# Transient Infrared Spectroscopy for Detection of Toxigenic Fungi in Corn: Potential for On-line Evaluation

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An urgent need for rapid sensors to detect contamination of food grains by toxigenic fungi such as *Aspergillus flavus* prompted research and development of Fourier transform infrared photoacoustic spectroscopy (FTIR−PAS) as a highly sensitive probe for fungi growing on the surfaces of individual corn kernels. However, the photoacoustic technique has limited potential for screening bulk corn because currently available photoacoustic detectors can accommodate only a single intact kernel at a time. Transient infrared spectroscopy (TIRS), on the other hand, is a promising new technique that can acquire analytically useful infrared spectra from a moving mass of solid materials. Therefore, the potential of TIRS for on-line, noncontact detection of *A. flavus* contamination in a moving bed of corn kernels was explored. Early test results based on visual inspection of TIRS spectral differences predict an 85% or 95% success rate in distinguishing healthy corn from grain infected with *A. flavus*. Four unique infrared spectral features which identified infected corn in FTIR−PAS were also found to be diagnostic in TIRS. Although the technology is still in its infancy, the preliminary results indicate that TIRS is a potentially effective screening method for bulk quantities of corn grain.

**Keywords:** *Aspergillus flavus; aflatoxin; fungi; corn; transient infrared spectroscopy; FTIR−photoacoustic spectroscopy*

## INTRODUCTION

A major problem facing American agriculture is the occurrence of deadly diseases in humans and animals caused by sporadic contamination of food and feed grains with mycotoxins from fungi such as *Aspergillus flavus* and *Fusarium moniliforme*. The *Aspergillus* strains which produce aflatoxins and the *Fusarium* strains that produce fumonisins are well-recognized health hazards (Goldblatt, 1969; Marasas et al., 1984; Smith and Moss, 1985; Sydenham et al., 1990b; Wicklow, 1991; Casteel and Braun, 1992). The most abundant aflatoxin, $B_1$, is known to be one of the most potent human liver carcinogens yet discovered (Hsieh, 1989; Sydenham et al., 1990a). Fumonisins, from the ubiquitous *Fusarium* strains, have been shown to induce liver tumors and hyperplasia in esophageal cells of rats (Marasas et al., 1984; Gelderblom et al., 1991) and have also been implicated in human esophageal cancer (Sydenham et al., 1990a,b). In addition to the health hazard, there is substantial economic loss when grain, destined for either animal feed or export commodity markets, is devalued due to mycotoxin contamination (Nichols, 1983).

There is an urgent need to develop rapid and efficient testing methods and strategies to reduce the extent of infestation of grains by these fungi and thus lower the levels of mycotoxins in the food and feed supply (Gorst-Allman and Steyn, 1984). Recently, the relatively new enzyme-linked immunosorbent assay (ELISA) has been used in screening corn samples for aflatoxins in laboratory as well as nonlaboratory situations (e.g., at elevators and off farms). Commercially available ELISA test kits are reliable aflatoxin detectors if the tests are conducted properly by trained personnel (Wilcke et al., 1990). Despite their advantage for quantitative analyses, detection and identification of mycotoxins by chemical methods such as ELISA is at present inconvenient and impractical for routine production use.

For several years, the bright greenish-yellow fluorescence (BGYF) test has been used throughout the corn industry to monitor corn for aflatoxin (Shotwell et al., 1975). The BGYF test detects the enzyme-activated fluorescence of kojic acid, a minor metabolite of *A. flavus*, when infected corn is irradiated with ultraviolet light. Although the BGYF test is rapid and facile, it is a presumptive test and therefore used only to visually identify questionable corn lots for further analyses by the more aflatoxin-specific ELISA method. If BGYF is the sole criterion for the presence or absence of aflatoxin, many false positive tests and a few false negative tests may occur (Shotwell et al., 1975, 1981; Shotwell and Hesseltine, 1981). For example, yellow corn lots from North Carolina in 1988 contained an average aflatoxin concentration of 49 ppb in the kernel fraction that appeared to be uninfected by the BGYF test. This was a potentially dangerous false negative test result since it exceeded the regulatory limit of 20 ppb (Dickens and Whitaker, 1981).

Recent technological advances in Fourier transform infrared (FTIR) spectroscopy have enabled infrared analyses of biological materials in their solid states. FTIR spectroscopy is extremely specific and provides a wealth of qualitative and quantitative information, especially in the mid-infrared absorption range where most biological materials have distinctive spectra (McDonald, 1986). Promising nondestructive FTIR spectroscopic methods are presently being studied at this

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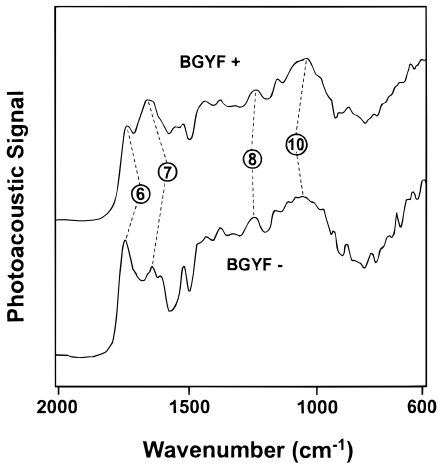


**Figure 1.** FTIR−PAS spectra of infected (BGYF+) and uninfected (BGYF−) corn kernels. Salient spectral features are noted by circled numbers.

USDA laboratory to detect *A. flavus* and *F. moniliforme* on corn grain and to obtain measurements from surfaces of individual infected kernels for possible use by plant pathologists and geneticists breeding corn hybrids for resistance to these and other mycotoxigenic fungi.

**Fourier Transform Infrared Photoacoustic Spectroscopy (FTIR−PAS).** Because of its inherent sensitivity to the near-surface region of the sample, FTIR−PAS is an excellent sensor of fungal infection on the surfaces of corn kernels (Greene et al., 1988, 1992; Greene and Gordon, 1992). In an earlier blind study (Gordon et al., 1997) it was found that FTIR−PAS spectra of 10 *A. flavus*-infected (BGYF-positive) corn kernels were distinct from spectra of 10 uninfected (BGYF-negative) kernels. These corn kernels which were previously classified as infected or uninfected by the BGYF test were then correctly and unambiguously classified by FTIR−PAS. BGYF-positive kernels, which showed the characteristic bright greenish-yellow fluorescence in the entire germ and endosperm, gave distinctive FTIR−PAS spectra from which a number of biochemically interpretable spectral features associated with fungal infection were identified. Four of these spectral features typical of infected and uninfected corn kernels are pointed out by circled numbers in Figure 1.

When these infrared spectral features were correlated with the BGYF data, it was observed that the FTIR−PAS and BGYF test results were identical. However, it was shown later (Gordon et al., 1998) that FTIR−PAS, with its multifactor classification scheme that detects several chemical and physical changes in the infected corn kernel, is considerably more reliable than the single-factor BGYF test for detecting *A. flavus* infection in corn. This is particularly important in cases where the kernels are lightly infected or *A. flavus* produces little or no kojic acid and false negative BGYF tests occur. Furthermore, FTIR−PAS can detect other mycotoxigenic fungi, such as *F. moniliforme* (Greene et al., 1992), while the BGYF test detects only *A. flavus* or *A. parasiticus* (Shotwell et al., 1975).

Despite these advantages, the FTIR−PAS technique is currently limited, by the design of commercially available photoacoustic detectors, to analysis of a single corn kernel in a small sealed sample cell. While this may be entirely adequate for use by plant pathologists or breeders studying individual seeds, any practical approach to safeguarding foods and feeds from mycotoxins must be capable of screening grain in scoopful or conveyor-belt quantities.

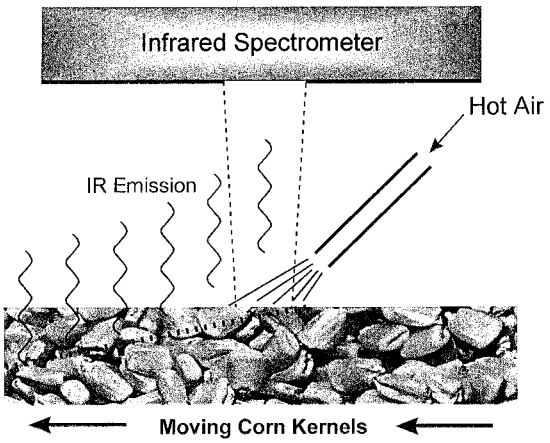


**Figure 2.** Schematic illustration of corn kernels moving past the detector in TIRS.

**Transient Infrared Spectroscopy (TIRS).** Transient infrared spectroscopy is a promising new technique that overcomes the limitations of photoacoustics and other midrange infrared methods by allowing, for the first time, noncontact analyses of a flat bed of solid materials moving at conveyor belt speed (Jones and McClelland, 1989, 1990a,b, 1993; McClelland and Jones, 1991a,b, 1992). TIRS works by heating or cooling the surface of the moving material to create a thin nonopaque surface layer that is ideal for infrared analysis. In TIRS an optically thin surface layer is optionally either rapidly heated or cooled for an instant before the sample passes through the field of view of an infrared spectrometer.

If, for example, the surface of a corn kernel is rapidly heated, thermal emission is collected for analysis from the surface layer before it cools. If the kernel surface is rapidly cooled, thermal emission from the bulk of the kernel passes through the chilled surface layer and is collected for analysis. When the surface layer is made hotter than the bulk of the kernel, as depicted in Figure 2, the technique is called transient infrared emission spectroscopy (TIRES). When the surface layer is made colder than the bulk, the technique is called transient infrared transmission spectroscopy (TIRTS). Both techniques work by creating a thin, short-lived, heated or cooled layer at the kernel surface and both allow infrared spectra to be obtained directly as transitory radiation from the sample kernels (Jones and McClelland, 1990a,b, 1992, 1993).

Although the TIRS technology is still in its infancy, early experimental results at the Ames Laboratory have shown that excellent quantitative infrared spectra can be obtained from both smooth and irregular materials moving from 4 to 1000 f/min (Jones and McClelland, 1989). Therefore, TIRS has the potential to provide online quality control of corn grain in a way not available before. This paper reports the first experiments in research to develop TIRS for detection of *A. flavus* in field-infected corn grain.

## MATERIALS AND METHODS

***Aspergillus flavus.*** Three aflatoxin-producing strains of *A. flavus* (NRRL 3357, NRRL 6412, and NRRL 6444) from corn harvested in North Carolina (Wicklow et al., 1981) were cultured on a slant of potato dextrose agar for 14 days at 25

°C and then freeze-dried. Conidia from cultures suspended in distilled water were used as a mixed spore inoculum.

**Corn Samples.** For initial TIRS experiments, individual corn kernels were collected from a commercial Illinois field corn as the control sample. These were kernels from a healthy yellow corn lot with no visible mold damage and presumably (from BGYF tests) no detectable aflatoxins. Infected kernels were collected from a Georgia field corn that was naturally contaminated with *A. flavus* and contained aflatoxins at average levels exceeding the permissible 20 ppb (Greene et al., 1992).

For subsequent TIRS experiments individual corn kernels were selected from two aflatoxin-susceptible experimental corn hybrids (Mycogen, L024 × L03 and L035 × L010) grown at Lincoln, IL in 1994. Maize ears of the hybrids were wound-inoculated in the late milk to early dough stage of kernel maturity (at 21 days after silking) with the *A. flavus* spore inoculum. Naturally dried-down ears (14−16% M.C.) were hand-harvested in October, and nondamaged kernels within two rows from individual points of wound inoculation were removed from the ear. Kernels were examined periodically under ultraviolet light (365 nm) and classified by visual observation of the bright greenish-yellow fluorescence as BGYF-positive (BGYF+) or BGYF-negative (BGYF−). BGYF-positive kernels showed full-kernel glowing under ultraviolet light and presumably were heavily infected with *A. flavus*.

The individual kernels were also classified based on the presence or absence of visible symptoms of fungal infection (kernel discoloration or damage). Mold damage to kernels caused by *A. flavus* invasion appeared as minute cracks or splits in the pericarp over the crown or along the side of the kernel but not over the germ. Heavily infected kernels naturally appeared more damaged than lightly infected kernels and showed visible *A. flavus* at points of damage to the kernel. Some infected kernels were not cracked or split but were classified as mold damaged because of the darkened or discolored appearance of the germ or crown due to fungal infection. Kernels that appeared undamaged and not discolored were classified as symptomless whether they were infected with *A. flavus*. While the uninoculated control kernels were classified as symptomless and BGYF-negative, some very lightly infected kernels were symptomless but tested BGYF-positive. Thus, the kernels in each hybrid corn were separated into four groups: mold damaged, BGYF+; symptomless, BGYF+; mold damaged, BGYF−; and symptomless, BGYF−.

**Sample Preparation for TIRS Analysis.** The selected corn kernels were stored in a freezer for several months before testing. All kernels were tested moist (19−22% M.C.) after being equilibrated to room temperature. Each group of several (20 to 30) kernels was analyzed separately. Stringent safety measures were observed in handling the samples because the infected kernels contained aflatoxin and viable *A. flavus* spores.

**Instrumentation.** In initial experiments TIRS spectra were obtained with a Perkin-Elmer 1800 FTIR spectrometer equipped with a wide-band liquid-nitrogen-cooled MCT detector. The spectrometer was operated at a 1.5 cm/s (OPD) scan speed and 8 $cm^{-1}$ resolution, coadding 256 scans. Spectra were acquired single beam and later ratioed against carbon black spectra obtained in the same manner. When needed, spectra were cleared of interference from water vapor by digital subtraction.

The intact corn kernels were epoxied single file in a 3 in. diameter circle on aluminum disks that were mounted on the shaft of a variable-speed DC motor. The infrared source of the spectrometer was removed, and the motor was mounted so that the corn was positioned where the source had been. A KCl window covered the port between the source position and the rest of the spectrometer. The disks were spun at 100 rpm, which corresponds to a linear sample speed of 80 ft/min. A hot jet of nitrogen from a 0.16 in. by 0.02 in. nozzle at 0.15 L/s was aimed onto the sample. The hot nitrogen was provided by a stream of nitrogen that passed through an improvised air heater incorporating the heating element of a heat gun running at 210 W. The tip of the nozzle was 2 mm upstream (in terms of sample motion) from the upstream edge of the spectrometer field of view. A jet of cold helium, produced by running 0.15 L/s of helium through a liquid-nitrogen bath, was directed onto the corn kernels about 45° downstream from the spectrometer field of view. This early TIRS instrumentation, which has been described in greater detail (Jones and McClelland, 1990a), produced the spectra in Figure 3.

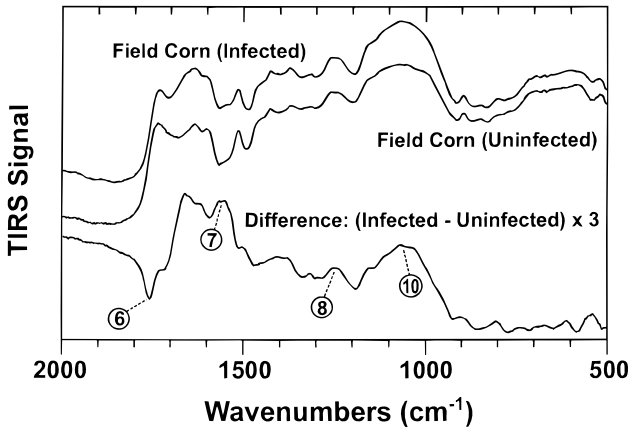


**Figure 3.** Normalized TIRS spectra of a uninfected field corn and field corn infected with *A. flavus*.

In subsequent experiments TIRS spectra were obtained with a Bomem MB100 FTIR spectrometer without an infrared source and with both an emission port and a wide-band liquid-nitrogen-cooled MCT detector. The emission port and the port to which the infrared source would normally attach were both covered by KBr windows. A cold source (liquid-nitrogen-filled Dewar with a window in its side) was positioned where the infrared source would normally be mounted. The Bomem spectrometer operates at 1.5 cm/s (OPD) scan speed. Coaddition of 64 scans at 8 $cm^{-1}$ resolution sufficed to give adequate signal-to-noise. Spectra were acquired single beam and normalized later. Water vapor bands were removed by normalization against a freshly acquired blackbody emission spectrum. A 5.5-in. focal-length gold mirror was mounted outside the emission port of the spectrometer so that it focused the spectrometer's gaze downward onto the moving sample.

The corn was mounted at the focal plane of the mirror. In this setup the corn was clamped to a brass disk having a 3-in. diameter circular groove cut in its face. A smaller brass disk and brass ring were screwed to the grooved disk so that there was a gap (a circular slot) between the ring and the small disk at the same diameter as the groove in the large disk. The corn kernels sat single file in this groove, and the ring and small disk acted as clamps, gripping the outer and inner edges, respectively, of the kernels. The disk was spun at a speed of 100 rpm, corresponding to 80 ft/min. The hot jet in this setup was a 0.47 L/s stream of air (from the laboratory compressed air system, which was not dehumidified or otherwise treated) that passed through a commercial hot-air tool (Leister model 700) operating at 198 W and was aimed onto the corn by a 2-mm-inner-diameter round nozzle. The nozzle tip was again slightly upstream (roughly 1 mm) from the spectrometer field of view. Since the gas flow rate was substantially higher, the hot jet was cooler in this setup than in the preliminary experiment but the heat flow (i.e., energy per time) was about the same. The cold jet was the same as that described above. This new TIRS instrumentation was a rugged prototype designed to operate under field conditions and to simulate analyses of solid materials moving on a process line in real time. It produced the spectra in Figures 4 and 5.

## RESULTS AND DISCUSSION

For comparison with the TIRS results, four of the most salient FTIR−PAS spectral features of *A. flavus*-infected corn reported earlier (Gordon et al., 1997) are significant. These features are those pointed out in Figure 1 by the circled numbers **6**, **7**, **8**, and **10**, the same

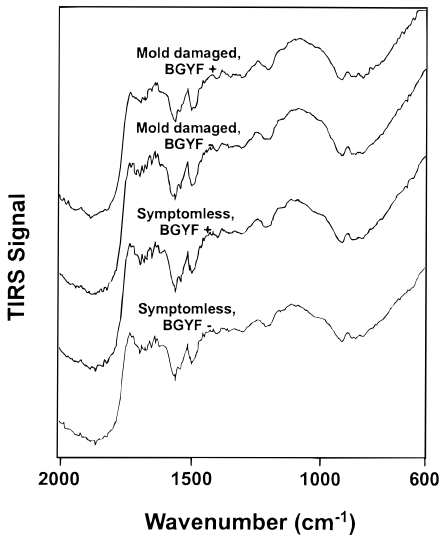


**Figure 4.** Normalized TIRS spectra of BGYF classified hybrid corn (L024 × L03).

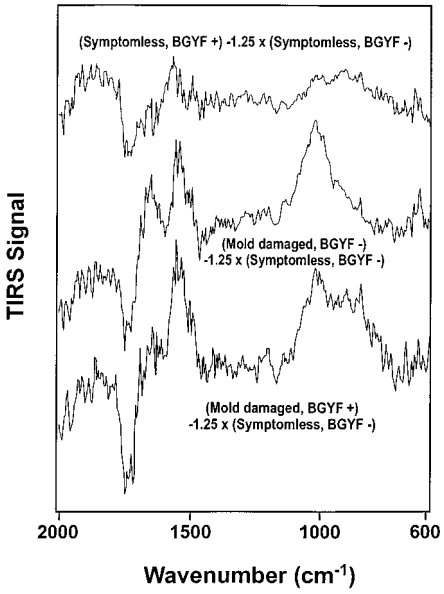


**Figure 5.** TIRS difference spectra of BGYF classified hybrid corn (L024 × L03).

numbers used in the earlier report. The four FTIR−PAS features correspond to features in the TIRS spectral region which were not confounded by unavoidable data processing differences between the two techniques.

Initial spectra recorded for field corn using the early TIRS instrument are shown in Figure 3. The difference between the spectra of infected field corn and uninfected field corn is shown magnified 3× in the bottom trace. Circled numbers point to the four TIRS features that correspond to FTIR−PAS features. As with the FTIR−PAS spectra, these TIRS features can be explained by effects expected from fungal growth on the surface of corn. The decreased carbonyl absorption (feature **6**) likely reflects consumption of corn lipids by the fungi, while the increased amide II absorption (feature **7**) and the changed C−O absorptions (features **8** and **10**) probably reflect increased protein and carbohydrate formation as the fungi grow. Another explanation for these feature changes might be that the damage caused by fungal invasion of corn kernels opens and exposes inner surfaces to the infrared light where relative carbohydrate, protein, and lipid composition differs from the outer surface. For both FTIR−PAS and TIRS, the increased porosity not only eases the passage of infrared light, but also eases passage of the thermal wave to (in TIRS) or from (in FTIR−PAS) 'inner surfaces', which would increase the 'inner surface' signal.

Since it was observed that both FTIR−PAS and TIRS showed these same four spectral effects, a reasonable estimation of the potential of TIRS for detection of *A. flavus* in corn could be predicted from the same spectral features and classification scheme used in the previous FTIR−PAS study.

In that work (Gordon et al., 1997) the spectral feature changes were the criteria for classification of 20 corn kernels (labeled A−T in Table 1) as infected with *A. flavus* or uninfected by comparison with BGYF tests. By this scheme each corn kernel was classified as infected (+), uninfected (−), or questionable (?) for each criterion from the FTIR−PAS spectral features. A simple unweighted voting strategy was used to compute a consensus from the spectral criteria. Each spectral test for each corn kernel was assigned a score of +1, −1, or 0, respectively, for infected, uninfected, or questionable classifications of each spectral feature. The sign of the sum (Σ) of the scores was taken as the consensus test result. For simplicity, in this classification scheme all criteria were thresholded before combining and given equal weight in the combination.

Applying this scheme, as shown in Table 1, to the four spectral features **6**, **7**, **8**, and **10** from the previous FTIR−PAS study which were also found to be diagnostic in the present TIRS experiments revealed that if TIRS had been applied to the 20 corn kernels in that study all 10 of the uninfected kernels would be correctly classified, 2 of the infected kernels would be classified as questionable and since questionable kernels can be counted as possibly infected, 7 or 9 of the infected kernels would be correctly classified by TIRS from these four features. This would therefore predict an immediate success rate of 85% (17/20) or 95% (19/20) for the TIRS technique in distinguishing infected from uninfected corn.

Further evidence of the potential of TIRS for detecting fungal contamination in corn was obtained in subsequent experiments using the prototype instrument

**Table 1. Classification of Infected and Uninfected Corn Kernels (A−T) from FTIR−PAS Spectral Features 6, 7, 8, and 10: Comparison with BGYF Tests[a]**

| | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **6** | − | + | + | − | + | − | − | − | − | ? | + | + | − | + | − | − | − | + | − | + |
| **7** | + | + | + | − | − | − | − | + | − | ? | + | + | + | + | + | + | − | + | + | + |
| **8** | − | + | + | − | − | − | − | − | − | − | + | + | − | + | − | − | − | + | + | + |
| **10** | + | + | + | − | − | − | − | − | − | − | + | + | − | + | − | − | − | − | − | + |
| Σ | ? | + | + | − | | − | − | − | − | − | + | + | − | + | − | − | − | + | ? | + |
| **BGYF** | + | + | + | − | − | − | − | − | − | − | + | + | + | + | − | − | − | + | + | + |

[a] FTIR−PAS and BGYF test results were extracted from data reported earlier (Gordon et al., 1997). +, Infected; −, Uninfected; ?, Questionable; Σ, Sum (unweighted).

developed recently at the Ames Laboratory. Tests of the new TIRS instrument were conducted on two aflatoxin-susceptible corn hybrids that were grown in experimental plots to study effects of *A. flavus* infection in the field. Each hybrid was classified according to BGYF and mold damage into four groups of kernels as described above.

When each group of kernels was moved at 80 ft/min past the field of view of the new TIRS instrument, spectra were obtained (Figure 4) that closely resembled the early TIRS spectra and also agreed with FTIR−PAS data. As seen in Figure 5, spectra of infected and uninfected kernels showed the four diagnostic feature differences in the lipid, protein, and carbohydrate regions. Interestingly, some kernels which were visibly damaged by *A. flavus* showed these same spectral differences even though they tested BGYF-negative. This would support the alternative explanation mentioned above, i.e., TIRS and FTIR−PAS spectral features may be measures of the damage inflicted by fungal hypha as they penetrate the surface and expose inner parts of the corn kernels. In fact, it is likely that spectral differences caused by fungal damage to the kernel are much larger and more evident than those due to the presence of the fungal mass itself. Even at fungal levels too low for BGYF detection, a small number of the microbes may leave destroyed or damaged kernel surface areas large enough to be detected by TIRS. As evidence, in the TIRS spectra of the hybrid corn in Figure 5, the mold-damaged BGYF-positive kernels show greater differences from symptomless BGYF-negative kernels than the mold-damaged BGYF-negative kernels, while, in the same hybrid, the symptomless BGYF-positive kernels showed significant but less difference from symptomless BGYF-negative kernels. Hence, these spectra indicate the relative levels of *A. flavus* infection in the four groups of kernels were as follows: mold-damaged, BGYF-positive > mold-damaged, BGYF-negative > symptomless, BGYF-positive > symptomless, BGYF-negative.

TIRS spectra similar to those of corn hybrid L024 × L03 in Figures 4 and 5 were obtained for both hybrids used in this study. Spectral differences for hybrid L035 × L010 were even more pronounced than for hybrid L024 x L03, but the former spectra are not presented here because only seven kernels were available in the symptomless BGYF-positive set, and these were too few to test by the TIRS instrument used. However, the TIRS spectra showed the same relative differences between kernels, including the interesting difference between mold-damaged BGYF-negative kernels and symptomless BGYF-negative kernels, as shown in Figure 5. Thus, besides correctly identifying both mold-damaged BGYF-positive and symptomless BGYF-positive corn, TIRS correctly indicated the fungally damaged corn was highly contaminated with *A. flavus* even though the BGYF test was negative.

The preliminary results presented in this paper demonstrate that TIRS is capable of detecting fungal contamination in corn grain moving on-line in real time. Although the technology is still in its infancy, these results indicate that TIRS promises to be an effective screening method for bulk quantities of corn grain. TIRS may also be useful for quality control of corn, peanuts, and other cereal products moving on process lines at conveyor-belt speed. More work is planned to determine the detection limits of fungal contamination as the budding TIRS technology matures. It should be possible to achieve greater sensitivity by including more infrared spectral features in the analysis. However, present data indicate the sensitivity is potentially excellent.

Furthermore, when differences between TIRS spectra of healthy grain and infected grain are subtle or small enough to escape detection by visual inspection, the spectra should be easily distinguishable by pattern recognition methods such as artificial neural networks. In an FTIR−PAS study of challenging samples of a commercial hybrid corn (Pioneer 3379) infected with *A. flavus*, neural networks correctly identified 96% of the infected kernels including 15% that were missed by the BGYF test (Gordon et al., 1998).

## CONCLUSIONS

The spectra obtained in this work prove that the TIRS technique effectively distinguishes healthy corn from corn infected with *A. flavus* at fungal levels comparable to the challenging levels successfully detected by FTIR−PAS. Since TIRS is a surface-sensitive technique, as well as a multifactor classification method, it is capable of providing all the advantages of FTIR−PAS but without the sample size limitation.

Although the emission technique, TIRES, may work successfully on corn kernels at normal room temperatures, it necessarily must rely on surface heating to produce thermal emission. Therefore, when the kernel surface is much hotter than the bulk or, more precisely, when the thermal gradient between the kernel surface and the bulk is high, the method will work best (Jones and McClelland, 1990b). This means that TIRES may not be applicable to corn grain under unusually hot initial conditions, as often occurs in industrial settings. However, in such situations the alternative transmission technique, TIRTS, which would chill the kernel surface, could then be applied.

At its early stage of development, TIRS technology already provides analytical results comparable to more well-known and widely used infrared techniques. The present signal-to-noise ratio in TIRS is excellent below 2000 $cm^{-1}$ but marginal above 2000 $cm^{-1}$. There are numerous modifications that could be made to optimize TIRS, and experiments are presently under way to improve the instrumentation. There is much incentive for developing TIRS technology further because of the myriad possible industrial applications for on-line infrared monitoring of solids. This is a specific goal of ongoing research at the Ames Laboratory. If this work continues to be successful, TIRS will emerge as a potentially important tool for safeguarding agricultural commodities from toxigenic and pathogenic fungi.

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## LITERATURE CITED

Casteel, S. W.; Braun, W. F. Mycotoxicoses. In *Encyclopedia of Microbiology*; Lederberg, J., Ed.; Academic Press: New York, 1992; Vol. 3, pp 217−227.

Dickens, J. W.; Whitaker, T. B. Bright greenish-yellow fluorescence and aflatoxin in recently harvested yellow corn marketed in North Carolina. *J. Am. Oil Chem. Soc.* **1981**, *58*, 973A−975A.

Gelderblom, W. C. A.; Kriek, N. P. J.; Marasas, W. F. O.; Thiel, P. G. Toxicity and carcinogenicity of *Fusarium moniliforme* metabolite, fumonisin B1, in rats. *Carcinogenesis* **1991**, *12*, 1247−1251.

Goldblatt, L. A. *Aflatoxin: Scientific Background, Control, and implications*; Academic Press: New York, 1969.

Gordon, S. H.; Schudy, R. B.; Wheeler, B. C.; Wicklow, D. T.; Greene, R. V. Identification of Fourier transform infrared photoacoustic spectral features for detection of *Aspergillus flavus* infection in corn. International Commission on Food Mycology. *IUMS. Int. J. Food Microbiol.* **1997**, *35*, 179−186.

Gordon, S. H.; Schudy, R. B.; Wheeler, B. C.; Wicklow, D. T.; Greene, R. V. Neural network pattern recognition of photoacoustic FTIR spectra and knowledge-based techniques for detection of toxigenic fungi in corn. *J. Food Protection*, **1998**, *61*, 221−230.

Gorst-Allman, C. P.; Steyn., P. S. Mycotoxins: Production, Isolation, Separation and Purification. In *Developments in Food Science*; Betina, V., Ed.; Elsevier: Amsterdam, 1984; Vol. 8, Chapter 6.

Greene, R. V.; Freer, S. N.; Gordon, S. H. Determination of solid-state fungal growth by Fourier transform infrared photoacoustic spectroscopy. *FEMS Microbiol. Lett.* **1988**, *52*, 73−78.

Greene, R. V.; Gordon, S. H. New nondestructive technology for evaluating corn. In *Proceedings: Corn Utilization Conference IV*, Peoria, IL, 1992.

Greene, R. V.; Gordon, S. H.; Jackson, M. A.; Bennett, G. A.; McClelland, J. F.; Jones, R. W. Detection of fungal contamination in corn: potential of FTIR−PAS and −DRS. *J. Agric. Food Chem.* **1992**, *40*, 1144−1149.

Hsieh, D. P. H. Potential human health hazards of mycotoxins. In *Mycotoxins and Phycotoxins '88*; Natori, S., Hashimoto, K., Ueno, Y., Eds.; Elsevier: Amsterdam, 1989; pp 69−80.

Jones, R. W.; McClelland, J. F. Transient infrared emission spectroscopy. *Anal. Chem.* **1989**, *61*, 650−656.

Jones, R. W.; McClelland, J. F. Quantitative analysis of solids in motion by transient infrared emission spectroscopy using hot-gas jet excitation. *Anal. Chem.* **1990a**, *62*, 2074−2079.

Jones, R. W.; McClelland, J. F. Transient infrared transmission spectroscopy. *Anal. Chem.* **1990b**, *62*, 2247−2251.

Jones, R. W.; McClelland, J. F. Transient IR spectroscopy: On-line analysis of solid materials. *Spectroscopy* **1992**, *7* (4), 54−58.

Jones, R. W.; McClelland, J. F. On-line analysis of solids and viscous liquids by transient infrared spectroscopy. *Process Control Qual.* **1993**, *4*, 253−260.

Marasas, W. F. O.; Nelson, P. E.; Toussoun, T. A. *Toxigenic Fusarium Species: Identity and Mycotoxicology*; The Pennsylvania State University Press: University Park, PA, 1984.

McClelland, J. F.; Jones, R. W. Apparatus and method for transient thermal infrared spectrometry. U.S. Patent 5,070,242,1991a.

McClelland, J. F.; Jones, R. W. Apparatus and method for transient thermal infrared emission spectrometry. U.S. Patent 5,075,552, 1991b.

McDonald, R. S. Infrared Spectrometry. *Anal. Chem.* **1986**, *58*, 1906−1925.

Nichols, T. E., Jr. Economic impact of aflatoxin in corn. In *Aflatoxin and Aspergillus flavus in corn*; Southern Cooperative Series Bulletin 279; Deiner, U. L., Asquith, R. L., Dickens, J. W., Eds.; Alabama Agricultural Experiment Station: Auburn, AL, 1983.

Shotwell, O. L.; Goulden, M. L.; Jepson, A. M.; Kwolek, W. F.; Hesseltine, C. W. Aflatoxin occurrence in some white corn under loan, 1971. III. Association with bright greenish-yellow fluorescence in corn. *Cereal Chem.* **1975**, *52*, 670−677.

Shotwell, O. L.; Hesseltine, C. W. Use of bright greenish yellow fluorescence as a presumptive test for aflatoxin in corn. *Cereal Chem.* **1981**, *58*, 124−127.

Shotwell, O. L.; Kwolek, W. F.; Hesseltine, C. W. Aflatoxin in freshly harvested 1979 Georgia corn and formation after collection. *J. Am. Oil Chem. Soc.* **1981**, *58*, 980A−983A.

Smith, J. E.; Moss, M. O. *Mycotoxins: Formation, Analyses and Significance*; John Wiley & Sons: Chichester, England, 1985.

Sydenham, E. W.; Gelderblom, W. C. A.; Thiel, P. G.; Marasas, W. F. O. Evidence for the natural occurrence of fumonisin B1, a mycotoxin produced by *Fusarium moniliforme*, in corn. *J. Agric. Food Chem.* **1990a**, *38*, 285−290.

Sydenham, E. W.; Thiel, P. G.; Marasas, W. F. O.; Shephard, G. S.; Schalkwyk, D. J. V.; Koch, K. R. Natural occurrence of some *Fusarium* mycotoxins in corn from low and high esophageal cancer prevalence areas of the Transkei, southern Africa. *J. Agric. Food Chem.* **1990b**, *38*, 1900−1903.

Wicklow, D. T. Epidemiology of *Aspergillus flavus* in corn. *North Central Regional Research Publication 329; Research Bulletin 599*; Iowa Agriculture and Home Economics Experiment Station. Iowa State University: Ames, IA, 1991; pp 315−327.

Wicklow, D. T.; Shotwell, O. L.; Adams, G. L. Use of aflatoxin-producing ability medium to distinguish aflatoxin-producing strains of *Aspergillus flavus*. *Appl. Environ. Microbiol.* **1981**, *41*, 697−699.

Wilcke, W. F.; Ehrich, M. R.; Ko, K. W. Using ELISA kits to test corn for aflatoxin. *Appl. Agric. Res.* ***1990***, *5*, 32−36.

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