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Detection of Scab in Single Wheat Kernels Using NIR Spectroscopy

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Summary:
Near-infrared spectroscopy (NIRS) was used to detect scab-damage and estimate deoxynivalenol (DON) and ergosterol levels in single wheat kernels. Results showed that all scab-damaged kernels identified by official inspectors were correctly identified by NIRS. DON and ergosterol were predicted with standard errors of about 50 and 100 ppm, respectively. This technology may provide a means of rapidly screening samples for potential quality problems related to scab damage.

Keywords: wheat, near-infrared spectroscopy, grading, grain quality, spectral analysis

INTRODUCTION

Scab damage in wheat (*Triticum aestivum* L.) can occur when damp and cool conditions during the maturing and harvesting seasons create a favorable environment for the growth of the mold *Fusarium graminearum*. The mold can cause kernels to appear dull, lifeless, or chalky and may produce the toxin deoxynivalenol (DON). The presence of scab adversely affects flour ash, flour color, glutenin levels, dough properties, and loaf volume (Dexter et al. 1996). In addition, the toxin can cause digestive disorders, diarrhea, refusal to eat, and death in animals. DON is also a suspected human carcinogen (Luo et al. 1990).

Besides adversely affecting grain quality and food safety, scab can reduce yields by 50% and crop losses in US in some years have exceeded $1 billion (Meronuck 1997; Liu 1985). Also, in years when DON levels are excessive, individual millers may spend more than $1 million as they attempt to blend wheat to meet Food and Drug Administration (FDA) guidelines (Anonymous 1998). FDA guidelines are one ppm for finished wheat products for human consumption, ten ppm for cattle or poultry feed, and five ppm for other animals (Herrman et al. 1995). Cleaning, milling, and baking can reduce DON levels, but concentrations in finished products may not be significantly less than levels measured before milling (Abbas et al. 1985; Nowicki et al. 1988; Scott et al. 1984; Seitz et al. 1986; Trigo-Stockli et al. 1996).

Several methods exist to detect scab-damaged kernels, DON, or ergosterol levels. Ergosterol indicates the presence of fungal invasion (Seitz et al. 1977). The Grain Packers and Stockyards Administration (GIPSA) includes visual scab detection as part of routine grain inspection procedures (USDA 1991). GIPSA inspection procedures consider kernels as scab-damaged if they have significant amounts of discolorations attributable to the fungus. However, kernels with little or no visible scab can have significant levels of ergosterol and DON (Seitz and Bechtel 1985) but not meet GIPSA criteria for damage. Chemical tests such as thin-layer chromatography, gas chromatography, or high performance liquid chromatography (HPLC) can measure DON or ergosterol levels (Miller et al. 1983; Nowicki et al. 1988; Seitz and Bechtel, 1985; Trigo-Stockli et al. 1996). However, these tests can take several hours to complete. Thus, a rapid, objective means of detecting scab-damaged kernels and indicating levels of DON and ergosterol is needed.

Ruan et al. (1988) showed that machine vision could estimate scab damage more accurately than a human expert panel. They did not attempt to use machine vision to estimate DON or ergosterol levels. Near-infrared spectroscopy (NIRS) can estimate wheat quality characteristics such as internal defects, color class, protein, and hardness (Delwiche and Massie 1996; Delwiche and Norris 1993; Dowell et al. 1998; Dowell 1998; Murray and Williams 1990). Thus, applying NIRS to the detection of scab-damaged kernels and the estimation of DON and ergosterol in wheat may be possible. The objective of this research was to assess the feasibility of single kernel spectral analysis as an objective method for measuring scab-damaged wheat kernels, and for measuring DON and ergosterol levels.

PROCEDURE

Ten samples of hard red spring wheat were collected from lots with high levels of scab damage. Lots originated from commercial sources and from scab nurseries. Samples were separated into scab-damaged and sound kernels by the GIPSA Board of Appeal and Review. Kernels determined as sound kernels by GIPSA criteria were further separated into kernels that appeared healthy and kernels with any visible scab damage. Table 1 shows the number of kernels used for subsequent NIR, DON, and ergosterol measurements. About 45 kernels per sample were used for subsequent tests.

NIRS

NIR spectra from all single kernels were collected using a diode-array spectrometer (DAS) integrated with a single kernel characterization system (SKCS) (Perten Instruments, Springfield, IL). Kernels were hand-placed into the viewing area where NIR spectra (400-1700 nm) were collected. Six spectra were collected per kernel and averaged. Absorption was measured in 7 nm increments in the 400-950 nm region, and 11 nm increments in the 950-1700 nm region. All data were interpolated to 5 nm increments. Dowell et al. (1998) and Martin et al. (1993) give details of the DA and SKCS. Spectra were analyzed using partial least squares (PLS) regression within the GRAMS/32 software package (Galactic Industries Corporation, Salem, NH).

Chemical Analyses

Single kernel DON and ergosterol levels were determined using reversed-phase high performance liquid chromatography to measure compounds in extracts from single kernels. DON and ergosterol were not measured on the same kernel. Detection limits were about four ng for DON and 90 ng for ergosterol.

RESULTS AND DISCUSSION

Relationship of DON and Ergosterol to Scab

Table 1 shows DON and Ergosterol levels in scab samples. Kernels determined by GIPSA as scab-damaged averaged 236.0 ppm DON and 568.4 ppm ergosterol. Kernels with visible scab but not meeting GIPSA criteria for scab damage contained an average of 50.8 and 157.8 ppm DON and ergosterol, respectively. Kernels with no visible scab had DON and ergosterol levels as high as 2.8 and 19.0 ppm, respectively.

Figures 1 and 2 show the distribution of DON and ergosterol levels in single kernels identified by GIPSA as sound or scab-damaged. All kernels identified by GIPSA as scab damaged had detectable DON and ergosterol. In addition, detectable amounts of DON or ergosterol were found in 60% and 84% of kernels identified by GIPSA as sound, respectively. Thus, current official grading procedures identify kernels with high levels of DON and ergosterol. However, many kernels not identified by current inspection procedures still contain significant levels of DON and ergosterol.

Figures 1 and 2 also show that more kernels had detectable ergosterol than DON. Ergosterol is produced by all fungi. Thus, the presence of ergosterol may indicate the presence of other fungi, that ergosterol was present before DON was produced, or that ergosterol detection methods were more sensitive than DON detection methods. O'Neill et al. (1993) reported that 14 days or more was required for DON to be detected after grain was inoculated with *F. graminearum*. Other fungi growing in the grain will also produce ergosterol.

Prediction of Scab-Damaged Kernels Using Spectral Characteristics

A calibration was developed from PLS regressions to predict scab damage using single kernel spectral characteristics. The calibration equation (11 factors) included only kernels determined by GIPSA as scab-damaged (31) and kernels with no measurable DON (102). All kernels (271), which included kernels not in the calibration, were predicted from this calibration equation.

Results showed that 100% of scab-damaged and 79% of kernels identified by GIPSA as sound were correctly identified by NIRS (Table 2). All sound kernels that were incorrectly identified had some amount of visible scab. All sound kernels with no visible scab were correctly identified. Thus, scab-damaged and sound kernels can be differentiated using spectral characteristics. However, as shown in Figures 1 and 2, this does not necessarily indicate the presence or absence of DON. The average DON levels in kernels predicted as sound or scab-damaged were 9.2 and 161.0 ppm, respectively. The average ergosterol levels in kernels predicted as sound or scab-damaged were 42.9 and 426.5 ppm, respectively.

Prediction of DON and Ergosterol Levels

A DON prediction equation (nine factors) was developed using only kernels with >5 ppm DON. The prediction equation was applied only to those kernels predicted previously as scab damaged. Figure 3 shows predicted DON levels (r^2=0.47, SE=53 ppm, n=92). Only one kernel was erroneously predicted as having 0 ppm DON.

Ergosterol was predicted using an equation (eight factors) developed using kernels with greater than 50 ppm ergosterol. As with the DON prediction, the ergosterol prediction equation was applied only to those kernels previously predicted as scab damaged. Figure 4 shows predicted ergosterol values (r^2=0.64, SE=108 ppm, n=46) where only one kernel was erroneously predicted as having zero ergosterol.

Calibrations were attempted using ng of DON or ergosterol instead of ppm. However, prediction results using ng resulted in lower r^2 values than those achieved when using ppm.

Relationship of DON to Ergosterol

Figure 5 shows the relationship of kernel weight to DON and ergosterol levels, adjusted by dividing by the respective mean ppm so y-axes would be of similar magnitude. For both DON and ergosterol, levels decreased as kernel weight increased. This relationship between DON and kernel weight agrees with results reported by others (Bechtel et al. 1985; Snijders and Perkowski 1990). This relationship may be due to smaller kernels having higher levels of DON and ergosterol, or to damaged kernels being less dense than sound kernels. Regner et al. (1994) reported that small kernels do tend to have higher ergosterol contents than larger kernels, but that small kernels have little influence on total ergosterol contents of the lot. Martin et al. (1998) reported that scab damaged kernels are less dense than sound kernels.

Wavelengths Used in Classifications

Beta coefficients calculated by PLS were examined to determine wavelengths used in scab, DON, and ergosterol predictions. Important wavelengths were noted throughout the 500-1700 nm indicating that absorption in the visible region and absorption arising from O-H (~750, 950, and 1400 nm), C-H (~1200, 1400, and 1650 nm), and N-H (~1050 and 1500 nm) overtones contributed to classifications. Figure 6 shows beta coefficients for DON only. Coefficients for ergosterol and scab were similar. Bechtel et al. (1985) reported that scab affects starch and protein, which absorb NIR radiation at the overtones noted above.

In summary, these results show that NIRS can detect scab-damaged kernels and estimate DON and ergosterol levels in single kernels. This technology could be used to rapidly screen incoming samples for potential wheat quality problems related to scab damage.

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Table 1. DON and Ergosterol levels (ppm) in scab samples.

Analysis Type	Scab Category	n	avg	min	max	std
DON	No Visible Scab	60	0.2	ND	2.8	0.5
	Visible Scab[a]	180	50.8	ND	558.1	88.3
	GIPSA Scab[b]	31	236.0	32.7	788.6	156.9
Ergosterol	No Visible Scab	40	4.6	ND	19.0	5.1
	Visible Scab	115	157.8	ND	1497.0	273.8
	GIPSA Scab	15	568.4	262.07	1232.0	285.3

[a] Kernels with visible scab, but not meeting GIPSA criteria for scab.
[b] Scab as determined by GIPSA standards.
ND=None detected.

Table 2. Deoxynivalenol and ergosterol levels in samples predicted as sound or scab damaged by near-infrared spectroscopy (NIRS).

GIPSA Classification	Kernels correctly classed by NIRS, %	DON, ppm		Ergosterol, ppm	
		avg	std	avg	std
Sound[a]	79	9.2	26.8	42.9	145.2
Scab[b]	100	161	140.9	426.5	329.9

[a] Kernels with no visible scab plus kernels with visible scab but not meeting GIPSA scab damage criteria.
[b] Scab as determined by GIPSA personnel
PLS=Partial Least Squares

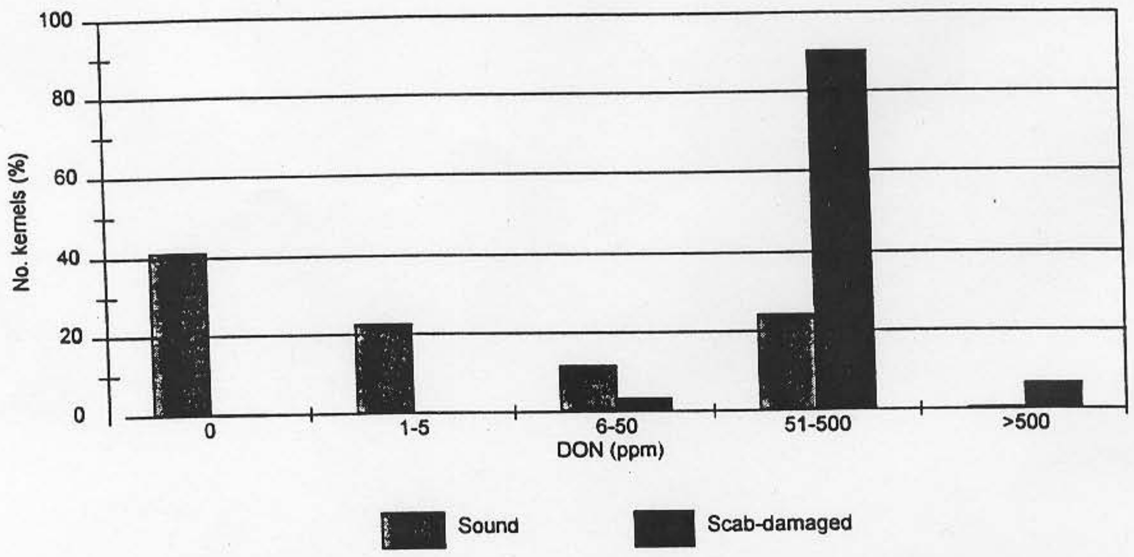

Figure 1. Distribution of deoxynivalenol in single wheat kernels determined as sound or scab-damaged by official grading procedures.

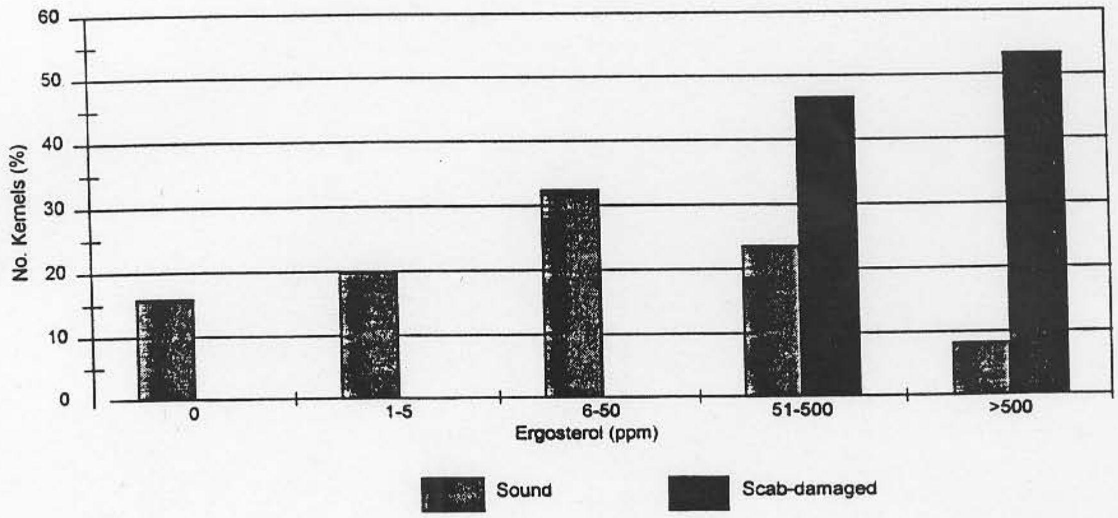

Figure 2. Distribution of ergosterol in single wheat kernels determined as sound or scab-damaged by official grading procedures.

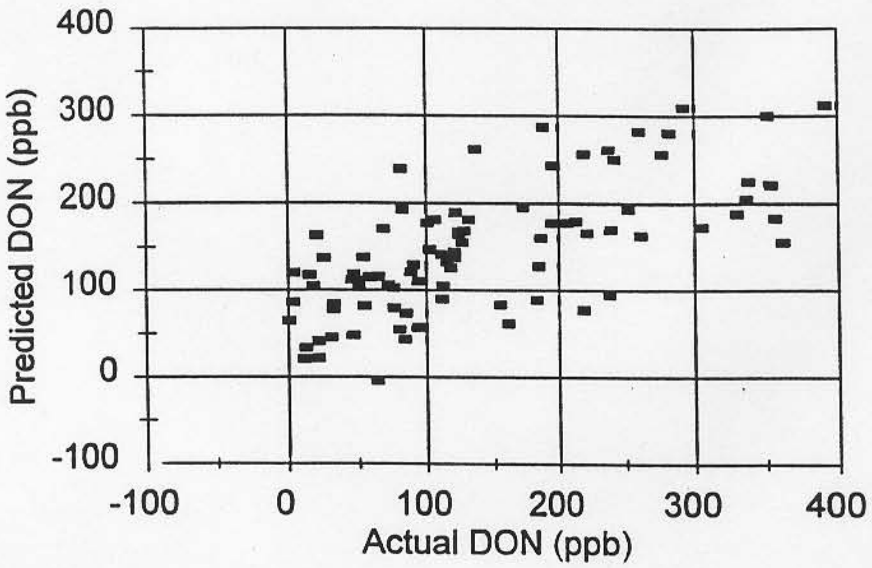

Figure 3. Single wheat kernel deoxynivalenol (DON) levels predicted from NIR spectra.

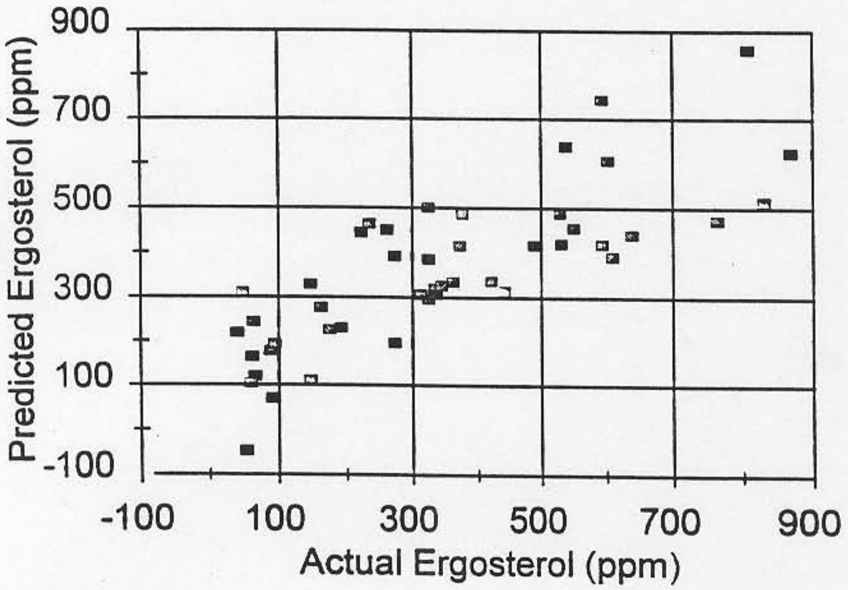

Figure 4. Single wheat kernel ergosterol levels predicted from NIR spectra.

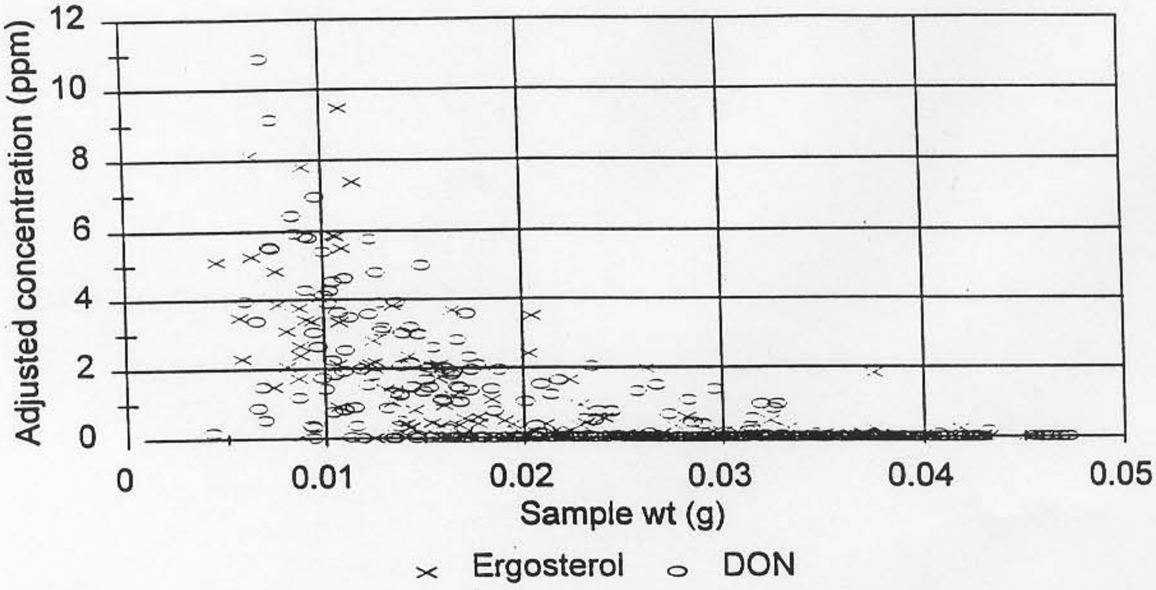

Figure 5. Relationship between single kernel weight and deoxynivalenol (DON) and ergosterol levels.

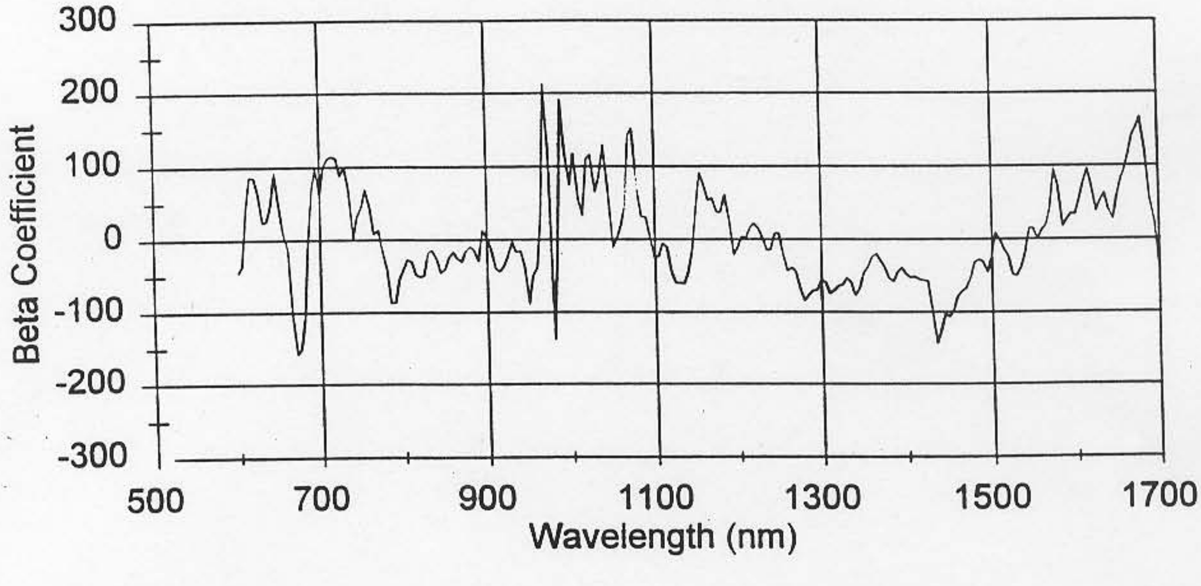

Figure 6. Partial least squares regression beta coefficients indicating important wavelengths for single kernel deoxynivalenol predictions from NIR spectra.