# Detection of *Sitotroga cerealella* (Olivier) Infestation of Wheat Kernels Using Hyperspectral Reflectance[1]

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**Abstract** Hyperspectral reflectance data were used to detect internal infestations of Angoumois grain moth, *Sitotroga ceralella* (Olivier), in wheat kernels. Kernel reflectance was measured with a spectroradiometer over a wavelength range of 350-2500 nm. Kernel samples were selected randomly and scanned every 7 d after infestation to determine the ability of the hyperspectral reflectance data to discriminate between infested and uninfested kernels. Immature stages of *S. ceralella* inside wheat kernels can be detected through changes in moisture, starch, and chitin content of the kernel. By using the spectrally-derived moisture variable ($Log[1/R_{972nm}]$-$Log[1/R_{1032nm}]$) and starch variable ($Log[1/R_{982nm}]$-$Log[1/R_{1014nm}]$), it was possible to discriminate between infested and uninfested wheat kernels with 100% classification accuracy based on 90% confidence intervals. Significant differences in the spectral reflectance between the infested and uninfested kernels were due to changes in moisture and starch content in wheat kernels. Three of the four chitin variables showed slight discrimination between the infested and uninfested wheat kernels based on 90% confidence intervals with 63.9%, 68.8%, 66.7%, and 41.6% classification accuracy of the three variables ($Log[1/R_{1130nm}]$-$Log[1/R_{1670nm}]$), ($Log[1/R_{1139nm}]$-$Log[1/R_{1320nm}]$), ($Log[1/R_{1202nm}]$-$Log[1/R_{1300nm}]$), and ($Log[1/R_{2046nm}]$-$Log[1/R_{2302nm}]$), respectively. Spectral reflectance changes as a function of wheat kernel position relative to the spectroradiometer sensor did not differ significantly ($P > 0.10$).

**Key Words** *Sitotroga ceralella*, hyperspectral data, spectroradiometer, pest detection, wheat, internal infestation, stored products

The Angoumois grain moth, *Sitotroga cerealella* (Olivier) (Lepidoptera; Gelechiidae), is a major pest of stored grain in tropical and subtropical regions and infests sorghum, maize, wheat, barley, and millets. Each female lays about 200 eggs during a 5 to 10 d period. Larvae feed inside the kernel, forming a chamber just under the grain seed coat and constructing an exit hole before pupation. The life cycle takes about 5 wks (Dean 1937, Singh and Pandey 1975). Population densities of more than 1 insect per kg of kernels present a serious risk to the quality of the grain during storage and can cause rejection of the grain during trading (Johnson 1979). The U. S. Standards for grain inspection consider wheat to be infested if ≥2 live insects injurious to wheat are found in a 1kg sample (USDA 1991).

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The current method for screening wheat kernels for infestation involves manual sieving and visual examination of the grains. Wilkin (1982) recommended sieving 1 kg per 20 tons of grain, but this method has been shown to be ineffective for detecting infestations of <5 insects per kg (Wilkin and Fleurat-Lessard 1990). In addition, this method is subjective and is not applicable to species whose immature stages develop inside grain kernels. Near-infrared (NIR) spectroscopy could offer a reliable, accurate, and rapid nondestructive detection method for stored cereal (Osborne and Fearn 1986, Chambers et al. 1993, Ridgway and Chambers 1998). NIR spectroscopy can determine both physical and chemical properties of the grain and may increase the classification accuracy of detecting infested grains (Wang et al. 2002, Maghirang et al. 2003). Reflectance spectra have been used to detect kernel protein content (Delwiche and Hruschka 2000), internal insects (Baker et al. 1999, Ridgway and Chambers 1996), pecky rice (Wang et al. 2002), and aflatoxin and fusonisin in corn (Pearson et al. 2001, Dowell et al. 2002). Research has identified several absorption peaks in the near-infrared spectral range of 780-2500 nm that appear to be important in the discrimination of uninfested and infested wheat kernels. Absorption at 972 nm and 1032 nm has been identified with kernel moisture, and absorption at 982 nm and 1014 nm appear to be related to starch content of the kernel (Ridgway et al. 1999). It is thought that insect feeding will cause a loss of kernel starch and an increase in moisture. Kernel moisture content also can increase due to the presence of water in the larva and because of larval respiration (Pederson 1992, Ridgway and Chambers 1996). Spectral changes at 1139 nm and 1320 nm appear to be due to insect cuticular chitin present in the kernels (Chambers et al. 1993, Ridgway and Chambers 1996). Dowell et al. (1999) reported that spectral changes at wavelengths 1130 nm and 1670 nm could be due to the presence of rice weevil cuticular lipids. Larval size also appears to be an important factor. Dowell et al. (1998) was able to detect 3$^{rd}$ and 4$^{th}$ instars of three different stored grain insect pests in wheat kernels with 95% confidence, but could not reliably detect smaller larvae.

The objectives of this research were to characterize the reflectance properties of wheat kernels using the portable field spectroradiometer FieldSpec Pro FR (Analytical Spectral Devices, Inc. Boulder, USA) and determine the effectiveness of several established spectral reflectance variables in detecting internal infestation of *S. cerealella* at four developmental stages.

## Materials and Methods

**Spectroradiometer.** Wheat kernel reflectance was measured from 350 nm to 2500 nm at 1 nm interval using the portable field spectroradiometer FieldSpec Pro FR (Analytical Spectral Devices, Inc. Boulder, USA) attached via a fiber optic cable to the Li-Cor 1800 integrating sphere (Li-Cor 1800-12, Li-COR, Inc., Lincoln, NE, USA). An integrating sphere is designed to collect all of the radiation reflected from or transmitted through a surface. The 1800-12 is an external integrating sphere meaning that the sample is held to the outside of the sphere with a small section of the sample acting as part of the sphere wall. The interior of the sphere is coated with barium sulfate to create a uniform diffuse reflector. An internal integrating sphere would have the sample entirely inside the sphere, generally near the center. This difference in type of integrating sphere may have an effect on the ability to detect internal pest infestation by spectral reflectance. A measurement of reflectance involves comparing the wall illumination caused by a focused beam of radiation reflected from the sample

material to that reflected from the white reference material. The two measurements are taken sequentially. The 1800-12 uses the same illumination for both sample and reference measurement by moving it from one port to the other between measurements. The integrating sphere was initialized following procedures given in the "RS3 Quick Reference for FieldSpec Pro". Each single kernel sample was scanned individually, taking 50 spectra for each single kernel to reduce spectral and detector noise. The collected hyperspectral data were stored on a hard disk for subsequent viewing and analysis with software provided with the spectroradiometer (ASD ViewSpecPro®, version 4.2). The measured reflectance spectra were used to calculate specific spectral variables that relate to the moisture, starch and chitin characteristics of the kernel. Table 1 lists the variables determined for each uninfested and infested kernel.

**Wheat sample.** To obtain wheat kernels with different-sized larvae of the *S. cerealella*, 250 g of a commercial hard red winter wheat were placed in a clear 3.1-L glass jar inoculated with 1 g of *S. cerealella* eggs and sealed with screen lids. Another 250 g of wheat kernels were placed in another 3.1-L glass jar but not inoculated, and used as an untreated control. The jars were replicated four times and held in an environmental chamber at 25°C, 60% RH, and 14-10 h light-dark photoperiod. Twelve infested kernels were selected randomly from the four infested jars (three kernels selected randomly from each infested jar) 7 d after inoculation and again every 7 d thereafter for 4 wks to be used for spectral measurements and data analysis. Another set of 12 uninfested kernels was selected randomly from the uninfested jars for the same purpose. Prior to scanning with the spectroradiometer, each selected kernel was cleaned gently using dry tissue paper and placed individually into a small plastic cup. Each cup was labeled with a number from 1-12 for the infested kernel sample. Another set of 12 cups was used for the sound kernels. Reflectance spectra were measured on individual kernels using the integrating sphere. Each kernel was scanned twice to determine if kernel position was important. The first scan was with the kernel's crease up toward the sensor, and the second position was with the kernel's crease down toward the sensor.

After the kernels were scanned, they were inspected under a binocular microscope to confirm the infestation. Each labeled kernel was inspected individually by cracking the kernel and removing the immature stage from the infested kernel. The removed

**Table 1. Variables evaluated for usefulness in discriminating between uninfested and infested wheat kernels**

| Variable | Expression | Reference |
|---|---|---|
| Moisture | $(\text{Log}[1/R_{972nm}] - \text{Log}[1/R_{1032nm}])$ | (Ridgway et al. 1999) |
| Starch-1 | $(\text{Log}[1/R_{982nm}] - \text{Log}[1/R_{1014nm}])$ | (Ridgway et al. 1999) |
| Starch-2 | $(\text{Log}[1/R_{990nm}])$ | (Osborne and Fearn 1986) |
| Chitin-1 | $(\text{Log}[1/R_{1130nm}] - \text{Log}[1/R_{1670nm}])$ | (Dowell et al. 1999) |
| Chitin-2 | $(\text{Log}[1/R_{1139nm}] - \text{Log}[1/R_{1320nm}])$ | (Chambers et al. 1993) |
| Chitin-3 | $(\text{Log}[1/R_{1202nm}] - \text{Log}[1/R_{1300nm}])$ | (Ridgway and Chambers 1996) |
| Chitin-4 | $(\text{Log}[1/R_{2046nm}] - \text{Log}[1/R_{2302nm}])$ | (Ridgway and Chambers 1996) |

larva or pupa was measured with a micrometric lens attached to the binocular microscope. Average larval size was 2.2, 3.8, 8.2, and 7.7 mm at 7, 14, 21, and 28 d after inoculations, respectively.

The data were analyzed using SAS (SAS Institute 1999). Paired *t*-tests were used to determine the possible effect of the kernel position. Analysis of variance was used to determine the ability to detect infestation. Prediction intervals were used to illustrate differences between infested and uninfested readings.

## Results and Discussion

The effects of kernel infestation and position on moisture, starch and chitin content of wheat kernels as determined by spectral reflectance is summarised in Table 2. Analysis of the data show that the moisture variable is the best parameter for detecting infestation and that kernel position is generally not a factor for indices that can effectively discriminate between infested and uninfested kernels. The interaction between infestation and position was not significant ($P > 0.10$).

**Moisture characteristics.** The moisture index based on the spectral reflectance at 972 nm and 1032 nm decreased as larvae developed (Table 3). There were significant differences between infested and uninfested kernels ($P < 0.0001$) confirming previous studies proposing that kernel moisture content increases are due to the presence of water in the larva and larval respiration. The classification accuracy in detecting hidden grain moth larval infestation using the moisture variable was 100% (12/12 kernels) based on a 90% confidence interval. Kernel position was not a significant factor.

**Starch variables.** The starch variable-1, based on the spectral reflectance at 982 nm and 1014 nm, decreased as larval development increased (Table 4). The differences between infested and uninfested kernels were highly significant ($P < 0.0001$). The classification accuracy in detecting hidden grain moth larval infestation using starch variable-1 was 100% (12/12 kernels) based on a 90% confidence interval. Kernel position was not a significant factor. The starch variable-2, which is based only on the spectral reflectance at wavelength 990 nm, did not significantly change as larvae developed. The differences between the indices for infested and uninfested

**Table 2. Analysis of variance summary for all data combined**

| Variable | Infestation | Kernel position | Infestation*position |
|---|---|---|---|
| Moisture | <0.0001 | NS | NS |
| Starch-1 | <0.0001 | NS | NS |
| Starch-2 | 0.0035 | NS | NS |
| Chitin-1 | NS | 0.0239 | NS |
| Chitin-2 | 0.0607 | 0.0163 | NS |
| Chitin-3 | 0.0259 | 0.0285 | NS |
| Chitin-4 | NS | NS | NS |

NS = Not significant at $P > 0.10$.

**Table 3. Discrimination between sound and *S. cerealella* infested wheat kernels using the moisture variable**

| Variable | Days after infestation | Mean moisture variable value ± std. dev | | *P* value infestation | *P* value kernel position | Classification accuracy (%) |
|---|---|---|---|---|---|---|
| | | Infested | Uninfested | | | |
| Moisture | 7 | 0.0101 ± 0.0021a | 0.0179 ± 0.0021a | <0.0001 | NS | 100 |
| | 14 | 0.0078 ± 0.0015b | 0.0187 ± 0.0045a | <0.0001 | NS | 100 |
| | 21 | 0.0051 ± 0.0032c | 0.0150 ± 0.0019b | <0.0001 | NS | 100 |
| | 28 | 0.0052 ± 0.0025c | 0.0128 ± 0.0014c | <0.0001 | NS | 100 |

NS = Not significant at $P > 0.05$.
Means in the same column followed by the same letter do not differ at $P = 0.05$ (LSD, SAS Institute 2003).

**Table 4. Discrimination between sound and *S. cerealella* infested wheat kernels using starch variables**

| Variable | Days after infestation | Mean variable value ± std. dev | | *P* value infestation | *P* value kernel position | Classification accuracy (%) |
|---|---|---|---|---|---|---|
| | | Infested | Uninfested | | | |
| Starch-1 | 7 | 0.0111 ± 0.0023a | 0.0194 ± 0.0022a | <0.0001 | NS | 100 |
| | 14 | 0.0087 ± 0.0017b | 0.0201 ± 0.0048a | <0.0001 | S | 100 |
| | 21 | 0.0070 ± 0.0028c | 0.0165 ± 0.0020b | <0.0001 | NS | 100 |
| | 28 | 0.0066 ± 0.0013c | 0.0136 ± 0.0013c | <0.0001 | NS | 100 |
| Starch-2 | 7 | 0.1815 ± 0.0076a | 0.1877 ± 0.0077a | 0.0077 | NS | 66.7 |
| | 14 | 0.1734 ± 0.0060b | 0.1705 ± 0.0071b | NS | NS | 33.3 |
| | 21 | 0.1724 ± 0.0076bc | 0.1779 ± 0.0059c | 0.0080 | NS | 75.0 |
| | 28 | 0.1687 ± 0.0056c | 0.1747 ± 0.0056c | 0.0008 | NS | 91.7 |

NS = Not significant at $P > 0.05$.
S = Significant at $P \leq 0.05$.
Means in the same column and variable followed by the same letter do not differ at $P = 0.05$ (LSD, SAS Institute 2003).

**Table 5. Discrimination between sound and infested *S. cerealella* wheat kernels using chitin variables**

| Variable | Days after infestation | Mean variable value ± std. dev | | *P* value infestation | *P* value kernel position | Classification accuracy (%) |
|---|---|---|---|---|---|---|
| | | Infested | Uninfested | | | |
| Chitin-1 | 7 | 0.0899 ± 0.0228ab | 0.0764 ± 0.0225a | 0.0362 | S | 66.7 |
| | 14 | 0.1006 ± 0.0166b | 0.0911 ± 0.0202b | 0.0790 | NS | 66.7 |
| | 21 | 0.0856 ± 0.0200a | 0.0930 ± 0.0258b | NS | NS | 58.3 |
| | 28 | 0.0974 ± 0.0135b | 0.1077 ± 0.0146c | 0.0074 | S | 91.7 |
| Chitin-2 | 7 | 0.0142 ± 0.0045ab | 0.0113 ± 0.0040a | 0.0201 | NS | 58.3 |
| | 14 | 0.0162 ± 0.0029a | 0.0133 ± 0.0038a | 0.0058 | NS | 66.7 |
| | 21 | 0.0128 ± 0.003b | 0.0134 ± 0.0042a | NS | NS | 58.3 |
| | 28 | 0.0148 ± 0.0030ab | 0.0158 ± 0.0025b | NS | S | 91.7 |
| Chitin-3 | 7 | 0.0168 ± 0.0036a | 0.0156 ± 0.0035a | NS | S | 50.7 |
| | 14 | 0.0185 ± 0.0031a | 0.0172 ± 0.0035ab | NS | NS | 66.7 |
| | 21 | 0.0147 ± 0.0038b | 0.0184 ± 0.0044bc | 0.0030 | NS | 75.0 |
| | 28 | 0.0168 ± 0.0028a | 0.0203 ± 0.0025c | <0.0001 | S | 75.0 |
| Chitin-4 | 7 | 0.0671 ± 0.0101a | 0.0688 ± 0.0141a | NS | NS | 33.0 |
| | 14 | 0.0805 ± 0.0150b | 0.0818 ± 0.0101a | NS | S | 50.0 |
| | 21 | 0.0621 ± 0.0134a | 0.0668 ± 0.0116a | NS | NS | 66.7 |
| | 28 | 0.0774 ± 0.0222b | 0.0804 ± 0.0294a | NS | NS | 16.7 |

NS = Not significant at $P > 0.05$.
S = Significant at $P \leq 0.05$.
Means in the same column and variable followed by the same letter do not differ at $P = 0.05$ (LSD, SAS Institute 2003).

**Table 6. Usefulness of infestation, position, and their interaction in predicting the variable reading**

| Variable | Day | Infestation* | Position | Interaction |
|---|---|---|---|---|
| Moisture | 7 | Yes | — | — |
| | 14 | Yes | — | — |
| | 21 | Yes | — | — |
| | 28 | Yes | — | — |
| Starch-1 | 7 | Yes | — | — |
| | 14 | Yes | — | — |
| | 21 | Yes | — | — |
| | 28 | Yes | — | — |
| Starch-2 | 7 | Yes | — | — |
| | 14 | — | — | — |
| | 21 | Yes | — | — |
| | 28 | Yes | — | — |
| Chitin-1 | 7 | Yes | Yes | Yes |
| | 14 | Yes | — | — |
| | 21 | — | — | — |
| | 28 | Yes | Yes | — |
| Chitin-2 | 7 | Yes | — | — |
| | 14 | Yes | — | — |
| | 21 | — | — | — |
| | 28 | — | Yes | — |
| Chitin-3 | 7 | — | Yes | — |
| | 14 | — | — | — |
| | 21 | Yes | — | — |
| | 28 | Yes | Yes | — |
| Chitin-4 | 7 | — | — | — |
| | 14 | — | Yes | — |
| | 21 | — | — | — |
| | 28 | — | — | — |

* "Yes" means the variable was useful, "—" means it was not useful. This is based on the $P > 0.10$.

kernels were less significant ($P = 0.0035$), and the classification accuracy ranged from 33-92% depending on larval development stage.

**Insect chitin variables.** Spectral reflectance in the wavelength range from 1130-2302 nm has been used to determine four variables related to chitin (Table 1). The

chitin variables did not change significantly as the larvae developed. The classification accuracy for discrimination between uninfested and infested kernels ranged from 17-92% depending on larval stage and chitin variable used. The best classification accuracy was achieved with the larger larvae. Chitin variable-4, which is based on spectral reflectance in the 2000-2302 nm range, was less able to detect infested kernels than the variables based on reflectance in the 1100-1670 nm wavelength range. This low classification accuracy may be due to the increased spectral noise above 2200 nm. The kernel position during reflectance measurement appeared to be important in most situations (Table 5).

This study shows that hyperspectral reflectance data can be used to detect internal infestation of *S. cerealella* in wheat kernels before they can be visually detected. The "usefulness" of the variables and the effect of position or interaction is shown in Table 6. The moisture variable ($\text{Log}[1/R_{972nm}]$-$\text{Log}[1/R_{1032nm}]$) and the starch variable ($\text{Log}[1/R_{982nm}]$-$\text{Log}[1/R_{1014nm}]$) were the most useful variables for detecting internal infestation in wheat kernels with 100% classification accuracy based on 90% confidence intervals compared with chitin variables which showed smaller classification accuracy. The presence of *S. cerealella* inside the wheat kernels increases the kernel's moisture content whereas a decrease in starch content is most likely due to insect feeding. As changes in moisture, water content and starch occur, significant changes in spectral reflectance also occur. The magnitude of spectral changes increased as the larvae developed and increased in size. Using hyperspectral reflectance data appears to be inexpensive, accurate, rapid, and useful method for detecting internal infestation in wheat kernels.

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