of means for comparing temperature × culture medium effects for chart a was 0.11 mm/day and strain × temperature interaction for chart b was 0.09 mm/day.

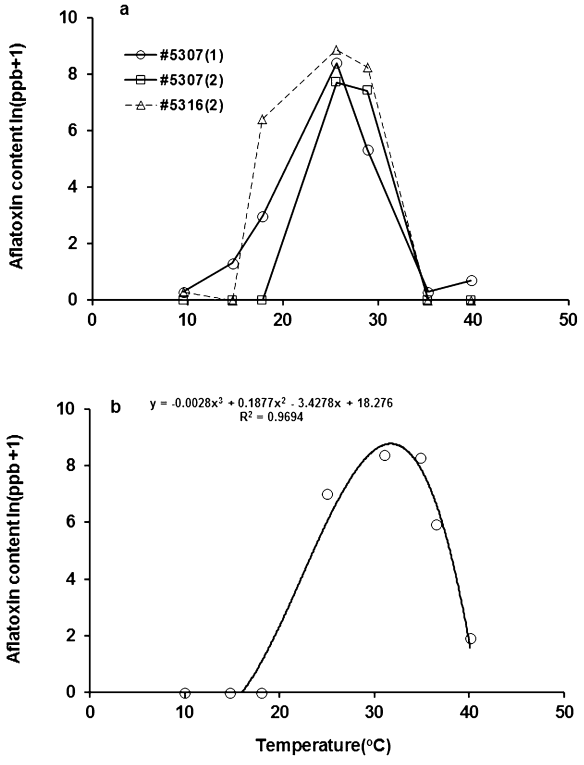

Fig. 2. Aflatoxin content on a) CYA medium in experiment 1 with single strain #5307, and in experiment 2 with two strains (#5307 and #5316), and b) in whole maize grains with strain #5307 of *A. flavus*. The temperature and strain × temperature interaction effects in experiments 1 and 2 and the temperature effect in experiment 2 were significant ($p \leq 0.01$). The SE value for comparing strain × temperature interaction in chart a was 0.17, and for temperature effect in chart b, 0.39.

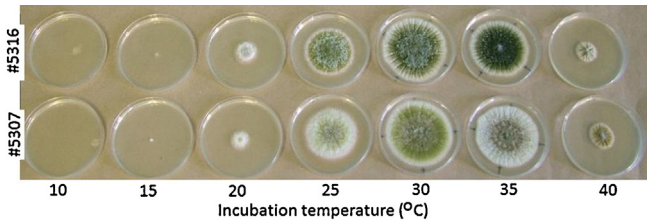

Fig. 3. A snapshot of the effect of temperature on the growth of *A. flavus* strains #5316 and #5307 on CYA medium in experiment 2. The strain × temperature interaction was highly significant for both colony growth and aflatoxin content.

experiments 1 and 2, but was similar to that observed for colony growth in both CYA and CMA media in these two experiments. The response pattern of aflatoxin production in intact grains could also be described by a 3rd order polynomial equation (Fig. 2b). The relationship between colony growth in CMA medium and aflatoxin production in intact grains between the 20 and 32 °C temperature range was highly significant ($R^2 = 0.996$, $p \leq 0.01$) for strain #5307. The cardinal temperatures derived for colony growth for culture media were therefore considered appropriate for aflatoxin production as well.

3.2. Aflatoxin risk index with pre-harvest aflatoxin contamination in the field

In the multi-location trials, where weather and soil moisture holding capacity varied greatly, the average aflatoxin content of the two hybrids ranged and <1 and 7143 ppb. In the two trials conducted at Kiboko aflatoxin content varied from <1 ppb in 2011 to 664 ppb in 2012 for early hybrid H513, and <1 ppb in 2011 to 1738 ppb in 2012 for medium hybrid H614. In trials conducted in 2012, the average aflatoxin content at Katumani was 53 ppb for H513 and 16 ppb for H614. At Mtwapa the average aflatoxin content was 956 ppb for H513 and 7143 ppb for H614, and at Perkerra 1559 ppb for H513 and 1682 ppb for H614. ARI computed with the new set of cardinal temperatures identified in this study and drought threshold varied between 0 and 100 (0 to 10 without the multiplier '10'). The linear relationship between ARI, which was simulated using the new set of cardinal temperatures and SDR as a new threshold, with aflatoxin contamination was highly significant ($p < 0.001$) with R^2 being 0.99 (Fig. 4). The root mean square error was 4.7% of the mean observed aflatoxin contamination.

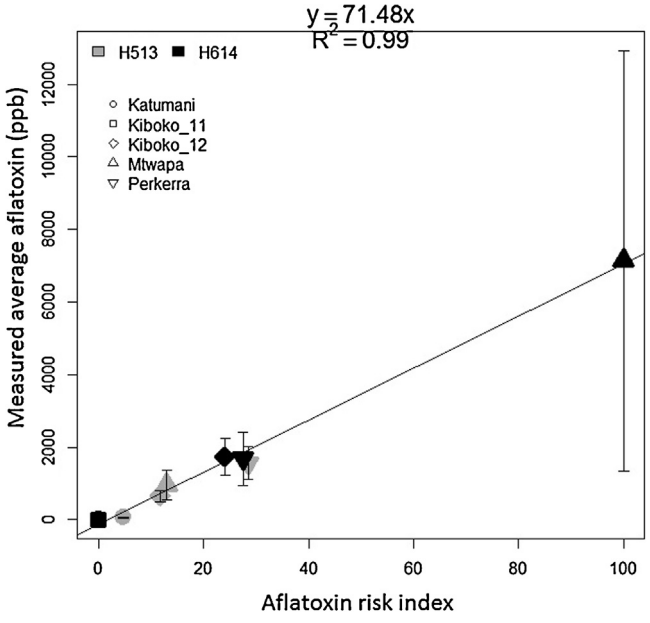

Fig. 4. Relationship between observed aflatoxin content (ppb) of early Hybred 513 (H513) and medium Hybred 614D (H614) and aflatoxin risk index simulated by the maize aflatoxin model in five trials conducted in different environments of Kenya. R^2 of the relationship was significant ($p < 0.001$). Vertical bars are standard errors of mean of observed aflatoxin contamination.

3.3. Differences in aflatoxin production potential between two maize types and two A. flavus strains

In experiment 4 both strains #5307 and #5316 were found to be colonizing the white maize more vigorously compared to the yellow maize (Fig. 5a). However the amount of aflatoxin produced by the two strains on each maize type varied significantly ($p \leq 0.01$). Strain #5316 produced 5.2 times more aflatoxin than strain #5307 (Fig. 5b). Aflatoxin production was 2.6 times more on the white maize than on the yellow maize under similar conditions. The differences between the natural log transformed means for both strain and the maize type were highly ($p \leq 0.01$) significant. The interaction between the strain and maize types was, however, not significant.

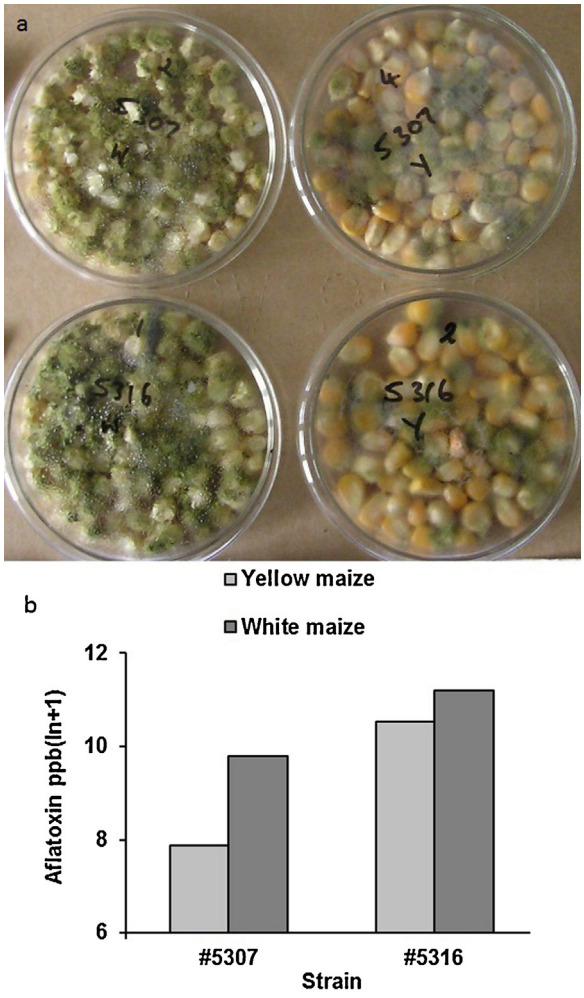

Fig. 5. A snapshot of white and yellow grains infected with #5307 and #5316 strains of *A. flavus* taken at 3 days after incubation at 32.5 °C in the laboratory (a) and the effect on aflatoxin production in the two maize types (b). The standard error of means for comparing strain and maize type is 0.215 ($p \leq 0.01$).

4. Discussion

4.1. Integrating drivers of aflatoxin contamination into the maize aflatoxin model

As aflatoxin contamination by *A. flavus* is significantly driven by climate it has led many researchers to explore the possibility of modelling contamination risk. Numerous laboratory models, which showed A. *flavus* growth being related to variation in temperature and humidity, have, however, have not translated well into the field where aflatoxin contamination occurs when conditions are hot as well as dry. In storage conditions these hot and dry conditions do not encourage aflatoxin production as they lower grain moisture content; nonetheless, storage remains a significant challenge for aflatoxin contamination in developing countries as well. Trenk and Hartman (1970) reported 18% moisture content to be a limit below which aflatoxin production will not occur. A prototype model of maize aflatoxin contamination (Chauhan et al., 2008) based on a peanut aflatoxin model (Chauhan et al., 2010) was developed in Australia that does not need water activity parameter as a driver for aflatoxin production. The model proposed by them for both peanut and maize used the onset of moisture stress (drought) represented by fractional available soil water being $\leq$0.20 during the reproductive stage to simulate susceptibility to *A. flavus* invasion and a set of equations based on cardinal temperatures to simulate aflatoxin risk. The risk computed by the model was found to be related to level of aflatoxin contamination for a limited number of locations. These cardinal temperatures were derived using peanut as a substrate and therefore needed to be verified for maize to increase accuracy of the model.

The results of experiments 1, 2 and 3 of this study showed that for both fungal growth and aflatoxin production in grains the base temperature was 11.5 °C, the optimum 32.5 °C, and the maximum 42.5 °C. These cardinal temperatures were very similar (within 0.5 °C) to those reported previously for *A. flavus* by Ayerst (1969), but slightly different than used in the peanut model by Chauhan et al. (2010) and Battilani et al. (2013). Unlike our study, Battilani et al. (2013) used two separate sets of cardinal temperatures for *A. flavus* growth and aflatoxin production as observed in culture medium studies. We also found that in culture medium the optimum temperatures for *A. flavus* growth and aflatoxin production were different, but the optimum temperature for aflatoxin production in intact grains was similar to that for growth in culture medium.

In laboratory experiments 2 and 3, we studied temperature responses at the highest level of relative humidity of 100% representing water activity of 1. Many studies in culture medium as well as with intact grains have found significant interactions between water activity and temperature (Samapundo et al., 2007; Mousa et al., 2011; Garcia et al., 2013). These interactions, which have been an essential component of *in vitro* modelling, have been known for a long time and have been modelled well (Ayerst, 1969; Niles et al., 1985; Abdel-Hadi et al., 2012). The inclusion of water activity as a parameter adds another level of complexity for field modelling of aflatoxin risk. Many models including APSIM do not have an input of relative humidity. The inability to reconcile with the requirement of the water activity parameter in field conditions could be one of the reasons why it has taken so long to produce a reliable and practical prediction model for pre-harvest aflatoxin contamination. While the inclusion of water activity may be important for culture medium studies and stored grains, we consider that it may not be as important for field grown maize where kernel moisture content before physiological maturity is almost always sufficiently high compared to the threshold of 24% for aflatoxin production (Payne et al., 1998; Ma and Dwyer, 2001).

Another complexity needed to be resolved for modelling aflatoxin risk was the use of appropriate indices to account for the occurrences of drought that trigger *A. flvaus* invasion and aflatoxin production. In our model fungal invasion of developing seeds was driven by a low SDR. Battilani et al. (2013) considered fungal invasion to occur when relative humidity was less than 80% and there was no rain on a particular day and subsequent colonization and aflatoxin production was dependent on temperature. Such days will invariably occur in arid and semi-arid environments where maize is grown and allow the crop to be infected although this infection may not necessary contaminate the crop with aflatoxin. Holtmeyer and Wallin (1981) found a reasonable number of *A. flavus* spores in the air in all the seasons and locations they compared. Bilgrami and Choudhary (1993) also could isolate toxigenic strains of *A. flavus* from maize on most occasions whether or not the maize sampled had aflatoxin. There was no apparent limitation or lag for their germination at temperatures maize is normally grown (Marín et al., 1998). The few differences between ours and the model proposed by Battilani et al. (2013) highlighted above were significant, but how these two models would compare in predicting aflatoxin risk in different environments was not known. This comparison was not attempted in the present study. It would be interesting to compare these on independent datasets to identify possible strengths and weaknesses of each of these two modelling approaches in predicting aflatoxin risk.

4.2. Evaluation and the potential applications of the maize aflatoxin model

With the use of the new set of cardinal temperatures and SDR as trigger point to simulate susceptibility of maize to *A. flavus* invasion and aflatoxin production, ARI simulated by the model was linearly related with the level of contamination. The model captured differences in aflatoxin contamination arising due to seasonal and locational weather conditions as well as those arising due to differences in the maturity of the hybrids. The major effect of seasonal weather, which was independent of soil, was captured well at Kiboko where differences due to season and hybrids varied from <1 ppb in 2011 to 1738 ppb in 2012. The aflatoxin risk index values for this location ranged from 0 to 24. Even though the hybrids differed in the extent of contamination with medium hybrid generally having greater contamination, a single relationship between ARI and aflatoxin contamination could describe the response of both the hybrids. Both hybrids are white seeded type, and could have similar sensitivity to *A. flvaus* if they experience similar growing conditions. Sometimes colonization of grains with *A. flavus* could be induced by insect damage. This effect of insects on aflatoxin production, though important, was not directly considered in our model. Once damaged, the temperature relations for aflatoxin production should still remain relevant.

For predicting risk in other varieties, they will need to be parameterized for APSIM before simulating the risk. They may also differ in relation to susceptibility to aflatoxin production as shown with the yellow and white maizes in this study. Hawkins et al. (2008) reported considerable differences in the level of aflatoxin production by three hybrids in the same environment over five consecutive seasons. When our model was applied to predict aflatoxin risk at their experimental site the model predicted ARI related well with the mean contamination levels of the three hybrids (R^2 = 0.90) (data not shown). However, the R^2 of the relationship of contamination observed in the individual hybrids and ARI ranged from 0.74 to 0.94. The best relationship (R^2 = 0.94) was obtained with the most susceptible hybrid.

Although ARI is not a probability parameter in a strict sense, its value could still indicate the likelihood of contamination and with its increased value the chances of detecting contamination

will progressively increase. This makes the model immensely suitable for individual farmers – or to extension and other related stakeholders – to monitor their crops and make informed decisions about various corrective steps to be undertaken to minimize aflatoxin contamination. The corrective steps could include improving weed management, application of irrigation if available to reduce drought intensity, and early harvest followed by rapid artificial drying (Hell and Mutegi, 2011) compared to the usual practice of leaving the crop in the field for dry down (Kaaya et al., 2006). The model could be turned into a decision support tool similar to Afloman being used by farmers in Australia (Chauhan et al., 2010). But the requirement of computers with internet connectivity could be to be a limiting factor in developing countries. The smart phone revolution, however, could effectively overcome this limitation. The widespread use of mobile phones is already beginning to revolutionize the livestock sector in Kenya (www.fao.org/news/story/en/item/170807/icode/). The maize industry may be particularly interested in learning how the composite value of ARI for an area relates to the proportion of grains above the legal limit that may enter the market in a given region. Such assessment in the developed countries (Battilani et al., 2013), where large volumes of the crop are received at the intake points, could provide useful guiding parameters that may also be applicable to developing countries.

5. Conclusions

This study was aimed at improving the prototype maize aflatoxin model integrated within the APSIM model (Chauhan et al., 2008), and verifying the new model's ability to simulate the risk of aflatoxin contamination in maize crop grown in diverse environments. The cardinal temperatures for the fungal growth and aflatoxin production used in the revised model were determined afresh which matched with a few published studies, but differed from some others. The other change that was made was to use of the water supply demand ratio as this parameter accounts for drought occurring due to limited moisture availability in the soil and water demand determined by the rate of crop growth and vapour pressure deficit. This change seems to have worked well.

We evaluated the ability of this model in using datasets generated multi-location trials in Kenya. The linear relationship of the ARI simulated by the model with the observed mean aflatoxin contamination which varied from <1 to 7143 ppb in five diverse environments was significant and explained a high degree of variation in the mean level of contamination. The model awaits further application as a research and decision support tool to minimize aflatoxin contamination in this important staple food crop of many developing countries.

Acknowledgements

Funding for this work was provided by the Australian Agency for International Development (AusAID) through CSIRO (Agreement no 2011125699) for the project 'Climatic risk of aflatoxin contamination in maize in eastern Africa (CRACMEA)', as part of the BecA-CSIRO partnership. Authors are grateful to Dr Brian Keating, Bruce Pengelly, and Peter Carberry of CSIRO for having supported this work under the African Food Security Initiative. They are also thankful for the assistance of Drs Nai Tran-Dinh, Mary Fletcher, James Gethi; the KALRO team implementing the field trials; and others involved in the sister project 'Capacity and action for aflatoxin reduction in eastern Africa'.

References

Abdel-Hadi, A., Schmidt-Heydt, M., Parra, R., Geisen, R., Magan, N., 2012. A systems approach to model the relationship between aflatoxin gene cluster expression, environmental factors, growth and toxin production by *Aspergillus flavus*. J. R. Soc. Interface 9, 757–767.

Amaike, S., Keller, N.P., 2011. *Aspergillus flavus*. Annu. Rev. Phytopathol. 49, 107–133.

Astoreca, A., Vaamonde, G., Dalcero, A., Ramos, A.J., Marín, S., 2012. Modelling the effect of temperature and water activity of *Aspergillus flavus* isolates from corn. Int. J. Food Microbiol. 156, 60–67.

Ayerst, G., 1969. The effects of moisture and temperature on growth and spore germination in some fungi. J. Stored Prod. Res. 5, 127–141.

Bandyopadhyay, R., Kumar, M., Leslie, J.F., 2007. Relative severity of aflatoxin contamination of cereal crops in West Africa. Food Addit. Contam. 24, 1109–1114.

Battilani, P., Barbano, C., Piva, G., 2008. Aflatoxin B1 contamination in maize related to the aridity index in North Italy. World Mycotoxin J. 1, 449–456.

Battilani, P., Camardo, L.M., Rossi, V., Giorni, P., 2013. AFLA-maize, a mechanistic model for *Aspergillus flavus* infection and aflatoxin B_1 contamination in maize. Comput. Electron. Agric. 94, 38–46.

Bilgrami, K.S., Choudhary, A.K., 1993. Impact of habitats on toxigenic potential of *Aspergillus flavus*. J. Stored Prod. Res. 29, 351–355.

Chauhan, Y.S., Solomon, K.F., Rodriguez, D., 2013. Characterization of north-eastern Australian environments using APSIM for increasing rainfed maize production. Field Crops Res. 144, 245–255.

Chauhan, Y.S., Wright, G.C., Rachaputi, R.C.N., Holzworth, D., Broome, A., Krosch, S., Robertson, M., 2010. Application of a model to assess aflatoxin risk in peanuts. J. Agric. Sci. 148, 141–151.

Chauhan, Y.S., Wright, G.C., Rachaputi, N.C., 2008. Modelling climatic risk of aflatoxin contamination in maize. Aust. J. Exp. Agric. 48, 358–366.

Chenu, K., Deihimfard, R., Chapman, S.C., 2013. Large scale characterization of drought pattern: a continent wide modelling approach applied to the Australian wheatbelt–spatial and temporal trends. New Phytol. 198, 801–820.

Cotty, P.J., Probst, C., Jaime-Gracia, R., 2008. Etiology and management of aflatoxin contamination. In: Leslie, J.F., Bandhyopadhyay, R., Visconti, A. (Eds.), Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade. CAB International, Oxfordshire, U.K., pp. 287–299.

Cotty, P.J., Jaime-Garcia, R., 2007. Influences of climate on aflatoxin producing fungi and aflatoxin production. Int. J. Food Microbiol. 119, 109–115.

Diener, U.L., Davis, N.D., 1977. Aflatoxin formation in peanuts by *Aspergillus flavus*. In: Agricultural Experiment Station Bulletin 493. Abrun University, Alabama, pp. 49.

Garcia, D., Ramos, A.J., Sanchis, V., Marín, S., 2013. Modeling kinetics of aflatoxin production by *Aspergillus flavus* in maize-based medium and maize grain. Int. J Food Microbiol. 162, 182–189.

Garcia, D., Ramos, A.J., Sanchis, V., Marín, S., 2009. Predicting mycotoxins in foods: a review. Food Microbiol. 26, 757–769.

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

Harrison, M.T., Tardieu, F., Dong, Z., Messina, C.D., Hammer, G.L., 2014. Characterizing drought stress and trait influence on maize yield under current and future conditions. Global Change Biol. 20, 867–878.

Hawkins, L.K., Windham, G.L., Williams, W.P., 2008. Occurrence of aflatoxin in three maize (*Zea mays* L.) hybrids over 5 years in Northern Mississippi. Mycopathologia 165, 165–171.

Hell, K., Mutegi, C., 2011. Aflatoxin control and prevention strategies in key crops of Sub-Saharan Africa. Afr. J. Microbiol. Res. 5, 459–466.

Hell, K., Fandohan, P., Bandyopadhyay, R., 2008. Pre- and postharvest management of aflatoxin in maize: an African Perspective. In: Leslie, J.F., Bandyopadhyay, R., Visconti, A. (Eds.), Mycotoxins: Detection Methods, Management, Public Health, and Agricultural Trade. CAB International, Oxfordshire, U.K., pp. 219–228.

Henry, S.H., Bosch, F.X., Troxell, T.C., Bolger, P.M., 1999. Policy forum: public health. Reducing liver cancer—global control of aflatoxin. Science 286, 2453–2454.

Holtmeyer, M.G., Wallin, J.R., 1981. Incidence and distribution of airborne spores of *Aspergillus flavus* in Missouri. Plant. Dis. 65, 58–60.

Jones, R.K., Duncan, H.E., Hamilton, P.B., 1981. Planting date, harvest date, and irrigation effects on infection and aflatoxin production by *Aspergillus flavus* in field corn. Phytopathology 71, 810–816.

Kaaya, A.N., Kyamuhangire, W., Kyamanywa, S., 2006. Factors affecting aflatoxin contamination of harvested maize in the three agroecological zones of Uganda. J. Appl. Sci. 6, 2401–2407.

Kang'ethe, E., 2011. Situation analysis: improving food safety in the maize value chain in Kenya. In: A Report Prepared for FAO., pp. 89, Download from ⟨www.fao.org/. . ./WORKING_PAPER_AFLATOXIN_REPORTDJ10thOctober.pdf⟩.

Keating, B.A., Carberry, P.S., Hammer, G.L., Probert, M.E., Robertson, M.J., Holzworth, D., Smith, C.J., 2003. An overview of APSIM, a model designed for farming systems simulation. Eur. J. Agron. 18, 267–288.

Keating, B.A., Godwin, D.C., Watiki, J.M., 1991. Optimising nitrogen inputs in response to climatic risk. In: Climatic Risk in Crop Production-Models and Management for the Semi-arid Tropics and Subtropics. CAB International, Wallingford, CT.

Kensler, T.W., Roebuck, B.D., Wogan, G.N., Groopman, J.D., 2011. Aflatoxin: A 50-year odyssey of mechanistic and translational toxicology. Toxicol. Sci. 120, S28–S48.

Kipkorir, E.C., Raes, D., Massawe, B., 2002. Seasonal water production functions and yield response factors for maize and onion in Perkerra. Kenya. Agric. Water Manage. 56, 229–240.

Lobell, D.B., Hammer, G.L., McLean, G., Messina, C., Roberts, M.J., Schlenker, W., 2013. The critical role of extreme heat for maize production in the United States. Nat. Climate Change 35, 497–501.

Luo, M., Liu, J., Lee, R.D., Scully, B.T., Guo, B., 2010. Monitoring the expression of maize genes in developing kernels under drought stress using oligo-microarray. J. Int. Plant Biol. 52, 1059–1074.
Ma, B.L., Dwyer, L.M., 2001. Maize kernel moisture, carbon and nitrogen concentrations from silking to physiological maturity. Can. J. Plant Sci. 81, 225–232.
Manjula, K., Hell, K., Fandohan, P., Abbas, A., Bandyopadhyay, R., 2009. Aflatoxin and fumonisin contamination of cassava products and maize grains from markets in Tanzania and Republic of the Congo. Toxin Rev. 28, 63–69.
Marín, S., Sanchis, V., Sáenz Ramos, A.J., Vinas, I., Magan, N., 1998. Ecological determinants for germination and growth of some *Aspergillus* and *Penicillium* spp. from maize grain. J. Appl. Microbiol. 84, 25–36.
Molina, M., Giannuzzi, L., 2002. Modelling of aflatoxin production by *Aspergillus parasiticus* in a solid medium at different temperatures, pH and propionic acid concentrations. Food Res. Int. 35, 585–594.
Mousa, W., Ghazali, F.M., Jinap, S., Ghazali, H.M., Radu, S., 2011. Modelling the effect of water activity and temperature on growth rate and aflatoxin production by two isolates of *Aspergillus flavus* on paddy. J. Appl. Microbiol. 111, 1262–1274.
Mutiga, S.K., Were, V., Hoffmann, V., Harvey, J., Milgroom, M.G., Nelson, R.J., 2014. Extent and drivers of mycotoxin contamination: inferences from a survey of Kenyan maize mills. Phytopathology 104, 1211–1231.
Mwangi, S.K.N., 1983. Soils of the Perkerra Agricultural Research Station, Marigat (Baringo District). In: Detailed Soil Survey Report No D30. World Data Centre for Soils.
Nandwa, S.M., Chege, A.G., 1996. Fertilizer use by maize in response to rainfall regimes. In: Maize Productivity Gains through Research and Technology Dissemination: Proceedings of the Fifth Eastern, Southern Africa Regional Maize Conference, Arusha, Tanzania, pp. 197–199, vol. 7.
Niles, E.V., Norman, J.A., Pimbley, D., 1985. Growth and aflatoxin production of *Aspergillus flavus* in wheat and barley. Trans. Br. Mycol. Soc. 84, 259–266.
Payne, G.A., Cassel, D.K., Adkins, C.R., 1986. Reduction of aflatoxin contamination in corn by irrigation and tillage. Phytopathology 76, 679–684.
Payne, G.A., Hagler, W.M., Adkins, C.R., 1998. Aflatoxin accumulation in inoculated ears of field-grown maize. Plant Dis. 72, 422–424.
Payne, G.A., Widstrom, N.W., 1992. Aflatoxin in maize. Crit. Rev. Plant Sci. 10, 423–440.
Pitt, R.E., 1993. A descriptive model of mold growth and aflatoxin formation as affected by environmental conditions. J. Food Prot. 2, 139–146.
Probert, M.E., Keating, B.A., Larkens, A.G., Siambi, M.M., 2001. Regional Assessment of Strategies for Maize Production in Semi-arid Eastern Kenya. CSIRO Tropical Agriculture.
Probst, C., Cotty, P.J., 2012. Relationship between *in vivo* and *in vitro* aflatoxin production: reliable prediction of fungal ability to contaminate maize with aflatoxins. Fungal Biol. 116, 503–510.
Probst, C., Njapau, H., Cotty, P.J., 2007. Outbreak of an acute aflatoxicosis in Kenya in 2004: identification of the causal agent. Appl. Env. Microbiol 73, 2762–2764.
Samapundo, S., Devlieghere, F., Geeraerd, A.H., De Meulenaer, B., Van Impe, J.F., Debevere, J., 2007. Modelling of the individual and combined effects of water activity and temperature on the radial growth of *Aspergillus flavus* and *A. parasiticus* on corn. Food Microbiol. 24, 517–529.
Trenk, H.L., Hartman, P.A., 1970. Effects of moisture content and temperature on aflatoxin production in corn. Appl. Microbiol. 19, 781–784.
Vicam Science and Technology, 1999. AflaTest. Instruction Manual. Vicam Science and Technology, Watertown, MA.
VSN International, 2011. GenStat for Windows, 14th ed. VSN Int, Hemel Hempstead, UK, Available: ⟨http://www.vsni.co.uk/software/genstat⟩ [2013 Jul. 09].
Wagacha, J.M., Muthomi, J.W., 2008. Mycotoxin problem in Africa: current status, implications to food safety and health and possible management strategies. Int. J. Food Microbiol. 124, 1–12.
Widstrom, N.W., 1996. The aflatoxin problem with corn grain. Adv. Agron. 56, 219–280.
Wild, C.P., Gong, Y.Y., 2010. Mycotoxins and human disease: a largely ignored global health issue. Carcenogensis 31, 71–82.
Williams, J.H., Grubb, J.A., Davis, J.W., Wang, J.S., Jolly, P.E., Ankrah, N.A., et al., 2010. HIV and hepatocellular and esophageal carcinomas related to consumption of mycotoxin-prone foods in sub-Saharan Africa. Am. J. Clin. Nutr. 92, 154–160.
Williams, J.H., Phillips, T.D., Jolly, P.E., Stiles, J.K., Jolly, C.M., Aggarawal, D., 2004. Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences and interventions. Am. J. Clin. Nutr. 80, 1106–1122.