

DETECTING AFLATOXIN IN SINGLE CORN KERNELS BY TRANSMITTANCE AND REFLECTANCE SPECTROSCOPY

T. C. Pearson, D. T. Wicklow, E. B. Maghirang, F. Xie, F. E. Dowell

ABSTRACT. *Transmittance spectra (500 to 950 nm) and reflectance spectra (550 to 1700 nm) were analyzed to determine if they could be used to distinguish aflatoxin contamination in single whole corn kernels. Spectra were obtained on whole corn kernels exhibiting various levels of bright greenish–yellow fluorescence. Afterwards, each kernel was analyzed for aflatoxin following the USDA–FGIS Aflatest affinity chromatography procedures. Spectra were analyzed using discriminant analysis and partial least squares regression. More than 95% of the kernels were correctly classified as containing either high (>100 ppb) or low (<10 ppb) levels of aflatoxin. Classification accuracy for kernels between 10 and 100 ppb was only about 25%, but these kernels do not usually affect total sample concentrations and are not as important. Results were similar when using either transmittance or reflectance, and when using either discriminant analysis or partial least squares regression. The two-feature discriminant analysis of transmittance data gave the best results. However, for automated high-speed detection and sorting, instrumentation that uses single-feature reflectance spectra may be more practically implemented. This technology should provide the corn industry with a valuable tool for rapidly detecting aflatoxin in corn.*

Keywords. *Corn, Aflatoxin, Near-infrared, Detection, Sorting, Aspergillus flavus.*

The fungus *Aspergillus flavus* produces aflatoxin and can invade corn kernels, especially if the plant has undergone some type of stress. For example, in the southeast United States in 1977, about 56% of the drought-stressed corn crop contained aflatoxin greater than the 20 ppb FDA limit for aflatoxin in foods (Olson and Stoloff, 1977). Corn samples with >20 ppb aflatoxin must be destroyed or blended for domestic livestock feed (U.S. Food and Drug Administration, 1988). In 1988, more than 30% of the corn samples from Iowa and Illinois contained aflatoxin at levels exceeding 20 ppb (Schmitt and Hurburgh, 1989; Hurburgh, 1991). Economic losses from aflatoxin occur at all levels in the production, marketing, and utilization process because of reduced prices, extra handling expenses, and poor performance of livestock and poultry on aflatoxin-contaminated feed. In addition, improperly handled high-aflatoxin corn has resulted in lawsuits filed against feed and grain firms (Nichols, 1983; CAST, 1989).

Currently, shipments of corn brought to grain elevators are screened for aflatoxin by counting the number of particles in a coarsely ground sample (4 to 5 kg) of kernels that exhibit a bright greenish–yellow fluorescence (BGYF) when

illuminated with a high-intensity ultra-violet light (Shotwell and Hesseltine, 1981; CAST, 1989; Hurburgh, 1991). It has been shown that the number of BGYF particles found in samples is related to the aflatoxin level in the load (Dickens and Whitaker, 1981). The incidence of aflatoxin-contaminated corn kernels is quite low, even during seasons when aflatoxin is a problem. Most of the aflatoxin is isolated in a very small fraction of kernels that are highly contaminated. Dickens and Whitaker (1981) reported that inspection of samples taken from 113 farm lots of corn grown in North Carolina in 1977 revealed that kernels exhibiting BGYF comprised only 0.38% of the weight of these samples but contained 41.9% of the aflatoxin. *A. flavus* has also been noted to produce kojic acid, which can convert to fluorescent compounds, but not produce aflatoxin (Basappa et al., 1970). As a result, the BGYF test is noted for giving a moderate amount of “false positive” results (i.e., BGYF kernel particles or whole kernels detected in grain samples with <20 ppb aflatoxin) and few “false negative” results (i.e., BGYF kernels were detected in grain samples with >20 ppb aflatoxin) (Shotwell et al., 1975; Shotwell and Hesseltine, 1981; Dickens and Whitaker, 1981; Schmitt and Hurburgh, 1989).

The highest concentrations of aflatoxin are usually found in heavily molded and damaged kernels where *A. flavus* can grow saprophytically in the damaged kernel tissues (Wicklow et al., 1988; Wicklow, 1999). Fortunately, such kernels are usually discarded during combine harvesting or can be removed through grain cleaning operations (Brekke et al., 1975). However, high concentrations of aflatoxin (>8,000 ppb) can also be found in whole corn kernels that are not readily removed from process streams by conventional equipment (Shotwell et al., 1974; Dickens and Whitaker, 1981; Wicklow, 1999). Over half of the aflatoxin is contained in kernels that only exhibit internal BGYF, which can be observed only after the kernel is coarsely ground or cracked

Article was submitted for review in November 2000; approved for publication by the Food & Process Engineering Institute of ASAE in April 2001.

The authors are Tom C. Pearson, ASAE Member Engineer, Agricultural Engineer, USDA–ARS–GMPRC, Manhattan, Kansas; Donald T. Wicklow, Research Microbiologist, USDA–ARS–NCAUR, Peoria, Illinois; Elizabeth B. Maghirang, ASAE Member Engineer, Research Associate, and Floyd E. Dowell, ASAE Member Engineer, Research Leader, USDA–ARS–ERU, Manhattan, Kansas; and Feng Xie, Research Assistant, Kansas State University, Manhattan, Kansas. Corresponding author: Tom C. Pearson, USDA–ARS–GMPRC, 1515 College Ave., Manhattan, Kansas 66502; phone: 785–776–2729; fax: 785–539–5550; e-mail: tpearson@gmprc.ksu.edu.

(Shotwell et al., 1974). The endosperm of kernels with the highest levels of aflatoxin becomes powdery and fluorescent. These friable kernels are a major source of BGYP particles in grain samples (Hesseltine and Shotwell, 1973; Lillehoj et al., 1976). The recommended sample size for testing aflatoxin in lots of shelled corn is 4.5 kg (GIPSA, 1999). Most of the aflatoxin is contained in a few highly contaminated kernels. For example, if twenty kernels in a 4.5-kg sample contain 15,000 ppb aflatoxin each, then the sample aflatoxin level would be 20 ppb. Kernels containing low levels of aflatoxin (<100 ppb) are unlikely to have a great effect on the aflatoxin level measured in a 4.5-kg sample because the incidence of kernels with this amount of contamination is about as rare as kernels with high levels of contamination (Shotwell et al., 1974).

Currently there is no economically viable method to remove whole intact corn kernels that are contaminated with aflatoxin. Even on whole intact kernels, *A. flavus* typically infects the kernel germ and can leave little indication of its presence on the kernel surface. Preliminary studies indicated that some whole BGYP corn kernels exhibit slight color and shape differences from non-BGYP kernels. Devices have been developed to detect BGYP on pistachio nuts and almonds for aflatoxin elimination (McClure and Farsaie, 1980; Brum, 1999). However, low levels of BGYP are difficult to detect accurately and rapidly. Corn exhibiting limited BGYP likely would not be detected by current systems (Brum, 1999). In addition, as mentioned earlier, many aflatoxin contaminated kernels only exhibit BGYP internally.

Near-infrared transmittance (NIRT) and near-infrared reflectance (NIRR) spectroscopy have been used to evaluate internal quality of many whole grains and nuts. NIRR and NIRT applications for measuring wheat characteristics include: wheat classification (Delwiche and Massie, 1996), color classification (Dowell, 1998), hardness measurement (Morris et al., 1999), protein content measurement (Delwiche, 1995), detecting internal insects (Ridgway and Chambers, 1996; Dowell et al., 1998), detecting scab damage and deoxynivalenol (Dowell et al., 1999; Williams, 1997), and detecting wheat-rye translocation (Delwiche et al., 1999). Corn characteristics measured by NIR spectroscopy include oil content (Orman and Schumann, 1992) and detecting transgenics (Kramer et al., 2000). Pearson (1999) developed a NIRT sorting system to detect internal chemical defects in whole almonds. Because aflatoxin occurs in extremely small concentrations, it is not likely that NIR can detect aflatoxin directly. However, prevalent infestation by *A. flavus* would likely affect other chemical and optical properties of whole kernels that can be detected with visible or NIR spectroscopy. Thus, it may be possible to automatically detect *A. flavus* or constituents directly correlated to aflatoxin levels in single corn kernels.

OBJECTIVE

The objective of this study was to determine if visible or NIR transmittance or reflectance spectra obtained from single whole corn kernels could be used to identify aflatoxin-contaminated corn kernels.

MATERIALS AND METHODS

CORN KERNEL PREPARATION

Pioneer 3394 corn kernels were harvested in 1998 from ears that were wound-inoculated per Wicklow (1999) with *Aspergillus flavus* NRRL A-27837 in the late milk to early dough stage of kernel maturity at the University of Illinois River Valley Sand Farm, Kilbourne, Illinois. Shortly after harvest on 19 October 1998, the corn kernels were examined under a black light (365 nm) and separated into the following categories based on their BGYP characteristics: (1) intact kernels with BGYP over most of the kernel, (2) intact kernels with BGYP limited to the germ region, (3) intact kernels with BGYP limited to the lower germ/tip cap, and (4) intact non-BGYP kernels. The actual wound-inoculated kernel was discarded so that only those kernels to which *A. flavus* spread naturally were studied. Friable kernels and fragments were not included in this study as they are usually removed by existing cleaning equipment at grain elevators. A total of 500 kernels were used for this study, 50 each from the above four categories and 300 randomly selected kernels from the same lot without examination under a black light.

Kernels were first shipped from the USDA ARS National Center for Agricultural Utilization Research (NCAUR) in Peoria, Illinois, to the USDA ARS Grain Marketing and Production Center in Manhattan, Kansas, for NIRR analysis. Kernels were stored at 4°C between experiments at this location. Kernels were then shipped overnight to the USDA ARS Western Regional Research Center in Albany, California, for NIRT analysis. The corn was stored at approximately -7°C between experiments at this location. Kernels were then shipped overnight to the USDA ARS NCAUR in Peoria, Illinois, for chemical analysis.

SPECTRA MEASUREMENT

NIR Transmission

Whole kernel transmission spectra from 500 to 950 nm were measured using a silicon photodiode-array fiber-optic spectrometer (Ocean Optics, Dunedin, Fla.) with a sensor resolution of approximately 0.48 nm and an optical resolution of approximately 3.5 nm. The spectrometer sampled ten complete transmission spectra and stored the average. The integration time of each photodiode element was 200 ms, for a total integration time of 2 seconds.

The light source was a 100 W quartz-tungsten-halogen lamp coupled to a 3 mm diameter fiber-optic bundle (Oriel, Stratford, Conn.). The transmittance spectrum of each kernel was measured from light emitted from a 3 mm diameter circle approximately in the middle of the kernel with the kernel lying flat, as shown in figure 1. Transmitted light through the kernel was collected by a collimating lens and transmitted to the spectrometer via a 0.50 mm fiber-optic bundle. A light standard and dark standard were obtained between the sampling of each kernel. The dark standard was obtained by blocking the light source with a steel shutter. The light standard was obtained by placing a glass neutral-density filter with a transmission of 0.1% (Ealing, Holliston, Mass.) in place of the sample.

NIR Reflectance

Whole kernel reflectance spectra from 550 to 1700 nm were measured using a diode-array near-infrared

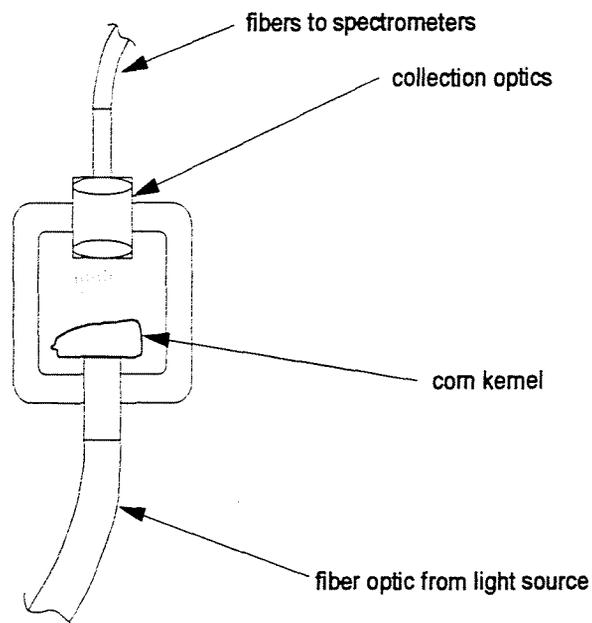


Figure 1. Transmittance sampling apparatus.

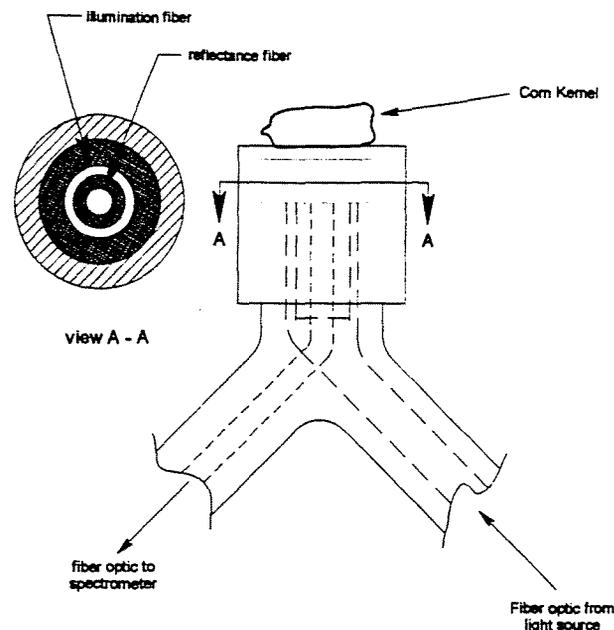


Figure 2. Reflectance sampling apparatus.

spectrometer, as shown in figure 2 (Perten Instruments, Springfield, Ill.). The spectrometer measures absorbance using an array of silicon (7 nm resolution) and indium-gallium-arsenide (11 nm resolution) sensors. The spectrometer sampled 15 spectra and stored the average. Each spectrum was collected in about 33 ms, for a total integration time of 0.495 seconds.

Kernels were manually placed on a bifurcated intertance probe attached to the spectrometer and light source (fig. 2). 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. All 500 kernels were viewed first at the germ-down position (germ facing the optical fiber bundle), and then a second set of spectra was collected for 206 kernels that were viewed germ-up. The spectra were stored on a hard disk for subsequent analysis.

DATA ANALYSIS

Discriminant Analysis

After acquisition of all spectra, each transmittance spectrum was smoothed by a 19-point Savitzky-Golay 2nd-order filtering operation (Hruschka, 1987). Absorbance values from the spectrometer were not equally spaced. Equally spaced data in 5 nm increments were computed by 19-point interpolation. From this reduced data set, all possible ratios of two absorbance values were computed. With the spectra parsed into 5 nm increments, there were a total of 91 absorbance values and 4095 ratio values for each kernel. For the reflectance spectra, only spectra from kernels arranged germ down were used. The spectra were parsed to 5 nm resolution from 550 nm to 1700 nm, resulting in 231 absorbance values. All possible ratios of absorbance values in 15 nm increments were computed, resulting in 2926 ratio values.

When considering the possible application of results in high-speed sorting operations, only a few of the computed

features can be economically measured in real time. An exhaustive search was performed for the best single feature and combination of two features to classify kernels as aflatoxin positive or negative. Kernels were considered aflatoxin positive if their measured aflatoxin concentration was greater than or equal to one of three different threshold levels (1 ppb, 10 ppb, and 100 ppb). Discriminant analysis was used as the classification procedure with both pooled and non-pooled covariance matrices (Huberty, 1994). The Mahalanobis distances were computed from each kernel to the aflatoxin positive and negative groups. A kernel was classified into the group with the lowest corresponding Mahalanobis distance. The sample means and co-variance matrices for each group were computed using the odd-numbered samples only. The feature set that obtained the lowest classification error rate, for each aflatoxin threshold level, on the even-numbered samples was recorded. The error rate was based on the number of false negatives with aflatoxin greater than 100 ppb and the number of false positives with zero aflatoxin.

PLS Analysis

Spectra were analyzed by using partial least squares (PLS) regression, a spectral decomposition technique similar to principal component regression (Martens and Naes, 1989). The PLS regression uses concentration data during the decomposition process and includes as much information as possible into the first few loading vectors. It also takes advantage of the correlation between the spectral data and the constituent concentrations. Cross-validation attempts to emulate predicting "unknown" samples by using the training set data itself (PLSplus/IQ, 1996). False positives and false negatives were calculated as described above.

CHEMICAL ANALYSES

Single-kernel aflatoxin levels were determined after the spectral transmittance and reflectance of all kernels were measured. Individual kernels were placed in an envelope of

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

RESULTS AND DISCUSSION

Of the 500 kernels individually analyzed for aflatoxin, 61 kernels contained aflatoxin greater than or equal to 10 ppb, 42 contained aflatoxin greater than or equal to 100 ppb, and 39 contained aflatoxin greater than or equal to 1000 ppb. One kernel with measured aflatoxin of 14,000 ppb exhibited no BGYF. All other kernels with measured aflatoxin greater than 1000 ppb exhibited some BGYF. Table 1 shows the distribution of aflatoxin from each of the categories of kernels analyzed. This distribution is similar to that found in Wicklow (1999). Although it was surprising to find an intact kernel showing no BGYF while having an aflatoxin level as high as 14,000 ppb, Shotwell et al. (1974) reported finding subsurface BGYF in a few of the kernels only after crushing, and three aflatoxin-positive kernels showed no evidence of subsurface BGYF. In the present study, the pulverized kernel fragments were not examined using the "black light" (365 nm).

Plots of the average transmittance and reflectance spectra from kernels with aflatoxin greater than 100 ppb, aflatoxin between 1 and 100 ppb, and no detectable aflatoxin are shown in figures 3 and 4. As can be seen in the transmittance spectra (fig. 3), the absorbance is generally much higher for kernels contaminated with high levels of aflatoxin. The absorbance of contaminated kernels in the reflectance spectra (fig. 4) is also higher below 850 nm, but from 850 nm to 1700 nm the absorbance of contaminated kernels is lower. (In this article, we use absorbance units to represent log(1/R) data). In general, the reflectance spectra from kernels having aflatoxin levels between 1 and 100 ppb follows the reflectance spectra of kernels having no detectable aflatoxin more closely than in the transmittance spectra. However, the variance of individual absorbance values at each wavelength is high

enough that no absorbance value from a group is significantly different from those of others groups at the 95% confidence level.

The differences in absorbance spectra for different aflatoxin levels can possibly be explained by the scattering and absorbance characteristics caused by the fungus in the kernel. The discoloration of the kernels would cause higher visible wavelength (<~750 nm) absorbance, as measured by transmittance or reflectance. A fungal-infected kernel would also scatter more light than a sound, vitreous kernel because the invasion of the fungus causes the kernel endosperm to become powdery (Hesseltine and Shotwell, 1973; Lillehoj et al., 1976). This scattering would cause more NIR (>750 nm) radiation to be absorbed in transmission mode, and less NIR radiation to be absorbed in reflectance mode. Powdery substances with refractive indices different than air, such as in the air-endosperm interface of infected kernels, cause more light to be reflected (Birth and Hecht, 1987), as opposed to the more crystal-like property of normal kernels.

CLASSIFICATION BY DISCRIMINANT ANALYSIS

Classification results using discriminant analysis are shown in table 2. All discriminant models used pooled covariance matrices, and the lowest error rates for kernels with 0 ppb and >100 ppb aflatoxin were achieved using an aflatoxin threshold of 10 ppb to define kernels as aflatoxin positive or negative. Features selected for the discriminant functions were ratios of absorbance values in all cases. When compared to results from reflectance spectra, the classifications based on the transmittance spectra have slightly higher false positive error rates among kernels having aflatoxin between 1 and 100 ppb, and lower false negative error rates among kernels having aflatoxin levels between 10 and 100 ppb. All models incorrectly classified one kernel having 120 ppb aflatoxin as aflatoxin negative. In addition, the single-feature classification using the transmittance spectra classified one kernel having 14,000 ppb aflatoxin as aflatoxin negative. It should be noted that this kernel did not exhibit any BGYF.

Figures 5 and 6 show the feature space for the transmittance spectra, and figures 7 and 8 show the feature space for the reflectance spectra. As can be seen from figure 8, the kernel containing 14,000 ppb is nearly classified as aflatoxin negative using the reflectance spectra and only one feature. It appears from figures 5 through 8 that while all the classification results are similar, the feature space variance of the aflatoxin positive and negative groups is lower and better separated in the transmittance cases. More experimentation with a much larger data set would be needed to determine which of the classifications (one or two variable,

Table 1. Distribution of aflatoxin from each category analyzed.

Category	n	Distribution of Aflatoxin (ppb)					
		Mean (ppb)	Not Detectable (%)	1 to 10 (%)	10 to 100 (%)	100 to 1000 (%)	Greater than 1000 (%)
Full kernel BGYF	50	6349	0.0	14.0	12.0	2.0	72.0
BGYF limited to germ	50	12.4	62.0	32.0	2.0	4.0	0.0
BGYF limited to lower germ/tip cap	50	86.6	88.0	8.0	2.0	0.0	2.0
Non-BGYF	50	0.66	72.0	26.0	2.0	0.0	0.0
Random selection	300	55.5	77.3	18.7	3.7	0.0	0.7
Total	500	678.1	68.6	19.2	4.0	0.6	7.8

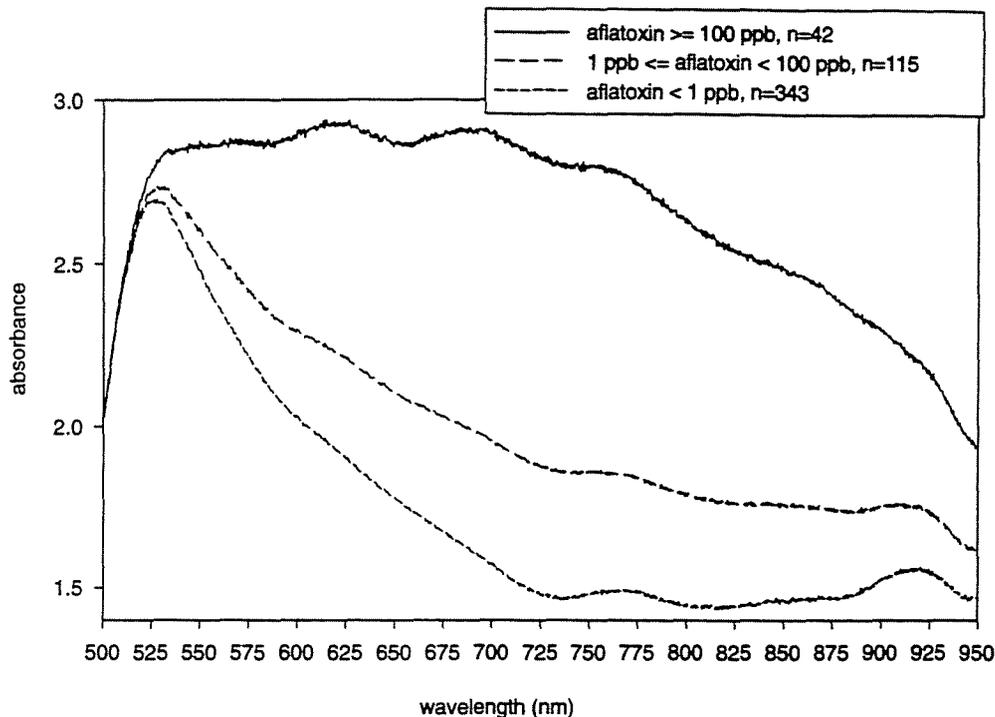


Figure 3. Plot of average whole kernel transmittance spectra.

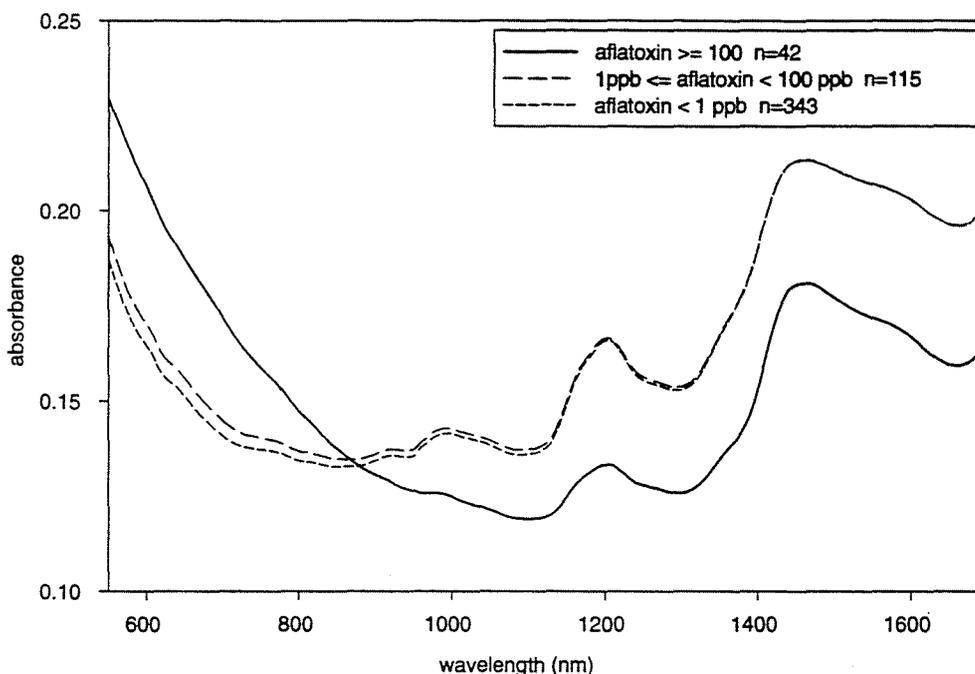


Figure 4. Plot of average whole kernel reflectance spectra.

reflectance or transmittance spectra) is most robust, but these figures suggest that transmittance might be. It should be noted that the orientation of the germ (toward the light source or the collection optics) while collecting the transmittance spectra was random, but this was not the case during reflectance measurements. It appears that, for reflectance measurements, a sorting system capable of determining kernel orientation may improve detection accuracy. When considering implementing reflectance or transmittance instrumentation in a high-speed online system, reflectance is generally easier to implement.

Commercial sorting machines are available that can measure transmittance of whole corn kernels at two discrete wavelengths and compute their ratios in a real-time sorting operation (Anzai et al., 1993). In addition, many machines can measure the reflectance of whole corn kernels at two discrete wavelengths (Brum, 1999). However, measuring transmitted or reflected light at four wavelengths and computing two ratios would require some hardware development. Further study would be needed to warrant development of a sorting system capable of computing two ratios using four different wavelengths.

Table 2. Classification results from the entire data set using discriminant analysis.

Spectra	Discriminant Function Features	Wavelengths Used for Ratios	False Positive Errors ^[a]		False Negative Errors ^[b]	
			0 ppb (%)	1 to 10 ppb (%)	10 to 100 ppb (%)	Over 100 ppb (%)
Transmittance	1	720/780	0.6	3.1	52.2	4.8
Transmittance	2	710/760	0.0	2.1	56.5	2.4
Reflectance	1	735/1005	0.0	1.0	65.2	2.4
Reflectance	2	1075/1135	0.3	1.0	87.0	2.4

[a] A false positive is defined as a kernel that was classified as aflatoxin positive having less than 10 ppb aflatoxin.

[b] A false negative is defined as a kernel that was classified as aflatoxin negative having 10 ppb or more aflatoxin.

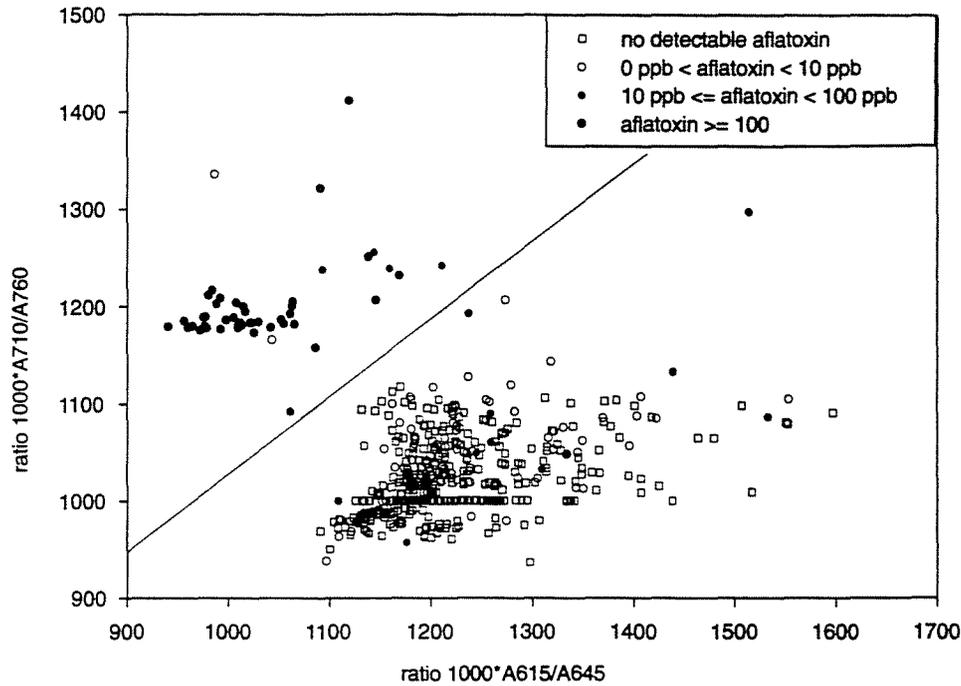


Figure 5. Plot of the ratio of absorbance values from the transmittance spectra at 615 nm and 645 nm versus the ratio of absorbance values at 710 nm and 760 nm. The line represents the boundary where kernels would be classified as aflatoxin positive or negative, given equal *a priori* probabilities.

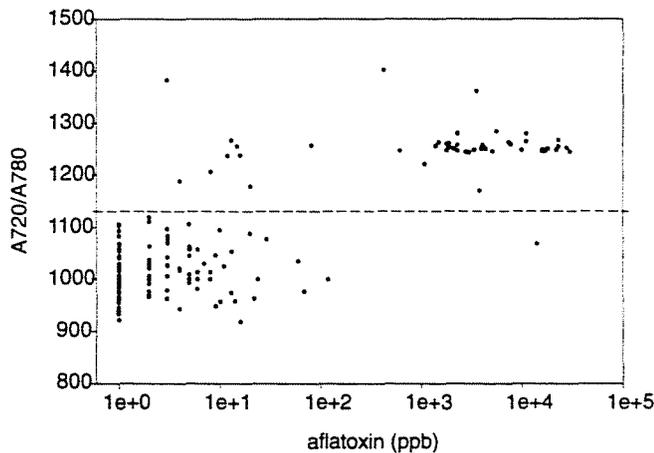


Figure 6. Plot of the ratio of absorbance values from the transmittance spectra at 720 nm and 780 nm versus measured aflatoxin content.

CLASSIFICATION BY PLS ANALYSIS

Table 3 shows the results of using PLS regression to classify kernels using all wavelengths. For both transmittance and reflectance measurements, kernels with

low (<10 ppb) and high (>100 ppb) aflatoxin levels could generally be correctly classified as aflatoxin positive or negative with greater than 95% accuracy when using a threshold of 10 ppb. Both techniques had difficulty in classifying kernels (<25% accuracy) with intermediate (10 to 100 ppb) aflatoxin levels. The classification results using PLS were comparable to discriminant analysis but not quite in all cases. This might be due to the nature of PLS being a regression technique rather than a classification technique. The germ-down position (germ facing the sensor) for reflectance measurements resulted in better classifications than the germ-up position, possibly because the fungus invades the germ first and thus should be more prevalent in the germ.

Classifications using transmittance spectra measured on randomly placed kernels were generally better than reflectance spectra from oriented kernels. The transmission spectra may yield better results than reflectance spectra because light must pass through the kernel, thus insuring that constituents inside the kernel have an opportunity to interact with the visible and NIR radiation. In reflectance mode, some energy penetrates into the kernel and is reflected back to the sensor, but most of the energy reaching the sensor may have interacted only with the kernel surface. If the *A. flavus* has

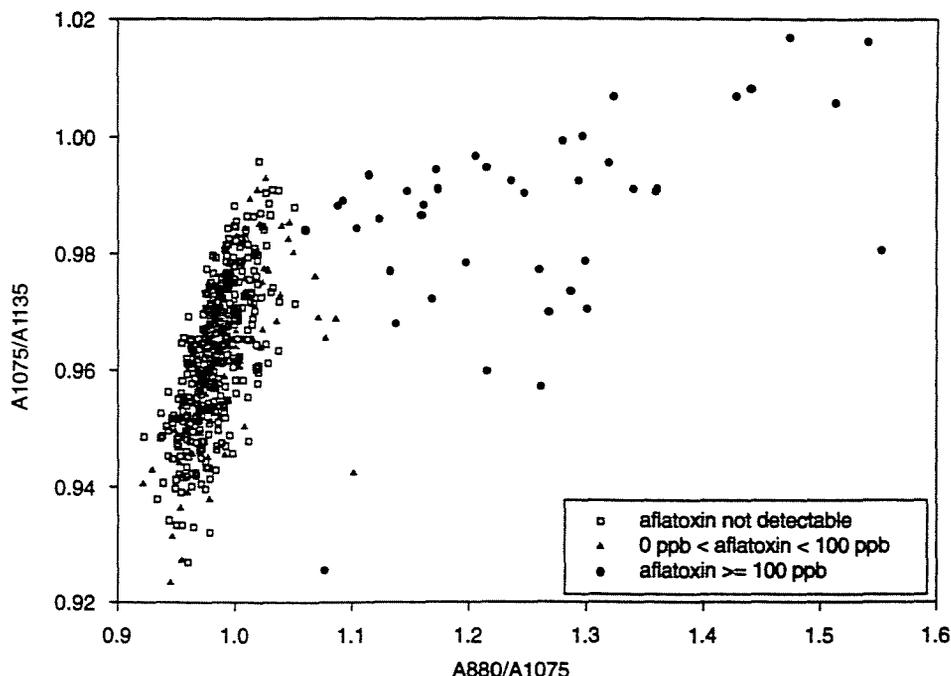


Figure 7. Plot of the ratio of absorbance values from the reflectance spectra at 1075 nm and 1135 nm versus the ratio of absorbance values at 880 nm and 1075 nm.

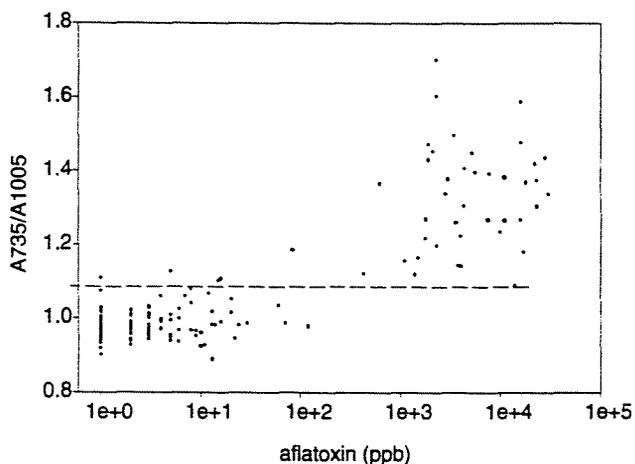


Figure 8. Plot of the ratio of absorbance values from the reflectance spectra at 735 nm and 1005 nm versus measured aflatoxin content.

had minimal influence on the kernel surface but has actively affected the kernel endosperm, then the transmission measurement may be more sensitive to effects of the fungal invasion. Similar classification results should be expected for

different varieties of corn. However, slightly different wavelength combinations may be required to give similar classification results for other varieties. Further experimentation would be required to verify classification accuracy on other corn varieties.

CONCLUSIONS

Transmittance and reflectance spectroscopy are both viable tools for detecting aflatoxin contamination in single corn kernels. Kernels with low (<10 ppb) or high (>100 ppb) levels could be detected with good accuracy, with transmittance yielding slightly better results. Classification accuracy of kernels with intermediate levels (10 to 100 ppb) was less than 25% for all techniques studied. However, intermediate levels of aflatoxin in individual kernels are unlikely to cause a sample to test above the FDA limit of 20 ppb. Discriminant analysis with one or two features gave slightly better classification results than full wavelength PLS analysis. These results indicate that this technology can potentially be used to automatically and rapidly detect aflatoxin in single corn kernels.

Table 3. Classification results for single corn kernel aflatoxin transmittance and reflectance spectra using PLS analysis.

Spectra	Number of PLS Factors	Number of Kernels	Wavelength Range (nm)	False Positive Errors ^[a]		False Negative Errors ^[b]	
				0 ppb (%)	1 to 10 ppb (%)	10 to 100 ppb (%)	Over 100 ppb (%)
Transmittance	4	495	500–950	0.6	2.1	78.9	5.1
Reflectance – Germ up	6	204 ^[c]	550–1700	2.6	4.7	91.7	14.7
Reflectance – Germ down	6	499	550–1700	0.0	2.1	84.2	4.9

^[a] A false positive is defined as a kernel having less than 10 ppb aflatoxin that was classified as aflatoxin positive.

^[b] A false negative is defined as a kernel having 10 ppb or more aflatoxin that was classified as aflatoxin negative.

^[c] The randomly-selected kernels from table 1 were not scanned in the germ-up position.

REFERENCES

- Anzai, K., A. Shibayama, S. Hirano, and H. Sasaki. 1993. Sorting apparatus utilizing transmitted light. U.S. Patent 5,190,163.
- Basappa, S. C., V. Sreenivasamurthy, and H. A. B. Paysia. 1970. Aflatoxin and kojic acid production by resting cells of *Aspergillus flavus* Link. *J. Gen. Microbiol.* 61(1): 81–86.
- Birth, G. S., and H. G. Hecht. 1987. The physics of near-infrared reflectance. In *Near-Infrared Technology in the Agriculture and Food Industries*, 1–15. P. Williams and K. Norris, eds. St. Paul, Minn.: American Association of Cereal Chemists.
- Brekke, O. L., A. F. Peplinski, G. E. N. Nelson, and E. L. Griffin. 1975. Cleaning trials for corn containing aflatoxin. *Cereal Chem.* 52(2): 198–204.
- Brum, J. W. 1999. Personal communication. Modesto, Calif.: Satake USA, Inc.
- CAST. 1989. Mycotoxins: Economic and Health Risks. Task Force Report No. 116. Ames, Iowa: Council for Agricultural Science and Technology.
- Delwiche, S. R. 1995. Single wheat kernel analysis by near-infrared transmittance: Protein content. *Cereal Chem.* 72(1): 11–16.
- Delwiche, S. R., and D. R. Massie. 1996. Classification of wheat by visible and near-infrared reflectance from single kernels. *Cereal Chem.* 73(3): 399–405.
- Delwiche, S. R., R. A. Graybosch, and C. J. Peterson. 1999. Identification of wheat lines possessing the 1A1.1RS or 1BL.1RS wheat-rye translocation by near-infrared reflectance spectroscopy. *Cereal Chem.* 76(2): 255–260.
- Dickens, J. W., and T. B. Whitaker. 1981. Bright greenish-yellow fluorescence in recently harvested yellow corn marketed in North Carolina. *J. Amer. Oil Chem. Soc.* 58(12): 973A–975A.
- Dowell, F. E., J. E. Throne, and J. E. Baker. 1998. Automated nondestructive detection of internal insect infestation of wheat kernels by using near-infrared reflectance spectroscopy. *J. Econ. Entomology* 91(4): 899–904.
- Dowell, F. E. 1998. Automated color classification of single wheat kernels using visible and near-infrared reflectance. *Cereal Chem.* 75(1): 142–144.
- Dowell, F. E., M. S. Ram, and L. M. Seitz. 1999. Predicting scab, vomitoxin, and ergosterol in single wheat kernels using near-infrared spectroscopy. *Cereal Chem.* 76(4): 573–576.
- GIPSA. 1999. *Grain Fungal Diseases and Mycotoxin Reference*. Kansas City, Mo.: GIPSA, Technical Services Division.
- Hesseltine, C. W., and O. Shotwell. 1973. New methods for rapid detection of aflatoxin. *Pure and Appl. Chem.* 35(3): 259–266.
- Hruschka, W. R. 1987. Data analysis: Wavelength selection methods. In *Near-Infrared Technology in the Agriculture and Food Industries*, 35–55. P. Williams and K. Norris, eds. St. Paul, Minn.: American Association of Cereal Chemists.
- Huberty, C. J. 1994. *Applied Discriminant Analysis*. New York, N.Y.: John Wiley and Sons.
- Hurburgh, C. R., Jr. 1991. Aflatoxin in midwestern corn. In *Aflatoxin in Corn: New Perspectives*, 343–350. Research Bulletin 599. O. L. Shotwell and C. R. Hurburgh, Jr., eds. Ames, Iowa: Iowa Agriculture and Home Economics Experiment Station.
- Lillehoj, E. B., W. F. Kwolek, R. E. Peterson, O. L. Shotwell, and C. W. Hesseltine. 1976. Aflatoxin contamination, fluorescence, and insect damage in corn infected with *Aspergillus flavus* before harvest. *Cereal Chem.* 53(4): 505–512.
- Kramer, K. J., T. D. Morgan, J. E. Throne, F. E. Dowell, M. Bailey, and J. A. Howard. 2000. Transgenic avidin maize is resistant to storage insect pests. *Nature Biotech.* 18(6): 670–674.
- Martens, H., and T. Naes. 1989. *Multivariate Calibrations*. Chichester, U.K.: John Wiley and Sons.
- McClure, W. F., and A. Farsaie. 1980. Dual-wavelength fiber optic photometer measures fluorescence of aflatoxin-contaminated pistachio nuts. *Trans. ASAE* 23(1): 204–207.
- Morris, C. F., V. L. DeMacon, and M. J. Giroux. 1999. Wheat grain hardness among chromosome 5D homozygous recombinant substitution lines using different methods of measurement. *Cereal Chem.* 76(2): 249–254.
- Nichols, T. E. 1983. Economic effects of aflatoxin in corn. In *Aflatoxin and Aspergillus flavus in Corn*, 67–71. Southern Cooperative Series Bulletin 279. U. L. Diener, J. W. Dickens, and R. L. Asquith, eds. Auburn, Alabama: Auburn University, Alabama Agricultural Experiment Station.
- Olson, M. C., and L. Stoloff. 1977. Analysis of aflatoxin problem in corn in the Southeast. Washington, D.C.: U. S. Food and Drug Administration.
- Orman, B. A., and R. A. Schumann Jr. 1992. Nondestructive single-kernel oil determination of maize by near-infrared transmission spectroscopy. *J. Amer. Oil Chem. Soc.* 69(10): 1036–1038.
- Pearson, T. C. 1999. Use of near-infrared light transmission to automatically detect almonds with concealed damage. *Lebensmittel-Wissenschaft und -Technologie* 32(2): 73–78.
- PLSplus/IQ. 1996. *PLSplus/IQ for Grams 32 and Grams 386: User's Guide*. Salem, N.H.: Galactic Industries Corporation and Microsoft Corporation.
- Ridgway, C., and J. Chambers. 1996. Detection of external and internal insect infestation in wheat by near-infrared reflectance spectroscopy. *J. Sci. Food Agric.* 71(2): 251–264.
- Schmitt, S. G., and C. R. Hurburgh, Jr. 1989. Distribution and measurement of aflatoxin in 1983 Iowa corn. *Cereal Chem.* 66(3): 165–168.
- Shotwell, O. L., M. L. Goulden, and C. W. Hesseltine. 1974. Aflatoxin: Distribution in contaminated corn. *Cereal Chem.* 51(4): 492–499.
- Shotwell, O. L., M. L. Goulden, A. M. Jepson, W. F. Kwolek, and C. W. Hesseltine. 1975. Aflatoxin occurrence in some white corn under loan, 1971: III. Association with bright greenish-yellow fluorescence in corn. *Cereal Chem.* 52(5): 670–677.
- Shotwell, O. L., and C. W. Hesseltine. 1981. Use of bright greenish yellow fluorescence as a presumptive test for aflatoxin in corn. *Cereal Chem.* 58(2): 124–127.
- U.S. Food and Drug Administration. 1988. Action levels for added poisonous or deleterious substances in food. Notice. *Federal Register* 53: 5043–5044.
- Wicklowsky, D. T., B. W. Horn, O. L. Shotwell, C. W. Hesseltine, and R. S. Caldwell. 1988. Fungal interference with *Aspergillus flavus* infection and aflatoxin contamination of maize grown in a controlled environment. *Phytopathology* 78(1): 68–74.
- Wicklowsky, D. T. 1999. *Aspergillus flavus* genotypic influence on aflatoxin and bright greenish-yellow fluorescence of corn kernels. *Plant Disease* 83(12): 1146–1148.
- Williams, P. C. 1997. Recent advances in near-infrared applications for the agriculture and food industries. In *Proceedings of the International Wheat Quality Conference*, 109–128. 18 to 22 May. Manhattan, Kansas.

Supplied by the U.S. Department of Agriculture, National Center for Agricultural Utilization Research, Peoria, Illinois.