# Detection of fungal contamination in corn: potential of FTIR-PAS and -DRS

**ARTICLE** *in* JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY · APRIL 2002

Impact Factor: 2.91 · DOI: 10.1021/jf00019a011 · Source: OAI



**4 AUTHORS**, INCLUDING:

Mark A. Jackson
United States Department of Agriculture
**100** PUBLICATIONS **1,872** CITATIONS

 Published in Jrl. of Agricultural & Food Chemistry, July 1992, 1144-1149, by the American Chemical Society 

# Detection of Fungal Contamination in Corn: Potential of FTIR–PAS and –DRS†

Richard V. Greene,* Sherald H. Gordon, Mark A. Jackson, and Glenn A. Bennett

Biopolymer Research, Fermentation Biochemistry Research, and Mycotoxin Research, National Center for Agricultural Utilization Research, Agricultural Research Service, U.S. Department of Agriculture, 1815 North University Street, Peoria, Illinois 61604

John F. McClelland and Roger W. Jones

Center for Advanced Technology Development and Ames Laboratory, U.S. Department of Energy, Iowa State University, Ames, Iowa 50011

Evaluation of agricultural grains, such as corn, suffers from a lack of techniques that can analyze solid materials. Two techniques, photoacoustic spectroscopy (PAS) and diffuse reflectance spectroscopy (DRS), were coupled to a Fourier transform infrared (FTIR) spectrometer to provide information about the mid-infrared absorption spectra of corn. Spectra generated from corn that was infected with *Fusarium moniliforme* or *Aspergillus flavus*, two mycotoxin producers, were dramatically different from those of uninfected corn. For *F. moniliforme*, enhanced spectral differences were associated with elevated culture toxicity. Preliminary studies to appraise the sensitivity of the methodology were conducted utilizing DRS. These indicated that spectra of corn contaminated at the 3% level (dry weight basis) with *F. moniliforme* were distinguishable from spectral variations associated with compositional divergence of different corn varieties. PAS was a more sensitive technique for detecting such fungal contaminations. Unfortunately, from a practical standpoint, PAS can presently analyze only one intact kernel at a time.

## INTRODUCTION

It is accepted that ingestion of microbially infected grains by humans and animals can result in acute toxicosis and that, additionally, some of the mycotoxins associated with fungally infected grains are carcinogenic (Marasas and Nelson, 1987). *Aspergillus flavus* and *Aspergillus parasiticus* are molds commonly found on grains and seeds, particularly corn and peanuts in the United States. Invasion of such crops occurs in the field during certain preharvest conditions. These fungi produce a group of related secondary metabolites known as aflatoxins, of which aflatoxin $B_1$ is the most commonly occurring. Numerous outbreaks of acute aflatoxicosis have been documented worldwide and several epidemiological studies have shown a highly significant, positive correlation between human hepatic carcinoma and dietary aflatoxin contamination (Marasas and Nelson, 1987; Van Rensburg, 1977; Shank, 1978; Van Rensburg et al., 1985). In countries where environmental conditions are not conducive to aflatoxin production, the problem is often imported by way of foreign animal feeds or foodstuffs.

*A. flavus* contaminated corn contains corn particles that exhibit bright, greenish-yellow fluorescence when irradiated with ultraviolet light (Shotwell and Hesseltine, 1981). This has led to the so-called "black-light test", in which the number of "glowers" is related to the presence of aflatoxins and is utilized to identify corn lots that should be analyzed by more specific methods, such as HPLC, HPTLC, and ELISA. Unfortunately, the above techniques are time-consuming, require considerable sample preparation, or have limited shelf life.

*Fusarium* contamination of grains and feeds is a common occurrence worldwide. These molds produce well over 100 secondary metabolites, of which more than 50 have been shown to be toxic (Vesonder and Golinski, 1989). Recently, *Fusarium moniliforme*, a ubiquitous fungal contaminant of corn, has received much scientific, as well as public, attention. Ingestion of *F. moniliforme* contaminated corn has been linked to equine leukoencephalomalacia (Wilson and Maronpot, 1971; Marasas et al., 1976; Pienarr et al., 1981), human esophageal cancer (Rheeder et al., 1992; Yang, 1980; Marasas et al., 1981), swine pulmonary hydrothorax (Harrison et al., 1990), and insidious liver disease (Jaskiewicz et al., 1987; Voss et al., 1990). Numerous secondary metabolites produced by *F. moniliforme* have been shown to be extremely toxic. Moniliformin and fusariocin are acutely toxic, fusarin C exhibits mutagenic activity, and fumonisin $B_1$ induces symptoms of leukoencephalomalacia in horses (Gelderblom et al., 1988; Wiebe and Bjeldanes, 1981; Marasas et al., 1988; Kellerman et al., 1990; Thiel et al., 1991).

The methods used to detect and measure *Fusarium* mycotoxins are as numerous and varied as the compounds produced. Difficulties encountered in separating the mycotoxin of interest from the multitude of interfering compounds found in corn make sample preparation laborious. While fusarin C can be detected with thin-layer chromatography, measurement requires analysis by high-performance liquid chromatography (Wiebe and Bjeldanes, 1981; Gelderblom et al., 1983; Jackson et al., 1990). Likewise, presumptive fumonisin detection can be accomplished with thin-layer chromatography but requires gas chromatography/mass spectral analysis for confirmation and measurement (Gelderblom et al., 1988; Jackson and Bennett, 1990). Recent efforts to measure fumonisins using high-performance liquid chromatography of fluorescent derivatives of the fumonisins show promise (Shepard et al., 1990; Ware et al., 1992). Gordon and Gordon (1990) have described a novel fluorescence assay for

† This work was supported in part by a grant (1-5-019) from the Biotechnology Research and Development Corp.

deoxynivalenol (vomitoxin) which is conducted after extraction with acetonitrile–water and partial purification on a minicolumn.

Several other fungi that infect corn, as well as other grains, are known to elicit toxic syndromes in animals and/or humans (Marasas and Nelson, 1987). Examples include *Penicillium viridicatum* and *Aspergillus ochraceus* (ochratoxicosis), *Fusarium sporotrichioides* and *Fusarium poae* (hemorrhagic syndrome), and *Fusarium graminearum* (estrogenic syndrome). Undoubtedly, other examples exist that have yet to be documented in scientific literature. Furthermore, different fungi growing on the same substrate can influence each other in various ways, and combinations of their secondary metabolites can act additively or synergistically in a toxic manner. Therefore, grain that is infected by any fungi could be regarded as potentially hazardous.

Unfortunately, there remains a lack of reliable analytical techniques that can detect the general presence of microbes on whole grains. This, in large part, is due to the solid nature of the kernel or bean itself. Indeed, few analytical techniques are capable of evaluating any material in the solid state. Two techniques, photoacoustic spectroscopy (PAS) and diffuse reflectance spectroscopy (DRS), have been coupled to Fourier transform infrared (FTIR) spectroscopy to provide information about the mid-infrared absorption spectra of solids. Since most biological compounds have distinct absorption patterns in the mid-infrared region, coupling FTIR with PAS or DRS could provide powerful tools for analyzing grains, such as corn. The techniques have the added advantages of requiring little sample preparation and being generally nondestructive. In this study, we examined the potential for utilizing FTIR–PAS and –DRS to detect fungal infections on grains. We chose as trial systems corn contaminated with *F. moniliforme* and *A. flavus*.

## MATERIALS AND METHODS

**Instrumentation.** Spectra were measured with a Laser Precision Analytical (Utica, NY) Model RFX-75 FTIR at 4-$cm^{-1}$ resolution over the infrared spectral region 400–4000 $cm^{-1}$. Interferograms were processed by use of trapezoidal apoidization with an APT-824 array processor. The interferometer, as well as the chambers that housed the detectors described below, was purged with dry nitrogen to remove spectral interference resulting from water vapor and carbon dioxide. Spectra were signal averaged from multiple interferometer scans as indicated in the text.

When PAS was employed, an MTEC (Ames, IA) Model 200 cell was utilized. Samples were placed in a 3 mm deep sample cup. The sample compartment was also purged with dry nitrogen to remove water vapor and carbon dioxide. Spectra were normalized against a carbon black background. DRS was conducted with a Spectra-Tech Collector accessory.

**Corn Samples.** Inbred varieties Ohio 43, A636, and W64A were grown aseptically in a sterile growth chamber, and whole kernels from mature plants were a generous gift of Dr. Frederick C. Felker (Seed Biosynthesis Research Unit, National Center for Agricultural Utilization Research). Sound, healthy corn kernels were from a yellow corn lot with no visible damage and contained no detectable mycotoxins. Corn kernels contaminated with *F. moniliforme* and to a lesser extent *F. graminearum* were from field samples of yellow corn visibly damaged by fungi and contained high levels of fusarin C, zearalenone, deoxynivalenol, and moniliformin. Corn samples contaminated with *A. flavus* were collected in Georgia and were contaminated with aflatoxins at levels exceeding the permissible level of 20 ppb.

***F. moniliforme* Cultures.** Submerged fungal cultures were grown from a single spore isolate of *F. moniliforme* NRRL 13616 under essentially the same conditions as were previously described (Jackson and Bennett, 1990; Jackson et al., 1989). Briefly, 500-mL baffled Erlenmeyer flasks containing 250 mL of a defined basal salts medium supplemented with 30 g/L glucose and 1.4 g/L ammonium sulfate were inoculated with *F. moniliforme* spores to give a final concentration of $5 \times 10^6$ spores/mL. Cultures were grown for 12 days at 28 °C and 220 rpm in a rotary shaker incubator. A pH of 5 was maintained by daily adjustment with 2 N HCl or 2 N NaOH. Cultures supplemented with zinc were at a final concentration of 3200 ppb. Mycelia were harvested by centrifugation, washed with deionized water, and lyophilized.

*F. moniliforme* growth on whole corn kernels was accomplished in 250-mL Erlenmeyer flasks containing 50 g of corn (Shissler hybrid GR8179) and 20 mL of deionized water. After autoclaving, flasks were inoculated with 5 mL of *F. moniliforme* NRRL 13616 spore suspensions ($5 \times 10^7$ spores/mL) and incubated at 28 °C. Duplicate flasks were harvested on days 10, 14, 21, and 35.

**Ergosterol and Fusarin C Determinations.** Ergosterol was measured according to the method of Martin et al. (1990), except that ergosterol extraction was accomplished with ethyl ether to avoid ethanol extraction of corn components which coeluted with ergosterol on the HPLC system. Fusarin C was measured according to the technique of Jackson et al. (1990).

**Sample Preparation for FTIR Analyses.** All corn samples were incubated at 60 °C in a vacuum desiccator for a minimum of 30 min to remove residual water. PAS analysis was conducted on whole kernels, unless otherwise indicated. For DRS analysis, kernels were pulverized in a Brinkman (Des Plaines, IL) Model MM2 ball mill. The chamber that contained the corn sample and the ball bearings was submerged in liquid nitrogen for 2 min to aid the pulverization process and to inhibit denaturation of protein. Pulverized corn samples were diluted to a final concentration of 5% with spectroscopic grade KBr (Spectra-Tech). Mixing was accomplished with a Wig-L-Bug (Lyons, IL) amalgamator at ambient temperature immediately prior to DRS analysis. The Wig-L-Bug amalgamator was similarly utilized to incorporate known quantities of *F. moniliforme* with pulverized corn when indicated in the text.

## RESULTS

Previous work (Greene et al., 1988) showed that solid-substrate fungal growth could be crudely monitored by Fourier transform infrared (FTIR)–photoacoustic spectroscopy (PAS), but improved instrumentation was required for practical application of the technology. Significant efforts were devoted toward enhancing the signal-to-noise ratio of FTIR–PAS, which were fully described in another paper (Gordon et al., 1992). For the purpose of this study, the signal-to-noise ratio of PAS was considered to be equivalent to that of diffuse reflectance spectroscopy (DRS). Therefore, our choice of analytical technique was dictated by sample morphology. PAS was selected for initial studies because it was the technique capable of evaluating whole kernels of corn and, other than drying, no sample preparation was necessary.

The top trace in Figure 1 is an FTIR–PAS spectrum of an intact corn kernel that was infected with *F. moniliforme*. This spectrum is dramatically different from one generated with uninfected corn (middle trace). The various absorption bands evident in the difference spectrum (bottom trace) should be indicative of specific biochemical changes that occur when *F. moniliforme* attacks corn. Strong amide I (1650 $cm^{-1}$) and amide II absorbances (1550 $cm^{-1}$) are evident in the difference spectrum, suggesting an increase in protein or acetylated amino sugar content. This is supported by the fact that the broad peak centered around 3400 $cm^{-1}$ in the uninfected corn spectrum shifts downfield subsequent to fungal infection. Such a shift is indicative of increased NH stretch absorptions relative to OH stretch absorptions. The two sharp peaks at 2855 and 2925 $cm^{-1}$ appear to result from an increase in lipid content. This doublet is typical of methylene CH stretches, which, in biological systems,

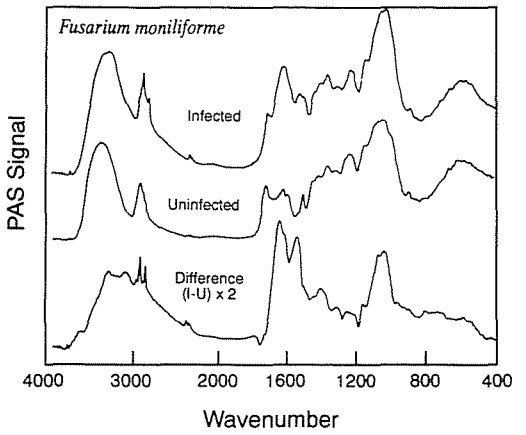


**Figure 1.** FTIR–PAS spectra of corn. (Top trace) Uninfected corn; (middle trace) corn infected with *F. moniliforme*; (bottom trace) difference spectrum generated by subtracting spectrum of uninfected corn from spectrum of infected corn. The resulting spectrum was normalized by a factor of 2.0.

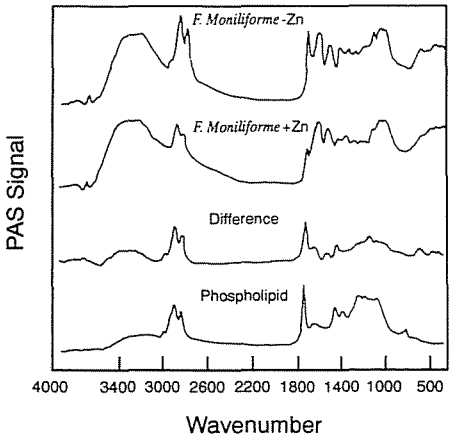


**Figure 2.** FTIR–PAS spectra of (top trace) *F. moniliforme* grown in the absence of zinc, (second trace) *F. moniliforme* grown in the presence of 3200 ppb of zinc, (third trace) zinc absent culture minus zinc present culture; and (bottom trace) soybean phospholipid preparation (azolectin).

generally arise from fatty acid tail hydrocarbons. A number of differences in the fingerprint region from 400 to 1500 $cm^{-1}$ are evident. Such absorbances can arise from numerous chemical moieties, but a change in carbohydrate composition appears to be one likely possibility.

Jackson et al. (1989) have shown that, in in vitro cultures of *F. moniliforme*, increased toxin production (fusarin C) is associated with elevated lipid content. This can be regulated by the zinc concentration. Figure 2 presents FTIR–PAS spectra of *F. moniliforme* grown in liquid in the absence (top trace) and presence (second trace) of zinc. The culture grown without zinc produced over 10-fold more fusarin C than did the one supplemented with zinc (data not shown). The difference between these two spectra (depicted by the third trace) exhibits a sharp doublet at 2855 and 2925 $cm^{-1}$, which, similar to the data presented in Figure 1, is indicative of increased lipid content. The distinct absorption band at 1740 $cm^{-1}$ is typical of an ester carbonyl, likely due to triglycerides or phospholipids. This notion is supported by the FTIR–PAS spectrum of soybean phospholipid (bottom trace), which closely resembles the difference spectrum in Figure 2.

Mid-infrared spectral changes resulting from *A. flavus* infection of corn were investigated by utilizing diffuse reflectance spectroscopy (DRS). The top trace in Figure 3 is an FTIR–DRS spectrum of infected corn that was

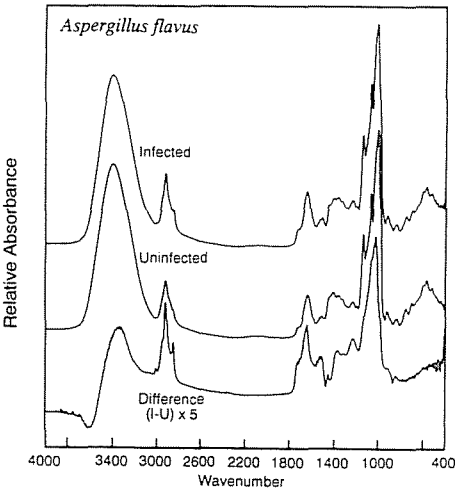


**Figure 3.** FTIR–DRS analysis of corn infected with *A. flavus*. (Top trace) Uninfected corn; (middle trace) corn infected with *A. flavus*; (bottom trace) difference spectra generated by subtracting uninfected corn from infected corn and normalizing by a factor of 5.0.

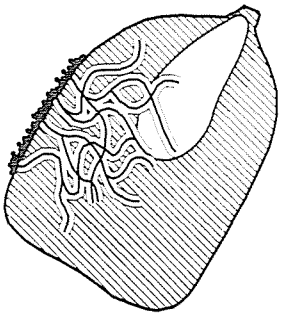

**Figure 4.** Model depicting fungal infection of corn.

pulverized in a ball mill. The middle trace is a spectrum generated from a similarly prepared sample of uninfected corn. The differences in these two spectra, represented by the bottom trace, resemble the differences observed in corn infected with *F. moniliforme* (Figure 1). Again, there are increased amide I and amide II absorbances in conjunction with a downfield shift of the broad peak located between 3000 and 3600 $cm^{-1}$. Furthermore, a doublet at 2855 and 2925 $cm^{-1}$ is readily apparent, as are several changes in the fingerprint region.

The spectral differences were not as pronounced for the data set of Figure 3 in comparison to those observed in Figure 1 (note normalization factors for Figures 1 and 3 difference spectra). However, when intact kernels infected with *A. flavus* were analyzed by PAS, the spectral differences relative to uninfected corn were as marked as those observed in Figure 1 (*F. moniliforme* infected corn). It is well accepted that PAS is a surface-sensitive technique. Apparently, as depicted in Figure 4, the bulk of the fungal mass is located at the surface of the kernel. Therefore, pulverizing the sample, which is necessary to obtain a suitable DRS signal from corn, in effect dilutes absorbances derived from fungal contaminations by exposing uncontaminated areas of the kernel. If mycelia have penetrated the interior of the kernel, they may remain accessible to PAS illumination due to a "tunnel-like" effect (Figure 4). "Tunneling" would also expose the interior of the kernel, which is known to be chemically dissimilar to the pericarp. This may also account for some of the enhanced sensitivity observed with PAS since pulverization would tend to average such compositional differences. The above notions appear valid. PAS analyses confirmed there were significant spectral dissimilarities between peri-

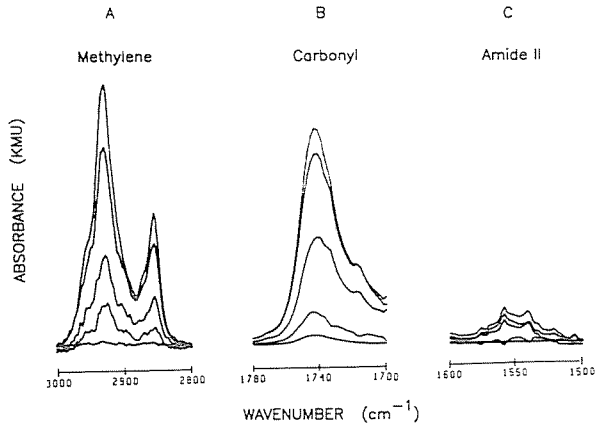


**Figure 5.** Sensitivity of FTIR–DRS for detecting corn contaminated with *F. moniliforme*. (A) Methylene CH doublet spectral region; (B) ester carbonyl spectral region; (C) amide II spectral region. The five traces in each region are spectra of the same five samples and represent, from bottom to top, the spectral difference between Ohio 43 corn and A636 corn and spectral differences between Ohio 43 and Ohio 43 corn supplemented with *F. moniliforme* at 5.1%, 16.1%, 29.7%, and 39.6% (dry weight basis).

carp and interior tissues (not shown). Also with PAS, much diminished infected vs uninfected difference spectra were acquired after kernel pulverization relative to whole kernel analyses. This latter result was obtained with both *F. moniliforme* and *A. flavus* infected corn (not shown).

While PAS appears to be a more sensitive technique for detecting fungal contaminations, the sample chamber currently used can only accommodate one kernel at a time. Spectrally analyzing an aliquot from a mixture of pulverized kernels offers the practical advantage of evaluating a representative sample. Preliminary experiments were conducted to determine the sensitivity of this methodology. FTIR–DRS difference spectra were generated from uninfected Ohio 43 corn and Ohio 43 corn supplemented with increasing percentages (dry weight basis) of *F. moniliforme*. Consistent with previously presented data, several mid-infrared spectral regions looked particularly promising for quantitating fungal contaminations. Difference spectra of the region that contains the methylene CH stretch doublet (2855 and 2925 $cm^{-1}$) are presented in Figure 5A. Analogous data for the ester carbonyl stretch (1740 $cm^{-1}$) and amide II (1550 $cm^{-1}$) regions are presented in parts B and C of Figure 5. The data in Figure 5C (amide II region) exhibit more noise than those in Figure 5A (methylene CH stretch doublet) and Figure 5B (carbonyl stretch), due to a lower signal intensity as well as residual water vapor absorbances.

The spectral divergence between different varieties of corn is an important parameter to consider since their compositional makeup can vary. The bottom traces in each part of Figure 5 represent the FTIR–DRS difference between two inbred corn lines (Ohio 43 and A636). The differences are minor relative to the spectral changes observed after fungal attack, particularly for the methylene doublet and the amide II region. Another uncontaminated inbred, as well as a hybrid strain, was examined in a similar manner. It was somewhat unexpected to find that relatively little variance in the mid-infrared absorption patterns of the four corns occurred, particularly considering that inbreds are genetically diverse strains from which hybrids arise. In fact, the average error at 2925 $cm^{-1}$ was only 0.034 absorbance unit, making the observed differences at >3% supplemented fungus easily distinguishable. Preliminary results indicated that *A. flavus* was also distinguishable by this methodology at the 2–5% level.

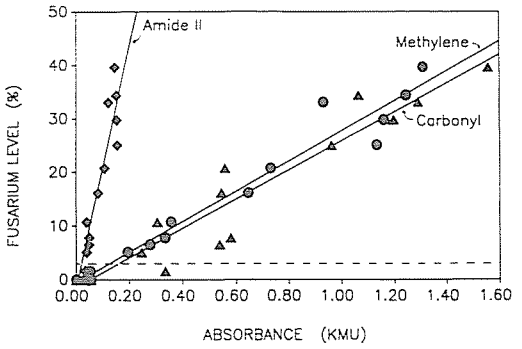


**Figure 6.** FTIR-DRS calibration curves generated from the complete set of data (16 samples) of which the spectra of Figure 5 are examples. Linear regression lines are drawn through three sets of data points: (▲) methylene (2927 $cm^{-1}$); (●) carbonyl (1742 $cm^{-1}$); (◆) amide II (1550 $cm^{-1}$). The dashed line represents the estimated lowest level of detection by this calibration relative to the spectral differences observed for four different corn varieties.

Figure 6 presents crude calibration curves generated with the raw data from 16 samples of which the data in Figure 5A are examples selected to illustrate the trend. The dashed line represents the uncertainty that arises from spectral variances associated with the different corn varieties thus far examined. Despite the considerable experimental error revealed by scatter in the data for Figure 6, linear regressions of *F. moniliforme* levels on either methylene or carbonyl absorptions exhibited virtually the same slope, while the regression on amide II absorption showed a significantly different slope. Correlation coefficients of the methylene, carbonyl, and amide II regressions were 0.980, 0.953, and 0.957, respectively. Since Beer's law holds for DRS spectra in Kubelka–Munk units, identical slopes of the methylene and carbonyl plots in Figure 6 might be expected, as they both appear to result from the lipid component of *F. moniliforme*. The different slope of the amide II plot would also be expected since it results from another component of *F. moniliforme*, namely, protein and/or acetylated amino sugars. Thus, the regressions verify the presence of at least two principal components in the system. Using recent advances in chemometrics, these data may be grouped together with data from other regions of the spectra and analyzed as a multivariate system. This would likely increase the accuracy of prediction over that indicated by the correlations.

A survey of corn samples known to contain high levels of various mycotoxins demonstrated that at least one and often several infrared spectral regions exceeded the uncertainty limits shown in Figure 6. The amide II region proved to be a very reliable indicator of fungal contamination. Figure 7 shows the increase in FTIR–DRS amide II absorption observed when corn becomes progressively invaded by *F. moniliforme*. Fungal content was calculated from relative ergosterol levels determined by HPLC analysis. The correlation coefficient for the infected corn amide II regression was 0.993, indicating a high reliability. Also, the slope of this line was noticeably larger than that of corn supplemented with *F. moniliforme*, suggesting that the methodology might be more sensitive for detection of fungal contaminants than would be predicted by supplementation experiments.

## DISCUSSION

The results presented in this study indicate that FTIR in conjunction with PAS or DRS is potentially a powerful technique for detecting corn infected with two hazardous

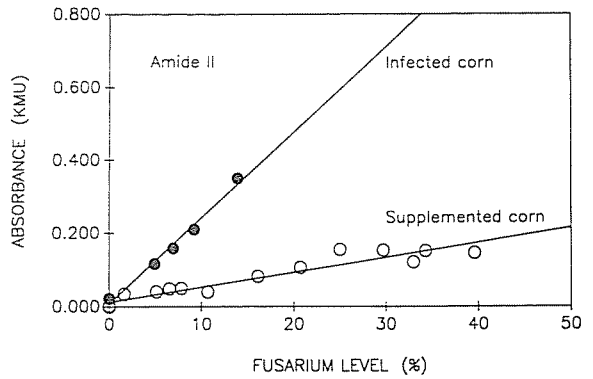


**Figure 7.** Amide II absorbance (1550 $cm^{-1}$) of corn invaded by or supplemented with increasing levels of *F. moniliforme*. Corn was infected with a spore solution and compared to uninoculated corn after 10, 14, 21, and 35 days of incubation. Supplemented corn was as in Figure 6. Fungal content was determined by comparison of ergosterol levels.

fungi, *F. moniliforme* and *A. flavus*. This instrumentation would also detect benign contaminations. In fact, FTIR–PAS was previously utilized to evaluate solid-state growth of *Phanerochaete chrysosporium* on cellulose (Greene et al., 1988). Methodology that detects the general presence of microbes on grains might be extremely beneficial given the complexities of toxin production in natural environments. Numerous toxins produced by many different fungi have been described. Undoubtedly, more will be discovered in the future. Furthermore, the environmental, genetic, and biochemical events that control microbial toxin production are poorly understood. Current quality control assays are toxin specific, and screening for numerous individual toxins can become quite costly. Preliminary FTIR evaluation could deem grain microbe "free" (within certain regulatory limits since microbes will always be present to some extent), in which case it would be unnecessary to assay for toxins. FTIR-questionable samples could then be further evaluated by specific assays which are currently available or are being developed. The magnitude of the problem is such that several complementary techniques appear to be necessary.

Commercial FTIRs are rather expensive instruments, generally costing in the tens of thousands of dollars. They offer several advantages, one of which is that an FTIR can almost instantaneously (1 s or less) scan an entire mid-infrared spectrum. However, provided a reliable spectral region that is indicative of microbial contamination can be identified, much less costly instrumentation can be developed. For *F. moniliforme* and *A. flavus* several regions appear promising. These include the amide I and amide II absorption bands (1650 and 1550 $cm^{-1}$), the broad absorption region that contains OH and NH stretches (from 3000 to 3600 $cm^{-1}$), the ester carbonyl (1740 $cm^{-1}$), and the methylene doublet (2855 and 2925 $cm^{-1}$).

As discussed previously and shown in Figure 7, the amide II region appears to be a reliable indicator of infected corn. However, the methylene doublet and the carbonyl absorption offer two possible advantages. First, they are areas in which no interference from water absorptions occur, making sample drying unnecessary. Second, in these studies using submerged cultures of *F. moniliforme*, stronger absorptions are associated with more toxic cultures. This resulted from an increased lipid content in more toxic cultures (Jackson et al., 1989). Perhaps, the metabolic pathway for toxin production proceeds through lipid anabolism. Indeed, Aflatoxin production by *A. parasiticus* and fusarin C and fumonisin $B_1$ production by *F. moniliforme* are notable examples of lipid-like compounds whose in vitro production was stimulated by nutritional conditions that enhanced lipid synthesis (Shih and Marth, 1974; Jackson et al., 1989; Jackson and Freer, 1991).

Numerous spectral changes were also observed from 400 to 1500 $cm^{-1}$ which, along with the amide and carbonyl regions, comprise what are generally considered to be fingerprint absorbances. Recently, Naumann et al. (1991) reported to have developed a prototype FTIR that can identify intact bacteria at the subspecies level by evaluating mid-infrared spectral fingerprints. It may also be possible to identify fungal contaminates of grains by such FTIR evaluation.

Application of PAS to infrared spectroscopy has lagged well behind that of other detectors. This results from the fact that PAS involves an acoustic element which makes the fundamental principles that govern the generation of the signal extremely different from those of other spectroscopic techniques. Therefore, it is not surprising that FTIRs are poorly designed for PAS applications. Previous efforts were devoted to increasing the signal-to-noise ratio of FTIR–PAS (Gordon et al., 1992) such that it approaches that of DRS. But, admittedly, the signal-to-noise ratio is potentially much higher for techniques that are solely dependent on photons due to physical constraints. However, PAS offers several advantages over DRS. PAS requires no sample preparation. It is a spectroscopic technique that can analyze an intact kernel. The majority of the signal arises from the surface of the kernel, and this leads to a greater sensitivity for detecting *F. moniliforme* and *A. flavus* infections, presumably due to the pattern of fungal growth as depicted in Figure 4. This pattern was confirmed by sectioning experiments. The major deficiency of PAS is that the PAS cell can accommodate only one kernel. Efforts are underway at this time to evaluate other surface-sensitive techniques that can instantaneously analyze large quantities of grain.

ACKNOWLEDGMENT

We are grateful to Christopher James, Dawn DeWispeleare, and June Stewart for their technical assistance and to Deborah Bitner for expert clerical assistance.

LITERATURE CITED

Gelderblom, W. C. A.; Thiel, P. G.; van der Merwe, K. J.; Marasas, W. F. O.; Spies, H. S. C. A Mutagen Produced by *Fusarium moniliforme*. *Toxicon* **1983**, *21* (4), 467–473.

Gelderblom, W. C. A.; Jaskiewicz, K.; Marasas, W. F. O.; Thiel, P. G.; Harak, R. M.; Vleggaar, R.; Kriek, N. P. J. Fumonisine—Novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. *Appl. Environ. Microbiol.* **1988**, *54*, 1806–1811.

Gordon, S. H.; Greene, R. V.; Pareja, C. Modification of a conventional Fourier transform infrared spectrometer for enhanced photoacoustic detection. In *Photoacoustic and Photothermal Phenomena III*; Springer-Verlag: New York 1992, in press.

Gordon, W. C.; Gordon, L. J. Rapid screening method for deoxynivalenol in agricultural commodities by fluorescent mini-column. *J. Assoc. Off. Anal. Chem.* **1990**, *73*, 266–270.

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.

Harrison, L. R.; Colvin, B. M.; Greene, J. T.; Newman, L. E.; Cole, J. R. Pulmonary edema and hydrothorax in swine produced by fumonisin $B_1$, a toxic metabolite of *Fusarium moniliforme*. *J. Vet. Diagn. Invest.* **1990**, *2*, 217–221.

Jackson, M. A.; Bennett, G. A. Production of fumonisin $B_1$ by *Fusarium moniliforme* NRRL 13616 in submerged culture. *Appl. Environ. Microbiol.* **1990**, *56*, 2296–2298.

Jackson, M. A.; Freer, S. N. The influence of carbon and nitrogen nutrition on fusarin C biosynthesis by *Fusarium moniliforme*. *FEMS Microbiol. Lett.* **1991**, *82*, 323–328.

Jackson, M. A.; Slininger, P. J.; Bothast, R. J. Effects of zinc, iron, cobalt and manganese on *Fusarium moniliforme* NRRL 13616 growth and fusarin C biosynthesis in submerged cultures. *Appl. Environ. Microbiol.* **1989**, *55*, 649–665.

Jackson, M. A.; Stewart, J. N.; Peterson, R. E.; Slininger, P. J. Fusarin C Purification and Measurement in Submerged Cultures of *Fusarium moniliforme* by High Performance-Liquid Chromatography. *J. Agric. Food Chem.* **1990**, *38*, 1511–1514.

Jaskiewicz, K.; Marasas, W. F. O.; Taljaard, J. J. F. Hepatitis in vervet monkeys caused by *Fusarium moniliforme*. *J. Comp. Pathol.* **1987**, *97*, 281–291.

Kellerman, T. S.; Marasas, W. F. O.; Thiel, P. G.; Gelderblom, W. C. A.; Cawood, M.; Coetzer, J. A. W. Leukoencephalomalacia in two horses induced by oral dosing of fumonisin $B_1$. *Onderstepoort J. Vet. Res.* **1990**, *57*, 269–275.

Marasas, W. F. O.; Nelson, P. E. Mycotoxiology: Introduction to the mycology, plant patholody, chemistry, toxicology and pathology of naturally occurring mycotoxicosies in animals and man. In *Mycotoxicology*; The Pennsylvania State University Press: University Park, 1987; pp 1–102.

Marasas, W. F. O.; Kellerman, T. S.; Pienarr, J. G.; Navde, T. W. Leukoencaphalomalacia: A mycotoxicosis of *Equidae* casued by *Fusarium moniliforme* Sheldon (fungi). *Onderstepoort J. Vet. Res.* **1976**, *43*, 113–121.

Marasas, W. F. O.; Wehner, F. C.; Van Rensburg, S. J.; van Schalkwyk, D. J. Mycoflora of corn produced in human esophageal cancer areas in Transkei, Southern Africa. *Phytopathology* **1981**, *71*, 792–796.

Marasas, W. F. O.; Kellerman, T. S.; Gelderblom, W. C. A.; Coetyer, J. A. W.; Thiel, P. G.; Van der Lugt, J. J. Leukoencephalomalacia in a horse induced by fumonisin $B_1$ isolated from *Fusarium moniliforme* Sheldon. *Onderstepoort J. Vet. Res.* **1988**, *55*, 197–203.

Martin, F.; Delaruelle, C.; Hilbert, J.-L. An improved ergosterol assay to estimate fungal biomass in ectomycorrhizas. *Mycol. Res.* **1990**, *94*, 1059–1064.

Naumann, D.; Helm, D.; Labischinski, H. Microbiological characterizations by FT-IR spectroscopy. *Nature* **1991**, *351*, 81–82.

Pienarr, J. G.; Kellerman, T. S.; Marasas, W. F. O. Field outbreaks of leukoencephalomalacia in horses consuming maize infected by *Fusarium verticillioides* (*Fusarium moniliforme*) in South Africa. *J. S. Afr. Vet. Assoc.* **1981**, *52*, 21–24.

Rheeder, J. P.; Marasas, W. F. O.; Thiel, P. G.; Sydenham, E. W.; Shephard, G. S.; van Schalkwyk, D. J. *Fusarium moniliforme* and fumonisins in corn in relation to human esophageal cancer in Transkei. *Phytopathology* **1992**, *83*, 353–357.

Shank, R. C. In *Mycotoxic Fungi, Mycotoxins, Mycotoxicoses: An Encyclopedic Handbook*; Wyllie, T. D., Morehouse, L. G., Eds.; Dekker: New York, 1978; pp 1–19.

Shepard, G. E.; Sydenham, E. W.; Thiel, P. G.; Gelderblom, W. C. A. Quantitative determination of fumonisin $B_1$ and $B_2$ by high-performance liquid chromatography with fluorescence detection. *J. Liq. Chromatogr.* **1990**, *13*, 2077–2087.

Shih, C. N.; Marth, E. H. Some cultural conditions that control biosynthesis of lipid and aflatoxin by *Aspergillus parasiticus*. *Appl. Microbiol.* **1974**, *27*, 452–456.

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.

Thiel, P. G.; Shephard, G. S.; Sydenham, E. W.; Marasas, W. F. O.; Nelson, P. E.; Wilson, T. M. Levels of fumonisins $B_1$ and $B_2$ in feeds associated with confirmed cases of equine leukoencephalomalacia. *J. Agric. Food Chem.* **1991**, *39*, 109–111.

Van Rensburg, S. J. In *Mycotoxins in Human and Animal Health*; Rodricks, J. V., Hesseltine, C. W., Mehlman, M. A., Eds.; Health Pathotoxicology Publishers: Park Forest South, IL, 1977; pp 699–711.

Van Rensburg, S. J.; Cook-Mozaffari, P.; Van Schalkwyk, D. J.; Van der Watt, J. J.; Vincent, T. J.; Purchase, I. F. Hepatocellular carcinoma and dietary aflatoxin in Mozambique and Transkei. *Br. J. Cancer* **1985**, *51*, 713–726.

Vesonder, R. F.; Golinski, P. Metabolites of Fusarium. In *Fusarium Mycotoxins, Taxonomy, and Pathogenicity*; Chelkowski, J., Ed.; Elsevier: Amsterdam, 1989; pp 1–39.

Voss, K. A.; Plattner, R. D.; Bacon, C. W.; Norred, W. P. Comparative studies of hepatotoxicity and fumonisin $B_1$ and $B_2$ content of water and chloroform/methanol extracts of *Fusarium moniliforme* strain MRC 826 culture material. *Mycopathologia* **1990**, *112*, 81–92.

Ware, G. M.; Francis, O.; Carmen, A.; Kuan, S. *J. Assoc. Off. Anal. Chem.* **1992**, in press.

Wiebe, L. A.; Bjeldanes, L. F. Fusarin $C_1$, a mutagen from *Fusarium moniliforme* grown on corn. *J. Food Sci.* **1981**, *46*, 1424–1426.

Wilson, B. J.; Maronpot, R. R. Causative fungus agent of leukoencephalomalacia in equine animals (*Fusarium moniliforme*). *Vet. Rec.* **1971**, 484–486.

Yang, C. S. Research on esophogeal cancer in China: A review. *Cancer Res.* **1980**, *40*, 2633–2644.

Received for review December 2, 1991. Revised manuscript received March 23, 1992. Accepted April 6, 1992. The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.