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Preharvest Management Strategies and Their Impact on Mycotoxigenic Fungi and Associated Mycotoxins

Lindy J. Rose, Sheila Okoth, Bradley C. Flett,
Belinda Janse van Rensburg and Altus Viljoen

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Abstract

Mycotoxigenic fungi that contaminate grain crops can lead to reduced grain quality, crop yield reduction and mycotoxicosis among humans and livestock. Preharvest management of fungi and mycotoxin contamination is considered among the most important mitigating strategies. Approaches include the breeding of resistant cultivars, use of microorganisms chemical control, production practises and the management of plant stressors. Resistant plants provide an effective and environmentally sound strategy to control mycotoxigenic fungi and mycotoxins; and have been documented. Their incorporation into commercial cultivars is, however, slow and complex. Therefore, emphasis should be placed on determining the resistance of cultivars and landraces currently used by producers. Chemical control has been successfully used for wheat; yet little to no research has been done on other important crops. Biological control strategies have focussed on *Aspergillus flavus* that produces aflatoxins and infects commercially important crops like maize and groundnuts. Commercial biological control products have been developed and field-tested in several African countries with promising results. The impacts of production practises are unclear under variable environmental conditions; but subsequent disease manifestation and mycotoxin contamination can be reduced. Each preharvest approaches contribute to managing mycotoxigenic fungi and their mycotoxins but integrating approaches may provide more effective management of fungal and mycotoxin contamination in crops.

Keywords: preharvest management, mycotoxins, tolerance, cereals, cultural practices

1. Introduction

The contamination of food and feed crops with mycotoxigenic fungi is a persistent problem contributing to food safety and security worldwide. The infection of crops by these fungal pathogens

affects crop yield and quality but of greater concern are the secondary metabolites they produce, collectively known as mycotoxins. Ingestion of mycotoxin-contaminated products has been associated with a wide range of noxious effects on humans and livestock. The major food and feed crops affected by mycotoxigenic fungi and mycotoxins include rice, maize, wheat, soybean, sorghum and groundnut, although several other crops are also affected. The association of these crops with mycotoxigenic fungi is ubiquitous, and crops are affected wherever they are produced. Three major groups of mycotoxigenic fungi are associated with mycotoxin contamination namely *Aspergillus*, *Fusarium* and *Penicillium*. They each produce a number of mycotoxins, but six mycotoxins have been studied extensively and are considered among the most important and they include the aflatoxins (AF), fumonisins (FUM), trichothecenes (TCT), zearalenone (ZEA), ochratoxin (OT) and patulin (PAT). Mycotoxin contamination levels in food and feed crops have therefore elicited numerous countries to institute regulations regarding the maximum permissible levels of these mycotoxins in unprocessed and processed products.

More than 100 countries have established mycotoxin regulations, including 15 African countries [1–3]. The European Union and United States Food and Drug Administration established maximum allowable levels for certain food contaminants, including mycotoxins, with the aim to reduce their presence in foodstuffs to the lowest levels reasonably achievable by means of good manufacturing or agricultural practices [4]. Most of the countries have mycotoxin regulations for at least AFB₁, produced predominantly by *Aspergillus* spp., to aid in minimising food safety concerns. Although fewer countries regulate *Fusarium* mycotoxins, a marked increase in the regulation of this mycotoxin has been observed recently. These regulations have globally significant implications for the importation and exportation of products. Regulatory infrastructure, however, does not enable inspection and enforcement [5], making the regulatory control of mycotoxins in Africa largely ineffective [6].

The management of mycotoxigenic fungi and their subsequent mycotoxins is therefore vital towards ensuring sustainable, safe food and feed production. Integrated management practices that reduce the incidence of mycotoxigenic fungi as well as the management of abiotic factors that contribute to mycotoxin contamination are required before and following harvest. However, preharvest management is considered the most important in limiting the overall contamination of crops. Therefore, the use of tolerant varieties is deemed the most proficient and environmentally sound approach to manage fungi and their toxins. In addition, several other management approaches such as optimal plant production, cultural practices, chemical control and the management of mycotoxigenic fungi by atoxigenic strains or bacteria could further reduce fungal incidence and subsequent mycotoxin contamination.

2. Management of mycotoxigenic fungi and their mycotoxins

Managing mycotoxigenic fungi and their mycotoxins in crop plants requires a proper understanding of the biology, epidemiology and genetics/genomics of the fungus and host plant. Major crops vary significantly in susceptibility to mycotoxigenic fungi and subsequent mycotoxin contamination. Maize is widely considered to be among the most susceptible of major crops to mycotoxins, while rice is considered among the least susceptible crop [7–9].

2.1. Tolerance to mycotoxigenic fungi

Crops with resistance to numerous mycotoxigenic fungi have been documented [10–12], but none of these are immune. Resistance to mycotoxigenic fungi therefore appears to be quantitative rather than qualitative. Breeding programmes at both public and private institutions are initiating and expanding their efforts to develop disease-resistant inbred and hybrid materials [13]. A number of international institutions such as the International Maize and Wheat Improvement Centre (CIMMYT) and the International Institute of Tropical Agriculture (IITA) in African countries including Kenya and Nigeria have established breeding programmes with the primary focus on producing inbred lines with improved resistance to *A. flavus* and AF. The development of tolerant cultivars, however, has been slow due to the polygenic, quantitative nature of resistance to mycotoxigenic fungi [14–17], the unavailability of immune germplasm [11, 15] and the effect of the environment on disease development and mycotoxin production [18–20]. The development of tolerant varieties, therefore, may be a long (8–10 years) and costly process that needs to be conducted as effectively as possible. Little to no commercial plant crop, completely resistant to mycotoxigenic fungi and mycotoxins, has been produced by conventional breeding, with the exception of wheat [21–23].

2.2. Conventional breeding strategies

Diallel analysis to determine the general combinability (GC) and specific combinability (SC) of resistant genotypes has been reported for *Aspergillus* and *Fusarium*, mostly performed on maize [24–27] and wheat [28–30]. The response of an inbred line to *F. verticillioides* and FUM, and the corresponding GC in hybrids, was significantly correlated. This indicates that an efficient way to improve resistance to *F. verticillioides* and FUM in maize hybrids, specifically, is to first evaluate and select resistant inbred lines that can be used to develop resistant hybrids [24]. This was also demonstrated for breeding resistance to Fusarium head blight (FHB) of wheat [30]. Maize hybrid performance for resistance to *F. graminearum* could, however, not be predicted based on the GC of inbred line parents [27]. Therefore, this relationship needs to be determined for each crop and fungal pathogen, respectively.

Inbred lines with resistance to aflatoxin contamination were evaluated for GCA and SCA for resistance to fumonisin accumulation, and two lines with resistance to FUM and AF were registered [25]. That research demonstrated the ability to breed resistance to multiple mycotoxigenic fungi and/or their mycotoxins. Furthermore, improved resistance to *F. verticillioides* and FUM in inbred lines derived from cross-pollination of resistant and elite maize lines has been demonstrated [31]. The subsequent hybrids produced from the crossing of improved lines with elite lines, however, did not demonstrate an improved activity against Fusarium ear rot (FER) and FUM accumulation, although some improved lines performed well as an inbred line and as a component of a hybrid [31]. To date, little to no research is reported on the development of tolerant varieties using recurrent selection breeding methods. Considering that resistance to mycotoxigenic fungi is polygenic and quantitative, recurrent selection presents a feasible breeding strategy; however, time and cost involved in this breeding strategy may be strong deterrent factors.

Quantitative trait loci (QTL) associated with resistance to mycotoxigenic fungi has been mapped in maize and wheat and can be used for marker-assisted selection [15, 16, 32–36]. Some QTLs, however, displayed pleiotropic effects, sometimes resulting in resistance to both traits [15, 32, 37]. QTL analyses have also demonstrated pleiotropic effects for resistance to other mycotoxigenic fungi and/or their associated mycotoxins. In QTL studies involving multiple ear rot pathogens, maize resistant to FER and FUM accumulation was also resistant to *F. graminearum* and/or *A. flavus*, with common loci for ear rots and FUM, respectively [15, 37, 38]. Research revealed that some of the genes involved in resistance to FER and *Aspergillus* ear rot (AER) of maize caused by *A. flavus*, as well as their associated mycotoxins (FUM and AF, respectively), were identical or genetically linked [38]. These studies highlighted common genes and/or resistance mechanisms to multiple mycotoxigenic fungi, demonstrating the potential for breeding resistance to one type of mycotoxigenic fungus, and its mycotoxin may lead to similar responses among other mycotoxigenic fungi and associated mycotoxin. The value of marker-assisted selection for improving *Fusarium* head blight resistance in wheat has been confirmed by numerous researchers and success stories from breeding programmes implementing MAS [39–47].

2.3. Unconventional breeding strategies

2.3.1. Genetic modification

Genetically modified crops are plants of which the DNA has been altered through the introduction of a foreign gene to express a trait not inherent to the modified plant. Three transgene-mediated strategies have been proposed for the management of mycotoxigenic fungi and mycotoxins in maize [48]. These include (1) the reduction of fungal infection, (2) the degradation of mycotoxins and (3) interfering with the mycotoxin biosynthetic pathway. To reduce infection by the fungus, the incorporation of antifungal and/or resistance genes, as well as the overexpression of defence-related genes, is required. Catabolic enzymes from microbes have been used to detoxify certain mycotoxins both *in vitro* and *in situ*, before they accumulate in the plant [49–51]. Fumonisin esterase and amine oxidase genes encoding FUM-degrading enzymes have been identified in *Exophiala spinifera* de Hoog and Hasse [48]. None of these genes have, however, been successfully introduced into maize. Maize plants have, however, been genetically engineered to interfere with the biosynthesis of AF and TCT [52, 53]. The best-known example of using genetically modified maize for reducing FER and FUM contamination of grain is Bt maize [54, 55]. This is due to the close association between kernel damage by insects and infection by *F. verticillioides* [56]. Bt maize plants that prevent insect damage, therefore, also reduce FUM contamination of maize grain. Genetically modified maize is not authorised in all countries and, consequently, conventional breeding efforts are still commonly used.

2.3.2. Mutation breeding

Exposure of seeds or other heritable materials to chemicals or radiation with the purpose to induce DNA changes (mutations) is known as mutation breeding. Nuclear technology for crop improvement makes use of ionising radiation, which causes induced mutations with a

high mutation frequency in plants [57]. These mutations might be beneficial and alter physiological characters of plants, including plant height, ear height and improved root architecture [58, 59]. The radiation of seeds may also cause genetic variability that enables breeders to select new genotypes with improved grain yield and quality [60]. Mutation breeding has been successfully used to generate genetic variation in cereal crops, including maize, for a number of aspects including enhanced yield and productivity, altered ear length, drought tolerance and enhanced stem structure [61–63]. It can thus potentially provide an attractive means for generating tolerance to mycotoxigenic fungi and their mycotoxins.

2.4. Host-plant resistance

The planting of disease-resistant plants is an effective, affordable and environmentally sound strategy to control ear rot diseases and mycotoxin accumulation [64]. Commercial hybrids differ in their ability to accumulate mycotoxins [64], while hybrids grown outside of their adapted range are more susceptible to mycotoxins than those grown within their adapted range [18]. Determining host-plant resistance to mycotoxigenic fungi and mycotoxin accumulation is a fundamental step towards developing commercially tolerant plant varieties. Several factors require careful consideration when screening materials for resistance to mycotoxigenic fungi and their mycotoxins. Inoculation technique significantly contributes to the efficacy of the screening protocol and should, therefore, be appropriate, produce consistent results and consider the disease cycle of the pathogen. Numerous studies relating to different crops report on the importance of screening for resistance under variable environmental conditions since genotype by environment interactions (GEI) plays such a vital role in disease development and mycotoxin contamination. Furthermore, GEI and stability indicators provide for the selection of material tolerant across a broad range of environments or alternatively exhibiting tolerance in specific environments.

Various countries have reported on the tolerance levels of maize and wheat cultivars to mycotoxigenic fungi and associated mycotoxins [65–67]. However, focus has been placed on the characterisation of inbred lines for the identification of appropriate breeding material towards resistance to mycotoxigenic fungi and their toxins [68–74]. Genetically modified maize, expressing *Bacillus thuringiensis* genes (BT maize), has been found to accumulate less FUM than its non-modified isolines [54].

2.5. Cultural preharvest management strategies

2.5.1. Planting recommendations

Adhering to planting dates and planting plants at lower or optimal densities reduces mycotoxin accumulation during production [75–77]. Plants should be planted at recommended row widths and densities to specifically reduce water stress [78] and ensure optimal nutrient availability. Maize ears should be harvested from the field as soon as possible because favourable conditions for ear rot and/or mycotoxin accumulation may occur if harvest is delayed, thus leading to elevated mycotoxin levels [79, 80].

2.5.2. Crop rotation

The primary objective of cultural control of mycotoxigenic fungi is to minimise factors that result in plant stress. Inoculum build-up on plant residues can be reduced by crop rotation practices, such as the rotation of maize with non-host crops [75, 81, 82]. Crop rotation with legumes, brassicas and potato could also significantly reduce *F. graminearum* contamination levels [83].

2.5.3. Tillage practises

Field preparation and cultivation practices play a central role in the management of *Fusarium* diseases and associated mycotoxins [84]. The burial of plant residues from a previous planting season by deep ploughing can reduce the primary inoculum that causes infections [85]. This is especially important when crops are affected by the same *Fusarium* species, such as *F. graminearum* on maize, wheat and sorghum grown in rotation [4]. While minimum tillage has significantly decreased stalk rot and increased grain yield of sorghum in South Africa [86], it has also increased inoculum build-up of mycotoxigenic fungi in maize cropping systems [84]. Alternate tillage practices, however, have had little effect on the incidence of FER in maize [87, 88].

2.5.4. Managing plant stressors

Limiting plant stress to increase plant vigour by adhering to optimum plant dates, preventing drought stress and the optimal use of fertilisers have reduced *Fusarium* infection in a number of grain crops [76, 89–91]. However, maize cultivated by means of organic agriculture does not accumulate less FUM than maize cultivated conventionally [92, 93]. Extended periods of heat and drought stress that lead to increased FUM levels could be managed with proper irrigation schedules [77, 94]. Managing plant stress conditions is also important as this is considered key in the symptomless endophytic relationship converting to a disease- and/or mycotoxin-producing interaction [95].

2.5.5. Chemical control

Fungicides have been shown to significantly reduce FHB and DON contamination of wheat grain. Triazole fungicides such as metconazole and tebuconazole have been shown to control FHB and DON contamination in wheat [96]. However, fungicides are neither effective in reducing *F. verticillioides* infection/FUM accumulation, nor *A. flavus* infection/AF accumulation in maize [97]. This may be due to the husks that cover maize kernels. FUM were, however, reduced by 95% *in vitro* when four fungicides and a biocontrol bacterium (Serenade, *B. subtilis*) were evaluated for the control of *F. verticillioides* and *A. flavus* [98]. No registered fungicides are available for the control of either *F. verticillioides* or *A. flavus* in any African country [98]. The use of insecticides can prevent insect wounds that contribute to fungal infection and mycotoxin accumulation in maize kernels [91].

Reduced FHB severity and mycotoxin contamination of wheat under field conditions using tannic acid and the botanicals, Chinese galls and buckthorn, have been shown [100]. These researchers also reported disease and mycotoxin reduction efficacy close to that observed with a synthetic fungicide, thereby demonstrating the potential use of natural compounds

in managing mycotoxigenic fungi and their toxins. Furthermore, several studies report on a reduced fungal growth and mycotoxin contamination for *Aspergillus* and *Fusarium* using natural oils and phenolic compounds *in vitro*; however, the commercial value of such products has not been explored and may not be feasible [101, 102].

2.5.6. Managing mycotoxigenic fungi with other microorganisms

The use of biological control agents to manage mycotoxigenic fungi has been reported. Atoxigenic *F. verticillioides* strains competitively excluded FUM-producing strains and prevented them from producing FUM [103]. When these strains were applied by themselves through the silk channel, however, they resulted in high levels of FER. The effective control of toxigenic *F. verticillioides* and *F. proliferatum* by non-toxigenic *Fusarium* species in maize residues has also been observed [104]. Most success, however, has been achieved with the use of atoxigenic strains of *A. flavus* to control toxigenic *A. flavus* and *A. parasiticus*. When introduced into the soil, these atoxigenic strains reduced AF contamination of peanuts in the USA by 74.3–99.9% [105]. Atoxigenic *A. flavus* strains are now widely used to control AF in maize in several African countries (www.aflatoxinpartnership.org). Endophytic bacteria have been reported to control FUM-producing fungi by competitive exclusion [106], while *Trichoderma* strains controlled them through competition for nutrients and space, fungistasis, antibiosis, rhizosphere modification, mycoparasitism, biofertilisation and the stimulation of plant-defence mechanisms [107].

2.5.7. Prediction systems

An epidemic can be described as a ‘change in disease intensity in a host population over time and space’ [108]. Mathematical modelling of crop disease is a rapidly expanding discipline within plant pathology [109] with the first models developed by Van der Plank [110, 111]. In epidemiology, modelling aims to understand the main determinants of epidemic development in order to address disease management in a sustainable and efficient manner. It can, therefore, serve as an instrument to monitor and assess the risk of mycotoxin contamination in crops that would drive agronomic decisions during cultivation, in order to enhance management strategies [112].

Most research regarding disease forecasting of mycotoxigenic fungi has focussed on FHB of wheat. This disease is considered well suited for risk assessment modelling because of the severity of epidemics, compound losses resulting from mycotoxin contamination and relatively narrow time periods of pathogen sporulation, inoculum dispersal and host infection [113]. This can be seen from the online forecasting model FusaProg [114], which is a threshold-based tool to control *F. graminearum* with the optimised timing of fungicide applications and forecasts of DON content during flowering. DONCast is a prediction model from Canada that has been extensively validated and commercialised for wheat [112], while an adaption of this model has been proposed for maize. This model predicts the variation in mycotoxin levels associated with the year and agronomic effects from simple linear models using wheat samples from farmers. The DONCast model accounts for up to 80% of the variation in DON and is commercially employed for the past 10 years.

Field-based models to predict FUM B1 contamination in maize grain have been elusive, most probably due to the complexity of interactions between numerous abiotic and biotic disease

factors [115]. The concentration and severity of FUM produced by *Fusarium* spp. varies with meteorological conditions, genotype and location [19]. In general, favourable conditions for *F. verticillioides* infection include high temperatures [56], drought stress [56, 116] and insect damage stress [56]. A mathematical simulation of the growth of *F. graminearum* and *F. verticillioides* in maize ears was developed; however, the model only simulates fungal growth and not mycotoxin accumulation [117]. A preliminary model developed in the Philippines and Argentina identified four weather periods near silking as critical to FUM accumulation at harvest [19]. This model accounted for 82% of the variability of total FUM across all locations in 2 years of study, but did not consider meteorological conditions during grain maturation when FUM are synthesised.

A risk assessment model (FUMAgrain) developed for FUM contamination of maize grain in Italy gives an initial risk alert at the end of flowering based on meteorological conditions [118]. A second alert follows at kernel maturation following assessments of grain moisture, European corn borer damage and FUM synthesis risk. FUMAgrain could simulate FUM synthesis in maize accounting for 70% of the variation for calibration and 71% for validation. The importance of meteorological conditions at flowering and the growth of *F. verticillioides* and FUM synthesis during grain maturation was emphasised as the most important factors contributing to FUM contamination [118]. Another model consistently identified mean maximum temperature and minimum humidity as driving variables in the colonisation of maize kernels by fumonisin-producing *Fusarium* spp [99]. Furthermore, *Fusarium* colonisation of grain and fumonisins were related to prevailing weather conditions during early post-flowering and dough stage of grain development, respectively [99]. A prediction model using variables such as cultivar, climate, management practice, soil type, phenological stages of the host plant and pathogen variation would be advantages in identifying areas with potentially dangerous levels of fungal contamination and associated mycotoxin production, enabling them to implement mycotoxin management strategies.

3. Conclusion

Food and feed crops are consistently threatened by mycotoxigenic fungi and compound their infection by depositing toxic metabolites, including mycotoxins. Preharvest management of mycotoxin contamination is vital to maintaining contamination levels below economically feasible and legislated thresholds. Planting genotypes with enhanced host resistance is considered the most practical, affordable and environmentally sound method of controlling mycotoxigenic fungi and their mycotoxins. However, integrating resistant varieties with good agricultural practises such as crop rotation, chemical/biological control and other strategies that optimise plant production by minimising stressors may further reduce the risks associated with mycotoxin contamination. Resistance to mycotoxigenic fungi exists and has been identified in appropriate breeding materials but such resistance needs to be introduced in high-yielding and locally adapted hybrids. To date, conventional breeding has not been able to introgress disease and/or mycotoxin resistance into important staple crops like maize. Therefore, further research is required into factors with a greater efficacy to reduce mycotoxigenic fungi and mycotoxins preharvest as resistant varieties are being developed.

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Conflict of interest

The authors declare no conflict of interest.

Author details

Lindy J. Rose^{1*}, Sheila Okoth², Bradley C. Flett³, Belinda Janse van Rensburg³ and Altus Viljoen¹

*Address all correspondence to: lindym@sun.ac.za

1 Stellenbosch University, Stellenbosch, South Africa

2 University of Nairobi, Nairobi, Kenya

3 Agricultural Research Council, Potchefstroom, South Africa

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