

Reduction of Aflatoxin and Fumonisin Contamination in Yellow Corn by High-Speed Dual-Wavelength Sorting

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ABSTRACT

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A high-speed dual-wavelength sorter was tested for removing corn contaminated in the field with aflatoxin and fumonisin. To achieve accurate sorting, single kernel reflectance spectra (500–1,700 nm) were analyzed to select the optimal pair of optical filters to detect mycotoxin-contaminated corn during high-speed sorting. A routine, based on discriminant analysis, was developed to select the two absorbance bands in the spectra that would give the greatest classification accuracy. In a laboratory setting, and with the kernels stationary, absorbances at 750 and 1,200 nm could correctly identify >99% of the kernels as aflatoxin-contaminated (>100 ppb) or uncontaminated. A high-speed sorter was

tested using the selected filter pair for corn samples inoculated with *Aspergillus flavus*; naturally infested corn grown in central Illinois; and naturally infested, commercially grown and harvested corn from eastern Kansas (2002 harvest). For the Kansas corn, the sorter was able to reduce aflatoxin levels by 81% from an initial average of 53 ppb, while fumonisin levels in the same grain samples were reduced an average of 85% from an initial level of 17 ppm. Similar reductions in mycotoxin levels were observed after high-speed sorting of *A. flavus* inoculated and naturally mold-infested corn grown in Illinois.

Mycotoxin contamination of corn (maize, *Zea mays* L.) affects the quality and safety of human food and animal feeds, thereby lowering the value of the corn and resulting in substantial economic losses to corn growers, livestock and poultry producers, grain handlers, and food and feed processors (Richard and Cole 1989). Prominent among the mycotoxins associated with such losses in corn are aflatoxins produced by *Aspergillus flavus* (Link) and fumonisin produced by *Fusarium verticillioides* (Sacc.) Nirenberg (syn.: *F. moniliforme* Sheld.), and *Fusarium proliferatum* (Matsushima) Nirenberg. To reduce the risks to animal and human health, the U.S. Food and Drug Administration (FDA) requires that corn intended for use in food, feed for dairy animals, or feed for immature animals contain <20 ppb of aflatoxin (U.S.FDA/CFSCAN 2000) and has issued guidance levels of 4.0 ppm for whole or partially degermed dry-milled corn products, dry-milled corn bran, cleaned corn intended for masa production, cleaned corn intended for popcorn, and guidance levels of 2 ppm for degermed dry-milled corn products intended for human consumption (U.S. FDA/CFSCAN 2001). If a sorter can simultaneously remove both aflatoxin and fumonisin from corn, it will improve the quality and safety of grain marketed domestically or internationally.

Seeds highly contaminated by aflatoxin are unevenly distributed in a seed lot and may be concentrated in a very small percentage of the product (Whitaker et al 1970; Hirano et al 1998; Pearson et al 2001). Therefore, removing a small percentage of contaminated kernels instead of discarding the entire lot is a reasonable approach for reducing aflatoxin contamination to satisfy statutory levels. Regional aflatoxin outbreaks are commonly accompanied by outbreaks in fumonisin (Mubatanhema et al 2002) and therefore aflatoxin and fumonisin can be present at unacceptable levels in the same grain samples (Chamberlain et al 1993; Chu and Li, 1994; Yoshizawa et al 1996; Shetty and Bhat 1997; Ali et al 1998; Medina-

Martinez and Martinez 2000; Ono et al 2001). The co-occurrence of these mycotoxins may increase the cancer risk of aflatoxin exposure. Fumonisin B1 was recently shown to promote aflatoxin B1-initiated liver tumors when fed to rainbow trout (Carlson et al 2001). Therefore, we also seek to simultaneously eliminate both aflatoxin-contaminated and fumonisin-contaminated kernels in a single pass through a commercial optical sorter.

Mycotoxin-contaminated kernels tend to be less dense than kernels not infested with molds. As such, physical methods have been developed to separate mycotoxin-contaminated kernels by flotation in water, sucrose, or salt solutions (Huff 1980; Huff and Hagler 1982; Huff and Hagler 1985; Rotter et al 1995; Shetty and Bhat 1999). The separation and removal of the buoyant kernel fractions significantly reduced aflatoxin, fumonisin, deoxynivalenol, and zearalenone contamination of nonbuoyant grain. Such procedures lack specificity and can result in segregation and removal of a substantial proportion (>20%) of the grain sample. In addition, flotation methods have an added cost of drying the uncontaminated grains if used commercially (Scott 1991). Finally, standard post-harvest cleaning operations (aspiration, gravity table separation, grain scouring, wet cleaning) are not entirely effective for separating mycotoxin-contaminated corn (Brekke et al 1975).

Aflatoxin contamination is potentially indicated by the black-light (365 nm) test used to screen corn lots for kernels exhibiting bright greenish-yellow fluorescence (BGYF) (Fennell et al 1973; Bothast and Hesselstine 1975; Shotwell and Hesselstine 1981). The fluorescence is produced by the oxidative action of heat-labile enzymes (peroxidases) in living plant tissue on kojic acid, which is produced by *A. flavus* (Marsh et al 1969). The BGYF method is not a definitive indicator of aflatoxin because contaminated corn sometimes does not exhibit BGYF (Wilson 1989), while kernels infected with *A. flavus* strains that produce kojic acid but do not produce aflatoxin exhibit BGYF and thus are aflatoxin “false positives” when corn grain is examined with a black light (Wicklow 1999). Tyson and Clark (1974) detected aflatoxin contamination of >20 ppm in pecan samples by BGYF detection. Farsaie et al (1981) developed an automatic sorter to remove BGYF pistachio nuts. It had an accuracy of 94% at an average rate of 18 pistachio nuts/sec. However, fumonisin-contaminated grain does not exhibit BGYF, so this mycotoxin is not detectable using this method. Birth and Johnson (1970) detected moldy yellow corn kernels with <5% classification error using the difference of logarithm of fluoresced energy at 442 and 607 nm.

Color changes can also be observed in grains when mold contamination develops to a later stage (Schmidt 1991; Wacowicz 1991). *A. flavus* yellow-green sporulation is visible in the germ region

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beneath the pericarp of some infected corn kernels (Jones et al 1980), and the fungus may cause other forms of kernel discoloration (Kumar and Agarwal 1997). Dickens and Whitaker (1975) reduced aflatoxin concentration in shelled peanuts by as much as 51% by cumulative removal of 10% of the kernels in five passes in an electronic color sorter followed by hand-picking. Color and form changes detectable visually are actually preceded by chemical changes in the grains caused by the fungus. Mycotoxin-producing molds initially infect the oil-rich germ using grain lipids for their growth and metabolism; thus lipid hydrolysis takes place faster than the degradation of protein or starch in stored grain (Wacowicz 1991; Pomeranz 1992). Lipids are broken down by lipases to free fatty acids and glycerol; thus the free fatty acid content of grain has been proposed as a sensitive index of incipient grain deterioration (Pomeranz 1992). Hirano et al (1998) found the transmittance differences between normal and moldy peanuts were highest at 700 and 1,100 nm, wavelengths believed to indicate fungal hydrolysis of triglycerides. Therefore, they used the transmittance ratio at 700–1,100 nm as a sorting criterion and found it could even separate surface-molded nuts from internally mold-infested nuts. Pearson et al (2001) found that the reflectance ratio (R_{735}/R_{1005}) from the germ side of the kernel could discriminate individual corn kernels highly contaminated (>100 ppb) with aflatoxin from corn with low levels of aflatoxin (<10 ppb). Two transmittance ratios at T_{710}/T_{760} and T_{615}/T_{645} also had similar results without the need to orient the kernels. Over 99% of the kernels with aflatoxin at >100 ppb and over 97% of the kernels with aflatoxin at <10 ppb were correctly classified using these techniques. While it is technologically feasible to develop a sorter that would segregate kernels based on transmittance at four different spectral bands or reflectance from only the germ side, no such sorters are commercially available, making transfer of this technology very difficult.

Given color and biochemical changes in mycotoxin contaminated kernels, electronic color sorters have the potential to segregate these from uncontaminated kernels. In the past, red optical filters were used to separate mold-contaminated products using monochromatic sorters (Dickens and Whitaker 1975). At present, dual-wavelength sorters have near-infrared detecting capability in addition to visible light capability, which may extend their usefulness for detecting mold-infected products based on both color and biochemical composition.

The objective of this study was to evaluate a commercial high-speed sorter equipped with dual-wavelength filters for reducing aflatoxin-contamination in shelled yellow corn. A secondary objective was to determine whether fumonisin levels in *Fusarium* infested grains were also reduced for the same corn samples.

MATERIALS AND METHODS

There were two phases of this project. The first phase involved inoculating corn ears in the field, recording reflectance spectra of individual kernels, then chemically measuring aflatoxin in each kernel so that the most discriminating pair of absorbance bands could be chosen to separate between aflatoxin-contaminated and uncontaminated kernels. Pearson et al (2001) reported the reflectance ratio (R_{735}/R_{1005}) from the germ side of the kernel could accurately distinguish contaminated corn. However, electronic sorters collect data from both sides of corn kernels, so the spectral data needed to be reanalyzed to account for this.

The second phase of the project involved application of the selected pair of absorbance bands in a commercial sorting machine for separating aflatoxin- and fumonisin-contaminated corn at high speeds. For this phase, *A. flavus* wound-inoculated and naturally infected corn from Illinois, as well as naturally infected grain, commercially grown and harvested in Kansas, was used. After sorting, bulk samples from the accept and reject streams were chemically analyzed for aflatoxin and fumonisin to evaluate the sorter's performance for removing kernels contaminated with these mycotoxins.

Corn Kernel Preparation

Pioneer 3394 corn kernels were harvested in 1998 from ears that were wound-inoculated as in Wicklow (1999) with *A. flavus* NRRL 32355 in the late milk to early dough stage of kernel maturity at the University of Illinois River Valley Sand Farm, Kilbourne, IL. Shortly after harvest (October 19, 1998), the corn kernels were examined under a black light (365 nm) and separated into categories based on BGYF characteristics: 1) intact kernels with BGYF over most of the kernel, 2) intact kernels with BGYF limited to the germ region, 3) intact kernels with BGYF limited to the lower germ/tip cap, and 4) intact non-BGYF kernels. Individual wound-inoculated kernels were discarded and only intact kernels surrounding each wound site, many of which had become infected by *A. flavus*, were studied. Heavily molded and light-weight kernels or kernel fragments were not included in this study, as they are usually dispersed during combine harvesting or removed by cleaning equipment at grain elevators. A total of 500 kernels were used for this study, 50 each from each of the four categories above, and 300 randomly selected kernels from the same lot without examination under a black light.

Spectra Measurement

Whole-kernel reflectance spectra from 500–1,700 nm were measured using a diode-array near-infrared spectrometer (DA7000, Perten Instruments, Springfield, IL). The spectrometer measures absorbance using an array of silicon (7 nm) and indium-gallium-arsenide sensors (11 nm). Each spectrum was collected in ≈ 33 msec and 15 spectra from each kernel were collected and averaged.

Kernels were manually placed on a bifurcated interconnectance probe attached to the spectrometer and light source (Fig. 1). The viewing area was 17 mm in diameter and 10 mm above the termination of the illumination and reflectance fibers. The illumination bundle was a 7-mm diameter ring, and the reflectance probe bundle was 2 mm in diameter. Spectra were first collected from all kernels oriented at the germ-down position (germ facing the optical fiber bundle), then a second set for kernels oriented germ-up. All spectra were stored on a hard disk for subsequent analysis.

Single-Kernel Chemical Analyses for Aflatoxin

Single-kernel aflatoxin levels were determined after the reflectance spectra of all kernels were measured. Individual kernels were placed in an envelope of folded weighing paper, weighed,

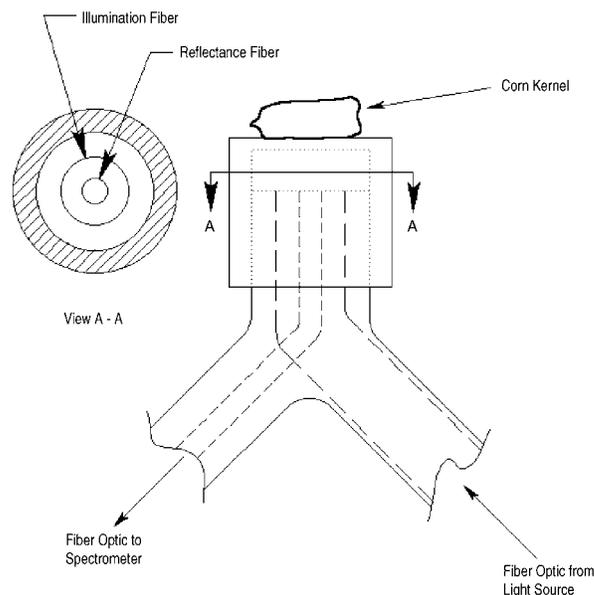


Fig. 1. Interconnectance probe used to collect reflectance spectra of individual kernels.

and crushed by striking with a hammer. The crushed kernels were then placed in small silitated vials and steeped for 2 hr in a volume of chloroform and water (0.0005% H₂O) equal to 5× the kernel weight and vortexed for 2 min; then the chloroform was transferred to a second silitated vial and dried for 1 hr. A solution of 80% methanol and water was added to each vial and the extract analyzed for aflatoxins following the USDA-FGIS Aflatest (Vicam, Watertown, MA) affinity chromatography procedures according to the manufacturer's instructions (0.5 ppb detection limit). Quantities used in the method equaled 2× the kernel weight. Dilutions 2, 10, or 100× the final filtrate were performed where required and fluorometer readings adjusted accordingly.

Spectra Data Analysis

When considering the application of high-speed sorting operations with the available dual-wavelength sorter, only two spectral bands, one band between 400 and 1,100 nm and the other between 1,100 and 1,700 nm, can be measured in real time. Furthermore, a high-speed sorter collects reflectance information from both sides of a kernel and the orientation of the kernel (germ-up or germ-down) is completely random. The sorter uses a pair of interference filters that need to be selected so they collectively give the greatest contrast between accept and reject product. The problem becomes one of selecting the most useful pair of absorbance bands given spectra from both sides of the kernel. For selecting absorbance bands, kernels with aflatoxin at >100 ppb were considered contaminated, while kernels with no detectable aflatoxin were considered uncontaminated. Spectra from ≈70% of kernels with aflatoxin detectable at <100 ppb are not different from kernels with no detectable aflatoxin (Pearson et al 2001).

All spectra were interpolated to 5-nm resolution between 500 and 1,700 nm, resulting in 241 absorbance values by the software (Perten Simplicity). The spectra were then convolved with Gaussian curves to simulate different full-width, half-maximum (FWHM) pass bands of interference filters 5, 10, and 20 nm wide. This increased the number of spectra threefold for a total of 723 potential features for identifying contaminated corn.

Discriminant analysis was used as the basis of a procedure to select the optimal pair of spectral bands for sorting. Both pooled and nonpooled covariance matrices (Huberty 1994) were considered. The means and covariances were computed using a random selection of half of the germ-down spectra. Spectra from the germ-down side of each kernel were used for this computation because the germ side was most useful for distinguishing contaminated from uncontaminated kernels (Pearson et al 2001). However, using these means and covariances, Mahalanobis distances were computed from all spectra from each kernel side (germ or endosperm) to the contaminated and uncontaminated groups. A kernel was classified as contaminated if the Mahalanobis distance from either the germ-up and germ-down spectra was closest to the contaminated group. This allowed kernels to be classified as contaminated if the spectra from only one side indicted contamination. This procedure was exhaustively performed for all possible pairs of absorbance values from the three convolved spectra where one absorbance was <1,100 nm and the other >1,100 nm. The pair of absorbance bands that obtained the lowest classification error rate was recorded and used for high-speed sorting.

Corn Kernel Preparation for High-Speed Sorting

Yellow dent corn (Pioneer 3394) grown at the University of Illinois River Valley Sand Field, Kilbourne, IL, in 1997 was inoculated with *A. flavus* NRRL 32355 using the inoculation procedure described above. However, corn ears were hand-harvested and the grain removed from the cob with a crank sheller (Black Beauty, Durbin-Durco, St. Louis, MO). *Aspergillus flavus* wound inoculations of the yellow dent corn ears (hybrid F S 7111) within six parallel 150-ft rows were performed in 2002 in a field near Kilbourne, IL. A field plot harvester with lateral bagging (Hege 125c,

Maschien, Waldenburg, Germany) was also used to harvest inoculated corn. Wound inoculations were performed using a knife to produce a 5-cm wound through the husk and damage to the underlying kernels. A pipe cleaner was first soaked in an *A. flavus* conidial suspension (1×10^5) and then inserted lengthwise into each wound site. Immediately before harvesting of ears, each row was surveyed, and all of the pipe cleaners were removed. Naturally infested portions of this field were harvested using both the Hege 125c and a commercial combine (Case IH 1688, Racine, WI). The Case IH 1688 was set up to harvest eight rows at a time and provide some cleaning of the corn, leaving behind 1–2% of the grain in the field. Mold and insect-damaged kernels are lighter in weight (Seitz et al 1982; Bennett et al 1988; Cardwell et al 2000) and are more readily dispersed with the dust and debris of blower discharge from combine cleaning fans (Wicklow et al 1984; Johnson and Lamp 1966). The Hege 125c was set up to harvest two rows at a time, collecting both intact kernels as well as the severely molded and damaged kernels in a lateral bag. This provided only minimal “cleaning” of the harvested grain.

A third source of aflatoxin-contaminated corn used to test the sorter was purchased from a grain elevator in northeast Kansas and was locally harvested in 2002. Northeast Kansas incurred a drought during this growing season that caused relatively widespread aflatoxin contaminations at >20 ppb and fumonisin contaminations at >10 ppm. All grain was shipped to Manhattan, KS, and randomly divided into two samples. Half of the Kansas grain was cleaned in a rotary cleaner (model ZP4; Simon-Carter Co., Minneapolis, MN) while the other half was sorted as received. The grain cleaner ran the corn through a tumbler constructed of perforated sheet metal with holes 4.6 mm in diameter. Cleaning removed ≈1% by weight of the incoming corn. The bulk of the removed material consisted of the glumes and paleas from fertile florets (Smart et al 1990) mixed with small pieces of broken kernels. All grain samples were placed in large paper containers and stored in a walk-in freezer at –20°C until retrieved for grain-sorting tests.

High-Speed Sorting

A high-volume optical sorter (ScanMasterII 2000 DE, Satake-USA, Houston, TX) was used in these experiments. This sorter uses two detectors, silicon and indium-gallium-arsenide (InGaAs), to give a broad sensitivity range from the visible to the near-infrared region of the spectrum. A dual-peak filter that matched the optimal pair of wavelengths for discriminating contaminated from uncontaminated product was fitted into the sorter. Signals from both silicon and InGaAs sensors were balanced by using white reflectors for bottom lamps and white backgrounds (the background enhances the difference between acceptable and non-acceptable products as seen by the sensors). The sorter has 10 inclined, parallel channels with 5-mm radius grooves that singulate corn kernels before each is viewed by front and rear sensors. Corn kernels were fed by a vibratory feeder at a rate of 30 kg/hr/channel. An air ejector diverts a kernel from its trajectory when the dual-wavelength signal, as seen by either one of the cameras, exceeds the set threshold. Diverted kernels fall into a reject container, while free-falling kernels fall into an accept container (hereafter, corn collected in the containers for accept and reject portions will be referred to as accepts and rejects, respectively).

The sorter was tested using two reject thresholds to evaluate mycotoxin removal when rejecting a relatively high percentage of corn (≈10%) compared with rejecting a relatively low percentage of corn (≈5%). There was considerable variation from sample to sample of the actual amount of corn rejected. Generally, clean, uncontaminated samples had very low reject levels (≈3% on the high-threshold setting), while heavily contaminated samples had higher reject levels (>9% on the low-threshold setting). Half of all samples were run at each reject threshold setting. For every sorter threshold, harvest type, and cleaning treatment, there were no fewer than four replicate samples, each consisting of 4 kg of corn.

Bulk Aflatoxin and Fumonisin Analysis

All rejects from each sample were chemically analyzed for aflatoxin and fumonisin, while only 500 g of the accepts from each sample was analyzed. The initial aflatoxin level of the incoming corn was computed using a mass balance of the accept and reject streams from the sorter. Each of the grain samples were first ground using a Stein mill (Steinlite, Atchison, KS). All samples were analyzed for aflatoxin by the Aflatest procedure according to the manufacturer's instructions. A total of 5 g of NaCl was added to 50 g of ground corn sample (≈ 150 kernels), which was then blended for 1 min with 100 mL of 80% MeOH in water (v/v) and filtered through Whatman 2V filter paper (filtrate no. 1 = 0.5 g equiv/mL). Next, 10 mL of filtrate no. 1 was diluted with 40 mL of distilled water (= 5 g equiv/50 mL) and filtered through a microfiber filter, after which 10 mL of filtrate no. 2 (=1 g equiv/10 mL) was passed through an Aflatest affinity column. The column was washed twice with distilled water and the aflatoxin concentrated in 1 mL of 100% MeOH. Following the addition of 1 mL of bromine developer, aflatoxin concentration was determined with a Vicam Series-4 fluorometer calibrated with Vicam test solutions. For samples weighing <50 g, the method was adjusted to scale for the quantity of sample.

Total fumonisin (B1, B2, and B3) was measured with a fluorometer after extracts were purified with immunoaffinity columns (Vicam Fumonitest) using the procedure recommended for corn, sorghum, and 17% protein poultry feed (Ware et al 1994). The quantity of diluted extract passed through the column was adjusted (0.1, 1, 2, or 5 mL) so as not to exceed a column capacity of 8 ppm of fumonisin. The column was then washed once with 10 mL of PBS/0.1% Tween-20 and washed a second time with 10 mL of PBS. The fumonisin was eluted from the affinity column using 1.0 mL of HPLC-grade methanol and collected into a glass cuvette to which 1 mL of Fumonitest Developer A and B mixture was also added. The cuvette was vortexed and placed into a calibrated fluorometer to read the fumonisin concentration.

RESULTS

Filter Selection

The discriminant analysis procedure selected the absorbance band pair of 750 nm and 1,200 nm, both from the spectra that were convolved with the 20-nm FWHM Gaussian curve. From the single-kernel spectral data, these two absorbance bands correctly classified 97% of the kernels with >100 ppb of aflatoxin as contaminated and 100% of the kernels with no detectable (nd) aflatoxin as uncontaminated. However, 70% of the kernels with aflatoxin detectable at <100 ppb were classified as uncontaminated. These classification errors were not seen as a severe problem as the incidence of these contamination levels are lower than highly contaminated kernels (Shotwell et al 1974; Lee et al 1980) and should not have a large effect on typical 5-kg test samples.

The same discriminant function developed for aflatoxin-contaminated kernels was applied to spectra taken from fumonisin-

contaminated corn kernels (Dowell et al 2002). Results showed that good separation of fumonisin-contaminated kernels should be possible as well. From this set of spectra, 100% of the kernels with <1 ppm of fumonisin were classified as uncontaminated, while 100% of the kernels with >100 ppm of fumonisin were classified as contaminated. For kernels contaminated with 1–10 ppm of fumonisin, 95% were classified as uncontaminated. Half of the kernels with a moderate amount of fumonisin (10–100 ppm) were classified as uncontaminated. These results indicate that good separation of kernels with high and low levels of aflatoxin or fumonisin can be achieved with the same sorting operation.

Sorting Results

Corn grown in northeastern Kansas (2002 harvest). Sorting results of commercially grown and harvested corn from northeastern Kansas are shown in Table I. The initial aflatoxin level for 16 subsamples of uncleaned grain used in sorting experiments averaged 51 ppb (range 22–76 ppb), while initial fumonisin levels averaged 18 ppm (range 12–27 ppm). Cleaning of the grain to remove dockage using a rotary grain cleaner did not reduce aflatoxin or fumonisin levels when compared with initial mycotoxin values determined for treatments using uncleaned grain. Sorting at the low-threshold setting removed an average of 5% of the incoming product, while the high-threshold setting removed 12%. Using the low-threshold setting, the sorter reduced aflatoxin in eight subsamples of uncleaned grain by an average of 79% with the accepted grain averaging 13 ppb (range nd–46 ppb), while reducing aflatoxin levels in eight subsamples of the cleaned grain by 83% with the accepted grain averaging 8 ppb (range nd–28 ppb). In treatments using the high-threshold setting, aflatoxin was reduced in eight subsamples of both cleaned and uncleaned grain by an average of 81%. Aflatoxin levels in the accepted cleaned grain averaged 11 ppb (range 2–30 ppb), while the uncleaned grain averaged 12 ppb (range 6–21 ppb). Thus, higher grain rejection rates did not significantly improve aflatoxin reduction ($P = 0.05$).

Similar trends were seen with fumonisin reduction. Sorting reduced fumonisin levels in the same cohort of subsamples of both cleaned and uncleaned grain by 82% using the low-threshold setting and 91% using the high-threshold setting. Fumonisin levels in all of the 16 subsamples of accepted grain sorted at the low-threshold setting averaged 3.3 ppm (range 0.9–8.4 ppm), while fumonisin levels in the accepted grain at the high-threshold setting averaged 1.6 ppm (range 0.7–2.3 ppm). The differences in means for the low- and high-threshold settings are not significantly different at the 95% confidence level with the data collected, but more data might indicate that higher reject rates help to further reduce fumonisin contaminations. Cleaning caused no apparent reduction in aflatoxin or fumonisin.

An examination of the aflatoxin and fumonisin levels in grain rejected at either low- or high-threshold settings offers further insight to the sorter's efficiency in rejecting mycotoxin-contaminated grains. At the low-threshold setting, aflatoxin levels in subsamples of both cleaned and uncleaned grain that were rejected by the

TABLE I
Aflatoxin (A) and Fumonisin (F) Sorting Results for Corn Harvested in Northeastern Kansas in 2002

Corn ^a	Fraction Rejected %		Mycotoxin Reduction After Sorting %		Aflatoxin (ppb)						Fumonisin (ppm)						
					Initial		Accepts		Rejects		Initial		Accepts		Rejects		
	Avg	Std	A	F	Avg	Std	Avg	Std	Avg	Std	Avg	Std	Avg	Std	Avg	Std	
Cleaned																	
LT	4.5	0.5	83.2	82.0	45	16	8.4	11	819	313	15	9.5	2.9	2.4	288	175	
HT	10.8	0.9	84.1	91.9	66	27	11	13	533	288	16	2.6	1.4	0.5	137	15	
Not cleaned																	
LT	5.0	0.3	79.1	80.8	49	16	13	16	745	226	19	4.5	3.6	2.3	306	83	
HT	12.7	0.3	77.4	90.7	53	9.7	12	7.1	343	103	18	1.7	1.7	0.1	131	9	

^a LT, low threshold (sorter set to reject 5% of the grain sample); HT, high threshold (sorter set to reject 10% of the grain sample).

sorter averaged 782 ppb (range 460–1,400 ppb), while averaging 438 ppb (range 129–954 ppb) at the high-threshold setting. Fumonisin levels in grain rejected at the low-threshold setting averaged 297 ppm (range 160–710 ppm), while averaging 134 ppm (range 116–155 ppm) at the high-threshold setting. The average mycotoxin levels in rejected grain from the high-threshold sort were significantly lower than the rejects from the low-threshold sort at the 95% confidence level. Variation in aflatoxin or fumonisin levels among samples removed from the same grain lot is well documented and is likely to have contributed to some of the variability in aflatoxin and fumonisin levels among the subsamples of sorted grain (Shotwell et al 1974; Whitaker and Dickens 1983; Murphy et al 1993; Whitaker et al 1998, 2001; Johansson et al 2000). Nevertheless, these results suggest that the additional volume of grain rejected at the high-threshold setting is contaminated with less aflatoxin and fumonisin, and this serves to dilute the aflatoxin and fumonisin concentrations recorded for the high-threshold setting.

Naturally infected corn grown in central Illinois (2002 harvest). Results from sorting uncleaned grain from naturally infested corn grown commercially in Central Illinois (2002 harvest) are shown in Table II. The initial aflatoxin level for 20 subsamples of the grain harvested with the Case IH 1688 and used in sorting experiments averaged 1.8 ppb (range nd–7 ppb). The sorter reduced aflatoxin levels by only 23% from the initial level for 10 subsamples sorted at the low-threshold setting and only 6% for 10 subsamples sorted at the high-threshold setting. Aflatoxin levels in grain rejected by the sorter averaged 14 ppb (range nd–125 ppb) at the low-reject setting and 6 ppb (range nd–20 ppb) at the high-threshold setting; however the differences were not significant. ($P = 0.05$). Initial fumonisin levels in the same samples averaging 2.3 ppm (range 1.3–3.0 ppm) were reduced by 86% at the low-threshold setting, with the accepted grain averaging 0.3 ppm (range nd–1.3 ppm) and 98% at the high-threshold setting, with the accepted grain averaging 0.23 ppm (range nd–1 ppm). At the high-threshold setting, the sorter rejected 266% more grain harvested with the Case IH 1688 than at the low-threshold setting. However, this did not significantly ($P = 0.05$) reduce the fumonisin content of the accepted grain, indicating that additional kernels rejected at the high-threshold sort had low levels of fumonisin.

The percentage of grain rejected from the Case IH 1688 harvested grain were significantly lower ($P = 0.05$) at each threshold setting when contrasted with grain rejects from the Hege 125c harvested grain (Table II). Given that corn from these two harvest methods came from the same field, many of the aflatoxin-contaminated kernels from Case IH 1699 harvested corn may have

been asymptomatic, at least to the classification scheme used with the sorting operation. Most of the contaminated kernels in the Case IH 1688 harvested samples might have had a low to moderate level of contamination (<100 ppb of aflatoxin and 100 ppm of fumonisin) that is not detected with high accuracy by the method used. This is explained by the fact that the commercial combine performs a cleaning step as grain is being harvested and shelled in the field. The Hege 125c collects these damaged and broken kernels, which are then removed by sorting, thus contributing to the greater percentage of rejects at both low- and high-threshold settings. The Hege 125c is equipped with a lateral cloth bag that collects nearly all of the grain shelled from each ear, including kernels that are small, light-weight, and damaged by insects or molds, as well as fragments from broken kernels. Thus, difference in initial fumonisin levels for grain harvested with the Hege 125c and the Case IH 1688 could be due to cleaning done in the Case IH 1688 and not in the Hege 125c. Sorting the grain harvested with the Hege 125c reduced fumonisin concentrations in the accepted grain to levels recorded for grain samples harvested with the Case IH 1688 (Table II).

The initial aflatoxin level for 24 subsamples of the naturally infected grain harvested with the Hege 125c averaged 2.7 ppb (range nd–22 ppb). For corn harvested with the Hege 125c, the sorter was able to reduce aflatoxin levels in 12 subsamples by 46% at the low-threshold setting with the accepted grain averaging 0.7 ppb (range nd–2.5 ppb), and by 70% at the high-threshold setting with accepted grain averaging 1.4 ppb (range nd–13 ppb). These grain samples include aflatoxin-contaminated, damaged grains, and grain fragments that might otherwise have been dispersed in the exhaust of the Case IH 1688. Aflatoxin levels in the grain harvested with the Hege 125c rejected by the sorter averaged 15 ppb (range 1.5–38 ppb) at the low-threshold setting and 27 ppb (range nd–120 ppb) at the high-threshold setting. However, the differences between low- and high-threshold averages were not significant ($P = 0.05$).

Initial fumonisin levels for 24 subsamples of naturally infected grain harvested with the Hege 125c averaged 19 ppm (range 10–31 ppm), while fumonisin levels for 20 subsamples of grain harvested using the Case IH 1688 averaged 2.3 ppm (range 1.3–3.0 ppm). This difference is presumably the result of cleaning in the Case IH 1688, with the removal of *Fusarium* rotted kernels (Munkvold and Desjardins 1997). The sorter reduced fumonisin levels in 12 subsamples of corn harvested with the Hege 125c by 82% at the low-threshold setting with the accepted grain averaging 2.9 ppm (range 1.4–5.9 ppm) and by 92% at the high-threshold setting,

TABLE II
Aflatoxin (A) and Fumonisin (F) Sorting Results for Corn Harvested in Central Illinois in 2002

Corn ^a	Fraction Rejected %		Mycotoxin Reduction After Sorting %		Aflatoxin (ppb)						Fumonisin (ppm)						
					Initial		Accepts		Rejects		Initial		Accepts		Rejects		
	Avg	Std	A	F	Avg	Std	Avg	Std	Avg	Std	Avg	Std	Avg	Std	Avg	Std	
Wound-inoculated ears ^b																	
Field plot harvester ^c																	
LT ^c	8.8	1.1	79.2	82.8	412	81	86	46	3,892	1,278	19	5.9	3.3	2.1	188	81	
HT ^c	17.2	1.4	92.7	88.6	398	47	30	19	2,185	319	22	8.2	2.4	1.6	117	51	
Natural infection																	
Field plot harvester																	
LT	4.7	0.5	46.3	81.9	1.3	1.2	0.7	0.8	15	15	16	4.5	2.9	1.5	281	89	
HT	10.2	1.0	70.2	92.3	4.1	6.6	1.4	3.7	27	40	23	6.2	1.7	0.6	214	63	
Commercial combine ^d																	
LT	1.2	0.2	23.3	85.7	1.2	1.5	1.0	1.6	14	39	2.1	0.5	0.3	0.4	148	42	
HT	3.2	0.3	6.2	97.5	2.4	2.7	2.3	2.6	6	8	2.5	0.4	0.2	0.3	70	14	

^a LT, low threshold (sorter set to reject 5% of the grain sample); HT, high threshold (sorter set to reject 10% of the grain sample).

^b *Aspergillus flavus* NRRL 32355.

^c Hege 125C, equipped with lateral bagging which collects all grain from the ear (whole, damaged, and broken fragments)

^d Case IH 1688.

with the accepted grain averaging 1.7 ppm (range 0.7–3.0 ppm). At the high-threshold setting, the sorter rejected 217% more grain harvested with the Hege 125c than at the low-threshold setting. However, the rejection of these additional kernels did not contribute to a further significant reduction in the aflatoxin or fumonisin content of the accepted grain ($P = 0.05$). There was little evidence of a grain-dilution effect on aflatoxin or fumonisin levels in grain samples rejected at the high-threshold setting. Fumonisin levels in grain rejected at the low-threshold setting averaged 281 ppm (range 170–445 ppm) and 214 ppm (range 120–320 ppm) at the high-threshold setting, indicating that the additional kernels rejected at the high-threshold setting contained high levels of fumonisin.

A. flavus wound-inoculated corn grown in central Illinois (2002 harvest). Results from sorting grain from ears (cultivar FS 7111) that were wound-inoculated with *A. flavus* and later harvested using the Hege 125c (2002 harvest) are shown in Table II. The initial aflatoxin level for 12 subsamples of the grain harvested with the Hege 125c and used in sorting averaged 405 ppb (range 268–497 ppb). The sorter was able to reduce aflatoxin levels in six subsamples by 79% at the low-threshold setting with accepted grain averaging 86 ppb (range 34–139 ppb), and by 93% at the high-threshold setting with accepted grain averaging 30 ppb (range 6–59 ppb). However, >17% of the initial grain sample was rejected at the high-threshold setting. At both the low- and high-threshold settings, the sorter rejected almost twice (170%) the amount of grain harvested from *A. flavus* wound-inoculated ears than grain harvested from uninoculated ears in the same field (Table II). Aflatoxin levels in grain that was rejected by the sorter averaged 3,892 ppb (range 2,511–5,214 ppb) at the low-threshold setting and 2,185 ppb (range 1,802–2,705 ppb) at the high-threshold setting, indicating that the rejected grain from the high-threshold sort was diluted with uncontaminated kernels. Wound-inoculation with *A. flavus* produced greater numbers of mold-damaged kernels, causing a greater percentage of grain to be rejected at each setting when contrasted with the uninoculated grain that was also harvested using the Hege 125c (Table II).

The initial fumonisin level for all subsamples of grain harvested with the Hege 125c averaged 21 ppm (range 11–31 ppm). For the corn harvested with the Hege 125c, the sorter reduced fumonisin levels by 83% at the low-threshold setting with accepted grain averaging 3.3 ppm (range 1.8–7.5 ppm), and 89% at the high-threshold setting with accepted grain averaging 2.4 ppm (range 1.3–5.7 ppm). The initial fumonisin levels in grain harvested with the Hege 125c from rows of *A. flavus* wound-inoculated ears were equivalent to those recorded for grain harvested from uninoculated ears. This was anticipated because natural *Fusarium* infestation and kernel rot damage occurred throughout the field. A larger proportion of the grain from *A. flavus* wound-inoculated ears was rejected (8.8–17.2%) than grain from uninoculated ears (4.7–10.2%) (Table II). Fumonisin levels in samples of the rejected grain averaged 188 ppm (range 98–330 ppm) at the low-threshold setting and 117 ppm (range 55–182 ppm) at the high-threshold setting. These results illustrate that the added volume of grain rejected at the high-threshold setting contains less aflatoxin and fumonisin.

A. flavus wound-inoculated corn grown in central Illinois (1997 harvest). To further investigate the limits of grain sorting in reducing aflatoxin levels for a highly aflatoxin-contaminated grain lot, sorter testing was performed on Pioneer 3394 that was wound-inoculated with *A. flavus* and later hand-harvested (1997 harvest) and continuously stored in the freezer at -20°C before sorting. Results from sorting this grain are shown in Table III. The initial aflatoxin level for eight subsamples averaged 1,268 ppb (range 805–1,632 ppb). At the low-threshold setting, the sorter was able to reduce aflatoxin levels in four subsamples by 76% with the accepted grain averaging 330 ppb (range 164–551 ppb), and by 88% at the high-threshold setting with accepted grain averaging 155 ppb (range 64–212 ppb). Aflatoxin levels in grain rejected at the low-threshold setting averaged 17,669 ppb (range 10,116–21,154 ppb) and 8,607 ppb (range 6,001–11,094 ppb) at the high-threshold setting. The hand-shelled grain from ears that were wound-inoculated at three points along the ear produced large numbers of *A. flavus* infested and discolored kernels that were probably rejected at the high-threshold setting but not at the low-threshold setting, causing a dilution effect in the rejects from the high-threshold samples. The initial fumonisin level for all subsamples of this same grain lot averaged 4.5 ppm (range 1.2–18 ppm). Sorting reduced fumonisin levels by 79% at the low-threshold setting with accepted grain averaging 0.4 ppm (range nd–1.0 ppm), and 76% at the high-threshold setting with accepted grain averaging 1.3 ppm (range 0.1–3.6 ppm). In this sorting experiment, there was 219% more grain rejected at the high-threshold setting than the low-threshold setting, while the fumonisin levels also increased in grain rejected at the high-threshold setting (48 ppm; range 15–134 ppm) versus the low-threshold setting (24 ppm; range 34–99 ppm). However, the initial fumonisin level for the samples used in the high-threshold experiments (7.3 ppm; range 2.2–18 ppm) were much higher than those for the low-threshold experiment (1.7 ppm; range 1.2–1.9 ppm), which might explain the higher fumonisin levels in the rejects from the high-threshold sort.

DISCUSSION

This research has shown that both aflatoxin- and fumonisin-contaminated grains are removed from grain samples based on absorbances of 750 and 1,200 nm. Coincidental removal of grains contaminated with fumonisin represents an added benefit when the primary objective of grain sorting is to remove aflatoxin-contaminated grains. The sorting results were consistent for corn grown in different years and different locations (Illinois and Kansas). Over 90% of corn samples with an initial aflatoxin level of 20–100 ppb and fumonisin of 4–100 ppm were reduced to levels below the U.S. FDA/CFSSAN action or guidance levels (1988, 2000, 2001) for these mycotoxins by removing $\approx 5\%$ of the incoming grain.

Sorting highly aflatoxin-contaminated grain (>1,000 ppb) failed to reduce aflatoxin levels to below the FDA limit of 20 ppb. However, for inoculated samples harvested with the Hege 125c, sorting reduced aflatoxin concentrations in the accepted grain below the U.S. FDA/CFSSAN action levels for corn intended for

TABLE III
Aflatoxin (A) and Fumonisin (F) Sorting Results for Corn Harvested in Central Illinois in 1997, Wound-Inoculated with *A. flavus* (NRRL 32355) and Harvested by Hand

Corn ^a	Fraction Rejected %		Mycotoxin Reduction		Aflatoxin (ppb)						Fumonisin (ppm)					
	After Sorting %		After Sorting %		Initial		Accepts		Rejects		Initial		Accepts		Rejects	
	Avg	Std	A	F	Avg	Std	Avg	Std	Avg	Std	Avg	Std	Avg	Std	Avg	Std
LT	5.8	1.0	75.6	78.6	1,301	293	330	164	17,669	5,106	1.7	0.4	0.4	0.4	24	11
HT	12.7	2.3	87.6	75.8	1,235	437	155	70	8,607	2,360	7.3	7.3	1.3	1.6	48	57

^a LT, low threshold (sorter set to reject 5% of the grain sample); HT, high threshold (sorter set to reject 10% of the grain sample).

finishing (feed lot) beef cattle (300 ppb); finishing swine of 100 lb or greater (200 ppb); and breeding beef cattle, breeding swine, or mature poultry (100 ppb).

Figures 2 and 3 show the percent reductions in aflatoxin and fumonisin, respectively, given an initial mycotoxin level. In Fig. 2, it appears that the sorter has a reduced ability to remove aflatoxin if the initial levels are <10 ppb. At >10 ppb, there does not appear to be a correlation between initial aflatoxin and percent reduction in aflatoxin due to sorting. When the initial level is >10 ppb, the sorter was able to reduce aflatoxin by $\geq 75\%$ in 75% of all the samples. Aflatoxin reduction of $\geq 90\%$ was observed in 40% of the samples when the initial level was >10 ppb. Failure of the sorter to reduce aflatoxin levels when the initial level was low and to completely eliminate aflatoxin may be due to the sorter's inability to detect kernels with low to moderate levels of aflatoxin. The spectra of kernels with aflatoxin at <100 ppb was more similar to that of uncontaminated kernels (Pearson et al 2001). However, this result limits the usefulness of sorting for aflatoxin in corn that is intended for food markets. Future research is needed to improve sorting of corn with low levels of aflatoxin. Additionally, the pair of filters used in this study were selected for optimal detection of aflatoxin. Future research is needed to select the optimal pair of filters for detection of fumonisin-contaminated kernels.

More consistent reductions in fumonisin were observed throughout the samples tested (Fig. 3): $\geq 81\%$ were observed in 80% of the samples and reductions of $\geq 90\%$ were observed in 50% of the samples. There was no correlation between initial fumonisin level and percentage of fumonisin removed by sorting. Initial fumonisin levels in the grain samples that were sorted in this study are representative of fumonisin levels recorded for grain produced in different regions of the world (Sydenham et al 1990, 1993; Murphy et al 1993; Schaafsma et al 1993; Doko et al 1995; Chulze et al 1996; Hirooka et al 1996; Viquez et al 1996; Munkvold and Desjardins 1997; Pineiro et al 1997; Orsi et al 2000; Almeida et al 2002).

Desjardins et al (1998) showed that while both symptomatic and symptomless kernels were extensively colonized with *F. moniliforme*, the highest levels of fumonisins were in the symptomatic kernels. There may be less variation in symptom expression for grains infected by *Fusarium* and contaminated with fumonisin (Dowell et al 2002) than with grains infected by *A. flavus* and contaminated with aflatoxin (Wicklow 1999; Pearson et al 2001).

Sorting results for removal of aflatoxin and fumonisin indicated that the sorter was usually able to reject >80–90% of the heavily contaminated kernels using the filter pair of 750 and 1,200 nm. In contrast, there was near-perfect classification of heavily contaminated and uncontaminated kernels using the same spectral bands extracted from near-infrared spectra of stationary kernels. The discrepancy in results from the sorter classifications and those derived from NIR spectra collected in a laboratory can be at least

partially explained by errors caused by material handling during sorting. Some kernels may be oriented on their sides as they pass through the optical sensor of the sorter, which decreases their ability to acquire useful spectral information. Additionally, some kernels may not be sliding at a consistent velocity, which renders proper removal with the air valve problematic. Another source of error with the sorter is that good kernels are occasionally in close proximity to contaminated kernels and are rejected with the contaminated kernel, causing a false-positive error. All of these types of errors are common to most sorting operations.

The sorter used in this study has a throughput of $\approx 7,000$ kg/hr and a cost of $\approx \$60,000$. By rejecting 5% of the incoming product, the sorter reduced aflatoxin and fumonisin in grain below the FDA limit of 20 ppb of aflatoxin for initial levels as high as 50 ppb, and fumonisin can be reduced below the FDA limit of 4 ppm for initial levels as high as 20 ppm. Other grain cleaning methods do not have the ability to reduce mycotoxins significantly or require the removal of >20% of the grain (Brekke et al 1975; Huff and Hagler 1985). If the 5% rejects are regarded as a complete loss of product, then the cost of sorting, excluding capital investment for the sorter, is \$0.10/bu based on a corn price of \$2.00/bu. This is competitive with shipping corn by rail. Any method that can clean corn to a desired quality and prevent 1% of the good grain from false rejection will quickly pay for itself if its throughput is on the order of this sorter. If corn has a wholesale price of \$2.00/bu (\$0.071/kg), then a 1% savings in lost corn will save nearly \$3,600/month if processed continuously at 7,000 kg/hr. The payback period of such a sorter would be <18 months. It appears the optical sorter used in this study can reduce mycotoxins to allowable levels by rejecting 5% of the grain, as compared with 20% required of other methods.

CONCLUSIONS

A method for selecting the optimal pair of filters for a dual-wavelength sorter was developed and applied to sorting corn for mycotoxins. By using spectra collected in a laboratory, spectral absorbances at 750 and 1,200 nm could distinguish kernels with aflatoxin contamination at >100 ppb from kernels with no detectable aflatoxin with >98% accuracy. When these two spectral bands were applied to sorting corn at high speeds, reductions in aflatoxin averaged 82% for corn samples with an initial level of aflatoxin at >10 ppb. For samples with initial levels of aflatoxin at <10 ppb, sorting reduced aflatoxin by an average of 38%. These results show that most of the aflatoxin is removed by rejecting $\approx 5\%$ of the grain. Higher reject rates did not significantly improve removal of aflatoxin-contaminated kernels ($P = 0.05$). Fumonisin is also removed along with aflatoxin during sorting. The sorter reduced fumonisin by an average of 88% for all samples, inclu-

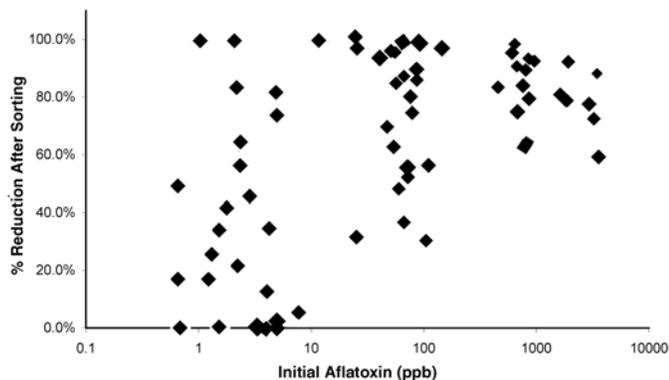


Fig. 2. Plot of initial aflatoxin levels vs. % aflatoxin reduction after sorting. Note there is a greater tendency for very low reduction in aflatoxin only when the initial level is <10 ppb.

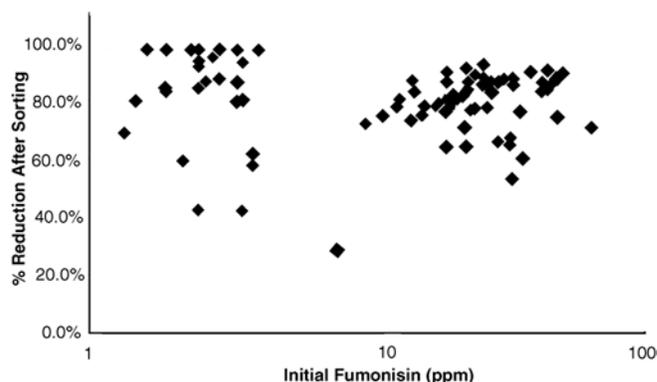


Fig. 3. Plot of initial fumonisin levels vs. % fumonisin reduction after sorting. Note there is little correlation between initial fumonisin level and % reduction.

ding those with low levels of fumonisin. However, rejecting $\approx 10\%$ of the incoming product improves fumonisin removal as compared with a reject rate of $\approx 5\%$. At the high-threshold setting, 91% of the fumonisin was removed, while 82% of the fumonisin was removed at a low-threshold setting.

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