

Researchers, donors, and governments are calling for the scale of the response to the aflatoxin problem to be informed by the scope of the risks involved. While current research establishes that a great deal of the global food supply is at risk, especially in developing countries, information on aflatoxin contamination and potential interventions is far from comprehensive, hindered in large part by a lack of information on the quantitative, geographic, and temporal occurrence of the toxins in various commodities. A set of diagnostic solutions is required that can span the dimensions of both scale (from smallholder farmer bags to large silos) and setting (from smallholder farms and village mills to large commercial mills). Of particular need is a new generation of inexpensive and portable diagnostics for testing on the front lines, particularly at the farm and village mill. These diagnostics must be underpinned both by sampling methods appropriate to developing countries and by reference labs accessible to key partners along the value chain on the ground.

The problem: Lack of diagnostics for use in the field

Two broad types of information are needed about aflatoxins: one is the scope and severity of the problem, as well as potential solutions, and the other is the quality, end use, and price differences of at-risk commodities. The aflatoxin problem cannot be addressed with the current network, which largely comprises a few minimally equipped laboratories using a non-standardized set of procedures.

A number of ongoing initiatives seek to address this lack of accessible, affordable, and context-appropriate diagnostics. One effort is the Australian Agency for International Development (AusAID)-funded Biosciences eastern and central Africa (BecA)-Commonwealth Scientific and Industrial Research Organisation (CSIRO) CAAREA project, encompassing a multidisciplinary team focused on diagnostics as one part of a multi-pronged approach to reducing aflatoxins. The project has established an aflatoxin research and capacity-building platform at the BecA-International Livestock Research Institute (ILRI) Hub in Nairobi, Kenya, which is open to biosciences researchers focused on improving food security in Africa.

Sampling issues

Aflatoxin risks are related to the concentration of mycotoxins in food commodities consumed by both humans and production animals. The first consideration for the development of appropriate diagnostics is sampling and scale. Measuring aflatoxin levels in grain products is complicated by the extremely skewed distribution of mycotoxin. Consequently sampling/sub-sampling is widely recognized as the largest source of error in aflatoxin measurement—accounting for up to 90 percent of the error in testing the variance in aflatoxin levels between the measured sub-sample and the whole sample, compared to variance from the analytical test itself (Whitaker 2003). Less than 1 percent of kernels may be contaminated, but these kernels can contain extremely high aflatoxin levels: up to

1,000,000 nanograms per gram (ng/g) for individual peanuts (Cucullu et al. 1986) and 400,000 ng/g for individual maize kernels (Shotwell, Goulden, and Hesselstine 1974). It is thus critical for the accuracy of any analysis that a "representative" sample is obtained for testing. Various sampling and sub-sampling protocols exist (Richard 2006), but these have generally been designed for container and truckload sampling and are not readily applicable to Africa's small-scale farming and village mills. Even the sampling of truckloads of 20 kg bags of grain is complicated when these bags have not originated from a single source, as occurs often with deliveries to commercial mills in Africa. Research efforts are underway to test a range of samples along the less-studied smaller end of the scale, from single kernels to ears in a field, to develop context- and diagnostic test-appropriate sampling procedures. Such sampling strategies are as important as the tests themselves.

Available diagnostic technologies

A number of established diagnostic technologies are already available. However, as shown in Table 1, they are typically expensive, have lower throughput, and are not portable and therefore not available for use in the field.

Available methods of analysis range from the in-field rapid diagnostic strips such as AgriStrips used in rapid test kits to competitive enzyme-linked immunosorbent assay (ELISA) with colorimetric detection to spectroscopic methods.

Aflatoxins possess significant ultraviolet (UV) absorption and fluorescence properties, and chromatographic methods—either high performance liquid chromatography (HPLC) or thin layer chromatography (TLC) with UV or fluorescence detection—are widely used. Such methods require sample extraction and extract clean-up by solid-phase extraction (SPE) or immunoaffinity chromatography (IAC) followed by chromatographic separation and aflatoxin detection. Total aflatoxins can also be measured by direct fluorescence measurements of these purified extracts (for example, VICAM).

Liquid chromatography-mass spectrometry (LC-MS) technology offers the advantage of "dilute and shoot" techniques where simple sample extracts are analyzed without clean-up, and with the added advantage of multi-mycotoxin analysis whereby a range of mycotoxins can be analyzed in the same sample analysis run (Sulyok et al. 2006).

A new generation of cheap and portable diagnostics is needed so researchers, regulators, the private sector, extension agents, and others can address the problem in developing countries. A few promising technologies under exploration include near infrared spectroscopy (NIR), electronic nose (e-nose), and paper microfluidics.

NIR is a rapid, non-destructive, predictive technology that has long been used routinely in plant breeding and in industrial applications to simultaneously predict multiple parameters. NIR can be used with solid or milled material and on liquids such as milk. NIR has identified correlation with aflatoxin levels and could

TABLE 1 Diagnostic technologies available commercially and under development

Diagnostic technology	Technology cost	Sample cost (\$)	Prep time (+)	Portable?	Discrimination at regulatory limits (10 ppb)?	Multi-mycotoxin analysis in same run?	Potential use for milled grain?	Potential use for whole grain?
VICAM	\$	\$\$\$	+++	No	Yes	No	Yes	No
ELISA	\$	\$	+++	No	Yes	No	Yes	No
UPLC	\$\$	\$	+++	No	Yes	No	Yes	No
LC-MS	\$\$\$	\$	+	No	Yes	Yes	Yes	No
TLC	\$	\$	++	No	Yes	No	Yes	No
NIR (proof of concept underway)	\$\$	\$	+	Yes	No	Potentially	Potential application > 200 ppb (in progress)	Potential application in kernel sorting (in progress)
E-Nose (proof of concept underway)	\$	\$	+	Potentially	No	Unknown	(In progress)	No
AgriStrips and other dipsticks	\$	\$	+	Yes	Yes	No	Yes	No

Source: Authors' calculations, 2013.

Note: \$ = low/\$\$\$ = High cost (relative within column); + = low effort/ +++ = high effort.

possibly be used in the screening of high levels of aflatoxins (above 200–500 parts per billion or ppb) in milled grains—though so far they have not been proven able to detect levels at regulatory limits for human consumption (10–20 ppb). Some developed countries have different limits for feeds (up to 300 ppb) for which NIR may be suitable. NIR may help in the removal of extremely contaminated kernels (above 1,000 ppb) via single-kernel sorters that have been developed based on spectral sorting (Pearson et al. 2004); spectral sorting for aflatoxins is already done commercially for groundnuts in the United States. Wet chemistry suggests that, if successfully developed, this approach could reduce the contamination levels of bags of maize grains from almost 100 times the legal limit to below the legal limit by removal of as little as three percent of the contaminated grains (Turner et al. 2013).

E-nose is a technology that uses an array of sensors to detect volatiles emanating from a sample. Like NIR, wet chemistry measurements are used to calibrate e-nose to predict a given chemical. E-nose, which is currently being used in a project designed to detect diseases in human breath (Berna et al. 2013), is also being adapted for possible use in aflatoxin detection. Advantages include that it could largely overcome sampling issues because the headspace is produced by the entirety of a sample; there is no sample preparation required, except for milling; and it could potentially be as portable and cheap as an inexpensive mobile phone.

Other recent developments include an immunoassay-based lateral flow device that can quantitatively determine four major aflatoxins in maize in only ten minutes (Anfosi et al. 2011). Paper microfluidics are also being developed for various food safety issues by organizations such as Diagnostics for All, and may provide inexpensive and rapid point-of-use diagnostics.

Challenges to policy and technology implementation

A wide range of potential diagnostics needs to be explored so that the right suite can be selected for use in the network of reference labs and field networks. Diagnostics need to be part of a system for monitoring and managing aflatoxin risk at all critical points, enabling the systems in developing countries to address the aflatoxin issue the way systems in the more-developed countries have largely addressed it. Decontamination procedures and changing regulations for variable limits according to use are also required to complement diagnostics. Otherwise, contaminated commodities are either stored in a state of limbo or re-enter the market via avenues that skirt monitoring and regulation, ultimately reaching the most vulnerable consumers whom the diagnostics were designed to protect in the first place.

In conclusion, a strategic and systemic approach is needed to ensure safe food for all citizens of the world.

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