
Insect species and infestation level determination in stored wheat using near-infrared spectroscopy

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Paliwal, J., Wang, W., Symons, S.J. and Karunakaran, C. 2004. **Insect species and infestation level determination in stored wheat using near-infrared spectroscopy**. Canadian Biosystems Engineering/Le génie des biosystèmes au Canada **46**: 7.17 - 7.24. Near-infrared spectroscopy (NIRS) technique was evaluated for detection of four different life stages (i.e. eggs, larvae, pupae, and adults) of *Sitophilus oryzae* (rice weevil) in artificially infested bulk samples of Canada Western Red Spring (CWRS) wheat at infestation levels from 0 through 50% at 5% infestation intervals. Sound wheat kernels were also infested with *Rhyzopertha dominica* (lesser grain borer) to create infestation levels of 0, 5, 10, 20, 25, 50, 75, and 100% and the spectral data from their pupae were compared to the pupal stage of rice weevil at corresponding levels of infestation. To distinguish wheat kernels infested with pupae of lesser grain borer from those infested with rice weevil, Principal Component Analysis (PCA) was employed and insect differentiation was done by examining score plots of spectral data. Original and difference spectra of infested wheat kernels and sound wheat kernels were tested. It was observed that differentiation of insect species becomes easier as infestation levels increase for difference spectra. Calibration models for quantitative determination of infestation levels were developed using Partial Least Square Regression (PLSR). Multiplicative Scatter Correction (MSC) and Standard Normal Variant (SNV) followed by detrending were found equally effective in removing irrelevant spectral information. Prediction performed well for high infestation levels but lower classification accuracies were obtained at low infestation levels. **Keywords:** near-infrared spectroscopy, wheat, *Sitophilus oryzae*, *Rhyzopertha dominica*, infestation, Principal Component Analysis, Partial Least Squares Regression, Multiplicative Scatter Correction, Standard Normal Variate and detrending.

La technique de spectroscopie à infrarouge rapproché a été évaluée pour la détection du *Sitophilus oryzae* (charançon du riz) à quatre différents stades de développement (oeuf, larve, chrysalide et adulte) dans des échantillons de blé Canada Western Red Spring (CWRS) en vrac artificiellement infestés à des taux d'infestation variant entre 0 et 50% par intervalles de 5%. Des grains de blé sains ont aussi été infestés avec des *Rhyzopertha dominica* (coléoptère bostrychidé) pour créer des infestations à des niveaux de 0, 5, 10, 20, 25, 50, 75 et 100% et les données spectrales des chrysalides de ces insectes ont été comparées au stade de chrysalide du charançon du riz à des niveaux d'infestation correspondants. Pour distinguer les grains de blé infestés par les chrysalides du charançon du riz de ceux infestés par le coléoptère bostrychidé, l'analyse des principales composantes (PCA) a été employée et la différenciation des insectes a été faite en examinant les courbes de taux des données spectrales. Les spectres originaux et différentiels des grains de blé infestés et des grains de blé sains ont été testés. Il a été observé que la différenciation des espèces d'insectes a été facilitée par une augmentation des niveaux

d'infestation pour différents spectres. Des modèles de calibration pour la détermination quantitative des niveaux d'infestation ont été développés en utilisant la régression des moindres carrés partiels (PLSR). La corrélation des écarts multiplicatifs (MSC) et la variante normale standard (SNV) suivies par la désorganisation se sont également avérées efficaces en enlevant les informations spectrales non pertinentes. Les prédictions ont été satisfaisantes pour les hauts niveaux d'infestation mais de faibles précisions de classification ont été obtenues à des taux bas d'infestation. **Mots clés:** spectroscopie à infrarouge rapproché, blé, *Sitophilus oryzae*, *Rhyzopertha dominica*, infestation, analyse des composantes principales, régression des moindres carrés partiels, corrélation des écarts multiplicatifs, variante normale standard et désorganisation

INTRODUCTION

Canada has a zero tolerance for insects in grain (Canada Grains Act 1975). About 10 to 30% of produced grains are lost every year worldwide during storage due to insect damage. In Canada, the storage losses in grain account for over a hundred million dollars each year (White 1995). Detection of insect pests is all the more difficult for species that develop inside of the kernels. At times, these species cannot be detected in grain until infestation reaches significant levels. The existing Berlese funnel method to detect these internal feeders is very time consuming and poses a practical problem at the elevators where grain movement cannot be stalled. Two of the most destructive pests of stored grain whose immature stages develop inside kernels are *Sitophilus oryzae* L. (rice weevil) and *Rhyzopertha dominica* F. (lesser grain borer). In the absence of a fast and reliable method to detect these internal feeders, infested grain, at times, is binned with uninfested lots causing cross-contamination. In other instances, very light infestations are heavily fumigated causing excessive chemical exposure to food grain.

Sitophilus oryzae is a primary pest of stored cereals such as rice, wheat, barley, and corn. Voracious feeding of whole grains by this insect causes weight loss, fungal growth, and quality loss which results in increased free fatty acid levels (Sinha and Watters 1985). The adult female bores a hole in a kernel of grain, lays an egg, and then seals the hole with a gelatinous material. The larvae feed and develop inside the kernel until they reach the adult stage. These internal feeders emerge by boring a hole in the kernel and cause further damage to grain kernels. The life cycle from egg laying to adult emergence at

70% relative humidity (RH) requires 26-28 days at 30°C. At low levels of *S. oryzae* infestation, visual inspection may produce the result that kernels are sound and uninfested (Arthur and Throne 2003).

Rhizopertha dominica is a very common stored product pest that feeds either on the germ or the endosperm. The adult female lays eggs on the external surface or crevices of kernels. The larvae bore holes into the kernels, feed, and pupate inside the grain kernels until the adult emerges (Hagstrum and Flinn 1994). The mean development time of *R. dominica* is 45 days at 27°C and 70% RH.

To detect internal infestations by species such as *S. oryzae* and *R. dominica* at an early stage, a fast and reliable tool is needed by the grain industry. Research has shown that the near-infrared spectroscopy (NIRS) technique has the potential to detect low levels of insect infestation in stored grain. Near-infrared spectroscopy has already established itself as a fast, reliable, accurate, and economical technique for compositional analysis and authentication of grains (Kim et al. 2003). The principle behind NIRS is that unique chemical composition causes molecules to absorb light in the near-infrared (NIR) region and vibrate at unique frequencies (Murray and Williams 1990). When using NIRS for compositional analysis, reflected or transmitted light is collected by a spectrometer that measures energy absorption by the sample. Near-infrared spectra carrying these amounts of absorption at multiple wavelengths can be related to the concentration of a particular constituent of the sample. Because the chemical composition of each insect species is unique (Lockey 1988) and different from that of grain kernels, spectral data can be used to differentiate between insect species (Dowell et al. 1999) and distinguish healthy kernels from infested ones (Baker et al. 1999; Dowell et al. 1998; Maghirang et al. 2003; Ridgway et al. 2001).

Most of the aforementioned research has concentrated on distinguishing sound kernels from infested ones, one kernel at a time. This method is inherently slow and has little practical applicability at grain handling facilities where large samples of grain are to be analyzed in a short time. There is a need to evaluate this technique for bulk sample analysis. Because it is highly unlikely that a sample of grain will have all the kernels infested, it is important to investigate what are the lowest levels of infestation that can be detected in a sample using NIRS. Also, much of the published research on either classification of sound and infested kernels or differentiation of insect species has invariably used Partial Least Squares Regression (PLSR) to identify insects by assigning integer values as reference data to the comparison of interest. The authors of the current research propose to use Principal Component Analysis (PCA) to spectrally distinguish the two infesting insect species by the clustering patterns in the score plots of the first few principal components (PCs).

The objectives of this study were:

1. to evaluate if NIR spectra can be used to distinguish between the pupal stages of *S. oryzae* and *R. dominica* at various infestation levels inside wheat kernels;
2. to evaluate the feasibility of NIRS in quantitatively determining the infestation levels of *S. oryzae* and *R. dominica* in Canada Western Red Spring (CWRS) wheat; and
3. to analyze the spectral data for wavelengths indicating specific constituents.

MATERIAL and METHODS

Kernel infestation

Adult *R. dominica* were reared in wheat flour and kept for 72 h at 30°C and 70% RH. Larvae were then collected from the flour by sieving out the adults and the larvae were used to infest CWRS wheat kernels. Each kernel was manually infested with a larva of *R. dominica* by puncturing a hole in the kernel and then placing a larva inside. These infested kernels were held for 3 weeks (until the larvae matured to become pupae) at 30°C and 70% RH in an incubator. Infested kernels were then mixed with sound (uninfested) kernels to create 0, 5, 10, 20, 25, 50, 75, and 100% levels of infestation.

When the same procedure of artificial implantation was used to infest wheat kernels with *S. oryzae* eggs, less than 10% of the eggs survived. Hence, *S. oryzae* adults were allowed to infest whole wheat kernels for three days at 30°C (70% RH) and the infested kernels were identified by the presence of egg plugs on the kernels under a microscope. These kernels were X-rayed using a Lixi fluoroscope (Model LX-85708, Lixi Inc., Downers Grove, IL) to make sure that the eggs were developing inside them. These egg-infested kernels were mixed with clean grain to obtain infestation levels of 0 (clean grain) through 50% at 5% intervals. Once the infested kernels were mixed with clean grain to obtain the required levels of infestation, it was impossible to salvage them from the mixture. So, the whole procedure of infesting wheat, analyzing kernels under microscope and X-ray fluoroscope, and mixing them with clean grain to create different infestation levels was repeated at 11, 21, and 26 days of development. These periods corresponded to the 2nd instar larvae (hereinafter referred to as larvae), pupae, and emerging adult stages, respectively, of the insect's life cycle. Because there was a possibility of the insect crawling out of the kernel at the emerging adult stage, the kernels were instantly frozen as soon as the adult was detected inside them under the microscope. These kernels were frozen for at least 48 h to make sure that the adults were dead and thawed at room temperature for at least 36 h before collecting spectral data.

Spectra measurement

A NIR spectrophotometer (Foss NIRSystems 6500, Silver Spring, MD) was used to collect reflectance spectra of the infested and uninfested bulk wheat kernels. The spectrometer was capable of scanning a wide wavelength range from 400 to 2500 nm under diffuse reflectance or diffuse transmittance mode. System spectral resolution was 2 nm with a wavelength accuracy of ± 0.5 nm. The instrument was equipped with a rectangular sample transport cell (175 mm long, 42 mm wide, and 17 mm deep). When preparing samples for measurement, the sample cell was filled to one-fourth of its height with about 35 g of grain. The samples were scanned at the rate of 32 spectra per second in reflectance mode. Each sample was loaded and analyzed 10 times, and 5 spectra replicates were saved as apparent absorbance (A) given by:

$$A = \log(1 / R) \quad (1)$$

where: R = reflectance from the wheat samples.

Data analysis

Two multivariate data analysis techniques were employed to explore the spectral data (Brereton 2003). To differentiate wheat

Table 1. Eigenvalues for covariance matrices of original spectra data and corresponding percentage of variance explained in principal component decomposition.

Infest. level	5%		10%		20%		25%		50%	
	PC No.	Eigenv.	Var. (%)	Eigenv.	Var. (%)	Eigenv.	Var. (%)	Eigenv.	Var. (%)	Eigenv.
1	0.979	72.83	1.370	81.06	3.311	95.62	2.685	88.39	5.169	92.96
2	0.326	24.23	0.288	17.05	0.103	2.98	0.307	10.12	0.359	6.46
3	0.027	1.98	0.018	1.07	0.029	0.84	0.027	0.90	0.021	0.38
4	0.006	0.43	0.007	0.40	0.010	0.30	0.011	0.37	0.008	0.14
5	0.004	0.27	0.005	0.28	0.007	0.21	0.004	0.12	0.003	0.05
6	0.002	0.18	0.002	0.14	0.002	0.05	0.003	0.10	0.001	0.01

Eigenv. - eigenvalues for corresponding Principal Component (PC)

Var. (%) - percentage of total variance in original spectral data explained by each Principal Component

kernels infested with pupae of *R. dominica* and pupae of *S. oryzae*, PCA was performed on the spectral data of wheat samples at the same infestation levels. The reason to use PCA was that it condensed the original data into the first few PCs that explain most of the variation present in the original data set. Each sample obtained scores when projected onto the first few PCs. These sample scores were plotted against one another and reveal grouping or clustering patterns within a data set. Detailed description of PCA and classification can be found in Kemsley (1998). For identification of infested wheat kernels based on infestation levels, PLSR was employed to quantitatively analyze spectral data for different infestation levels. The PLSR decomposition is a regression technique similar to Principal Component Regression (PCR). When iterating through the PLSR algorithm, concentration block and spectral data block are decomposed simultaneously and have their scores exchanged in calculation, and this exchange of scores provides a better inner relation between two data blocks (Geladi and Kowalski 1986). The calibrated model was validated by leave-one-out full-cross-validation. All data were mean centered before analysis. For identification of infestation levels, all spectra were preprocessed with Multiplicative Scatter Correction (MSC) (Geladi et al. 1985) or Standard Normal Variant (SNV) followed by detrending (Barnes et al. 1989).

Analysis using PCA and PLSR regression was performed using GRAMS/AI version 7 (Thermo Galactic, Woburn, MA). All analysis of the spectral data set was done by the add-on software package PLS Plus/IQ (Thermo Galactic, Woburn, MA). For PCA analysis, score and loading values for the first few PCs are calculated by PLS Plus/IQ, exported, and plotted in Excel. For prediction of infestation levels, PLS Plus/IQ provides several parameters to define the performance of calibration models. Coefficient of determination (r^2), Standard Error of Cross Validation (SECV), beta coefficient, optimum factor number, Predicted Residual Error Sum of Squares (PRESS), and concentration residuals are used to evaluate prediction performance of the calibrated model. The optimum number of independent variables in building a calibration equation was determined using PRESS and SECV. Model fit to the data was determined based on the r^2 values and concentration residual was measured as the difference between concentration value predicted by the model and reference concentration. Concentration residual describes the accuracy of the calibrated

model in predicting infestation levels in our study. Positive and negative peaks in the beta coefficient plot correspond to the significant wavelength variables in the calibration models.

RESULTS and DISCUSSION

Differentiation of infesting insect species

A comparison was made between spectral data of wheat kernels infested with *R. dominica* and wheat samples infested with *S. oryzae* at the pupal stage. An exploratory study using PCA on the raw spectral data set was first conducted. All original spectra of wheat kernels infested with the two different species were preprocessed by MSC before performing PCA. Because the CWRS wheat samples came from the same growing region and harvest year, the spectra of sound kernels for two batches of samples were assumed to be identical. Examination of the first two PC scores of original data set showed that there existed an obvious difference between spectra of sound wheat kernels. All subsequent PCA score plots for different infestation levels indicate the same clustering pattern of spectral data. The first component accounts for more than 95% of the variation for most infestation levels. Wheat kernels infested with *R. dominica* could easily be distinguished from those infested with *S. oryzae* by the first PC scores for all infestation levels. Since the two batches of spectral data were acquired on different dates, a possible explanation is that changes in measurement conditions (instrumental and environmental factors) and wheat samples occurred during that period. This variation is dominant and could have complicated spectral interpretation. To circumvent this, we used spectral subtraction between the spectra of wheat at different infestation levels and spectra of sound wheat kernels with a subtraction factor of 1 (Friese and Banerjee 1992). The spectra of sound wheat kernels used in subtraction were taken to be the average of five replicates of original spectra of sound wheat kernels. It was assumed that after spectra subtraction these difference spectra only carry information on insect species and infestation levels. Difference spectra of wheat samples infested with different insects at the same infestation levels were then compared. Grouping of samples in the score plots illustrated that spectra infested with different insects tend to separate from each other more obviously as the infestation levels increase (Fig. 1). Eigenvalues of the covariance matrix of the original spectra and percentage of variance explained by the corresponding eigenvectors are listed in Table 1. As can be

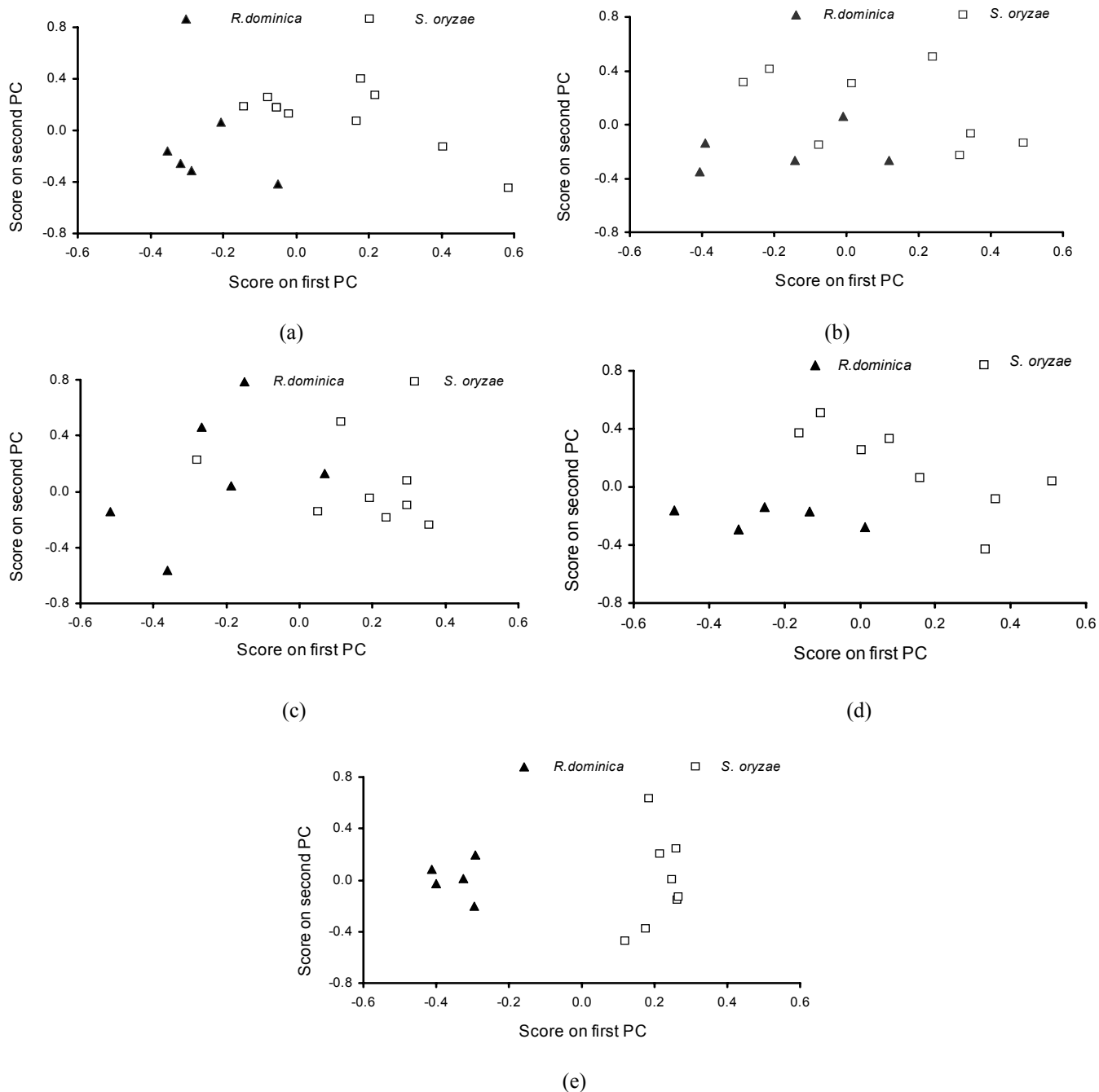


Fig. 1. Score plots on first and second PCs obtained by PCA analysis of difference spectral data at (a) 5%, (b) 10%, (c) 20%, (d) 25%, and (e) 50% infestation level.

seen, the first PC only accounts for about 85% of the total and more PCs were needed. At low infestation levels (5 and 10%), the spectra of wheat kernels infested with *R. dominica* and *S. oryzae* are less distinct from each other. This is true since instrumental variations (e.g. mechanical vibration and electronic noise) and sample difference (e.g. origin of growth and environmental factors) were only partially removed by spectra subtraction. After spectra subtraction, the two groups of spectra have more even score distributions along the first and second PC. At 20% infestation level, two groups of spectra for different

insects in the first two score space tend to separate with some overlapping at the decision boundary. At 25% infestation level, the first two PC scores could easily differentiate wheat kernels infested with *R. dominica* from those infested with *S. oryzae*. Checking the plot of PC scores at the 50% infestation level (Fig. 1), we find that the first PC is sufficient to discriminate these two kinds of infested kernels. The difference spectra, although it tends to include subjective factors due to spectral subtraction, intuitively illustrate how infestation levels might affect insect differentiation.

Table 2. Regression statistics for calibration models of spectra preprocessed by Multiplicative Scatter Correction (MSC).

Infesting insects	Optimum factor number	PRESS	SECV	r ²
<i>R. dominica</i> (pupae)	1	0.0064	0.028	0.99
<i>S. oryzae</i> (larvae)	2	0.0133	0.035	0.92
<i>S. oryzae</i> (pupae)	1	0.0068	0.025	0.98
<i>S. oryzae</i> (adult)	1	0.0079	0.027	0.97

PRESS - predicted residual error sum of squares
 SECV - standard error of cross validation
 r² - coefficient of determination

Predicting infestation levels within insect life stages

Calibration models were set up for wheat infested with pupae of *R. dominica* at eight infestation levels (0, 10, 20, 25, 50, 75, and 100%) using PLSR. Regression analysis was also performed on spectra of wheat infested with *S. oryzae* at four different life stages (egg, larva, pupa, and adult). For *S. oryzae* eleven infestation levels (0, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50%) were used.

Rather than assigning logic or integer values to spectra of sound and infested wheat samples for classification, infestation levels were employed as reference data in our regression analysis. For each infestation level, ten spectra replicates were averaged to achieve better Signal-to-Noise-Ratio (SNR) and regressed against reference data. Two preprocessing techniques namely, MSC and SNV followed by detrending of the averaged spectra were evaluated for their performance in the training sets. Two sets of calibration models on spectra treated with aforementioned two preprocessing methods were built and compared for their prediction performance. Full spectra calibration was utilized for all calibration models. No wavelength selection was done in our study and we tried to extract important wavelength variables within the NIR spectral range in the beta coefficient plots. Outlier detection was performed in the score plots and no outlier was found as a result of strict sampling procedures and stable instrumental performance.

Model statistics are summarized in Tables 2 and 3. Regression equations for eight calibrated models are plotted in Figs. 2 and 3. The regression statistics revealed that both preprocessing methods rendered equivalent prediction

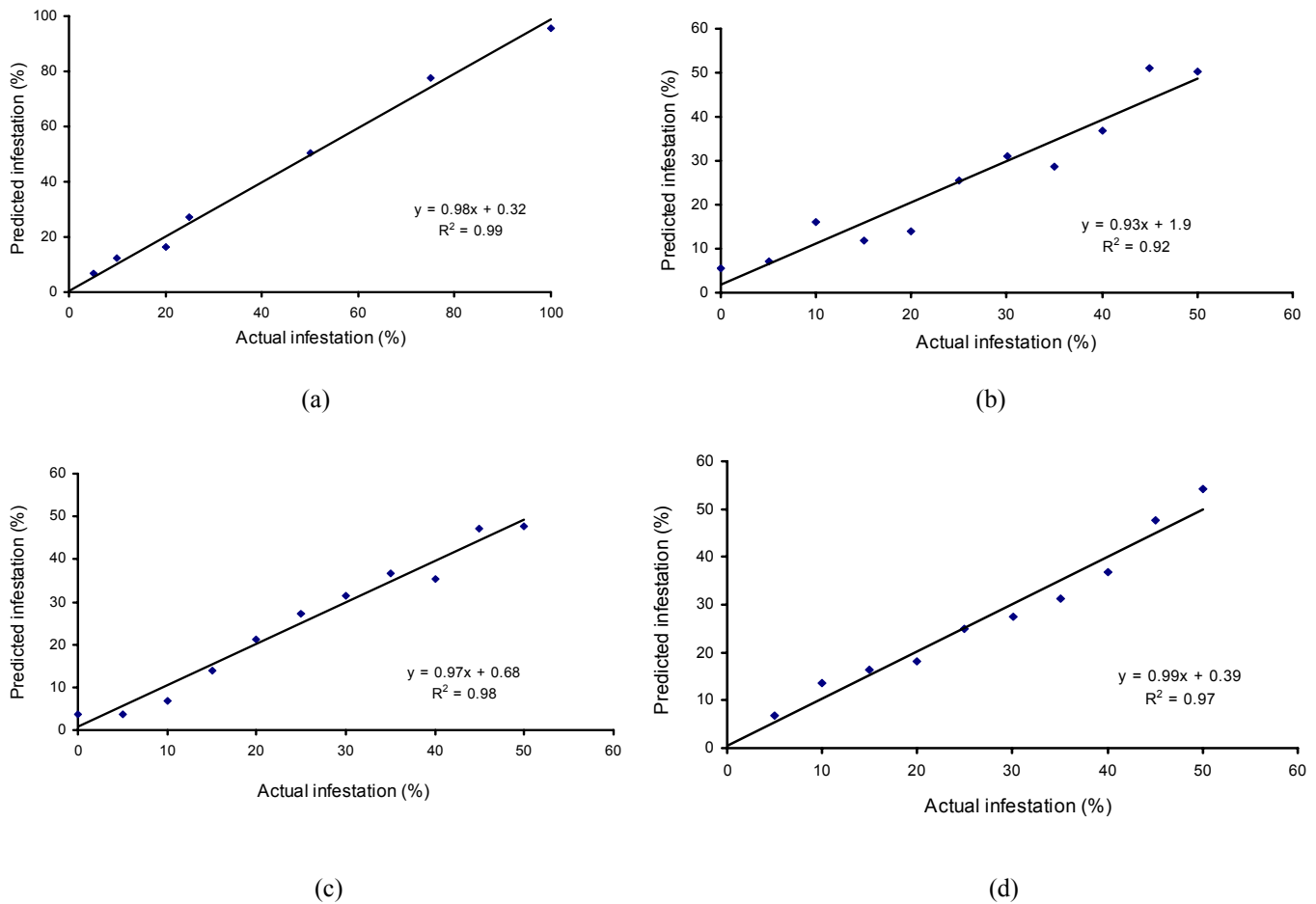


Fig. 2. Regression equations of spectra preprocessed by MSC for wheat samples infested with (a) pupae of *R. dominica*, (b) larvae of *S. oryzae*, (c) pupae of *S. oryzae*, and (d) adults of *S. oryzae*.

Table 3. Regression statistics for calibration models of spectra preprocessed by Standard Normal Variate (SNV) followed by detrending.

Infesting insects	Optimum factor number	PRESS	SECV	r^2
<i>R. dominica</i> (pupae)	1	0.0053	0.026	0.99
<i>S. oryzae</i> (larvae)	1	0.0143	0.036	0.95
<i>S. oryzae</i> (pupae)	1	0.0079	0.027	0.97
<i>S. oryzae</i> (adult)	1	0.0092	0.029	0.97

PRESS - predicted residual error sum of squares

SECV - standard error of cross validation

r^2 - coefficient of determination

performance. Calibration model using MSC performed slightly better for wheat kernels infested with *S. oryzae* and SNV followed by detrending performed better for wheat samples infested with *R. dominica*. One advantage of SNV followed by detrending is that it provides beta coefficients that are much easier to interpret (Barnes et al. 1989). The low number of optimum factors (1 or 2 factors) and high r^2 values also show that most variations in the spectral data correlated with the infestation levels well. This suggests that MSC and SNV followed by detrending could effectively remove the light

scattering effect and physical properties of samples that interfere with model performance on prediction of infestation levels. Regression results reveal that for samples infested with eggs of *S. oryzae*, it is impossible to differentiate different infestation levels. This could be because the tiny eggs do not have enough mass to alter the chemical composition of the host kernel to detectable levels. For the larvae, pupae, and adult life-stages, the infestation levels could be easily differentiated. However, the models generally do not perform well for low infestation levels. It is obvious from the plot of concentration residuals (Fig. 4) that for low infestation levels, the residuals are as large as 6%. Such large prediction residuals suggest that classification of sound and infested wheat samples at low infestation levels will not be very reliable. For the larval, pupal, and adult life-stages of *S. oryzae*, the prediction performance remains good for high infestation levels. This is true since there exists a detection limit regardless of the development stage of the insect. The PLSR beta coefficients obtained on regression models of wheat kernels infested with *R. dominica* and *S. oryzae* at different stages using optimum number of factors are provided in Fig. 5.

Examination of the beta coefficient plots indicates that wavelength variables 1028, 1214, 1938, and 2244 nm are important for the calibrated model of wheat kernels infested with *R. dominica*. Absorption bands at these wavelengths have their corresponding chemical assignments (Osborne et al. 1993).

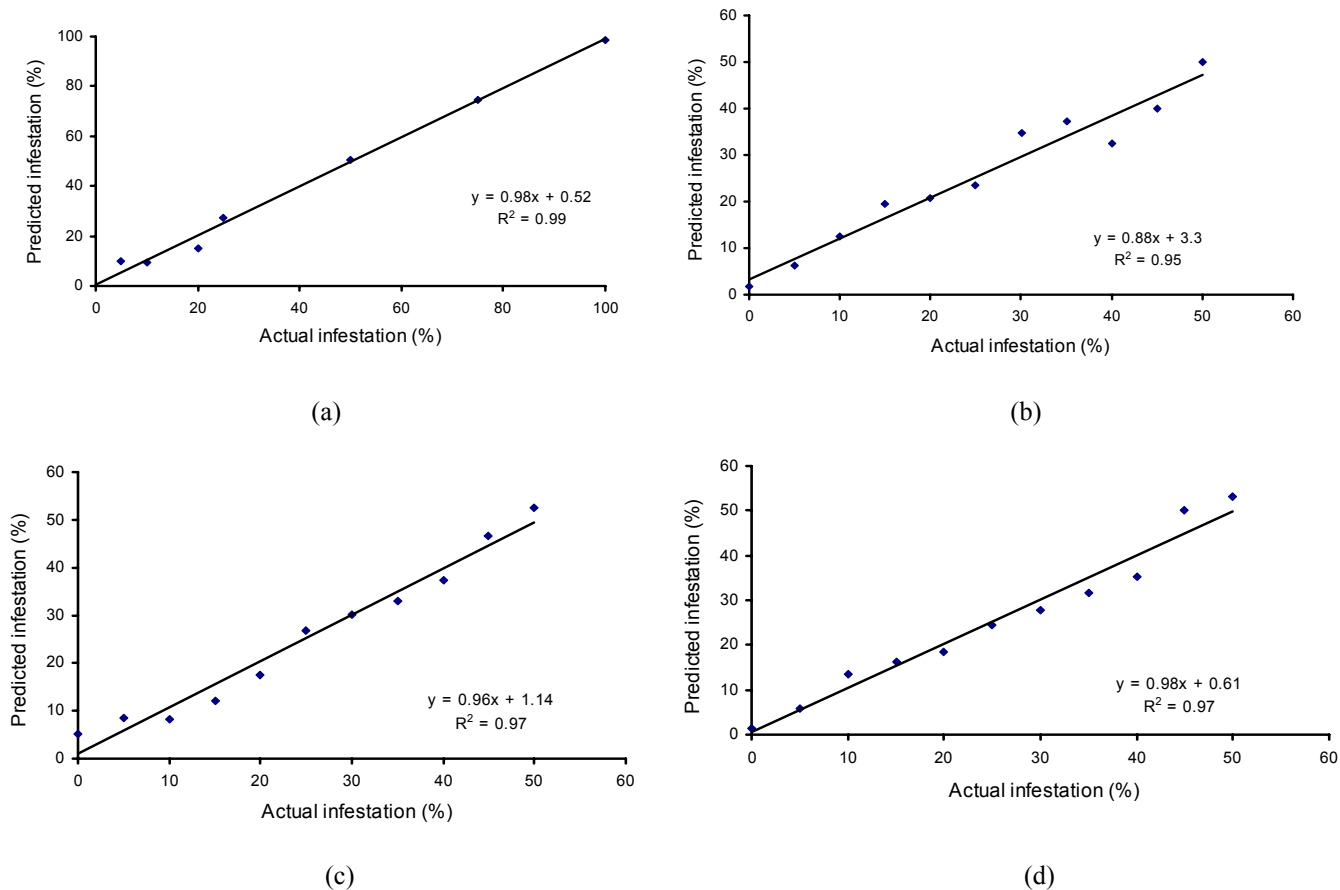


Fig. 3. Regression equations of spectra preprocessed by SNV followed by detrending for wheat samples infested with (a) pupae of *R. dominica*, (b) larvae of *S. oryzae*, (c) pupae of *S. oryzae*, and (d) adults of *S. oryzae*.

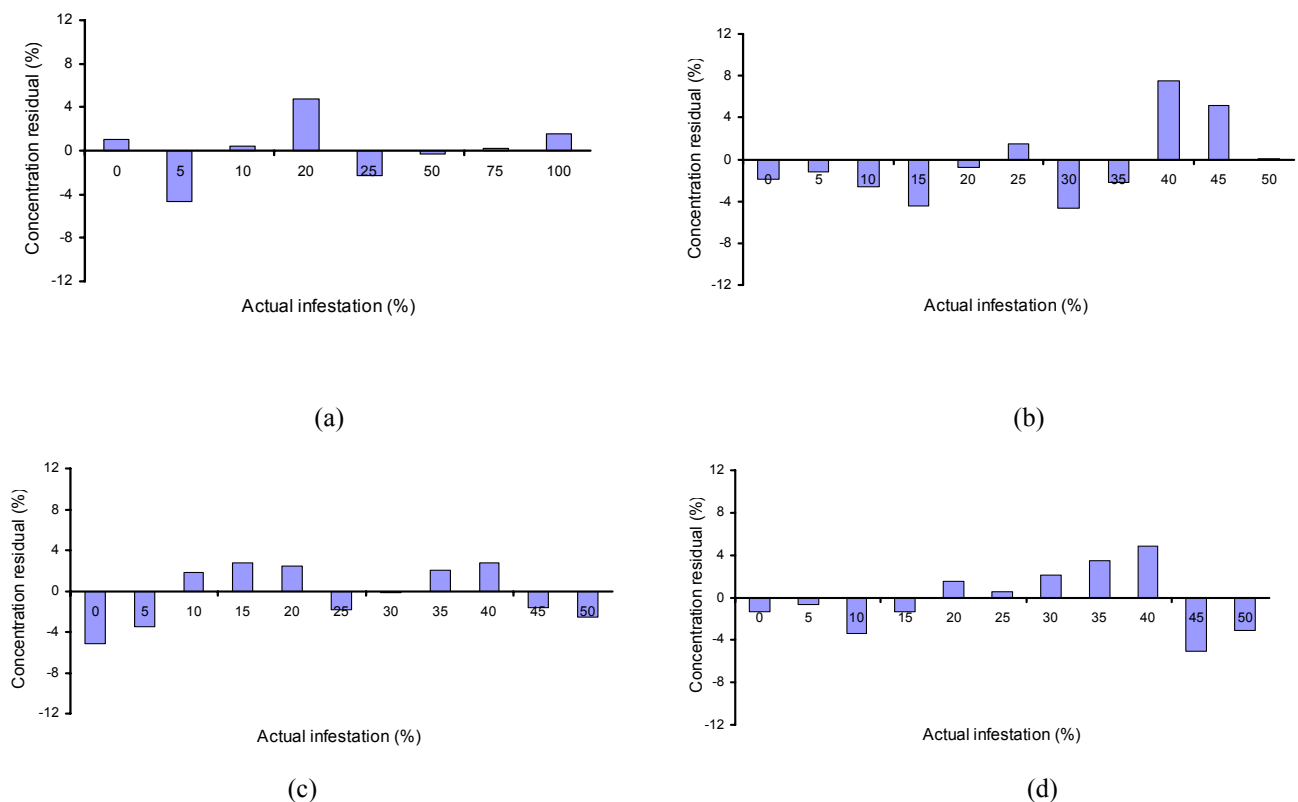


Fig. 4. Concentration residual plots for wheat samples infested with (a) pupae of *R. dominica*, (b) larvae of *S. oryzae*, (c) pupae of *S. oryzae*, and (d) adults of *S. oryzae*.

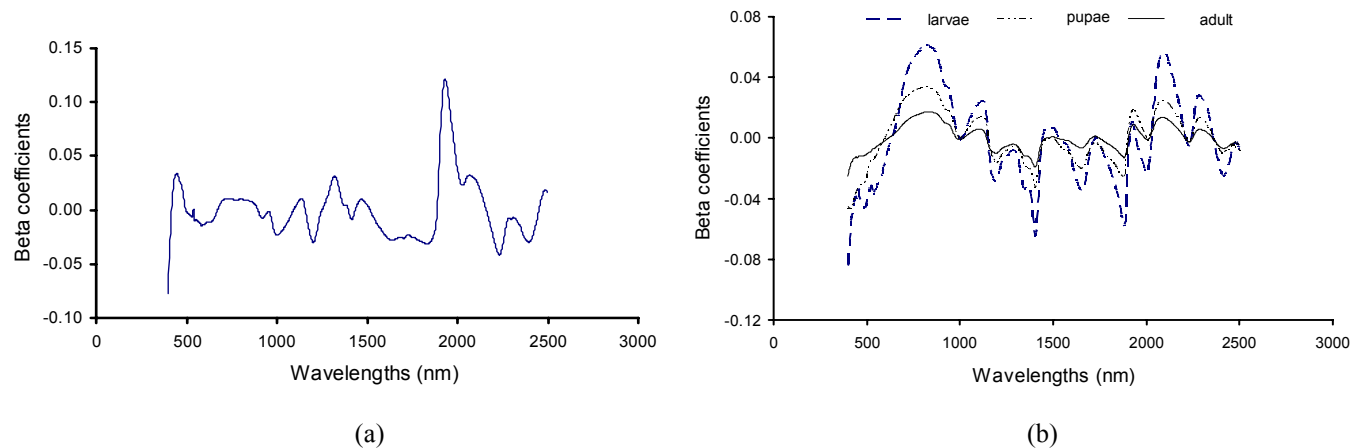


Fig. 5. Beta coefficient plots for wheat samples infested with (a) *R. dominica* and (b) *S. oryzae*.

In Fig. 5a, the 1028 nm negative peak corresponds to the nitrogen-hydrogen (N-H) stretch second overtone and the 1214 nm negative peak corresponds to the carbon-hydrogen (C-H) stretch second overtone. The positive peak at 1938 nm corresponds to moisture content in infested wheat kernel. Moisture content could be important in determining infestation levels because insect respiration indirectly raises kernel moisture (Ridgway and Chambers 1996). The negative peak at 2244 nm corresponds to a response to amino acid. Figure 5b provides PLSR beta coefficients of regression models on wheat kernels infested with *S. oryzae*. Positive and negative peaks

occur at 852, 1132, 1200, 1408, 1668, 1884, 2102, and 2302 nm. The peaks at 1132 and 1668 nm corroborate the finding reported by Dowell et al. (1999) that attribute the absorption in the C-H region to the presence of *S. oryzae* cuticular lipids. The peak at 1200 nm corresponds to C-H stretch second overtone and the peaks at 1884 and 2102 nm correspond to starch content. Another peak at 1408 nm corresponds to oxygen-hydrogen stretch first overtone. Absorption at this band is due to water molecules in bonded form as the hydrogen bonding environment affects positions of overtone bands (Osborne et al. 1993).

CONCLUSIONS

Spectra of wheat kernels infested with *Rhyzopertha dominica* and *Sitophilus oryzae* were analyzed and classified. This study has demonstrated that it is possible to differentiate wheat samples infested with pupae of *R. dominica* and *S. oryzae* at different infestation levels. It was observed that although it is possible to detect infestation levels above 25% using near-infrared spectroscopy, lower levels of infestation might not be detected using this technique. Due to instrumental and sample differences, PCA on the difference spectra provides better interpretation of results than using the original spectra. Partial Least Squares Regression was performed to quantitatively determine the infestation levels. It was demonstrated that preprocessing methods such as Multiplicative Scatter Correction and Standard Normal Variate followed by detrending can effectively remove information irrelevant to insect infestation levels and ensure equally good prediction performance. Wavelengths corresponding to moisture, starch, amino acids, and cuticular lipids of insects could be clearly identified from the beta coefficient plots of the data. These wavelength peaks were in agreement with previously published research.

ACKNOWLEDGMENTS

We thank Dr. N.D.G. White and Mr. C. J. Demianyk at the Cereal Research Centre, Agriculture and Agri-Food Canada, Winnipeg, Manitoba for their help in rearing the insects. We also thank Dr. M.S. Borhan, Mr. T. Leung, and Ms. T. Volkart at the Department of Biosystems Engineering, University of Manitoba, Winnipeg, Manitoba for their help in infesting grain kernels. Thanks also to Ms. H. Cordiero and Ms. D. Sobering of the Canadian Grain Commission, Winnipeg, Manitoba who helped us in scanning the samples and collecting the data. Partial funding for this study was provided by the Canada Research Chair program.

REFERENCES

- Arthur, F.H. and J.E. Throne. 2003. Efficacy of diatomaceous earth to control internal infestations of rice weevil and maize weevil (Coleoptera: Curculionidae). *Journal of Economic Entomology* 96(2): 510 – 518.
- Baker, J.E., F.E. Dowell and J.E. Thorne. 1999. Detection of parasitized rice weevils in wheat kernels with near-infrared spectroscopy. *Biological Control* 16:88-90.
- Barnes, R.J., M.S. Dhanoa and S.J. Lister. 1989. Standard normal variate transformation and de-trending of near-infrared diffuse reflectance spectra. *Applied Spectroscopy* 43(5):772-777.
- Brereton, R.G. 2003. Chemometrics: Data analysis for the laboratory and chemical plant. Hoboken, NJ: John Wiley & Sons Ltd.
- Canada Grains Act. 1975. Canada Grain Regulations. Canada Gazette, Part II, Vol. 109, No. 14.
- Dowell, F.E., J.E. Thorne and J.E. Baker. 1998. Automated nondestructive detection of internal insect infestation of wheat kernels by using near-infrared reflectance spectroscopy. *Journal of Economic Entomology* 91(4):899-904.
- Dowell, F.E., J.E. Throne, D. Wang and J.E. Baker. 1999. Identifying stored-grain insects using near-infrared spectroscopy. *Journal of Economic Entomology* 92(1):165-169.
- Friese, M.A. and S. Banerjee. 1992. Lignin determination by FT-IR. *Applied Spectroscopy* 46(2):246-248.
- Geladi, P., D. Macdougall and H. Martens. 1985. Linearization and scatter-correction for near-infrared reflectance spectra of meat. *Applied Spectroscopy* 39(3):491-500.
- Geladi, P. and B. Kowalski. 1986. Partial least-squares regression: A tutorial. *Analytica Chimica Acta* 185:1-17.
- Hagstrum, D.W. and P.W. Flinn, 1994. Survival of *Rhyzopertha dominica* (Coleoptera: Bostrichidae) in stored wheat under fall and winter temperature conditions. *Environmental Entomology* 23(2):390-395.
- Kemsley, E.K. 1998. *Discriminant Analysis and Class Modeling of Spectroscopic Data*. Chichester, UK: Wiley & Sons.
- Kim, S.S., M.R. Phyu, J.M. Kim and S.H. Lee. 2003. Authentication of rice using near infrared reflectance spectroscopy. *Cereal Chemistry* 80(3):346-349.
- Lockey, K.H. 1988. Lipids of insect cuticle: Origin, composition and function. *Comparative Biochemistry and Physiology B* 89:595-645.
- Maghirang, E.B., F.E. Dowell, J.E. Baker and J.E. Throne. 2003. Automated detection of single wheat kernels containing live or dead insects using near-infrared reflectance spectroscopy. *Transactions of the ASAE* 46(4): 1277-1282.
- Murray, I. and P.C. Williams. 1990. Chemical principles of near-infrared technology. In *Near-infrared Technology in the Agricultural and Food Industries*, ed. P.C. Williams and K.H. Norris, 17-34. St. Paul, MN: American Association of Cereal Chemists.
- Osborne, B.G., T. Fearn and P.H. Hindle. 1993. *Practical NIR Spectroscopy: with Applications in Food and Beverage Analysis*, 2nd edition. New York, NY: J. Wiley.
- Ridgway, C. and J. Chambers. 1996. Detection of external and internal insect infestation in wheat by near-infrared reflectance spectroscopy. *Journal of the Science of Food and Agriculture* 71(1):251-264.
- Ridgway, C., J. Chambers and I.A. Cowe. 2001. Detection of grain weevils inside single wheat kernels by a very near infrared two-wavelength model. *Journal of Near Infrared Spectroscopy* 7:213-221.
- Sinha, R.N. and F.L. Watters. 1985. *Insect Pests of Flour Mills, Grain Elevators, and Feed Mills and Their Control*. Ottawa, ON: Agriculture Canada.
- White, N.D.G. 1995. Insects, mites and insecticides in stored grain ecosystems. In *Stored-Grain Ecosystems*, eds. D.S. Jayas, N.D.G. White and W. E. Muir, 123-168. New York, NY: Marcel Dekker Inc.