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Detecting Single Wheat Kernels Containing Live or Dead Insects Using Near-Infrared Reflectance Spectroscopy

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Abstract *An automated NIR system was used over a two-month storage period to detect single wheat kernels that contained live or dead internal rice weevils at various stages of growth. Correct classification of sound kernels and kernels containing live pupae, large larvae, medium-sized larvae, and small larvae averaged 94, 92, 84, and 62%, respectively. Pupae + large larvae calibrations were developed using live or dead internal insects. Validation results showed correct classifications ranging from 86 to 96% over the two-month storage period. Thus, wheat kernels containing either live or dead insects can be used to develop calibrations for detecting both live and dead insects in wheat.* **Keywords.** *Wheat quality, Internal insect, Hidden insects, Rice weevil, Single kernel, Single kernel characteristics, Grading, Inspection*

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Introduction

Rice weevil (*Sitophilus oryzae* L.) is a primary pest of hard red winter wheat (*Triticum aestivum* L.). Adult insects feed on the kernel surface, but deposit eggs inside kernels. Weevil larvae feed and complete development inside the kernel until they mature and emerge as an adult. The presence of internal insects in wheat is a major problem for the wheat industry. The insects may eventually emerge and cause further damage to kernels and contribute to fragments in flour. While a wheat lot may visually appear to be sound or uninfested, internal insects may be present in some kernels. The presence of live or dead internal insects in wheat kernels equates to lower wheat quality. In the United States, the Food and Drug Administration (FDA) has imposed the defect action level for insect contamination to be 32 or more insect damaged kernels per 100 g of wheat and 75 or more insect fragments per 50 g of flour (FDA, 1998). To coincide with FDA's defect action levels, the U.S. Standards for wheat consider wheat containing 32 or more insect-damaged kernels per 100 g as U.S. sample grade. Those containing less than 32 insect-damaged kernels can be given a designation of U.S. Grades 1 to 5 based on other set criteria; it may be certified with a special grade of "Infested" based on the presence of live weevils or other live insects injurious to stored grains (USDA, 1997).

Numerous studies have focused on the development of methods for detecting internal insects, which are needed because visual inspection cannot detect internal insects. For example, visual inspection showed that 4% of wheat samples from 79 U.S. grain elevators were infested with insects, while incubation of the same wheat samples over 3 to 6 weeks showed 16% insect infestation (Storey et al., 1982). Other detection techniques include: (a) selective fluorescent stains (Milner et al., 1950a), (b) x-ray inspection (Milner et al., 1950b; Schatzki and Fine, 1988; Keagy and Schatzki, 1993; Throne, 1994; AACC, 2001; and Haff, 2001), (c) cracking and flotation (AACC, 2001), (d) acid hydrolysis test (Trauba et al., 1981; AACC, 2001), (e) immunological technique (Kitto, 1991; Quinn et al., 1992; Schatzki et al., 1993), (f) machine vision (Zayas and Flinn, 1998; Ridgway et al., 2001), and (g) near-infrared spectroscopy (NIR) (Chambers and Ridgway, 1996; Ridgway and Chambers, 1996, 1998; Ghaedian and Wehling, 1997; Dowell et al., 1998, 1999; Baker et al., 1999; Ridgway et al., 1999; Cheewapramong and Wehling, 2001; Ridgway et al., 2001). Pedersen (1992) and Brader et al. (2002) reviewed some of these screening methods for insect contamination in wheat.

NIR spectroscopy has the advantage of being a rapid and accurate method that can be adapted for non-destructive and automated detection. Previous studies show that live (e.g., Dowell et al., 1998; Ridgway and Chambers, 1999) and dead internal insects (e.g., Cheewapramong and Wehling, 2001) can be detected using NIR. The current study builds further on the potential of using NIR for detecting single kernels of wheat containing dead or desiccated insects. Considering that several NIR instruments capable of handling single kernels are already being used for measuring other quality attributes, adding the capability to detect kernels containing live and dead internal insects automatically and non-destructively will be highly beneficial. Also, no published research has validated whether calibrations developed using wheat kernels containing either live or dead internal insects can be used to detect kernels containing live and dead insects over a storage period. The objectives of this research were:

- To evaluate the potential of a commercially-available automated NIR system for detecting wheat kernels containing live and dead internal insects (rice weevil) at varying stages of growth in wheat stored over a two-month period, and

- To develop and validate calibrations using wheat samples containing either live or dead internal insects to predict the presence of both live and dead internal insects in single wheat kernels.

Methodology

Wheat Sample

A commercial hard red winter wheat sample was adjusted to 13.5% moisture content (wet basis) by addition of a pre-determined amount of water. After a 7-day equilibration period, adult weevils were allowed to oviposit into the wheat for 10 days, after which the weevils were removed by screening. After 21 days at 27°C and 55 to 60% relative humidity, a portion of randomly picked wheat kernels were x-rayed following the procedure outlined by Throne (1994) to obtain:

- (a) 200 uninfested or sound kernels - free of internal insects
- (b) 100 kernels containing pupae - pronounced or visible limbs, snout, and/or wings
- (c) 100 kernels containing large larvae - fourth larval instar stage
- (d) 100 kernels containing medium-sized larvae - second or third larval instar stage , and
- (e) 100 kernels containing small larvae - first larval instar stage.

All infested kernels chosen contained one internal insect per kernel.

After the spectra of wheat kernels were obtained (see below), 100 sound kernels were set aside while the remaining samples were treated with phosphine to kill the internal insects. Phosphine treatment involved placing each set of samples in separate labeled jars that were positioned in a dessicator together with 100 mg shavings of a commercially available aluminum phosphide, Phostoxin tablet (Degesch America, Inc., Weyers Cave, VA). Fumigation in the dessicator was done in a fume hood for 3 days. A 100% insect kill was achieved as evidenced by the non-emergence of insects from any of the phosphine-treated infested kernels during the two-month storage period. After fumigation, the samples were placed in individual compartments of labeled pillboxes and allowed to equilibrate under ambient room conditions (18 to 21°C and about 35 to 40% relative humidity) for 4 days prior to the next spectra collection. All samples were stored under these conditions between all tests.

Spectra Measurement

The Single Kernel Characterization System (SKCS) 4170 (Perten Instruments, Springfield, IL) was used to collect spectra of individual wheat kernels. The system and data collection described by Dowell et al. (1998) was used.

Six sets of spectral data were collected. The first set, referred to as the Day 1 sample, was obtained immediately after the samples were x-rayed and sorted. Day 1 samples include sound and internally infested kernels containing live rice weevils at various stages of growth. Spectral data were obtained on day 7, 14, 28, 42, and 56 samples, respectively. These samples include untreated sound, phosphine treated sound, and those containing rice weevils at various stages of growth that have been dead for 7, 14, 28, 42, or 56 days.

Data Analysis

Spectra were analyzed by partial least squares (PLS) regression using PLSPlus/IQ software (Galactic Industries, Salem, NH). Sound kernels were assigned a score of 1 and kernels with internal rice weevils were assigned a score of 2. The coefficient of determination (r^2), standard

errors of cross validation (SECV), and percentage of correct classification were used to evaluate the potential of the NIR technique. Within a spectral data set, cross-validation was used to determine classification accuracy. The percentage classification accuracy refers to the combined sound and infested kernels that were correctly classified. Dowell et al. (1998) showed that the visible or very near infrared regions do not contribute to the classification information for detecting internal insects in wheat. Thus, calibrations were developed in the 950 to 1690 nm wavelength range for (a) pupae, (b) large larvae, (c) medium-sized larvae, (d) small larvae, (e) pupae + large larvae + medium-sized larvae + small larvae, (f) pupae + large + medium-sized larvae, and (g) pupae + large larvae models. Based on statistical measures, a model was chosen for each spectral data set. Each of these calibrations was then validated against another spectral data set. For example, the Day 1 calibration was used to validate classification accuracy for Days 7, 14, 21, 28, 42, and 56; Day 56 calibration was used to validate Days 1, 7, 14, 21, 28, and 42 spectra.

Results and Discussion

Figures 1a and 1b show the absorbance ($\log 1/R$) of sound (uninfested) and infested wheat kernels (pupae, large larvae, medium-sized larvae, and small larvae) for Days 1 and 56. Spectra of wheat samples containing dead rice weevils at Days 7, 14, 28, and 42 (spectra not shown) had the same trends as those for Days 1 and 56. Day 1 samples contain live internal rice weevils while Day 56 samples contain dead internal rice weevils that were stored for 56 days after being killed with phosphine treatment. Absorbance was generally highest for sound wheat kernels and decreased at later growth stages. These results agreed with results of Dowell et al. (1998) and Ridgway et al. (1999) for live internal insects.

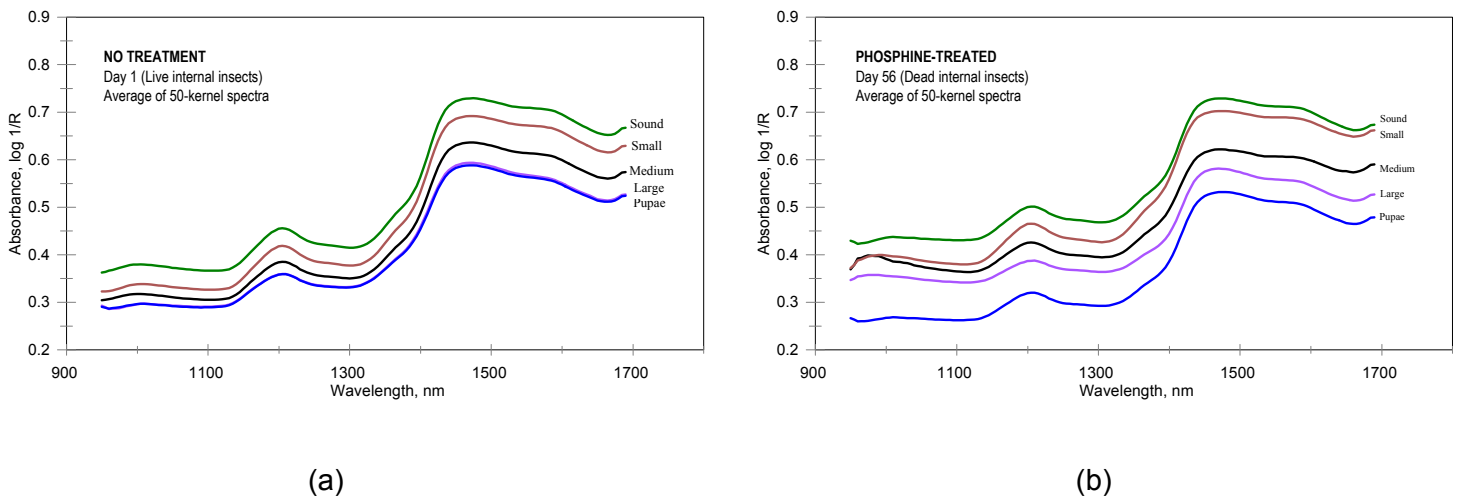


Figure 1. Comparison of reflectance spectra of wheat kernels that are sound or internally infested by (a) live or (b) dead internal rice weevils at various stages of growth. Day 56 spectra were used to represent kernels containing dead internal insect (similar trends were found for Days 7, 14, 21, 28, and 42). Each plot represents the average spectra of 50 single wheat kernels.

Figure 2 provides PLS beta coefficient output of three representative sampling times (Days 1, 28 and 56) at the optimum number of PLS factors. Important wavelength bands for detecting presence of internal insects in wheat kernels (across live or dead internal insects and across storage periods) indicated by the beta coefficients generally occurred around 990, 1135, 1210, 1325, 1370, 1395, 1425, 1610, and 1670 nm. The 990 nm starch band agrees with the findings of Ridgway et al. (1999) indicating that the ability to detect wheat kernels containing insects may be due to the loss of starch from the kernel that was replaced and/or consumed by the developing larvae. The wavelength region around 1425 nm (moisture band) was also identified by Ridgway and Chambers (1996) and is likely a response from insect moisture. The other regions correspond to C-H 1st and 2nd overtones and C-H combination bond vibrations (Shenk et al., 1992). Absorption in the C-H region may be attributed to the presence of rice weevil cuticular lipids, which Dowell et al. (1999) reported had peaks at 1130 and 1670 nm. The data provide evidence that the physical or biochemical differences detected by NIR for live insects are generally the same factors detected by NIR for dead insects over a two-month storage period. These results indicate that calibrations can be developed using wheat samples containing either live or dead internal insects regardless of whether or not the wheat sample has been stored over a two-month period. These findings will impact how calibration sample sets can be handled. Based on these findings, immediate sample processing may no longer be necessary; internal insects can be killed and calibrations can be created at a later time without sacrificing accuracy. Additionally, these same calibration samples can be shared across locations or laboratories resulting in savings in time and resources.

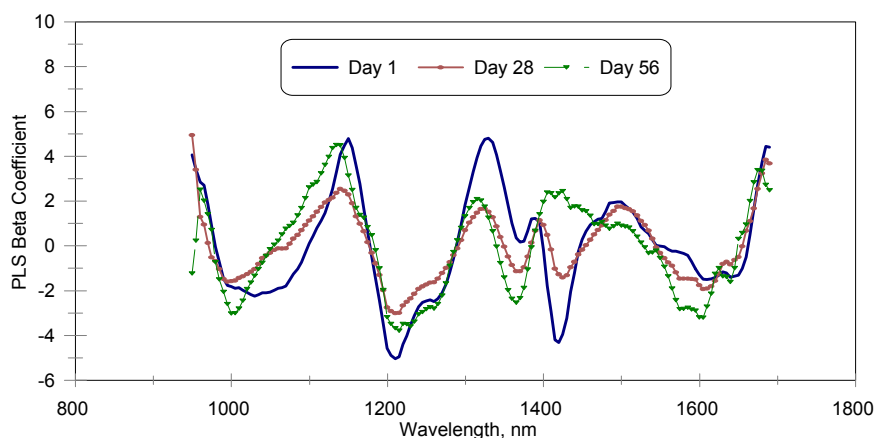


Figure 2. Partial least squares regression beta coefficients (Days 1, 28, and 56 samples) used for indicating important NIR wavelengths for detecting kernels containing internal rice weevils (Number of factors = 6).

Model Calibration

Table 1 provides a listing of calibration models developed and corresponding cross-validation results for differentiating sound versus infested kernels. Wheat samples containing pupae or large larvae were correctly classified against sound kernels with greater than 90% accuracy and the models had r^2 values ranging from 0.63 to 0.78. Samples containing medium larvae had r^2

Table 1. Summary of performance of calibration models developed for detecting wheat kernels that are sound or infested by rice weevil.

Calibration Model	Number of Factors	r^2	SECV	Correct Classification, %
Day 1 (Live)				
Pupae	7	0.73	0.21	94
Large larvae	7	0.74	0.25	95
Medium larvae	6	0.40	0.39	84
Small larvae	6	0.22	0.44	71
Pupae + Large larvae (PL1)	6	0.71	0.27	93
Pupae + Large + Medium larvae	6	0.44	0.35	87
Pupae + Large + Medium + Small larvae	6	0.22	0.38	78
Day 7 (Dead)				
Pupae	7	0.78	0.23	93
Large larvae	7	0.76	0.25	93
Medium larvae	6	0.52	0.35	88
Small larvae	5	0.02	0.50	68
Pupae + Large larvae (PL7)	7	0.71	0.25	95
Pupae + Large + Medium larvae	8	0.54	0.29	92
Pupae + Large + Medium + Small larvae	5	0.22	0.35	80
Day 14 (Dead)				
Pupae	6	0.78	0.23	95
Large larvae	6	0.73	0.26	93
Medium larvae	6	0.48	0.36	86
Small larvae	6	0.08	0.49	62
Pupae + Large larvae (PL14)	6	0.72	0.25	96
Pupae + Large + Medium larvae	6	0.51	0.30	92
Pupae + Large + Medium + Small larvae	6	0.25	0.35	81
Day 28 (Dead)				
Pupae	6	0.72	0.27	94
Large larvae	6	0.65	0.30	92
Medium larvae	6	0.50	0.36	86
Small larvae	6	0.08	0.48	62
Pupae + Large larvae (PL28)	6	0.65	0.28	93
Pupae + Large + Medium larvae	9	0.54	0.30	92
Pupae + Large + Medium + Small larvae	9	0.30	0.34	80
Day 42 (Dead)				
Pupae	5	0.76	0.24	95
Large larvae	6	0.63	0.30	90
Medium larvae	5	0.38	0.40	80
Small larvae	3	0.03	0.50	57
Pupae + Large larvae (PL42)	5	0.66	0.28	91
Pupae + Large + Medium larvae	5	0.43	0.33	89
Pupae + Large + Medium + Small larvae	3	0.21	0.36	80
Day 56 (Dead)				
Pupae	6	0.74	0.26	95
Large larvae	6	0.67	0.29	92
Medium larvae	6	0.43	0.38	82
Small larvae	2	0.04	0.49	56
Pupae + Large larvae (PL56)	6	0.67	0.28	91
Pupae + Large + Medium larvae	6	0.47	0.32	89
Pupae + Large + Medium + Small larvae	3	0.22	0.36	82

r^2 – coefficient of determination

SECV – standard error of cross validation

values ranging from 0.38 to 0.52 and correct classification (CC) ranging from 80 to 88%. Samples containing small larvae were not well differentiated from sound kernels; r^2 values ranged from only 0.02 to 0.22 and CC's from 56 to 71%.

Based on these parameters, the pupae + large larvae model was chosen. While the CC for the pupae + large larvae + medium-sized larvae ranged from 87 to 92%, the r^2 values only ranged from 0.43 to 0.54. Samples containing small larvae and medium larvae were not included in the final calibration models. The calibration models presented are thus limited by their ability to predict the presence of pupae and large larvae only. However, considering that a wheat lot would likely have many different stages of internal insects at a given time, the model's ability to detect pupae and large larvae will provide critical information for determining if the wheat is infested.

Model Validation

The pupae + large larvae model for each spectral data set was validated against the pupae and large larvae spectral data obtained from different storage times. Table 2 summarizes the percentage of CC for each validation model. When the calibration developed using live pupae + large larvae (Day 1) was used to detect dead pupae + large larvae (Days 7 to 56), CC's ranged from 86 to 96%. Calibrations that used dead pupae + large larvae (Days 7 to 56) were also able to detect the presence of live pupae + large larvae (Day 1) with CC's ranging from 92 to 93%. These results indicate that calibrations developed from wheat kernels containing live internal insects can be used to detect dead internal insects, and vice-versa.

Table 2. Correct classification of wheat samples validated using a calibration developed from pupae + large larvae.

Calibration Model	Percentage of Correct Classification for Validation Sample					
	Day 1 (Live)	Dead				
		Day 7	Day 14	Day 28	Day 42	Day 56
Day 1 (Live)	-	89.8	94.5	87.2	88.6	86.5
Day 7 (Dead)	93.3	-	95.5	89.1	90.0	88.3
Day 14 (Dead)	93.0	93.7	-	92.6	91.8	92.2
Day 28 (Dead)	91.7	93.0	92.1	-	91.2	90.0
Day 42 (Dead)	93.0	90.9	91.8	91.2	-	90.0
Day 56 (Dead)	91.7	86.3	93.2	89.1	92.9	-

Conclusion

An automated NIR system detected the kernels containing either live or dead rice weevils in single hard red winter wheat kernels during a two-month storage period. Correct classification of sound kernels and kernels containing live insects at pupal, large, medium-sized and small larval stages averaged 94, 92, 84, and 62%, respectively.

A calibration model was developed for pupae + large larvae (r^2 values ranging from 0.65 to 0.72 and CC of 91 to 96%) for each sample set containing live (Day 1) and dead (Days 7, 14, 21, 28, 42, and 56) rice weevils. The live pupae + large larvae (Day 1) calibration yielded an 86 to 96% correct classification for dead pupae + large larvae validation samples. Calibrations that used dead pupae + large larvae over a two-month storage period correctly detected the presence of live pupae + large larvae with an accuracy of 92 to 93%.

These findings will impact how calibration sample sets can be handled. Thus, immediate sample processing may no longer be necessary; internal insects can be killed and calibrations created at a later time without sacrificing accuracy. Additionally, laboratories can share these same calibration samples saving time and resources.

The data presented herewith included only phosphine-treated samples. Results from a parallel study done for low temperature (freezing) treatment for killing internal insects in wheat samples will be incorporated later. Further studies on how other methods of killing internal insects (e.g., high temperature treatment) will affect the detection capability of NIR will be beneficial. Additionally, based on the current findings on important wavelengths, calibration models using narrower wavelength range will be developed and evaluated.

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