APPLIED SPECTROSCOPY REVIEWS, 36(4), 399–416 (2001)

SORTING SYSTEMS BASED ON OPTICAL METHODS FOR DETECTING AND REMOVING SEEDS INFESTED INTERNALLY BY INSECTS OR FUNGI: A REVIEW*

M. C. Pasikatan† and F. E. Dowell

Engineering Research Unit, USDA-ARS, Grain Marketing Research and Production Research Center, 1515 College Avenue, Manhattan, KS 66502

ABSTRACT

Sorting systems based on optical methods have the potential to rapidly detect and physically remove seeds severely contaminated by fungi, or infested internally by insect larvae or pupae. Thus, the literature on sorting systems based on optical methods for detecting and sorting seeds with these attributes was reviewed. Sorting indices based on wavelengths useful for detecting these attributes were emphasized. Surface characteristics of seeds, like discoloration caused by fungi, are generally detectable in the visible range of the electromagnetic spectrum, whereas internal attributes are detectable in the near-infrared range. The

*Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Dept. of Agriculture.

†Corresponding author. Fax: (785) 776-2792; E-mail: choypc@gmprc.ksu.edu

Copyright © 2001 by Marcel Dekker, Inc. www.dekker.com

spectral differences between sound and infested seeds are usually subtle, but full-spectrum and two-wavelength classification models have succeeded in detecting and classifying seeds based on these attributes. For high sorting accuracies, wavelength identification and proper selection of a sorting criterion are important. Color, chitin, ergosterol, or hydrolysis of triglycerides have been identified as indicators of seed fungal contamination whereas chitin, protein, phenolic compounds, or changes in starch have been useful indicators of internal insects in seeds.

Key Words: Seeds; Insects; Fungi; Internal infestation; Fungal infection; Near-infrared (NIR) spectroscopy

I. INTRODUCTION 400

II. DETECTING AND SORTING FOR FUNGAL CONTAMINATION 402
- A. Early Studies On Mold Detection 402
- B. Aspergillus Flavus and Aflatoxin 403
- C. Fusarium 405

III. DETECTING AND SORTING FOR INTERNAL INSECTS 407

IV. FACTORS THAT LIMIT CLASSIFICATION OR SORTING ACCURACY 410
- A. Threshold Setting and Sorting Criterion 410
- B. Moisture Content 410
- C. Kernel Orientation and Biological Variation 410

V. SUMMARY AND CONCLUSIONS 411

REFERENCES 412

I. INTRODUCTION

Detection and removal of internal insects and fungal contamination from seeds (grains, beans, and nuts) are important control measures for ensuring storage longevity, seed quality, and food safety. Insect feeding and respiration may create conditions favorable for fungal growth during storage [1]. A few insects can increase rapidly and give rise to a serious infestation later [2]. Postharvest grain losses caused by pests and poor storage practices total more than $1 billion

per year in the U.S. [3] Mycotoxins, such as aflatoxin or fumonisin, from fungi-contaminated grains have been linked to equine leukoencephalomacia, porcine pulmonary edema, liver cancer and esophageal hyperplasia in rats, and human esophageal cancer [4,5]. Hence, regulatory limits of seed contamination for food and feed products are being strictly enforced in the grain trade. However, internal insects and fungal contamination in seeds may not be removed completely by typical cleaning equipment.

Internal insect infestation of grains may be detected by staining of kernels, flotation, radiographic techniques, acoustic techniques, uric acid measurement, ninhydrin-impregnated paper, nuclear magnetic resonance, and immunoassays [1]. These detection methods are either slow, labor intensive, expensive, or difficult to automate. Mycotoxins may be detected from ground samples by high-performance liquid chromatography, thin-layer chromatography, mass spectroscopy, and immunochemical methods [6]. None of these methods are amenable to rapid and simple automation. The method based on blue-green-yellow fluorescence indicates the presence of molds in seeds but is not a definitive indicator of the aflatoxin. Promising results in photoacoustic spectroscopy (PAS) and transient infrared spectroscopy (TIRS) have been reported for detecting fungal infection in seeds [7–9]. However, PAS takes at least 10 minutes to get the photoacoustic signal from a single corn kernel8. TIRS, on the other hand, is useful for detection from a mass of solid materials moving at conveyor belt speeds [9,10]. Neither PAS nor TIRS were designed for removal of infected seeds which requires singulation and high speeds. There is, therefore, a need for a rapid means of detection and removal of fungal or insect-infested seeds from a seed lot.

High-speed sorting systems based on optical properties are being used in some commodities, such as beans, peanuts, coffee, peas, and rice [11]. These sorters depend mainly on color differences between "accept" and "reject" seeds and other quality factors that are detectable visually. While surface fungal contamination was shown to be detectable in the visible range of the light spectrum (400–750 nm) [12,13], symptomless fungal contamination and insect larva or pupae in seeds were not [14–18]. The unique absorption at specific wavelengths in the near-infrared region has potential to detect these internal attributes.

Optical sorting machines use sorting indices for product segregation. A sorting index is the form of the measured optical properties of a product that maximizes the difference between acceptable and objectionable products. It should correlate well with the quality attribute being evaluated, not be easily affected by other physical parameters of the product, and should vary little with the equipment variables [11]. Sorting indices for various products have been formulated from single wavelengths [19], or two wavelengths in the form of a ratio, difference, normalized difference, or normalized sum [19–24]. Advantages and disadvantages of these indices have been reported [12,14,19,21,25–

26]. Commercial sorters use either monochromatic (single wavelength) or bichromatic (two wavelengths) mode, or both, to sort for product defects [27–29].

Complexity of the sorting process and introduction of various spectral pretreatments have led to the development of more sorting indices, many of those are not applicable to high-speed implementation such as: (1) discriminant analysis based on full-spectrum or regions of the spectrum [17] and (2) eigenvector-based models (multiple linear regression, principal component analysis, and partial least-squares regression) that use regions of the spectrum [30, 31]. These models may not be useful for high-speed sorting, but help identify specific wavelengths that could be used later for developing few-wavelength models.

An extensive review of optical, nondestructive quality evaluation encompassing various agricultural and biological materials was reported by Gunesakaran [32]. A later review by Chen and Sun [11] covered all nondestructive methods, including optical methods, for quality evaluation of agricultural products. This review continues from where previous reviews stopped but focuses only on seeds and two economically important quality factors: fungal contamination and internal insects in seeds. The objective of this review was to integrate information related to optical seed sorting for internal insects and fungal contamination in order to identify sorting indices and principles relevant to these applications.

II. DETECTING AND SORTING FOR FUNGAL CONTAMINATION

A. Early Studies on Mold Detection

Using a research spectrometer described by Norris [20], Birth [25] observed that the slope of optical density (log 1/T) curves between 750 and 1000 nm was proportional to the amount of smut in the wheat sample. He assumed therefore, that optical density at any two wavelengths within that range, excluding the water absorption band at 970 nm, was a good measure of total smut content. He developed a smut meter based on this principle. It was essentially a photometer for measuring differences in optical density (log 1/T) at two wavelengths in the red or infrared region of the electromagnetic spectrum. A 28 V, 20 W reflector-type bulb was used as the light source. Two 5.1 x 5.1 cm narrowband interference filters isolated the desired wavelengths. A 55-g wheat sample was placed in a 7 cm-diameter aluminum cup and inserted between the interference filter and photomultiplier tube. Johnson [33] studied five different methods of detecting smut content in bulk wheat. The methods were light transmittance, sedimentation, catalase activity, light reflectance, and light absorption. For the

light absorption method, the smut meter designed by Birth [25] was used. Smutty wheat ranged from 0.5 to 3%. Johnson [33] found that the light absorption method was the fastest and most suitable method for routine smut testing. Smut spores adhering to the wheat kernels increased the absorption of light at the shorter wavelengths (700–850 nm), relative to the longer wavelengths (850–1000 nm). The presence of smut was indicated by a lower transmittance at 800 nm and a higher value at 930 nm, compared to non-smutty wheat. Therefore, the ratio of transmittance at 800 to 930 nm was found to be the best measure of smut content in wheat (r=0.95).

Birth and Johnson [12] evaluated four optical properties (total fluorescence, spectral characteristics of fluorescence, transmittance, and reflectance) for detecting mold contamination in shelled yellow corn. The corn kernels varied in mold contamination from slight to severe on a scale of 1 to 10. Sound kernels fluoresced more than moldy kernels; molds quenched the natural fluorescence of corn. The best results were obtained for a difference of logarithm of fluoresced energy at 442 and 607 nm (<5% classification error). They observed that a certain level of contamination must be reached in order to detect fluorescence characteristics.

These early studies were concerned mainly with mold detection, not with specific mycotoxins. But these principles were followed in ensuing studies that aimed to detect specific mycotoxins.

B. Aspergillus Flavus and Aflatoxin

Aspergillus, Penicillium, and Fusarium species are the most prevalent mycotoxigenic fungi in stored products [6]. Mycotoxins of the greatest economic concern are the aflatoxins, which are produced by A. flavus [6]. A. flavus-contaminated corn can have particles that exhibit bright, greenish-yellow (BGY) fluorescence when irradiated with UV light [34]—the basis for the "black light test" to identify contaminated corn lots. A count of one BGY particle per kg obtained from a given corn sample is an indication that the sample should be tested for aflatoxin by chemical means such as high-performance liquid chromatography (HPLC) or enzyme-linked immunosorbent assay (ELISA). However, corn contaminated with aflatoxin sometimes does not exhibit BGY fluorescence [35].

The presence of internal fungal contamination may not be visible on the kernel surface [36, 37]. X-ray scans could not detect internally moldy peanuts, but nuclear magnetic resonance (NMR) scans could [36]. However, an NMR scan is expensive and takes 10 min or more. Moreover, aflatoxin is unevenly distributed in a seed lot and may be concentrated in a very small percentage of the product [36, 38–40]. For example, aflatoxin is found in < 0.1% of the

shelled peanuts, but the contamination level on a single kernel may be as high as 1,000,000 ppb [38]. High levels of aflatoxin in edible peanuts can therefore be reduced by removing contaminated kernels, which may comprise a small percentage of the total lot. Hirano et al. [36] reported a study in which the removal of just 5% of the peanuts lowered the aflatoxin content of an entire lot of peanuts from 4.5 to 0.2 ppb.

Tyson and Clark [13] developed a technique based on fluorescence to determine aflatoxin contamination in pecans. Pecan halves were soaked and incubated for two days in a chloroform-aflatoxin B1 solution of varying concentrations (0.095, 0.95, 9.5, and 95 ppm). The top and bottom of each pecan half was subjected to ultraviolet radiation at 365 nm and the intensity and wavelength of light coming from each side of the pecan halves were recorded. Flourescence was measured at 10 nm intervals from 410 to 610 nm. Thin layer chromatography was used to analyze aflatoxin in each sample. The flourescence ratios 440 to 490 nm (F_{440}/F_{490}) or 450 to 490 nm (F_{450}/F_{490}) measured from the bottom side of the pecan halves yielded the highest correlation coefficients (0.86 and 0.83). They detected aflatoxin contamination 20 ppm with 90% confidence, using these fluorescence ratios. Farsaie et al.[41] used the bright-greenish-yellow (BGY) fluorescence under UV excitation to detect aflatoxin in pistachio nuts. They showed that the ratio of the fluorescence of pistachio at 490 and 420 nm could be a basis for detecting BGY fluorescence. These two wavelengths were used in an automatic sorter for aflatoxin-infected pistachio nuts [42,43]. Each nut was viewed by the dual wavelength detectors for about 30–40 ms. The average sorting rate was 18 nut/s.

A sorting machine for effectively separating peanuts infected by aflatoxin-producing molds from good nuts was reported by Fraenkel et al [44]. It detects both red and green light reflected from a peanut being viewed. The reflected red light produces a first signal indicative of the amount of mold in the peanut. The reflected green light produces a second signal indicative of the extent of mechanical damage to the nut. The two signals are compared and the resulting signal is passed to an ejector. When the extent of mechanical damage exceeds a predetermined level, the ejector is actuated by the comparison signals. Hirano et al.[36] used the transmittance ratio of peanuts at 700 to 1100 nm as a sorting criterion between sound and moldy (surface and internal) peanuts. These are the wavelength regions where spectral differences between the two classes were highest. The transmittance ratio could even separate surface-moldy from internally moldy nuts. The hydrolysis of triglycerides in peanuts by the fungus seemed to be the best explanation for changes in NIR spectra.

Pearson et al. [37] found that one reflectance ratio (R_{735}/R_{1005}) could discriminate individual corn kernels highly contaminated (>100 ppb) with aflatoxin from corn with low levels of aflatoxin (<10 ppb). The corn kernels were all oriented so that reflectance was measured from the germ side of the kernel.

Transmittance ratios at T_{710}/T_{760} and T_{615}/T_{645} were also found to have similar results without the need to orient the kernels. Over 95% of the kernels with aflatoxin >100 ppb or <10 ppb were correctly classified using these techniques. Fungal discoloration of the kernels caused higher absorbance in the visible wavelengths (<750 nm) as measured by either reflectance or transmittance. Transmission measurements yielded slightly better results than reflectance. Fungal invasion causes the kernel endosperm to be more powdery, thus a fungal-infected kernel tends to scatter more light than a sound, vitreous kernel [45].

C. Fusarium

The fungi Fusarium monoliforme, F. proliferatum, F. graminearum, and other fusaria produce a number of toxins of which fumonisins are of current importance; these can infect wheat, corn, rice, sorghum, millet, and other food products, both in the field or in storage, and infection can be symptomless [5,16]. The genus Fusarium produces over 100 biologically active metabolites, of which more than 50 have been shown to be toxic [46]. The presence of any fungal invasion can be indicated by ergosterol. Presumptive fumonisin detection uses thin-layer chromatography but requires gas chromatography and spectral analysis for confirmation [47]. Existing laboratory methods for detecting fumonisin are expensive (>$50,000) or laborious; recent techniques may require about 30 minutes to process a sample [48]. Thus, there is a need for a sensitive, rapid, and less expensive fumonisin-detecting procedure.

Greene et al.[7] identified features from Fourier transform infrared-photoacoustic (FTIR-PAS) spectra of corn that were indicative of attack from F. moniliforme. These were the strong amide I (1650 cm^{-1}) and amide II (1550 cm^{-1}) absorbances, suggesting an increase in protein or acetylated amino sugar content, methylene CH stretch (2855 and 2925 cm^{-1}), and ester carbonyl stretch (1740 cm^{-1}). Absorption at the amide II region is linearly related to progressive F. moniliforme invasion as determined by ergosterol levels. Dowell et al.[48] evaluated reflectance and transmittance spectroscopy to detect fumonisin in single corn kernels infected with Fusarium verticillioides at three levels of infection (<10 ppm, <100 and >10 ppm, and ≥ 100 ppm). PLS regression was used to classify kernels as fumonisin positive (10 ppm) or fumonisin negative (<10 ppm). Discriminant analysis was also performed using single feature (ratio of two wavelengths) and two features (two ratios of two wavelengths) to classify kernels as fumonisin positive or negative based on three threshold levels (10 ppm, 50 ppm, and 100 ppm). The reflectance spectra of kernels infected with high levels of fumonisin showed higher absorbance at <750 nm; whereas, transmittance spectra showed generally higher absorbance at wavelengths <900 nm. It appeared that F. verticillioides caused kernels to darken and decrease vitreous-

ness. In transmittance mode this is measured as more energy absorbed at visible wavelengths, because light does not pass through an infected kernel as readily as through a healthy, vitreous kernel [48]. In reflectance mode, this is measured as less absorption in the NIR region because infected kernels scatter more energy back to the sensor [48]. The classifications (>97% accuracy) were relying on kernel color, vitreousness, and kernel composition related to fumonisin content and fungal damage, not entirely on scattering.

Dowell et al. [48] also showed that classification for fumonisin levels of >100 ppm and <10 ppm was excellent, but was generally poor for kernels having fumonisin between 10 and 100 ppm (23.5–73.0%). Wavelength pairs which performed well in discriminant analysis (for thresholds >100 ppm and <10 ppm) were 640 and 910 nm (transmittance, kernels randomly oriented), 625 and 1555 nm (reflectance, kernels germ up), 835 and 1030 nm (reflectance, kernels germ down), and 730 and 1360 nm (reflectance, randomly oriented). The classifications could be improved by redefining the classification threshold, measuring fumonisin in each kernel instead of five-kernel groups, and placing kernels in germ-up orientation [48]. Fumonisin-infected kernels tend to weigh less than uninfected kernels; hence, accounting for kernel weight might improve separation accuracy. Beta coefficients for PLS models showed that wavelengths around 450–500 nm contributed most to classifications between kernels infected by Fusarium or A. flavus. This region corresponds to the blue-green region of the spectrum and implies that the color of F. verticilioides differs significantly from A. flavus.

The fungus Fusarium graminearum can cause scab damage in wheat. The mold causes kernels to appear dull or chalky and may produce the toxin deoxynivalenol (DON), commonly called vomitoxin. Dowell et al. [49] developed a six-factor calibration based on partial least-squares (PLS) regression from absorbance at 400–1700 nm wavelength region that classified scab-damaged and undamaged wheat kernels. The kernels were determined by Grain Inspection, Packers and Stockyards Administration (GIPSA) inspectors as either scab-damaged or undamaged. Many kernels with DON were predicted by the calibration as scab-damaged, and these had been classified by GIPSA inspectors as sound. Thus, the visible-NIR system could identify scab-damaged kernels with significant levels of DON more accurately than human inspectors. Equations predicting levels of DON (>5 ppm) and ergosterol (>50 ppm) were developed that could be used as screening tools. Relevant wavelengths that contributed to scab, DON, and ergosterol predictions are the visible region, and the absorption from O-H (750, 950, and 1400 nm), C-H (1200, 1400, and 1650 nm), and N-H (1050 and 1500 nm) overtones.

Chitin is one compound that may be responsible for NIR absorption differences in mold-infected and sound products [50–52]. It is the major carbohydrate in the cell walls of molds and is found in insects, diatoms, arachnids, nematodes,

crustaceans, and some other organisms, but not in other plants or animals used as food [53]. Chitin content was the reference for mold quantification in ground barley [51,52]. Chitin, determined by NIRS, was correlated with the relative mold index (scale of visual mold damage from 0 to 5; r=0.86); chitin determined chemically was correlated with chitin determined by NIRS (r=0.98) [51,52]. Optical properties of chitin were reported by Muzzarelli [53]. Chitin as a measure of mold contamination of agricultural and food products was reviewed by Cousin [54].

III. DETECTING AND SORTING FOR INTERNAL INSECTS

The first study on NIRS for detecting insects in bulk samples was done by Wilkin et al.[55]. They detected the presence and predicted the number of mites (Acarus siro L.) in pig feed using four principal components derived from reflectance spectra (1100–2500 nm). The most important spectral feature that could be related with presence of mites was an absorption peak at 1926 nm, probably due to the mite haemolymph [56]. Wilkin et al. [55] also used four-(1494, 1548, 1896, and 1914 nm) and two-wavelength (1706 and 1840 nm) calibration models to predict levels of mites in pig feed. The two-wavelength regression, which used first derivative of log 1/R, performed better than the four-wavelength model (r=0.93 vs. 0.86).

Chambers et al. [2] followed up this study to detect external adult insects mixed with whole wheat grain. Adult insects of four species were studied: grain weevils (Oryzaephilus surinamensis), saw-toothed beetles (Sitophilus granarius), red flour beetles (Tribolium castaneum), and foreign grain beetles (Ahasverus advena). The insects were either live or freshly killed and presented in sample cells in increasing numbers from 10 to 100 in steps of 10. The four species gave similar log 1/R spectra, and all showed large absorptions at 1450 and 1940 nm attributed to water and at 1520 nm attributed to –NH. Two to five principal component models yielded r^2 of 0.99 and standard error estimate of 0.46- 1.60, depending on insect species. The shape of the first principal component was similar for all four species but became increasingly different in subsequent components. Second derivative spectra over the range 1450–1600 nm showed distinct changes with different numbers of insects.

Further studies on detection of external saw-toothed grain beetles (O. surinamensis) in bulk wheat and internal beetles in wheat kernels using NIRS were conducted by Chambers et al [57]. The spectral difference between uninfested and infested grains showed that absorption at 2290 nm increased with level of infestation and was attributed to insect lipids. For internally infested kernels, absorbance increased at 1139, 1320, and 1935 nm. The absorption at 1935 nm

was probably due to the high water content of the larvae, while the absorbance at 1139 and 1320 nm might be associated with insect chitin. Chambers et al.[58] hypothesized that feeding, excretory, and other actions of the internal larvae affected the spectral characteristics of the infested kernels.

Ridgway and Chambers [15] used NIRS (1100–2500 nm) to detect insect (O. surinamensis) larvae in single-wheat kernels and external infestations in bulk samples. Their best calibration equation was obtained from PLS regression of standard, normal variate-transformed data (1100–2500 nm) against the infestation level. Differences in absorption intensity appeared to be due to insect cuticular chitin present in the kernels. They also plotted the derivative of spectral response at two wavelengths (2046 and 2302 nm) that achieved an almost compete classification. Discrimination of sound kernels and kernels infested with pupae was also achieved by using the criterion log1/R(1194 nm)—log1/R(1304nm), without any form of scatter correction [15]. The starch band at 1194 nm was less pronounced in the log 1/R spectrum of infested kernels, leading them to conclude that the spectral response could likely be due to wheat starch lost as a consequence of insect feeding.

Ghaedian and Wehling [17] used discriminant analysis based on loadings derived from principal component analysis (PCA) of full (1100–2498 nm) or partial NIR spectra (1100–1900 nm) to classify sound and granary weevil-infested wheat. Individual wheat kernels were placed in a parabolic reflector, both crease up and down, to collect the radiation reflected from the entire surface of the kernel. PCA of NIR spectra from sound kernels was used to construct calibration models by calculation of Mahalanobis distances (MD). Kernels with MD of <3 standard deviations from the training set center were classified as sound, and those with MD 3 standard deviations were classified as infested. The models were then applied to spectra obtained from both sound and infested kernels in a separate validation set. A five-factor PCA model using data from NIR spectra in the 1100–1900 nm region and transformed by first-derivative was the best model for correctly classifying kernels. They found that the region 1980 to 2498 nm did not contain enough information for reliable classification. Scatter-corrected spectra improved the prediction rate and used fewer factors. They further used discriminant analysis based on MD applied to selected wavelengths. Best results were obtained with a 12-wavelength model (1200, 1240, 1300, 1320, 1360, 1400, 1420, 1440, 1660, 1680, 1880, and 2220 nm). The best models were based on spectra of kernels positioned with crease down. PCA factors and discrete wavelengths with high correlation against internal insects pointed to protein, lipid (1200, 1360, 1440 and 1660 nm), and phenolic (1420 nm) compounds as the major source of variation between sound and infested wheats.

Dowell et al. [30] identified the wavelength ranges 1000–1350 nm and 1500–1680 nm as important for internal insect detection in wheat kernels. These corresponded to the C-H overtones and combinations. The C-H overtone regions

corresponded to chitin absorption regions. They observed that absorbance peaks of ground insect cuticle or chitin were at 1178 nm and 1500 nm, which confirmed their findings. Thus, chitin present in insect tissues may explain differences between sound kernels and those containing larvae. The effect of larval size on detection accuracy was also determined. They were able to detect 3rd and 4th instars in wheat kernels with 95% confidence but could not reliably detect smaller larvae. Insect larvae could be detected independent of the effects of wheat class, protein content, or insect species.

Baker et al. [31] used NIRS to rapidly differentiate single-wheat kernels containing the parasitoid Anisopteromalus calandrae from rice weevils, and to separate sound kernels from those containing weevil or parasitoid larvae or pupae. Using 13 PLS factors, their model correctly classified 97.4% of uninfested kernels and correctly classed as infested 90–100% of those kernels with either host or parasitoid larvae or pupae. When differentiating weevils from parasitoids, kernels containing weevil larvae and pupa could be differentiated from parasitoid pupa with 100% accuracy. The method could not reliably classify parasitoid larva, probably because of their small size. Spectral differences between adult, pupal, and larval stages of the saw-toothed beetle were earlier reported by Chambers et al. [2].

The studies of Dowell et al.30 and Baker et al.31 used a single-kernel characterization system coupled with a diode-array spectrometer for automated kernel handling and collection of spectra. With integration of NIR spectroscopy and seed-sorting instrumentation, kernels containing parasitoid pupae could be automatically collected from cultures of beneficial insects for mass rearing and for later optimized release at a specific age and stage [31]. Automatic separation of single-wheat kernels containing weevil larvae or pupae could also be done using this technology.

In order to identify wavelengths for NIR imaging in the very near-infrared, where sensitivity is better, and instrumentation is generally cheaper, Ridgway et al.[59] evaluated the wavelength region 700–1100 nm for a sorting criterion. Single wheat kernels infested with S. granarius larvae (3–4 weeks after egg-laying) or pupae (4–5 weeks after egg-laying), as well as uninfested kernels, were scanned using a reflectance cup. They have shown that two-wavelength classification models, based on either log1/R (982 nm) log1/R(1014 nm) or log1/R(972 nm)- log 1/R(1032 nm), could correctly classify wheat infested by S. granarius larvae and uninfested wheat (>96% accuracy) as well as that of a full-spectrum model. They have confirmed the earlier hypothesis of Chambers et al.[58] that the spectral difference arose from the actions of the developing insects, such as decreasing grain starch, for log1/R (982 nm) – log1/R(1014 nm), or increasing grain moisture, for log1/R(972 nm)- log 1/R(1032 nm), due to insect activity. The NIR method can detect infestation up to about seven weeks before insects emerge as adults.

IV. FACTORS THAT LIMIT CLASSIFICATION OR SORTING ACCURACY

A. Threshold Setting and Sorting Criterion

In many sorting situations, products do not classify neatly into "accept" and "reject" categories. This is because acceptable and unacceptable products can have spectral characteristics that range from very pronounced to very subtle, thus making identification of the cutoff point difficult. Increasing the cutoff increases the "accepts" in the reject portion, while decreasing the cutoff increases the "rejects" in the accept portion. McClure and Farsaie [42], reported the overlapping distributions in BGY and non-BGY fluorescent pistachio nuts. Various methods have been proposed to define the cutoff or threshold, such as the 99.5% reference value set by an adjustment knob [60], and adding 2.6 standard deviations to the highest mean reflectance of good light and good dark fruits [61]. Often the most that can be done is to maximize the acceptance of good products and minimize the acceptance of rejects. Since this is mainly an economic decision, some commercial sorters leave this decision to the operator with a threshold-adjusting knob or button to define acceptable shades of color, as well as acceptable sizes of defects [28, 29].

A non-sensitive sorting criterion, not equipment limitation, was the main cause of poor performance of existing sorters [19]. A criterion that worked in one quality factor may not necessarily work on another quality factor; hence, wavelength identification and proper selection of a sorting criterion are important.

B. Moisture Content

Water absorption bands have been observed to interfere with spectral bands of other constituents in detecting internal insects [17] and fungal contamination [7.] This source of variation was eliminated by excluding water-absorbing wavelengths in the prediction model and using other indicator wavelengths [7]. Excluding water-absorption wavelengths (950 nm and between 1350–1500 nm) improved the classification of insect-infested and sound kernels [30].

C. Kernel Orientation and Biological Variation

There seemed to be a preferred orientation for NIR presentation of seeds that is related to seed geometry. Chambers and Ridgway [62] reported that although detection of internal insects in wheat kernels was successful regardless

of orientation, the classification accuracy was greater when the kernels were placed crease down. Ghaedian and Wehling [17] reported that crease-down orientation yielded better predictions for internal insects. Tyson and Clark [13] obtained higher correlation coefficients between aflatoxin contamination and fluorescence when fluorescence was measured from the bottom side of pecan halves. In corn kernels, the fungal infecting point may be important. Molds tend to invade the germ more readily than other parts of the corn kernel [12]. Within the corn kernel the fungus F. moniliforme is associated with the tip-cap end, the point of attachment to the cob [5]. Variation in the shape of corn kernels made it difficult to restrict transmittance measurements to the germ area of the kernel, thus contributing to errors [12]. Random placement of the wheat kernel in the instrument viewing area is a major source of classification error [30, 63]. However, they reasoned that because a commercial, automated larval detection device probably would not orient kernels, their results represent the potential for larval detection with a commercial unit.

Biological variations such as lipid properties within a single insect64; pigments in yellow corn [12]; and thickness of corn kernels [65] can also cause spectral variations that lead to misclassifications.

V. SUMMARY AND CONCLUSIONS

Models that used spectral properties in the visible and near-infrared regions, with or without spectral pretreatments, have performed well in detecting and classifying seeds infested by internal insects and fungi. Some sorting models based on ratio or difference of absorbance or fluorescence at two wavelengths in these regions performed equally well and was of more practical importance. Performance of sorting models varied depending on the seed, its orientation or geometry, extent of infestation or contamination, and thresholds used. Wavelengths from both visible and near-infrared regions are useful in detecting fungal contamination of seeds, which may indicate that visible damage or discoloration, as well as chemical constituents, are different between infected and sound seeds. For internal insects, the wavelengths for detection are mainly in the near-infrared. Wavelengths for detection depend on the infecting fungus or internal insect. For fungal contamination in various seeds, the following wavelength pairs for detection and sorting have been reported: (a) 440 and 490 nm; 442 and 607 nm (by fluorescence), (b) 800 and 930; 700 and 1100; 710 and 760; 615 and 645; 640 and 910 nm (by transmittance), and (c) 735 and 1005; 625 and 1555 nm; 835 and 1030; 730 and 1360 nm (by reflectance). For internal insects in wheat, the following wavelength pairs have been reported: (a) 982 and 1014, and (b) 1194 and 1304 nm (by reflectance). Further studies are needed for optical detection of internal insect and fungal contamination in other cereal grains. The prob-

able constituent that yielded the unique spectral response, from which detection models were based, varied according to each study.

REFERENCES

1. Pedersen, J.R. Insects: Identification, damage, and detection. In Storage of Cereal Grains and their Products, 4th ed. D.B. Sauer, Ed., St. Paul, MN: AACC, 1992, 435–489.
2. Chambers, J.; Cowe, I.A.; van Wyk, C.B.; Wilkin, D.R.; Cuthbertson, D.C. Detection of insects in stored products by NIR. In Near-Infrared Spectroscopy: Bridging the Gap Between Data Analysis and NIR Applications; K.I. Hildrum, T.Isaksson, T. Naes, and A. Tandberg, Eds.; Ellis-Horwood, Chichester, U.K., 1992; 203–208.
3. Cuperus, G.; Krischik, V. Why stored product integrated pest management is needed. In Stored Product Management. Oklahoma State Univ. Ext. Coop. Serv. Circ. E-912. 1995, 199.
4. Marasas, W.F.O. Mycotoxicology: Introduction to the mycology, plant pathology, chemistry, toxicology and pathology of naturally occurring mycotoxicosies in animals and man. Pennsylvannia State Univ. Press: University Park, 1987.
5. Bacon, C.; Nelson, P.E. Fumonisin production in corn by toxigenic strains of Fusarium moniliforme and Fusarium proliferatum. J. Food Prot. 1994, 57 (6), 514–521.
6. Wilson, D.M.; Abramson, D. Mycotoxins. In Storage of Cereal Grains and their Products, 4th ed. D.B. Sauer, Ed., St. Paul, MN.:AACC, 1992, 341–391.
7. Greene, R.V.; Gordon, S.H.; Jackson, M.A.; Bennett, G.A. Detection of fungal contamination in corn: potential of FTIR-PAS and DRS. Journ. Agric. Food Chem. 1992, 40 (7), 1144–1149.
8. Gordon, S.H.; Wheeler, B.C.; Schudy, R.B.; Wicklow, D.T.; Greene, R.V. Neural network pattern recognition of photoacoustic FTIR spectra and knowledge-based techniques for detection of mycotoxigenic fungi in food grains. Journ. Food Prot. 1998, 61 (2). 221–230.
9. Gordon, S.H.; Jones, R.W.; McClelland, J.F.; Wicklow, D.T.; Greene, R.V. Transient infrared spectroscopy for detection of toxigenic fungi in corn: potential for online evaluation. Journ. Agric.Food Chem. 1999, 47 (12), 5267–5272.
10. Jones, R.W.; McClelland, J.F. Transient IR spectroscopy: Online Analysis of Solid Materials. Spectroscopy 1992, 7 (4): 54–58.
11. Chen, P.; Sun, Z. A review of non-destructive methods for quality evalua-

tion and sorting of agricultural products. Journ. Agric. Eng. Res. 1991, 49 (2), 85–89.
12. Birth, G.S.; Johnson, R.M. Detection of mold contamination in corn by optical measurements. Journ. Assoc. Official Anal. Chem. 1970, 53 (5), 931–936.
13. Tyson, T.W.; Clark, R.L. An investigation of the fluorescent properties of aflatoxin-infected pecans. Trans. ASAE 1974, 17 (5), 942–944, 948.
14. Johnson, R.M. Determining damage in yellow corn. Cereal Sci. Today 1962, 7 (1), 14–15.
15. Ridgway, C.; Chambers, J. Detection of external and internal insect infestation by near-infrared spectroscopy. Journ. Sci. Food Agric. 1996, 71, 251–264.
16. Munkvold, G.P.; Desjardins, A.E. Fumonisins in maize: Can we reduce their ocurrence? Plant Disease 1997, 81 (6), 556–565.
17. Ghaedian, A.R.; Wehling, R.L. Discrimination of sound and granary-weevil larva-infested wheat kernels by near-infared diffuse reflectance spectroscopy. Journ. AOAC Internl. 1997, 80 (5), 997–1005.
18. Chambers, J.; Ridgway, C.; Davies, E.R.; Mason, D.R.; Bateman, M.W. Rapid automated detection of insects and certain other contaminants in cereals. Home-Grown Cereals Authority (HGCA) Project Report No. 156, U.K., 1998.
19. Powers, J.B.; Gunn, J.T.; Jacob, F.C. Electronic color sorting of fruits and vegetables. Agric. Eng. 1953, 34 (3), 149–154, 158.
20. Norris, K.H. Measuring light transmittance properties of agricultural commodities. Agric. Eng. 1958, 39 (10), 640–643, 651.
21. Birth, G.S. Agricultural applications of the dual-monochromator spectrophotometer. Agric. Eng. 1960a, 41(7), 432–435, 452.
22. Birth, G.S.; Norris, K.H. The difference meter for measuring interior quality of foods and pigments in biological tissue. Technical Bull. 1341. USDA-ARS, Washington, D.C., 1965.
23. Birth, G.S.; Zachariah, G.L. Spectrophotometer for biological applications. Trans. ASAE 1973, 16 (2), 371–373.
24. Birth, G.S. Radiometric measurement of food quality – a review. Journ. Food Sci. 1979, 44 (4), 949–953, 957.
25. Birth, G.S. Measuring the smut content of wheat. Trans. ASAE 1960b, 3, 19–21.
26. Gillispie, R.M. Equipment for sorting food products by light reflectance. In Quality Detection in Foods. ASAE Publication 1–76. 1976, 175–176.
27. Sortex Inc. Sortex 3000 Series Service Manual 1994. Sortex Inc. Newark, CA.
28. Key Technology, Inc. 2000. Product Catalog. Walla Walla, WA.

29. Satake. ScanMaster High-Capacity Sorters, 2000. Satake-USA Corp., Houston, TX.
30. Dowell, F.E.; Throne, J.E.; Baker, J. E. Automated nondestructive detection of internal insect infestation of wheat kernels by using near-infrared reflectance spectroscopy. Journ. Econ. Entom. 1998, 91 (4), 899–904.
31. Baker, J. E.; Dowell, F. E.; Throne, J. E. Detection of parasitized rice weevils in wheat kernels with near-infrared spectroscopy. Biological Control 1999, 16, 88–90.
32. Gunesakaran, S.; Paulsen, M.R.; Shove, G.C. Optical methods for nondestructive quality evaluation of agricultural and biological materials. Journ. Agric. Eng. Res. 1985, 32, 209–241.
33. Johnson, R.M. Five methods for determining smut content in wheat. Cereal Chem. 1960, 37 (3), 289–308.
34. Shotwell, O.L.; Hesseltine, C.W. Use of bright greenish-yellow fluorescence as a presumptive test for aflatoxin in corn. Cereal Chem. 1981, 58 (2), 124–127.
35. Wilson, D. Analytical method for aflatoxin in corn and peanuts. Arch. Environ. Contam. Toxicol 1989, 18, 304–314.
36. Hirano, S.; Okawara, N.; Narazaki, S. Near-infrared detection of internally moldy nuts. Biosci. Biotechnol. Biochem. 1998, 62 (1), 102–107.
37. Pearson, T. C.; Wicklow, D. T.; Maghirang, E. B.; Xie, F.; Dowell, F. E. Detecting aflatoxin in single corn kernels by using transmittance and reflectance spectroscopy. Trans. ASAE 2001, in press.
38. Cucullu, A.F.; Lee, L.S.; Mayne, R.Y.; Goldblatt, L.A. Detection of aflatoxin in individual peanuts and peanut sections. Journ. Amer. Oil Chem. Soc. 1966, 43 (2), 89–92.
39. Ayers, J.C.; Lillard, H.S.; Lillard, D.A. Mycotoxins:methods for detection in foods. Food Technol. 1970, 24(2), 55–60.
40. Whitaker, T.B.; Dickens, J.W.; Wiser, E.H. Design and analysis of sampling plans to estimate aflatoxin concentrations in shelled peanuts. Journ. Amer. Oil Chem. Soc. 1970, 47 (12), 501–504.
41. Farsaie, A.; McClure, W.F.; Monroe, R.J. Development of indices for sorting iranian pistachio nuts according to fluorescence. Journ. Food Sci. 1978, 43(5), 1550–1552.
42. McClure, W.F.; Farsaie, A. Dual-wavelength fiber optic photometer measures flourescence of aflatoxin-contaminated pistachio nuts. Trans. ASAE 1980, 23 (1), 204–207.
43. Farsaie, A.; McClure, W.F.; Monroe, R.J. Design and development of an automatic electro-optical sorter for removing BGY fluorescent pistachio nuts. Trans. ASAE 1981, 24(5), 1372–1375.
44. Fraenkel, H.; Gough, P.B.; Maughan, W.S. Method and apparatus for sorting agricultural products. US Patent 4203522, 1980.

45. Shotwell, O.L.; Goulden, M.L.; Hesseltine, C.W. Aflatoxin: Distribution in contaminated corn. Cereal Chem. 1974, 52, 670–677.
46. Vesonder, R. F.; Golinski, P. Metabolites of Fusarium. In Fusarium Mycotoxins, Taxonomy, and Pathogenicity, Chelkowski, J. Ed., Amsterdam: Elsevier, 1989, 1–39.
47. Jackson, M.A.; Bennett, G.A. Production of fumonisin B1 by Fusarium monoliforme NRRL 13616 in submerged culture. Appl. Environ. Microbiol. 1990, 56, 2296–2298.
48. Dowell, F.E.; Pearson, T.C.; Maghirang, E.B.; Xie, F.; Wicklow, D.T. Reflectance and transmittance spectroscopy applied to detecting fumonisin in single corn kernels infected with Fusarium verticillioides. Cereal Chem. 2001, in press.
49. Dowell, F.E.; Ram, M.S.; Seitz, L.M. Predicting scab, vomitoxin, and ergosterol in single wheat kernels using near-infrared spectroscopy. Cereal Chem.1999, 76 (4), 573–576.
50. Nilsson, M.; Elmqvist, T.; Carlsson, U. Use of near-infrared reflectance spectrometry and multivariate data analysis to detect anther smut disease (Microbotryum violaceum) in Silene dioica. Phytopathology 1994, 84 (7), 764–770.
51. Roberts, C.A.; Marquardt, R.R.; Frohlich, A.A.; McGraw, R.L.; Rotter, R.G.; Henning, J.C. Chemical and spectral quantification of mold in contaminated barley. Cereal Chem. 1991, 68 (3), 272–275.
52. Roberts, C.A. Quantification of fungal contaminants in forage and grain. In Making Light Work: Advances in Near-Infrared Spectroscopy. I.A. Murray and I.A. Cowe, Eds., VCH Publishers, N.Y., 1992, 352–356.
53. Muzzarelli, R.A.A. Chitin. Pergamon Press, NY., 1977.
54. Cousin, M.A. Chitin as a measure of mold contamination of agricultural commodities and foods. Journ. Food Prot. 1996, 59 (1), 73–81.
55. Wilkin, D.R.; Cowe, I.A.; Thind, B.B.; McNicol, J.W.; Cuthbertson, D.C. The detection and measurement of mite infestation in animal feed using near-infrared reflectance. Journ Agric. Sci. Camb. 1986, 107 (2), 439–448.
56. Wilkin, D.R.; Cowe, I.A. The detection of infestation in stored products using NIRS. In Analytical Applications of Spectroscopy. C.S. Creaser and A.M.C. Davies, Eds., London: Royal Society of Chemistry, 1988, 91–97.
57. Chambers, J.; van Wyk, C.B.; Baker, C.W.; Barnes, R.J. NIR analysis for the detection of insect pests in cereal grains. In Proc. of the 6th International Diffuse Reflectance Conference, Chambersburg, Penn., 9–14 August. 1993a, 96–100.
58. Chambers, J.; Ridgway, C.; Baker, C.W.; Barnes, R.J. Improving quality by rapid pest detection. In Cereal Quality III: Aspects of Applied Biology, Churchill College, Cambridge, 13–15 December, 1993; U.K. 1993b, 465–470.

59. Ridgway, C., Chambers, J., and Cowe, I.A. Detection of grain weevils inside single wheat kernels by a very near-infrared two-wavelength model. Journ. NIR Spectrosc. 1999, 7:213–221.
60. Moini, S.; O'Brien, M.; Chen, P. Spectral properties of mold and defects of processing tomatoes. Trans. ASAE 1980, 23 (4), 1062–1064.
61. Burkhardt, T.H.; Mrozek, R.F. Light reflectance as a criterion for sorting dried prunes. Trans. ASAE 1973, 16 (4), 683–685.
62. Chambers, J.; Ridgway, C. Rapid detection of contaminants in cereals. In Near-Infrared Spectroscopy: The Future Waves, A.M.C. Davies and P. Williams, Eds.; NIR Publications, Chichester, U.K., 1996, 484–489
63. Dowell, F.E.; Martin, C.R.; Steele, J.L.; Wang, D.; Baker, J.E.; Throne, J.E.; Delwiche, S.R. Automated single wheat kernel quality measurement using near-infrared reflectance. 1997; ASAE Paper 97–3022, St. Joseph, MI.
64. Gibbs, A.; Crowe, J.H. Intra-individual variation in cuticular lipids studied using fourier transform infrared spectroscopy. Journ. Insect Physiol. 1991, 37 (10), 743–748.
65. Paez, A.V.; Helm, J.L.; Zuber, M.S. Quantitative measurement of light transmission through corn endosperm. Cereal Chem. 1968, 45 (6), 595–599.