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Title Page

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Authors: Matthew J. Stasiewicz[a1*], Titilayo D.O. Falade[b], Murithi Mutuma[c], Samuel K. Mutiga[d2], Jagger J W Harvey[e3], Glen Fox[b], Tom C. Pearson[f], James W. Muthomi[c], Rebecca J. Nelson[d]

Author Affiliations:

[a]Department of Food Science. Cornell University. Ithaca, NY 14850. USA. E-mail: ms984@cornell.edu.

[b]QAAFI Centre for Nutrition and Food Science. The University of Queensland. Coopers Plains 4108, Australia. Falade E-mail: titilayo.falade@uq.net.au. Fox E-mail: g.fox1@uq.edu.au

[c]Department of Plant Science and Crop Protection. University of Nairobi. P. O. Box 29053-00625, Nairobi, Kenya. Mutuma E-mail: murithimutuma@yahoo.com. Muthomi E-mail: james_wanjohi@yahoo.com.

[d]School of Integrative Plant Science, Cornell University, Ithaca, NY 14853. Mutiga E-mail: skm88@cornell.edu. Nelson E-mail: rjn7@cornell.edu.

[e]Biosciences eastern and central Africa-International Livestock Research Institute (BecA-ILRI) Hub, ILRI. P.O Box 30709, Nairobi 00100, Kenya.

[f]Center for Grain and Animal Health Research, US Department of Agriculture - Agriculture Research Service. 1515 College Ave, Manhattan, KS, USA. E-mail: pearsontomc@gmail.com

*Corresponding Author: M.J. Stasiewicz. Department of Food Science and Human Nutrition. University of Illinois at Urbana Champaign. Bevier Hall. 905 S. Goodwin Ave. Urbana, IL. 61801. mstasie@illinois.edu. +1 (217) 265-0963.

Present Addresses:

[1]Department of Food Science and Human Nutrition. University of Illinois at Urbana Champaign. mstasie@illinois.edu.

[2]Department of Plant Pathology. University of Arkansas. smutiga2008@gmail.com.

[3]Kansas State University, Feed the Future Innovation Lab for the Reduction of Post-Harvest Loss. jjharvey@ksu.edu

Abstract

Maize, a staple food in many African countries including Kenya, is often contaminated by toxic and carcinogenic fungal secondary metabolites such as aflatoxins and fumonisins. This study evaluated the potential use of a low-cost, multi-spectral sorter in identification and removal of aflatoxin- and fumonisin-contaminated single kernels from a bulk of mature maize kernels. The machine was calibrated by building a mathematical model relating reflectance at nine distinct wavelengths (470-1,550 nm) to mycotoxin levels of single kernels collected from small-scale maize traders in open-air markets and from inoculated maize field trials in Eastern Kenya. Due to the expected skewed distribution of mycotoxin contamination, visual assessment of putative risk factors such as discoloration, moldiness, breakage, and fluorescence under ultra-violet light (365 nm), was used to enrich for mycotoxin-positive kernels used for calibration. Discriminant analysis calibration using both infrared and visible spectra achieved 77% sensitivity and 83% specificity to identify kernels with aflatoxin > 10 ng g^{-1} and fumonisin > 1,000 ng g^{-1}, respectively (measured by ELISA or UHPLC). In subsequent sorting of 46 market maize samples previously tested for mycotoxins, 0-25% of sample mass was rejected from samples that previously tested toxin-positive and 0-1% was rejected for previously toxin-negative samples. In most cases where mycotoxins were detected in sorted maize streams, accepted maize had lower mycotoxin levels than the rejected maize (21/25 accepted maize streams had lower aflatoxin than rejected streams, 25/27 accepted maize streams had lower fumonisin than rejected streams). Reduction was statistically significant ($p<0.001$), achieving an 83% mean reduction in each toxin. With further development, this technology could be used to sort maize at local hammer mills to reduce human mycotoxin exposure in Kenya, and elsewhere in the world, while at once reducing food loss, and improving food safety and nutritional status.

Key words: aflatoxin, fumonisin, maize, spectral sorting, food safety

1. Introduction

Mycotoxins are toxic secondary metabolites of fungi that contaminate food crops such as cereals and nuts globally (Wild & Gong, 2010). The best-studied are aflatoxins, to which more than 5 billion people in developing countries are chronically exposed through food (Wild & Gong, 2010; Wu, Narrod, Tiongco, & Liu, 2011). Acute exposure to high levels of aflatoxin causes potentially fatal aflatoxicosis (Nyikal et al., 2004) and chronic exposure to naturally-occurring aflatoxins causes liver cancer (IARC, 2012). The mycotoxin fumonisin frequently co-occurs with aflatoxin in maize (Magoha et al., 2014; Mutiga et al., 2014; Mutiga, Hoffmann, Harvey, Milgroom, & Nelson, 2015; Torres et al., 2014; Wild & Gong, 2010) and chronic exposure has been associated with esophageal cancer and neural tube defects (Wild & Gong, 2010). Additionally, exposure to both mycotoxins is correlated with childhood stunting (Khlangwiset, Shephard, & Wu, 2011; Shirima et al., 2015; Wu, Groopman, & Pestka, 2014), possibly by inducing environmental enteropathy, an intestinal condition that leads to reduced absorption of nutrients (Smith, Stoltzfus, & Prendergast, 2012).

The Kenyan maize value chain, dominated by self-provisioning, purchase from open-air markets, and local milling (Hellin & Kimenju, 2009; Kang'ethe, 2011), is unable to protect consumers from foodborne exposure to mycotoxins. Aflatoxin and fumonisin are endemic in household maize supplies in Kenya (Hoffmann, Mutiga, Harvey, Nelson, & Milgroom, 2013a; Mutiga et al., 2014; Mutiga et al., 2015). Maize brought by Kenyans for local milling showed contamination above Kenyan regulatory limits of 10 ng g^{-1} aflatoxin and 1,000 ng g^{-1} fumonisin in 39% and 37% of samples, respectively (Mutiga et al., 2014). Further, Eastern Kenya region has repeatedly been host to acute aflatoxicosis outbreaks shortly after the major maize harvest, including a severe outbreak in 2004 in which 125 Kenyans died (Daniel et al., 2011; Nyikal et al., 2004).

The focus of this study was to adapt a relatively-simple, multi-spectral sorter to reduce aflatoxin and fumonisin contamination in Kenyan maize. Such a device could be part of an integrated approach to mycotoxin management that empowers consumers to personally ensure food safety. Sorting exploits the fact that mycotoxin distribution is generally highly skewed: a relatively small proportion of kernels contain the majority of the toxin (Kabak, Dobson, & Var, 2006). For food-insecure populations, sorting could directly improve food security by removing the few highly-contaminated kernels in a grain lot, while retaining the majority of the healthy grain for consumption. Sorting at the individual consumer level could also help overcome the problem of misaligned incentives for mycotoxin control between producers, who often bear the costs but not the benefits of pre- and post-harvest interventions, and consumers, who are less able to demand control since the toxins are generally undetectable by human consumers (Hoffmann, Mutiga, Harvey, Nelson, & Milgroom, 2013b). This approach would represent an improvement over ineffective test-and-reject strategies that reduce an already marginal food supply, such as when 2.3 million bags of maize were condemned by the Kenyan government in 2010 due to aflatoxin contamination, and much of the contaminated maize may have been illicitly returned to the market (Ng'erich & Gathura, 2010).

Existing sorting methods to remove aflatoxins and fumonisins from maize have been summarized in larger reviews focusing on mycotoxin reduction in grains (Grenier, Loureiro-Bracarense, Leslie, & Oswald, 2013), aflatoxin detection and quantification (Yao, Hruska, & Di Mavungu, 2015), and non-biological aflatoxin remediation (Womack, Brown, & Sparks, 2014). The last review includes a table of existing applications of hand-sorting, infrared spectrometry, and ultraviolet fluorescence to the

reduction of aflatoxin in tree nuts, peanuts, and maize. Low-cost spectral-sorting, such as developed in this study, was not represented. Two general approaches to sorting for mycotoxin reduction exist: sorting to remove low-quality kernels in general or sorting by algorithms calibrated to remove mycotoxin contaminated kernels specifically.

Sorting to remove low-quality, possibly fungal-infected, grains in general, which can be achieved through sieving, density separation, and removal of discolored kernels (Grenier et al., 2013). To improve maize quality, Kenyan consumers often manually sort maize using large sieve tables prior to local milling, which can be effective at reducing levels of fumonisin but may have little effect on aflatoxin levels (Mutiga et al., 2014). Alternatively traditional processing though sorting, winnowing, and washing has been shown to reduce aflatoxin and fumonisins in traditional food products in Benin (Fandohan et al., 2006; Fandohan et al., 2005). We would put into this category the 'black light' or Bright Greenish Yellow Fluorescence (BGYF) test (Grenier et al., 2013), where kernels are viewed under 365 nm ultraviolet light for fluorescence characteristic of *A. flavus* infection, specifically fluorescence of peroxidase transformed kojic acid.

Recently developed approaches use some combination of infrared, visible, and ultraviolet light imaging calibrated to detect maize kernels known to be contaminated with aflatoxin or fumonisin. Hyperspectral imaging of ultraviolet light fluorescence can classify kernels as having undetectable, low, medium, or high aflatoxin contamination (bins of < 1, 1-20, 20-100, or > 100 ng g^{-1} aflatoxin, (Yao et al., 2010). Combining visible and near-infrared transmittance or reflectance spectra can classify maize by aflatoxin level (Pearson, Wicklow, Maghirang, Xie, & Dowell, 2001). Implementing this approach in high-speed sorting has been shown to reduce both aflatoxin and fumonisin contamination in maize from Texas, USA by over 80% (Pearson, Wicklow, & Pasikatan, 2004). While modern imaging approaches are effective, there is a need for improved sorting technology designed for lower-resource markets in which small samples are processed.

In this study, we calibrated a laboratory-scale, multi-spectral sorter (Haff, Pearson, & Maghirang, 2013) to remove aflatoxin- and fumonisin-contaminated kernels from diverse maize samples. Samples included maize purchased from open-air markets in Eastern Kenya and kernels from a field trial of *Aspergillus flavus*-inoculated maize. We chose to evaluate this specific sorting technology because the basic circuitry is relatively inexpensive (<US$100 in components), and throughput is modest (20 kernels/s, theoretically around 25 kg/h), providing an opportunity to adapt the design for application in small-scale milling in developing countries such as Kenya.

We tested the major hypothesis that mycotoxin levels in market maize can be significantly reduced by removing the kernels contaminated at the highest levels using a relatively simple optical sorting technology. In the process of testing this hypothesis, we also generated data on the skewed distribution of and risk factors for aflatoxin or fumonisin contamination at the single-kernel level.

2. Materials and Methods

This study focused on calibrating an existing single-kernel optical sorter for the purpose of removing aflatoxin and fumonisn contaminated kernels from bulk samples of Kenyan market maize. To develop the calibration algorithms, we sourced single kernels from two concurrent mycotoxin-related studies in Kenya. Given prior knowledge that aflatoxin (Lee, Lillehoj, & Kwolek, 1980; Turner et al., 2013) and fumonisin (Mogensen et al., 2011) contamination in single-kernels is skewed, we expected

aflatoxin and fumonisn contamination in our samples to also be skewed towards few individual kernels being contaminated. If we analyzed a simple random sampling of kernels from these studies, we anticipated we would not analyze sufficient contaminated kernels to develop a statistically robust calibration. Therefore, we employed multiple stages of sample selection designed to enrich for toxin-contaminated kernels in the final data set. A summary of the kernel selection process is summarized in **Table 1** along with the critical analytical methods applied to each sample subset.

2.1 Bulk maize samples.

Samples of shelled maize kernels were obtained from two mycotoxin-related studies in Kenya. The first source was a survey of shelled maize purchased in < 1 kg lots from open-air markets in Meru, Machakos, and Kitui counties of Eastern Kenya , comprising 204 unique samples in total (Eliphus, 2014). Some samples were locally dehulled. The second source was shelled maize collected immediately after harvesting ears previously inoculated with an aflatoxin-producing strain of *A. flavus*. Kernels from 17 highly aflatoxin-contaminated bulk samples were selected for ultra-high performance liquid chromatography (UHPLC) analysis for aflatoxin levels (Falade et al., 2014).

2.2 Selection, enrichment, and visual characterization of maize kernels.

Maize samples from the two studies were selected, enriched, and characterized separately. Individual kernels from the market survey were enriched for mycotoxin prevalence by selecting, first, contaminated bulk samples and, second, kernels within those samples that exhibited fluorescence under ultraviolet (UV) light. A total of 25 bulk samples were randomly selected from the population of all bulk samples for which 5-g subsamples had previously tested above 10 ng g^{-1} aflatoxin or 1,000 ng g^{-1} fumonisin. Kernels from these samples were visualized under 365 nm light for bright greenish-yellow fluorescence (BGYF) or bright orange fluorescence (BOF) (Pearson, Wicklow, & Brabec, 2010). All kernels that fluoresced, and three that did not, were selected for further analysis. In total, 233 kernels were selected from the 25 samples. Kernels were visually inspected for three factors previously associated with aflatoxin or fumonisin contamination: breakage (Mutiga et al., 2014), insect damage (Pearson et al., 2010), and discoloration (Pearson et al., 2010). An additional factor, mass in the lower 10^{th} percentile of the set, was calculated during risk factor analysis because aflatoxin-contaminated maize kernels have lower average mass than uncontaminated kernels from the same ear (Lee et al., 1980).

Individual kernels from the *A. flavus* inoculated field trials were selected at random from 17 aflatoxin-contaminated bulk samples: ten kernels each from the first 12 samples, and 20 kernels each from the second five samples (Falade et al., 2014). The first set of 120 kernels were available for visual assessment of all the same risk factors as the market samples, except that both BGYF and BOF were aggregated as fluorescence under UV.

2.3 Single kernel spectroscopy.

Limited-spectra collection. Individual kernels from both the market survey (n=233) and the field trials (n=220) were scanned by passing through the sorter three times. During operation of the sorter, a single stream of kernels fell past a circuit board that cycled through a ring of light-emitting diodes (LEDs) with 9 distinct emission wavelengths; reflectance from each of the 9 individual LEDs was captured by a photodiode. If the machine was operating in sorting mode, calibrated software triggered removal of

contaminated kernels by a pulse of compressed air. To mimic the orientation differences that would occur in real-time sorting, individual kernels were allowed to fall through the sorter in random orientation.

Two separate sorter circuit boards were used, each with distinct analytical ranges. The first was a low wavelength board (nirL) that used LEDs with peak emission wavelengths of 470 (blue), 527 (green), 624 (red), 850, 880, 910, 940, 1070 nm. The second was a higher wavelength board (nirH) that used LEDs of 910, 940, 970, 1050, 1070, 1200, 1300, 1450, 1550 nm. Composite features (n=205 features) were calculated: bulk reflectance from each LED (reflectance minus background, n=9 features), total visible and total infrared reflectance (n=2), all pairwise differences (n=55), all pairwise ratios (n=55), and all second derivatives of the combination of three features (n=84). Hardware and software has been comprehensively described previously (Haff et al., 2013).

High-resolution spectra collection. To inform future development of the limited-spectra sorting technology, Fourier transformed near infrared (FT-NIR) reflectance spectra from 800 to 2,780 nm in 1,154 steps were captured, in duplicate, for each individual kernel (on a Multi-Purpose FT-NIR Analyzer; Bruker Optics Inc. Billerica, MA, USA). Each scan captured reflectance from one of the two broadest faces of each kernel.

2.4 Mycotoxin analysis.

In this study we analyzed all market maize kernels for aflatoxin and fumonisin levels using ELISA methods and also analyzed the inoculated field trial kernels for fumonisin. The inoculated field trial kernels had been analyzed for aflatoxin by UHPLC in a parallel study (Falade et al., 2014).

From the market maize survey, single kernels were selected for wet chemistry mycotoxin analysis in a two-tiered process. To maximize diversity among the spectra with associated wet chemistry, a principal components analysis was performed on the FT-NIR data. Eighty-one kernels were sampled across the first principal component, spanning all 22 markets. Subsequently another 77 kernels were selected by stratified random sampling of kernels from the 22 markets. From the inoculated field trials, all 220 kernels selected for aflatoxin analysis in (Falade et al., 2014) were also subject to fumonisin analysis.

Individual maize kernels were milled for 10 s at 30 Hz to a fine powder (< 1 mm particle size) in a ball mill with 5 mL stainless steel jars (MM301 mill, manufacturer jars; Retsch Haan, Germany). Between samples, jars were cleaned with absolute ethanol and wiped with a dry cloth. Kernels were assayed for total aflatoxin and fumonisin levels using toxin-specific ELISA kits (Total Aflatoxin ELISA Quantitative and Fumonisin ELISA Quantitative, respectively; Helica Biosystems Inc., Santa Ana, CA). The manufacturer's protocol was followed with minor modifications to toxin extraction. To eliminate sub-sampling variation, mycotoxins were extracted from the entire ground maize kernel. Mycotoxins were extracted using standardized volumes of 80% methanol ranging from 400 to 1,500 µl according to initial kernel mass; extractions targeted a manufacturer recommended 1:5 nominal dilution. Aliquots of the same extractions were diluted 20-fold in 80% methanol for fumonisin ELISA. Samples with contamination above the highest ELISA standard were diluted and retested. Manufacturer performance data correlating results from Helica ELISA to HPLC analysis suggested only minor bias; the reported correlation implies that an ELISA measurement of aflatoxin = 10 or 100 ng g^{-1} and fumonisin = 1,000 or

10,000 ng g^{-1} would measure by HPLC as 9.4 or 95.5 ng g^{-1} aflatoxin or 1,020 or 9,360 ng g^{-1} fumonisin, respectively.

Inoculated field trial kernels were assayed by UHPLC for aflatoxin levels for a parallel study (Falade et al., 2014). Briefly, toxins were extracted from the entire ground maize sample with 70% methanol. Extracts were assayed using a Phenomenex Synergi 2.5u Hydro – RP (100 mm x 3.00 mm) column at 3500 psi. Toxin was detected with excitation/emission wavelengths of 365/455 nm and peaks compared to standard curves of aflatoxin B1, B2, G1, and G2 for quantification. Total aflatoxin values for comparison to ELISA results were calculated by summing the individual aflatoxin quantities multiplied by the reported antibody cross-reactivity rates, as follows: B1 – 100%, B2 – 77%, G1 – 64%, and G2 - 25%. After UHPLC analysis for aflatoxin, extractions were passed to the fumonisin ELISA assay as described above.

2.5 Statistical analysis of mycotoxins and kernel characteristics.

The association between kernel characteristics and mycotoxin contamination was first evaluated with univariate statistics. Binary mycotoxin values of aflatoxin > 10 ng g^{-1} or fumonisin > 1,000 ng g^{-1} were included as responses in a Chi-Square test, or a Fisher's Exact test for sample sizes < five. Significant factors were included in multivariate logistic regression to predict the odds of aflatoxin or fumonisin contamination. Sample region (Meru, Machakos, and Kitui) was included as a covariate. The best model was identified based on a stepwise regression. All analyses were performed in R v.3.1.0 (R Core Team, 2014), separately for each mycotoxin.

2.6 Sorting algorithm calibration and assessment.

The linear discriminant analysis (LDA) software distributed with the sorter (Haff et al., 2013) was used to calibrate the sorter to detect single kernels with either aflatoxin > 1, 10, or 100 ng g^{-1} or fumonisin > 100, 1,000, or 10,000 ng g^{-1}, in all dual-toxin pairs. For example, there was one calibration to identify kernels with aflatoxin > 10 ng g^{-1} or fumonisin > 1,000 ng g^{-1}. This required nine separate calibrations for both the low (nirL) and high (nirH) wavelength circuit boards.

To generate the calibrations, a training file was created by associating the mycotoxin levels with the first two of each individual-kernel spectra. A discriminant analysis exhaustive search selected three optical features that minimized overall classification error rate using the first scan for training and the second scan for cross-validation. The full data set was required for training. Cross-validation sensitivity (Sn, $n_{\text{toxin positive kernels rejected}} / n_{\text{toxin positive kernels}}$) and specificity (Sp, $1 - n_{\text{toxin negative kernels rejected}} / n_{\text{toxin negative kernels}}$) were calculated.

2.7 Alternative sorting algorithm assessment.

To evaluate the extent to which selected hardware or software limitations affected sorting performance, three separate limiting components were evaluated (i) the classification algorithm, (ii) the detector, and (iii) the LED emission wavelengths. While the default software used linear discriminant analysis for classification, random forest (RF) and support vector machine (SVM) algorithms were also evaluated in R (packages randomForest and kernlab, respectively). Classification performance was evaluated identically as for LDA.

Existing detector hardware required separate circuit boards to gather reflectance spectra wavelengths of either 470-1,070 nm (the nirL board) or 910-1,550 nm (the nirH board). To evaluate if this range limitation decreased performance, data for an *in silico* 'composite' board (nirHL) were calculated using all the features from all 14 unique LEDs present across both boards (four of the nine LEDs were present on both boards). The same set of optical features were calculated including ratios, differences, and second derivatives (n = 816 total features). This larger set of features was used for classification by LDA, RF, and SVM algorithms. Although limited spectra are more useful for high-throughput sorting, we also evaluated the performance of higher-resolution spectral data, the FT-NIR data, using the RF and SVM algorithms for classification.

2.8 Maize sorting validation.

Market maize samples not used for selecting calibration kernels (n=46) were selected for physical sorting to validate the best classification algorithm. Samples were stratified by previous bulk analysis of maize by ELISA. Categories were 'high fumonisin' (> 1,000 ng g^{-1}), 'high aflatoxin' (> 10 ng g^{-1}), 'medium aflatoxin' (> 1 and < 10 ng g^{-1}), 'medium fumonisin' (> 100 and < 1,000 ng g^{-1}), and 'control' (no detected toxins). Whatever mass of the sample remained was sorted, up to a maximum of 75 g. To isolate the analytical accuracy of the machine, samples were sorted manually rather than mechanically (the air diversion was disabled). Kernels were dropped through the machine and software indicated if the kernel should be rejected or not (≤ threshold = accept; > threshold = reject). Manual sorting validated the theoretical performance of the multi-spectral sorting process, without noise from misclassification due to the mechanical errors (*e.g.* the air mechanism failing to divert the kernel).

Rejection rates were calculated from the bulk mass of the accepted and rejected kernels and modeled with a linear model of the logit-transformed reject proportion by bulk aflatoxin and fumonisin detection status. The minimum non-zero rejection rate was added to all values to accommodate rejection rates of zero in the analysis (Warton & Hui, 2011). Accepted and rejected maize streams were ground and assayed by ELISA for aflatoxin and fumonisin levels. A general linear model of bulk toxin levels was used to test the effect of sorting as the change in toxin level in the accepted versus rejected stream, with blocking by sample. All samples without detectable toxin in both the accepted and rejected kernels were excluded as no sorting effect was observable.

3. Results

The goal of this study was to evaluate the potential for multi-spectral sorting to remove aflatoxin and fumonisin from Kenyan market maize as a proof of concept for maize in similar agricultural systems globally. To do so, we calibrated an existing laboratory-scale, multi-spectral sorting device to identify kernels contaminated with mycotoxins above thresholds of concern. Then, we used the device to sort samples of Kenyan market maize and show that toxin levels are reduced in maize accepted by the machine compared to maize rejected from the same sample. To guide future improvements of the sorting technology, we then compared results to calibrations achievable using other classification algorithms and with higher-resolution spectral data. Finally, we used this opportunity to assess the observed skewness of the distribution of mycotoxins in the single-kernels results and asses risk factors associated with single-kernel contamination.

3.1 *Discriminant analysis can differentiate aflatoxin or fumonisn contaminated kernels from uncontaminated kernels.*

Overall, we scanned and measured aflatoxin and fumonisin levels in 378 individual maize kernels from a market maize survey and *A. flavus* inoculated field trials; in total 158 and 54 kernels had measured aflatoxin > 10 ng g^{-1} or fumonisin > 1,000 ng g^{-1}. We associated measured mycotoxin levels with the spectral features for each kernel from circuit boards with lower range (470-1,070 nm, nirL) or higher range (910-1,550 nm, nirH) LEDs. Then we calibrated a linear discriminant analysis (LDA) algorithm to classify kernels based on various mycotoxin thresholds.

The discriminant analysis achieved a maximum cross-validation sensitivity (Sn) and specificity (Sp) of around 80% to reject kernels with mycotoxin levels at various thresholds (**Fig. 2**). As expected, classification performance showed a trade-off between increasing the true positive rate (Sn) and increasing the true negative rate (Sp), for a maximum of around 80% Sn and Sp when balancing both performance metrics. The lower wavelength board (nirL) showed a trend towards greater classification sensitivity and the higher wavelength board (nirH) showed a trend towards greater classification specificity. The *in silico* combination of the two boards (nirHL) did not dramatically improve classification relative to either existing board (nirL or nirH), as Sn and Sp values for each threshold fell within the range of values for the existing boards. Therefore, wavelength limitations of the existing hardware did not likely limit classification performance.

The calibration chosen for sorting was the nirL board rejecting kernels with aflatoxin > 10 ng g^{-1} and fumonisin > 1,000 ng g^{-1} (Sn = 0.77 and Sp = 0.83, **Fig. 2**). The next best calibration, also using the nirL board, lowers the fumonisin rejection threshold to 100 ng g^{-1} for Sn = 0.82 and Sp = 0.80. If it were physically possible, use of the nirHL board at the AF > 10 ng g^{-1} and FM > 100 ng g^{-1} thresholds would provide marginally better discrimination (Sn = 0.78 and Sp = 0.85). Both infrared and visible features were used in the 3-feature discriminant analyses at aflatoxin > 10 ng g^{-1} and fumonisin > 1,000 ng g^{-1} thresholds (Table 3).

3.2 *Optical sorting reduces aflatoxin and fumonisin in accepted maize.*

For a direct test of the potential for optical sorting to reduce mycotoxin levels, 46 market maize samples were sorted kernel-by-kernel with the nirL board calibrated to identify and then reject kernels with aflatoxin >10 ng g^{-1} or fumonisin >1,000 ng g^{-1}. Kernels were manually binned into accept or reject streams to isolate the theoretical sorting performance from mechanical error, such as imperfect reject kernel diversion.

The rejection rate was significantly greater for samples for which previous bulk tests detected either aflatoxin ($p = 0.014$) or fumonisin ($p < 0.001$, **Fig. 4**). No significant interaction was detected between aflatoxin and fumonisin contamination and rejection rate. In almost every case in which aflatoxin or fumonisin were detectable in the sorted maize, the accepted maize had lower aflatoxin levels than the rejected maize (**Fig. 5**). In 21 of 25 cases (84%), the accepted maize fractions had lower aflatoxin levels than those of the rejected maize fractions. In 14 cases (56%), the accepted maize had aflatoxin < 10 ng g^{-1} and the rejected maize had > 10 ng g^{-1}. In 25 of 27 cases (93%), accepted maize had lower fumonisin levels than rejected maize, while in 15 cases (56%) the accepted maize had fumonisin < 1,000 ng g^{-1} and the rejected maize had fumonisin > 1,000 ng g^{-1}. Toxin levels were significantly lower in

the accepted maize than the rejected maize by 0.78 log(ng g^{-1}) for aflatoxin and 0.79 log(ng g^{-1}) for fumonisin, $p < 0.001$) for each toxin, blocking by sample. These estimates corresponded to an 83% and 84% reduction in aflatoxin and fumonisin, respectively. Sorting efficacy was not affected by the district the samples were purchased from or by the sorting reject rate ($p > 0.05$ for each parameter).

3.3 Evaluation of alternative classification algorithms and spectral data do not suggest any major limitations to the existing sorter software or hardware.

In addition to evaluating the effect of the detector hardware (by comparing nirH, nirL and the *in silico* nirHL board as discussed above), we assessed two other potential software and hardware limitations: (i) the choice of classification algorithm, and (ii) the choice of LED peak emission wavelengths.

We compared the existing discriminant analysis algorithm with random forest (RF) and support vector machine (SVM) algorithms for classifying kernels based on spectra captured by the nirL and nirH boards. These machine learning algorithms were chosen because (i) they classify using all 205 features simultaneously, unlike the LDA algorithm which uses only 3 selected features, and (ii) SVMs have previously been used for classifying aflatoxin levels in single corn kernels (Samiappan et al., 2013) and RFs have outperformed LDA in other contexts (Cutler et al., 2007). For the nirL board, neither RF nor SVM improved upon LDA (Fig. **3**). For the nirH board, RF models were marginally superior to LDA for rejecting aflatoxin > 10 ng g^{-1} (Fig. S1). Nonetheless, even the best performing alternative nirH board calibration (RF rejecting aflatoxin > 10 or fumonisin > 100 ng g^{-1}, Sn = 0.76 and Sp = 0.81) was inferior to the best nirL LDA calibration. In other research, full-spectrum partial least squares regression did not improve upon LDA to classify single kernels as having high (> 100 ng g^{-1}) or low (<10 ng g^{-1}) aflatoxin levels (Pearson et al., 2001). These results suggest these machine learning models do not provide sufficient performance increases to justify their increased complexity.

To test the potential impact of building circuit boards with LEDs at alternative peak emission wavelengths, FT-NIR spectra from 800 nm to 2,800 nm was used in RF and SVM models. Use of RF and SVM models with these spectral data to classify kernels at the aflatoxin > 10 and fumonisin > 1,000 thresholds using only wavelength intensity values gave poor classification performance (RF Sn = 0.50 and Sp = 0.76, SVM Sn = 0.39 and Sp = 0.80). It was not computationally feasibly to enumerate all the multi-spectral features used as candidate features in the previous limited-spectra analysis, i.e. all pairwise differences, ratios, and second-derivatives, of > 1,000 spectra for exhaustive search classification. While feature selection strategies prior to classification have been used for classification of aflatoxin-contaminated hazelnuts (Kalkan, Beriat, Yardimci, & Pearson, 2011) and chili pepper (Ataş, Yardimci, & Temizel, 2012), a study classifying bulk maize samples as having aflatoxin > 20 ng g^{-1} using spectrophotometric instruments with spectral ranges of 400-2,500 nm and 1,100-2,500 nm achieved cross-validation error rates of 15-25% (Fernández-Ibañez, Soldado, Martínez-Fernández, & de la Roza-Delgado, 2009). Those results are not superior to than the limited spectra results reported here.

3.4 Aflatoxin and fumonisin levels in single kernels of Kenyan maize are skewed even under conditions of heavy selection.

In this study, we attempted to heavily enrich our single kernel sample for mycotoxin contamination by selecting kernels from bulk maize known to be contaminated with aflatoxin or

fumonisn (both market and inoculated field trial) and preferentially selecting kernels that fluoresced under ultraviolet light (market maize sample). Of the 159 kernels from the market maize survey, 54 (34%) showed fluorescence under ultraviolet light. Only a small proportion had high levels of contamination (**Fig. 1**). Only 17% and 3.2% of kernels, respectively, were contaminated with aflatoxin > 10 ng g^{-1} or fumonisin > 1,000 ng g^{-1}. A few kernels contained very high mycotoxin levels, up to 7,200 ng g^{-1} total aflatoxin or 93,000 ng g^{-1} total fumonisin.

From the 220 kernels selected from *A. flavus*-inoculated field trials, contamination rates of kernels were higher and less skewed (**Fig. 1**). Overall, 59% and 22% of kernels were contaminated with aflatoxin and fumonisin above levels of concern, respectively. While 25% of kernels had no detectable aflatoxin, the toxin distribution in kernels with detectable aflatoxin was bimodal with peaks near 10 and 10,000 ng g^{-1}. While 53% of kernels had no detectable fumonisin, the toxin distribution in kernels with detectable fumonisin peaked near 1,000 ng g^{-1} with a longer tail than the distribution for the market maize samples. The most contaminated kernels contained 1,454,000 ng g^{-1} aflatoxin and 237,000 ng g^{-1} fumonisin. The much higher rates and levels of aflatoxin contamination in the kernels from the field trial is unsurprising given the field trial inoculated with a highly toxigenic strain of *A. flavus*. Relatively higher odds of fumonisin contamination could be partially explained by a previous study that found a weak but significant correlation between fumonisin and aflatoxin prevalence in a bulk maize from Eastern Kenya (Mutiga et al., 2014).

These results show skew in mycotoxin contamination even among samples selected to enrich for greater rates and levels of mycotoxin. The true distribution of contamination in a random sampling of market kernels, or naturally infected field maize kernels, would likely be even more skewed than reported here.

3.5 Discoloration, insect damage, and fluorescence under ultraviolet light are associated with aflatoxin and fumonisin contamination of single maize kernels.

To extend the limited research in single kernel risk factors for mycotoxin contamination, kernels were scored for previously identified risk factors for mycotoxin contamination prior to grinding for mycotoxin analysis. In univariate analysis, kernel brokenness, discoloration, insect damage, and fluorescence under UV light, were associated with mycotoxin contamination (Supplemental Table 1). Bright Greenish Yellow Fluorescence (BGYF) was significantly associated with aflatoxin contamination above 10 ng g^{-1} ($p = 0.028$). Bright Orangish Fluorescence (BOF) was marginally associated with fumonisin contamination above 1,000 ng g^{-1} ($p= 0.078$) and undifferentiated fluorescence had a stronger association ($p = 0.003$). Light kernels, those with mass in the lower 10^{th}, were significantly associated with aflatoxin contamination ($p < 0.001$). Contamination with aflatoxin was non-independent from contamination with fumonisin ($p < 0.001$, odds ratio (OR) = 4.6), with 10% of kernels in this study having both aflatoxin and fumonisin above levels of concern.

While almost all highly contaminated kernels showed the presence of at least one factor associated with mycotoxin contamination, a few asymptomatic kernels had aflatoxin above the maximum tolerable limits. Out of the 92 kernels with aflatoxin > 10 ng g^{-1}, 4 kernels had aflatoxin levels ranging 14 to 481 ng g^{-1} and did not exhibit any of the factors associated with mycotoxin contamination. None of the 27 kernels with fumonisin > 1,000 ng g^{-1} were asymptomatic. A previous single-kernel study that investigated the relationship between mycotoxin contamination, discoloration, and fluorescence

under ultraviolet light, reported a few asymptomatic kernels with aflatoxin levels up to 17 ng g^{-1} and fumonisin levels up to 1,300 ng g^{-1} (Pearson et al., 2010).

In multivariate logistic regression (**Table 2**), factors significantly associated with higher odds of both aflatoxin > 10 ng g^{-1} and fumonisin > 1,000 ng g^{-1} included discoloration (aflatoxin OR = 4.6, fumonisin OR = 4.2), insect damage (aflatoxin OR = 5.3, fumonisin OR = 3.2), and toxin-specific fluorescence under UV light (aflatoxin OR = 2.6; fumonisin OR = 3.8). In addition, the lightest kernels in each sample set had higher odds of aflatoxin presence ($p < 0.001$, OR = 9.7), and kernels with breakage had borderline significant higher odds of fumonisin presence ($p = 0.051$, OR = 2.8).

4. Discussion

4.1 Sorting strategies to reduce aflatoxin and fumonisin can meet a real need in African maize value chains.

The efficacy of sorting Kenyan market maize with a relatively simple multi-spectral sorter are consistent with results that have been reported based on sorting maize with more sophisticated spectrometry. High-speed dual–wavelength sorting reduced aflatoxin and fumonisin levels in commercial yellow maize samples by around 80% (Pearson et al., 2004), and identified over 95% of extensively discolored, fungus-infected grains (Wicklow & Pearson, 2006). In dual-wavelength sorting of white maize samples, using reflectance of 500 nm and 1,200 nm, fixed reject rates of 4 to 9% achieved an average reduction of aflatoxin by 46% and fumonisin by 57% (Pearson et al., 2010). One remaining challenge for is sorting technology is that while mycotoxin levels were reduced, on average, by just over 80%, in some cases aflatoxin or fumonisin levels remaining in the accepted fraction were still above levels of concern. This shows that while the current technology could improve food safety, it is not yet sufficient to ensure mycotoxins levels are below concern. Overall, this relative simple, multi-spectral sorter has shown potential to reduce mycotoxins in Kenyan maize. Follow-up for this study should work to (i) improve the theoretical performance of the machine, e.g. by improving hardware or software, and (ii) better adapt the sorting technology to the conditions in the local hammer mills where it is intended for use, e.g. by reducing the cost of components and increasing throughput.

The use of this type of optical sorting technology in local hammer mills could improve upon classic food processing operations for mycotoxin reduction in maize. In resource-constrained households, many of these traditional food processing operations are labor intensive and do not integrate directly into the preferred maize value-chain involving local hammer milling. Traditional food processing steps of winnowing, washing, crushing, and dehulling were responsible for aflatoxin and fumonisin removal rates between 40-90% for traditional food products in Benin (Fandohan et al., 2005). Manual sorting of kernels to remove visibly infected or damaged maize can remove up to 70% of the fumonisin in the maize under laboratory conditions, and addition of a washing step with ambient temperature water is able to remove an additional 13% of fumonisin (van Der Westhuizen et al., 2011). When carried out by residents of subsistence farming communities, a similar procedure reduced fumonisin in maize by 84% and in porridge by 65% (van der Westhuizen et al., 2010). Traditional sorting prior to milling reduced fumonisin in post-milling maize flour by a mean of 65%, but was ineffective at reducing aflatoxin levels (Mutiga et al., 2014). We have found that density-based sorting can also remove a substantial proportion of aflatoxin from maize samples (RJN, unpublished).

The sorting technology evaluated here, perhaps combined with density-based sorting, could be integrated directly into existing local hammer milling infrastructure as a grain cleaning unit operation directly prior to milling, perhaps strategically located at the entrance to local open-air markets. With access to such technology, consumers would able to apply an inexpensive intervention to remove the most heavily mycotoxin-contaminated kernels and then consume the majority of their existing food with minimal exposure to mycotoxins. Coupled with information access, this could enhance consumer awareness of the issues and thus provide incentives for implementation of mycotoxin management measures throughout the maize value chain.

4.2 Opportunities to improve the performance of multi-spectral sorting.

Further improvements to the performance of this multi-spectral sorting technology could be driven by hardware improvements and further research to overcome some limitations to sorting algorithm.

Concerning hardware, increasing or optimizing the emission spectra range of the sorter may increase performance; it is not a given the discrete LEDs evaluated in the study (with spectral ranges from 470 to 1,070 nm or 700 to 1,550 nm) are the best for this particular application, although previous literature supports their use. Previous sorting work, which selected the best features from a full-spectrum scan experiment, used 500 nm (blue-green) and 1,200 nm spectra to discriminate white maize kernels with high levels of aflatoxin (> 100 ng g^{-1}) or fumonisin (> 40 ppm) from those with low levels (< 10 ng g^{-1} or < 2 ng g^{-1} aflatoxin or fumonisin, respectively) (Pearson et al., 2010). In contrast, only near-infrared spectra, 750 and 1,200 nm, were optimal for high-speed sorting of yellow maize (Pearson et al., 2004). Maize samples in this study included both white and yellow kernels and the best performing algorithm used the full range of LEDs from 470-1,070 nm.

One potential improvement supported by data would be to incorporate ultraviolet light into the panel of emission LEDs. Our results found that fluorescence under ultraviolet light was a risk factor for aflatoxin and fumonisn contamination. In addition, in the hyperspectral imaging work described above, peak fluorescence from 365 nm excitation was characteristic of aflatoxin contamination (Yao et al., 2010) and subsequent work showed that 260 nm excitation of aflatoxin extracts from maize kernels showed a 600 nm peak that was free from interference by kojic acid (Hruska et al., 2014). Given the current hardware setup it would be relatively simple to add UV LEDs to the circuitry to evaluate ultraviolet fluorescence in real-time sorting applications.

There are also a few limitations to the sorting algorithm itself, which could be addressed by further research. We chose to sort maize based on a calibration that was about 80% accurate to identify kernels with aflatoxin >10 ng g^{-1} and fumonisin >1,000 ng g^{-1}, but we do not know the optimum classification threshold. Choice of an optimal classifier for this mycotoxin sorting problem is difficult because it would require both knowledge of expected proportion of kernels in each class (class skew) and the costs associated with misclassification of both contaminated and uncontaminated kernels (error costs) (Fawcett, 2006). While there is strong prior knowledge (supported by our results) that naturally-occurring mycotoxin contamination is highly skewed towards low rates of contamination, misclassification costs for this problem are more difficult to quantify. One would have to balance the impact of low specificity on food security (through increased sorting losses) with the impact of low sensitivity on health (through consuming a larger number of highly-contaminated maize kernels).

Because this technology is intended for use among food insecure populations, we chose to prioritize minimizing food loss. Therefore, we chose the best calibration as one with maximum specificity for which further increases in specificity would dramatically reduce sensitivity. An additional advantage of using the aflatoxin >10 and fumonisin >1,000 ng g^{-1} thresholds for sorting is those thresholds nominally target kernels that exceed levels of concern for each mycotoxin. In contrast, use of a more stringent threshold would reject additional kernels that are unlikely to negatively impact health and may increase increased food losses.

Two more caveats should be noted. The first is that the full set of single kernel data was used for training, with sensitivity and specificity calculated from cross-validation. While the algorithm was validated by sorting novel bulk maize samples, additional work could validate the single kernel performance of the classification algorithm. One approach would be to analyze single kernels from the sorted bulk maize samples that are classified as toxin positive or negative to determine empirical false negative and positive rates.

The second caveat is that calibration kernels were taken from bulk samples known to be mycotoxin positive. This selection creates a bias towards analyzing samples where *Aspergillus* of *Fusarium* fungi are capable of producing mycotoxins. One well-accepted method of aflatoxin biocontrol is to inoculate fields with *Aspergillus* incapable of producing aflatoxin that are then able to exclude aflatoxin producing strains (Wu & Khlangwiset, 2010). A biocontrol product being promoted in Africa as "Aflasafe" (Aflasafe.com) has strong potential for adoption in Kenya (Marechera & Ndwiga, 2015). Further work is needed to develop sorting algorithms that could accommodate maize treated with atoxigenic strain(s) .

4.3 Single kernel phenotyping reveals multiple targets for sorting-based mycotoxin management.

Our results showed a skewed distribution of aflatoxins and fumonisins in market samples and confirmed that phenotypes of discoloration, insect damage, and fluorescence under ultraviolet light are associated with mycotoxin contamination. The skewed rates and levels of contamination observed here in kernels from Kenyan market maize samples are consistent with existing literature, although the precise nature of the distribution are likely to vary. In a study of single kernels from intact ears of U.S. corn with visibly evident contamination characteristic of *A. flavus*, only 23%, 27%, and 41% of single kernels in three samples were contaminated with aflatoxin above 100 ng g^{-1} (Lee et al., 1980), and contaminated kernels had levels up to 80,000 ng g^{-1}. Similarly, studies using wound inoculated corn found that 13 of 300 (4%) of randomly selected kernels contained aflatoxin above 10 ng g^{-1}, two of those kernel above 1,000 ng g^{-1} (Pearson et al., 2001), and 13% of non-BGYF kernels contained aflatoxin above 20 ng g^{-1} (Yao et al., 2010). A study with Kenyan maize sampled from *A. flavus* inoculated field trials found only 6 and 20% of single kernels in two samples were contaminated with aflatoxin above 20 ng g^{-1} (Turner et al., 2013), but toxin-positive kernels contained up to 85,000 ng g^{-1} total aflatoxin (Turner et al., 2013). Single-kernel analysis of fumonisn in maize found that only 20% of visibly infected kernels contained detectable fumonisins, and 15 of the 300 kernels contained more than 100 mg kg^{-1} fumonisins (Mogensen et al., 2011). These data support the general view that naturally-occurring aflatoxin and fumonisin contamination of maize kernels is highly skewed. Given the biases in our kernel selection strategy to enrich for contaminated kernels (fluorescence screening and artificial inoculation), further work is required to understand the underlying variability in rates and levels of mycotoxin contamination

in single kernels. Future studies should involve larger random samples of single kernels from a more diverse set of market and field conditions.

Finding that fluorescence under ultraviolet light is a risk factor for aflatoxin and fumonisin contamination in Kenyan maize builds upon a body of literature that has evaluated BGYF as indicator of kojic acid, an imperfect indicator of aflatoxin contamination (Shotwell & Hesseltine, 1981). Single-kernel maize studies have shown that BGYF (Pearson et al., 2001; Yao et al., 2010) and BGYF with discoloration (Pearson et al., 2010) are risk factors for aflatoxin. Additionally, bright orangish fluorescence (BOF) with discoloration has been identified as a risk factor for fumonisin (Pearson et al., 2010). Another line of research has used hyperspectral reflectance in the 400-600 nm range of single kernels excited with 365 nm light to determine aflatoxin contamination, with an 84% and 91% accuracy to classify kernels with aflatoxin >20 or 100 ng g^{-1} aflatoxin (Yao et al., 2010). Results from this study suggest that fluorescence under ultraviolet light could be useful not only as an indicator of aflatoxin contamination, but simultaneously for fumonisin contamination as well. This has significant relevance for the African maize value chain, where the two toxins frequently co-occur.

Our study also confirms that general indicators of low-quality maize, such as insect damage, discoloration, breakage, and low mass, can be specifically useful features for managing mycotoxin contamination. A commercial, speed-sorting study of white corn in the USA, intended to remove aflatoxins and fumonisins, specifically highlighted insect-damaged BGYF kernels as a critical challenge for optical sorting (Pearson et al., 2010). The germ portion of the kernel was entirely consumed without other external symptoms such as moldiness or discoloration. Insect damage is a vector for both *A. flavus* and *F. verticillioides* contamination and subsequent mycotoxin-contamination (Miller, 2001; Wicklow, 1994). Consistent with this observation, aflatoxin-contaminated maize kernels have previously been shown to have lower average mass than uncontaminated kernels from the same ear (Lee et al., 1980). And in Kenyan maize, single kernel breakage was previously associated with aflatoxin and fumonisin levels (Mutiga et al., 2014). One possible advantage of the visible to infrared spectra employed in this study was the simultaneous ability to assess visible discoloration (through differences in visible light reflectance) and possibly assess density changed (though difference in NIR reflectance). In addition, these observations suggest that grain cleaning operations, removing low mass or low density kernels, could complement the multi-spectral sorting as an integrated approach to mycotoxin management.

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721 **Tables**

722 **Table 1.**

723 Selection process for individual maize kernels sourced from two concurrent studies in Kenya.

Kernel selection process component	Maize sample source study	
	Kenyan market maize survey [a]	*A. flavus* inoculated field trial [a]
Method to enrich for bulk samples with mycotoxin contamination	Randomly selected bulk samples that previously tested above 10 ng g^{-1} aflatoxin or 1,000 ng g^{-1} fumonisin	Field maize was inoculated with an aflatoxin-producing *A. flavus*.
Method to enrich for mycotoxin contamination among of individual kernels that are scanned	Select all kernels exhibiting BGYF[b] or BOF[b] and randomly select 3 non-fluorescent kernels from the same bulk sample; n=233	None deemed necessary, due to the field inoculation. Kernels were selected randomly; n=220
Number of bulk and individual kernels selected	25 bulk samples from sites in Meru (68 kernels), Machakos (46 kernels), or Kitui (44 kernels)	17 bulk samples from distinct genetic lines selecting 10 kernels from each of 12 samples and 20 kernels from each of 5 samples
Single kernel spectroscopy	Triplicate scan through sorting machine using low (nirL) and high (nirH) wavelength circuit board (9 primary features on each board, 470-1,070 and 910-1,150 nm, nirL and nirH boards) Duplicate scan by FT-NIR (1,154 primary features, 800-2,780 nm)	
Method to enrich for spectral diversity among scanned kernels assayed by wet chemistry	Principal component analysis of FT-NIR spectra. Sample across first principal component, stratified by site.	None. The source study had resources to assay all kernels.
Number of single kernels subject to mycotoxin analysis	158 kernels	220 kernels
Aflatoxin analytical method	ELISA (this study)	UHPLC[b] (concurrent study)
Fumonisin analytical method	ELISA (this study)	ELISA (this study)
Phenotypes scored in physical examination	BGYF/BOF	Fluorescence under UV light[c]
	Insect damage	Insect damage
	Discoloration	Discoloration
	Mass	Mass
	Whole kernel or dehulled	All whole kernel
Kernels used for sorter calibration	All assayed (n=158)	All assayed (n=220)
Bulk samples used for sorter validation	46 bulk samples from the market maize survey	None used

724 [a] References: Market survey (Eliphus, 2014). Field trial (Falade, et al., 2014).

725 [b] BGYF, Bright Greenish Yellow Fluorescence. BOF, Bright Orangish Fluorescence. UHPLC, Ultra-High
726 Performance Liquid Chromatography

727 [c] Only 120 kernels were available for physical examination, and in this examination fluorescence under
728 UV light was not differentiated between BGYF or BOF.

729

730 **Table 2.**

731 Logistic regression for factors associated with contamination of single kernels with aflatoxin or
732 fumonisin.

	Model for Aflatoxin > 10 ng g^{-1}			Model for Fumonisin > 1,000 ng g^{-1}		
Model Parameter	Parameter Estimate	Odds Ratio (95% CI)	P-value	Parameter Estimate	Odds Ratio (95% CI)	P-value
Intercept	-3.61	0.03 (0.01, 0.08)	<0.001	-4.40	0.01 (<0.01, 0.03)	<0.001
Discoloration [a]	1.52	4.58 (2.11, 10.31)	<0.001	1.44	4.22 (1.42, 15.55)	0.016
Insect Damaged [a]	1.67	5.31 (2.21, 13.51)	<0.001	1.16	3.18 (1.21, 8.36)	0.018
Mass in Lower 10th [a]	2.27	9.70 (2.89, 38.23)	0.001	-	-	-
Toxin Specific Fluorescence	0.96	2.62 (1.27, 5.52)	0.010	1.35	3.84 (1.48, 10.82)	0.007
Site: Field trial [b]	1.79	6.00 (1.90, 22.2)	0.004	-	-	-
Site: Kitui [b]	1.56	4.76 (1.38, 18.86)	0.018	-	-	-
Site: Meru [b]	-0.71	0.49 (0.09, 2.38)	0.383	-	-	-
Breakage [a]	- [c]	-	-	1.02	2.78 (0.98, 7.75)	0.051
Full model	-	-	<0.001	-	-	<0.001

733 [a] Parameter estimates are for the presence of the factor.

734 [b] Parameter estimates contrast with the category reference of Site: Machakos.

735 [d] -, Parameter not estimated in the best fitting final model.

736 **Table 3.**

737 Optical features used to identify and reject kernels with aflatoxin or fumonisin above given thresholds.

Nominal Feature Number	Individual Feature Component 1	2	3	Operation
nirL board rejecting aflatoxin > 10 ng g^{-1} or fumonisin > 1,000 ng g^{-1}				
1	880 nm	Infrared sum	- [a]	Difference
2	910 nm	Infrared sum	-	Difference
3	910 nm	Color sum	-	Ratio
nirH board rejecting aflatoxin > 10 ng g^{-1} or fumonisin > 1,000 ng g^{-1}				
1	700 nm	-	-	Absolute
2	940 nm	1070 nm	-	Difference
3	700 nm	970 nm	1070 nm	2nd derivative
nirHL *in silico* board rejecting aflatoxin > 10 ng g^{-1} or fumonisin > 100 ng g^{-1}				
1	940 nm	Infrared sum	-	Difference
2	Blue [b]	Red [b]	Color sum	2nd derivative
3	700 nm	970 nm	1070 nm	2nd derivative

738 [a] -, component not used for the calculation of this feature

739 [b] Blue (470 nm), Green (527 nm), and Red (624 nm)

740 **Figure Legends**

741 **Fig. 1.** Distribution of aflatoxin and fumonisin levels in single kernels from both the market maize
742 survey (n=158) and the *A. flavus* inoculated field trial (n=220). Kernels below the level of detection for
743 each mycotoxin (LOD of 1 ng g^{-1} for aflatoxin and 100 ng g^{-1} fumonisin) were counted in the bar just to
744 the left of the level of detection. Light grey bars, kernels tested below LOD; dark grey bars, kernels
745 tested below level of concern; black bars, kernels test above levels of concern of 10 ng g^{-1} aflatoxin or
746 1,000 ng g^{-1} fumonisin.

747 **Fig. 2.** Performance of the three-feature, linear discriminant analysis algorithm to reject single
748 maize kernels contaminated with aflatoxin or fumonisin at various thresholds. The algorithm was trained
749 to classify all 378 kernels using measured aflatoxin and fumonisin contamination and the best 3 of 205
750 features from the spectra captured by the lower range (470-1,070 nm, nirL) and higher range (910-1,550
751 nm, nirH) circuit board, as well as the *in silico* composite board (470-1,154 nm, nirHL with 816 features).

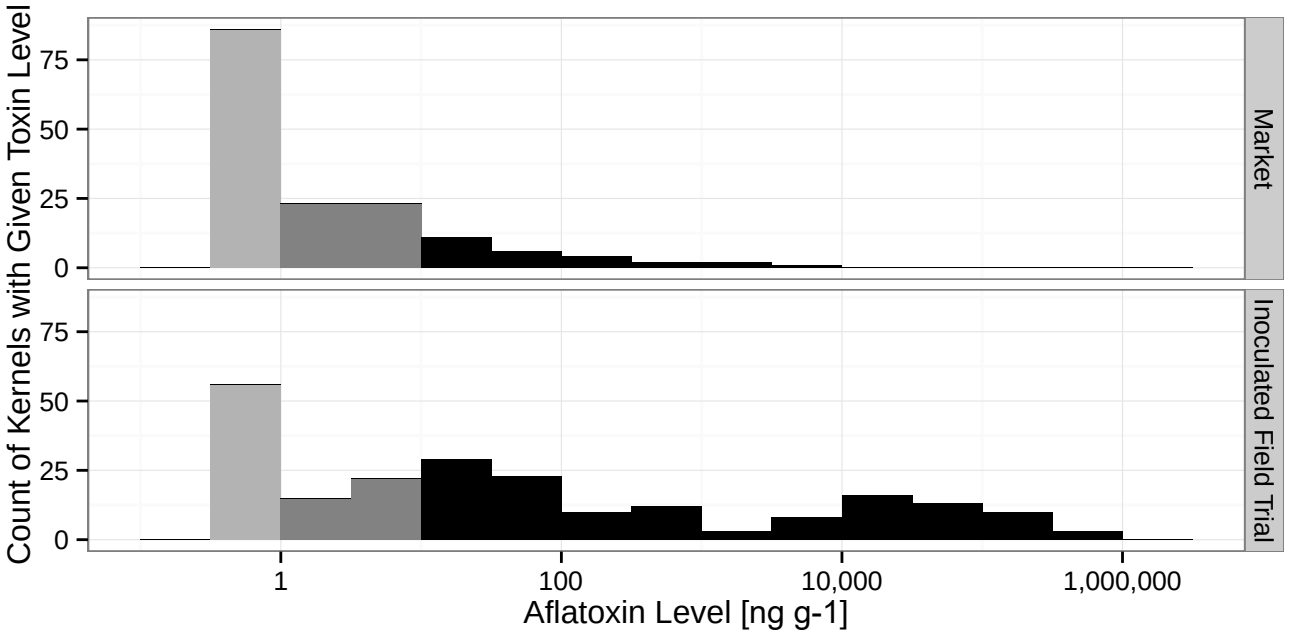
752 **Fig. 3.** Performance of alternative classification algorithms to reject single kernels contaminated
753 with aflatoxin or fumonisin at various thresholds. Each algorithm was trained to classify all 378 kernels
754 using measured aflatoxin and fumonisin contamination and the appropriate combination of 205
755 features from the spectra captured by the lower range (470-1,070 nm, nirL) circuit board. Algorithms
756 evaluated are: LDA, linear discriminant analysis; RF, random forest; and SVM, support vector machines.

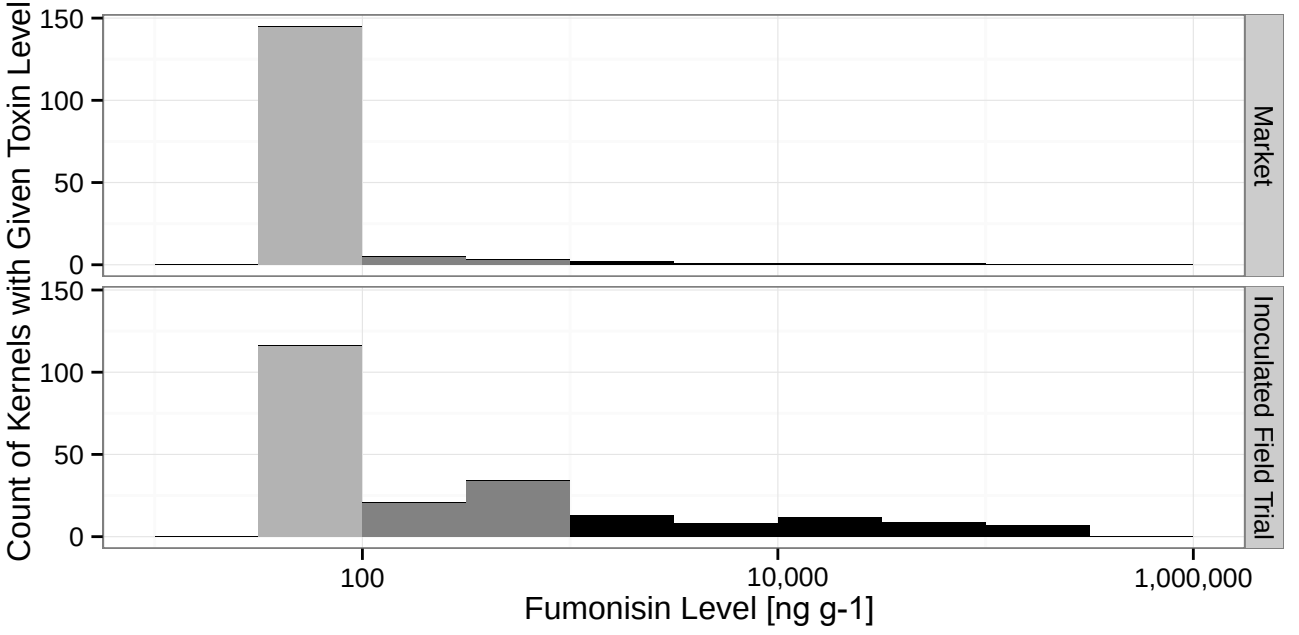
757 **Fig. 4.** Reject rates in decimal mass fraction of sorted maize samples as classified by mycotoxin
758 presence in previous bulk sample ELISA. For each box the solid line is the median reject rate, box height
759 is the inner quartile range, whiskers extend to the most extreme value within 1.5*IQR of the box, and
760 outliers are plotted as points.

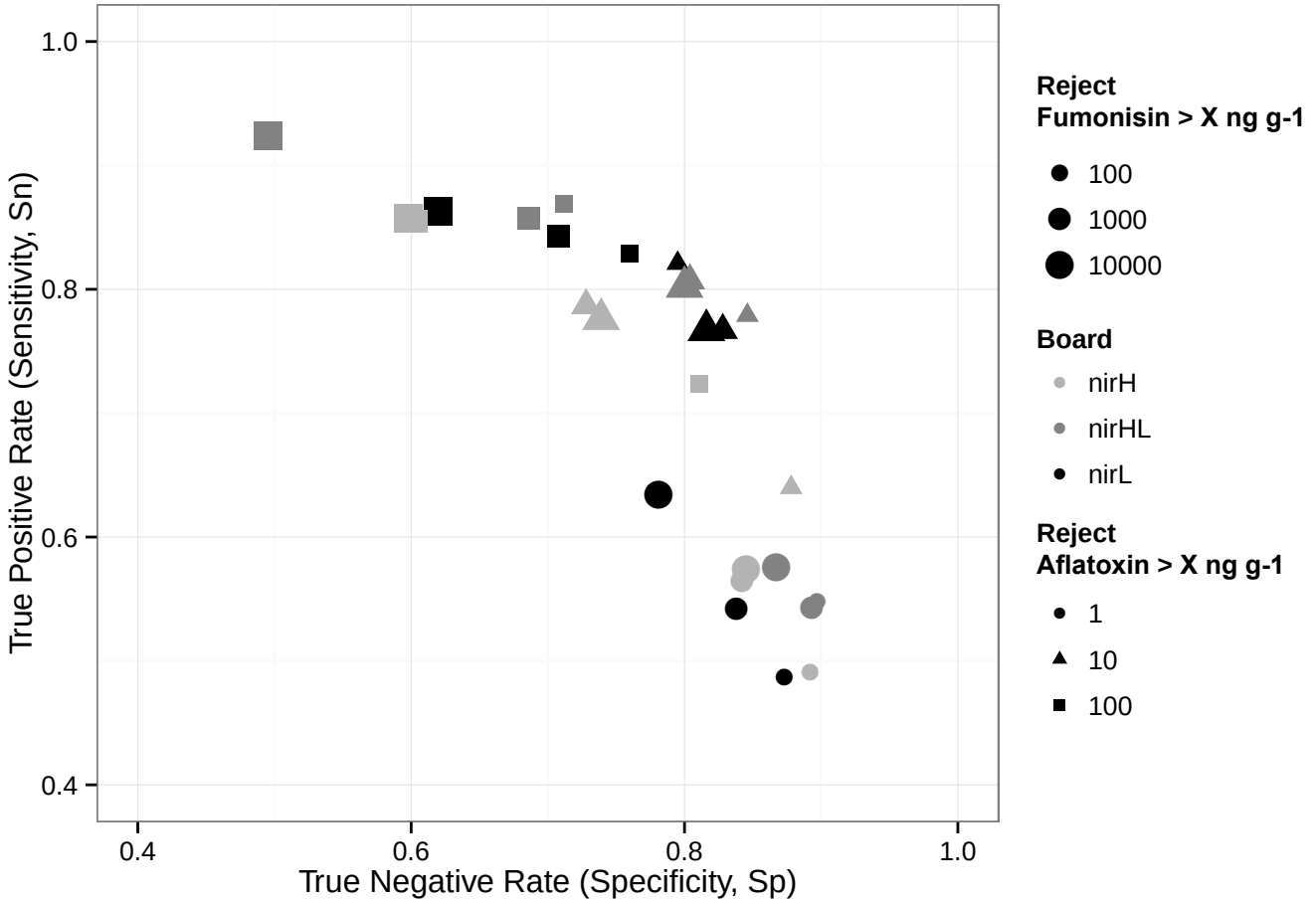
761 **Fig. 5.** Aflatoxin (top) and fumonisin (bottom) levels in the accept and reject streams of 46 Kenyan
762 market maize samples sorted to removed single kernels with aflatoxin > 10 ng g^{-1} or fumonisin > 1,000
763 ng g^{-1}, using the visible to infrared spectrum board (nirL). A downward pointing arrow indicates that
764 mycotoxin levels were reduced in the accept stream of the sorted maize sample. Points with no
765 detectable mycotoxin are plotted at the LODs.

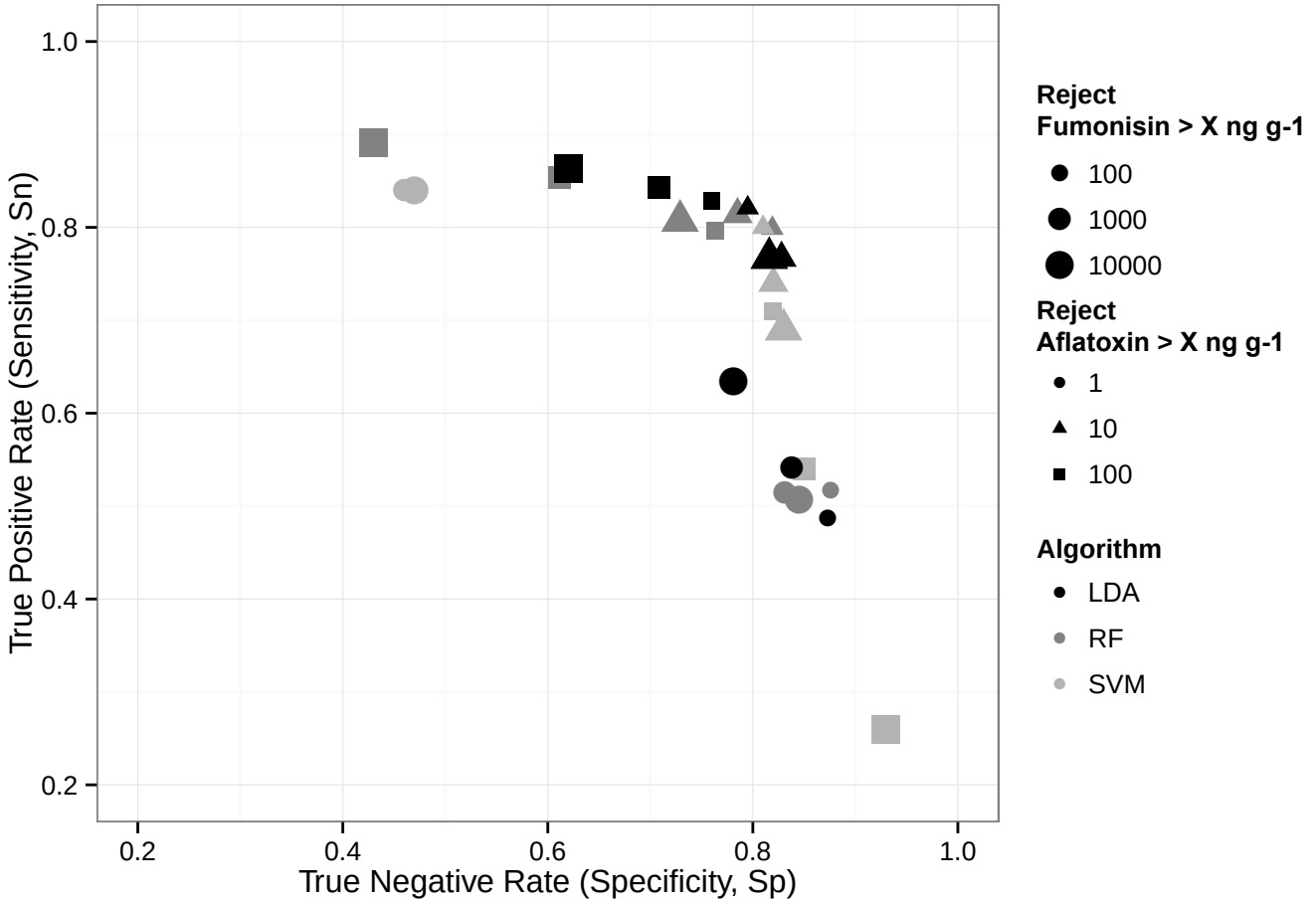
766 **Supplemental Figure Legends**

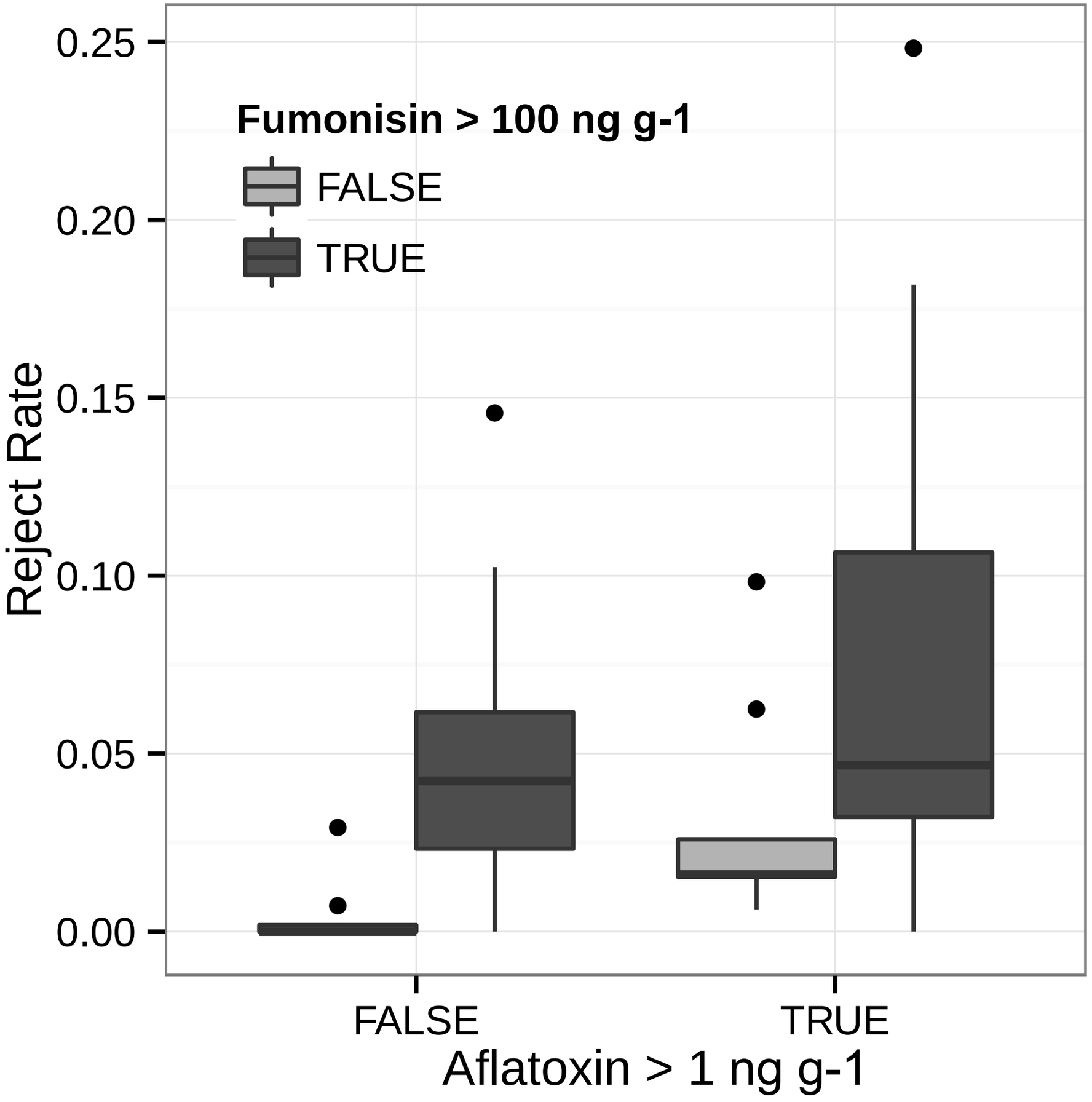
767 **Fig. S1.** Performance of the alternative classification algorithms to reject single kernels
768 contaminated with aflatoxin or fumonisin at various thresholds. Each algorithm was trained to classify all
769 378 kernels using measured aflatoxin and fumonisin contamination and the appropriate combination of
770 205 features from spectra captured by the higher range (910-1,550 nm, nirH) circuit board. Algorithms
771 evaluated are: LDA, linear discriminant analysis; RF, random forest; and SVM, support vector machines.


Count of Kernels with Given Toxin Level
75
50
25
0
Market
Inoculated Field Trial
1
100
10,000
1,000,000
Aflatoxin Level [ng g-1]


Count of Kernels with Given Toxin Level
150
100
50
0
Market
Inoculated Field Trial
100
10,000
1,000,000
Fumonisin Level [ng g-1]


1.0
0.8
0.6
0.4
True Positive Rate (Sensitivity, Sn)
0.4
0.6
0.8
1.0
True Negative Rate (Specificity, Sp)
Reject
Fumonisin > X ng g-1
100
1000
10000
Board
nirH
nirHL
nirL
Reject
Aflatoxin > X ng g-1
1
10
100


1.0
0.8
0.6
0.4
0.2
True Positive Rate (Sensitivity, Sn)
0.2
0.4
0.6
0.8
1.0
True Negative Rate (Specificity, Sp)
Reject
Fumonisin > X ng g-1
100
1000
10000
Reject
Aflatoxin > X ng g-1
1
10
100
Algorithm
LDA
RF
SVM


Fumonisin > 100 ng g-1
FALSE
TRUE
Reject Rate
0.25
0.20
0.15
0.10
0.05
0.00
FALSE
TRUE
Aflatoxin > 1 ng g-1

Aflatoxin Level [ng g−1]
1000
10
Stream
Accept
Reject
K9−M3−L3 (4.1%)
M2−M3−L2 (18.2%)
K9−M3−L1 (6.3%)
C4−M1−L1A (2.2%)
M8−M2−L1A (7.4%)
M3−M3−L3 (13.3%)
M10−M1−L1A (4.3%)
C2−M1−L5 (1.5%)
K6−M3−L1A (2.3%)
C9−M2−L1A (6.4%)
C10−M1−L1 (10.2%)
M4−M2−L1 (5.4%)
M8−M3−L2A (24.8%)
K10−M2−L1 (4.7%)
K3−M1−L3 (9.8%)
C10−M2−L3 (1.6%)
M3−M3−L2 (14.6%)
K7−M1−L1A (6.3%)
K5−M1−L1A (0.6%)
K10−M3−L1A (2.6%)
M2−M1−L1A (5.5%)
C10−M1−L2 (8.0%)
C4−M2−L1 (0.7%)
C7−M2−L3 (1.6%)
C8−M2−L1 (2.9%)
K1−M2−L2A (1.1%)
K2−M1−L3 (4.3%)
K3−M2−L1 (2.3%)
K7−M2−L2 (1.1%)
K7−M3−L1A (2.5%)
M1−M2−L1A (9.1%)
M5−M3−L2 (5.2%)
M5−M3−L3 (1.1%)
M6−M1−L3 (4.2%)
M6−M2−L2 (2.4%)
M7−M1−L1 (1.3%)
M7−M1−L2 (2.3%)
M9−M3−L3 (1.2%)
K7−M1−L2 (0.0%)
C9−M3−L2A (0.0%)
K10−M2−L4 (0.0%)
K3−M2−L2 (0.0%)
K5−M2−L1A (0.0%)
K6−M2−L2 (0.0%)
K8−M2−L1 (0.0%)
K8−M2−L2A (0.0%)
Sample (Reject %)

Fumonisin Level [ng g−1]
1e+05
1e+03
M2−M3−L2 (18.2%)
C8−M2−L1 (2.9%)
M6−M2−L2 (2.4%)
C9−M2−L1A (6.4%)
K6−M3−L1A (2.3%)
K9−M3−L3 (4.1%)
M2−M1−L1A (5.5%)
M7−M1−L2 (2.3%)
M3−M3−L2 (14.6%)
K5−M1−L1A (0.6%)
K10−M2−L1 (4.7%)
M4−M2−L1 (5.4%)
C4−M1−L1A (2.2%)
M10−M1−L1A (4.3%)
M7−M1−L1 (1.3%)
K7−M2−L2 (1.1%)
M5−M3−L2 (5.2%)
M6−M1−L3 (4.2%)
M5−M3−L3 (1.1%)
M3−M3−L3 (13.3%)
K2−M1−L3 (4.3%)
M9−M3−L3 (1.2%)
C10−M1−L2 (8.0%)
C10−M1−L1 (10.2%)
M8−M3−L2A (24.8%)
K9−M3−L1 (6.3%)
K7−M1−L1A (6.3%)
C10−M2−L3 (1.6%)
C2−M1−L5 (1.5%)
C4−M2−L1 (0.7%)
C7−M2−L3 (1.6%)
K10−M3−L1A (2.6%)
K1−M2−L2A (1.1%)
K3−M1−L3 (9.8%)
K3−M2−L1 (2.3%)
K7−M3−L1A (2.5%)
M1−M2−L1A (9.1%)
M8−M2−L1A (7.4%)
K7−M1−L2 (0.0%)
C9−M3−L2A (0.0%)
K10−M2−L4 (0.0%)
K3−M2−L2 (0.0%)
K5−M2−L1A (0.0%)
K6−M2−L2 (0.0%)
K8−M2−L1 (0.0%)
K8−M2−L2A (0.0%)
Sample (Reject %)

- Multi-spectral sorting can reduce aflatoxins and fumonisin mycotoxin in Kenyan market maize
- Simple multi-spectral sorting may facilitate mycotoxin management by the consumer
- Mycotoxin distribution in single-kernels of Kenyan market maize is skewed even under UV fluorescence enrichment.