2950 Niles Road, St. Joseph, MI 49085-9659, USA
269.429.0300 fax 269.429.3852 hq@asabe.org www.asabe.org

An ASABE Meeting Presentation

Paper Number: 131592957

Determination of Degree of Infestation of Triticale Seed Using NIR Spectroscopy

Mahmoud K. Khedher Agha [a, b], Won Suk Lee [a], Chuan Wang [e], Richard W. Mankin [c], Nikolay Bliznyuk [e], Ray A Bucklin [a]

a. Agricultural and Biological Engineering, University of Florida, Gainesville, FL, United States.

b. Agricultural Machinery and Equipment, University of Baghdad, Baghdad, Iraq.

c. ARS, CMAVE, USDA, Gainesville, FL, United States.

e. Statistics, University of Florida, Gainesville, FL, United States.

Written for presentation at the

2013 ASABE Annual International Meeting

Sponsored by ASABE

Kansas City, Missouri

July 21 – 24, 2013

Abstract. *Insect infestation of triticale (Triticosecale) seed causes extraordinary storage losses as a consequence of vulnerability of triticale seed to insect infestation and its soft coat. Rice weevil, Sitophilus Oryzae (L.), is a common insect that causes infestation in Florida, which was the focus of this research. The objective of this research was to develop a method to determine the degrees of infestation (DI) in the seed at two growth stages by measuring their spectral reflectance. To achieve this goal, triticale samples with eleven DI (0, 6, 11… and 62%) were prepared with two growth stages of larvae 2nd instar, and adult outside seed with three to four replications. The reflectance was measured from 400 nm to 2500 nm. The DI results were compared to manual inspection in order to evaluate the accuracy. The data was analyzed using stepwise multiple linear regression (SMLR). The result showed that the DI for larvae 2nd instar stage could be detected using an average reflectance in 400 - 410 nm, with an R^2 of 0.87 using the SMLR method. The adult outside stage also resulted in a good prediction using the SMLR method, where it yielded four wavelengths (400, 783, 791, and 967 nm) that provided an acceptable result with an R^2 of 0.87 for the adult outside stage. The prediction of early growth stages was more challenging than for late growth stages, due to the smaller size of larvae 2^{nd} instar stage compared to the adult stage. Overall, NIR spectroscopy was proved to be useful to detect insect infestation in triticale seed.*

Keywords. *Rice Weevil, Spectral reflectance, stepwise multiple linear regression (SMLR).*

Introduction

Triticale, *Triticosecale*, is a hybrid between wheat and rye, where this crop is used as animal feed. Meanwhile, this seed is susceptible to insect infestation. Detecting the Infestation of triticale seed by rice weevils, *Sitophilus Oryzae (L.)* was hard with unaided eyes because most of their life cycles are completed inside the seed without a visual sign. Therefore, using near infrared spectra is a reasonable approach to detect the developing stages for these weevils without destroying the seed. However, spectroscopy data tends to be one of the more challenging data to analyze.

Min and Lee, (2005) used two methods for prediction of nitrogen concentration in citrus leaves using the spectroscopy approach: partial least squares (PLS) and stepwise multiple linear regression (SMLR). They found that SMLR gave a better prediction with low collinearity. Using this method with an average of 20-point wavelengths led to reduce the collinearity. Jones, et al. (2010) conclude that disease severity of tomatoes can be diagnosed using near-infrared (NIR) spectra with acceptable result while using PLS and SMLR methods, where SMLR gave higher R^2 than PLS. Both of the previous studies used root mean square difference (RMSD) to evaluate their result.

Paliwal, et al. (2004) used PLS to predict the infestation percentage and they achieve a reasonable result. When they used principal component analysis (PCA) to reduce the dimension, they obtained a better reduction. They also used a beta coefficient plot to visualize the wavelength that was affected by insect activity, such as eating the starch of the seed.

The purpose of this research was to distinguish between insect infested and non-infested seed using nondestructive inspection and without using chemicals. Therefore, the goals in this paper were to predict the degree of infestation at early growth stage of rice weevils inside triticale seed using spectral signatures, to determine the best wavelengths to represent the infestation degree, and to develop prediction models of the infestation using statistical parameters that can evaluate each prediction model.

Materials and methods

Sampling and testing

Triticale seed samples were collected randomly from a bulk seed that was harvested in May 2012 from North Florida Research and Education Center (NFREC) in Quincy, Florida. The seed was cleaned using U. S. standard sieves to insure uniformity and clarity from foreign materials. Adult rice weevils (RW) insects were added to the seed sample for laying eggs, where for every 188 g of seed there were 600 insects added. Then the adults were removed after five days to insure a good percent of infestation occurrence. This mix was kept in these conditions at 26 ° C and 60% RH for ordinary growth. The infested seed was mixed with non-infected clean seed in different percentages of infestation where the experimentally designed percentages were from 10 to 100 in increments of ten, but the actual was from 6 to 62.5% where these percentages are shown Table 1 below. This variation in seed infestation occurred due to the variation in female and male percentages and others factors such as the ability of each female to lay eggs in those five days.

Table 1. Experimentally designed and Actual DI.

Experimentally designed DI %	0	10	20	30	40	50	60	70	80	90	100
Actual DI %	0	6.33	11.4	18.7	24	30.8	36.3	41.4	50.3	59	62.5

The actual infestation percentages were measured using a manual counting method. When the adults emerged from the seed, they were collected and separated into a different container in order to count them. After spectral measurements (described below), the holes in the seed were counted and compared with the number of adults in each container.

Spectral measurement was conducted in August 2012, using a spectrophotometer (CARY 500 SCAN, Varian Inc., Palo Alto, California) and an external integrating sphere (diffuse reflectance accessory (DRA)-CA-5500D, Labsphere, Inc., North Sutton, NH.). Mercury lamps were the source of light within the spectrophotometer. Sample reflectance was measured from 400 to 2500 nm with increments of 1 nm. These wavelengths covered the visible and near infrared ranges to cover a wide range of bands and predict the infestation. Each sample had three to four replications, where the capacity of the sample holder was only 15 grams of triticale seed.

DI = Zero% (Control) L2	DI = 62.5 % L2

Figure 1.Triticale seed samples with 62.5% infestation of larvae 2nd instar growth stage of rice weevils vs. zero infestation as a control.

Analysis Method

The data obtained from the spectrophotometer had noise in part of the spectrum, and so in order to reduce the noise, Savitzky–Golay smoothing method was applied. In order to give a reasonable smoothing for the data, 41 points were chosen using the MATLAB program version 7.14.0 (MathWorks, Inc., Natick, MA, USA). After that the smoothed data was divided into two sets, the first set as a calibration set containing 2/3 of the data and the second set with the remaining 1/3 of the data as a validation set. Random selection was used to separate the data.

The calibration set was analyzed using the SAS program version 9.2 (SAS Institute Inc. Cary, NC, USA), and stepwise regression method was used to determine the best wavelengths giving the best prediction of the infestation. The chosen wavelengths were tested using the validation data set to evaluate the result while using the following parameters:

- Standard error of calibration (SEC).
- Root mean square of the differences (RMSD) where it measures the accuracy of the calibration equation.
- Standard error of prediction (SEP), where it is the standard deviation (SD) of variances within predicted and measured values and the equations for calculation these parameters was used by (Min and Lee, 2005) .
- RPD is the quotient of SDx to SEP, where SEP will be tested in terms of SD of the measured data. And it had the reference values 0-2.4 = very poor prediction, 2.4-3=poor, 3-5=fair, and 5-6.4= good prediction.
- Coefficient of determination, R^2 (Williams and Norris, 2001).

Results and discussion

Larvae 2nd instar

The result for the correlation coefficient between the degree of infestation (DI) and the reflectance shows wavelengths having a high correlation, as shown in figure 1. The wavelengths from 400 to 830 nm have a high correlation higher than 0.5. Also, the wavelengths from 946 to 1340 nm have a higher correlation more than 0.5 and these wavelengths were chosen as starting points in analyzing the data.

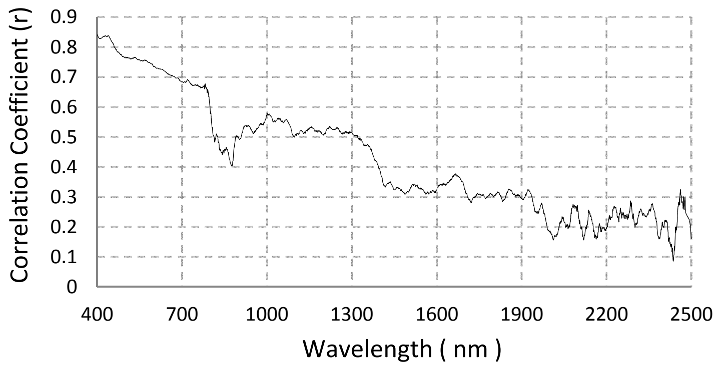

Figure 1. Correlation coefficient between reflectance and degree of infestation for larvae 2 instar stage. Reflectance in 400-830 nm and 941-1323 nm shows correlation higher than 0.5 with the degree of infestation.

Three methods were used for the larvae second instar stage to choose important wavelengths and to do the prediction, as shown in table 2. The Maximum R^2 Improvement (MAXR) method yielded a very low R^2 and RPD values. While, the best R^2 value of 0.87 was obtained using stepwise regression method with an average of 10 wavelengths, the next one was a stepwise regression without an average and gave an R^2 value of 0.49.All the parameters that were previously mentioned are shown in table 2.

Table 2. Prediction evaluation three methods while testing Larvae second instar stage.

Analysis Method	Selected Wavelengths (nm)	SEC	RMSD	SEP	RPD	R^2
Maximum Regression	400, 420, 421, 503, 712, 1918 , 1961, 1963 , 2497	32.48	23.99	25.36	0.91	0.08
Stepwise Regression	400	17.47	15.8	16.55	1.40	0.49
Stepwise Regression with Average 10 wavelengths	Average (400-410)	16.35	14.79	21.25	0.99	0.87

Figure 2 shows the prediction vs. actual degree of infestation for the larvae 2nd instar using a stepwise multiple liner regression with an average of 10 wavelengths. This method yielded the best R^2 value of 0.867, with the consideration of the smaller size of larvae 2nd instar compared to the size of the seed.

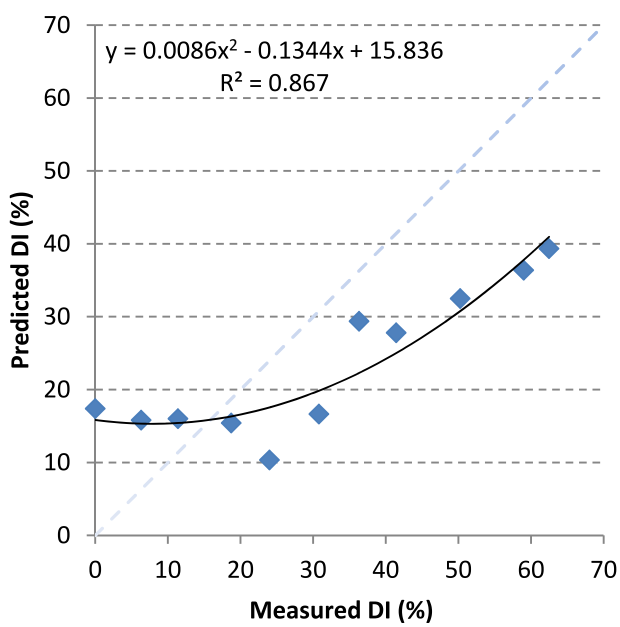

Figure 2. Measured vs. Predicted degree of infestation percentages for larvae second instar growth stage using a stepwise multiple liner regression with 0.01 significant level.

Adult outside the seed stage

The result for the correlation coefficient between the degree of infestation (DI) with the adult outside stage and the reflectance shows wavelengths having a high correlation with the DI, as shown in figure 3. The wavelengths from 400 to 579 nm have a high correlation in the visible range. In addition, the wavelengths higher than 972 nm have a high correlation higher than 0.5. The highest value was found at 400 nm with a coefficient of 0.85.

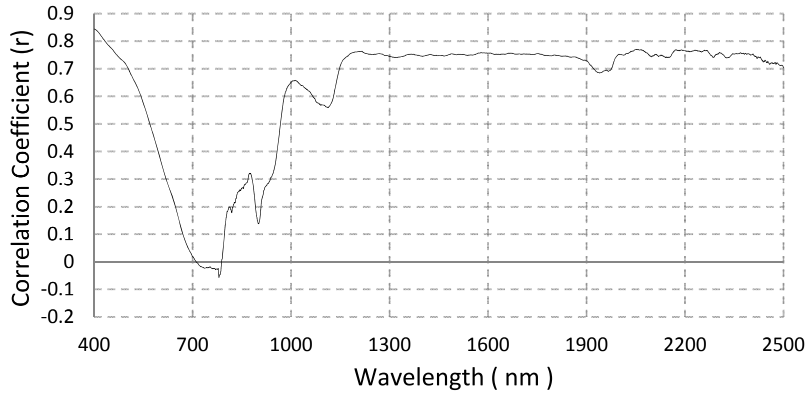

Figure 3. Correlation coefficient between reflectance and degree of infestation for Adult outside stage. Reflectance in 400-580 nm and above 972 nm shows correlated higher than 0.5 with the degree of infestation.

Two methods were used for the adult outside growth stage to choose the wavelengths and to do the prediction, which are shown in table 3. The best result was yielded using the stepwise regression using the following wavelengths: 400, 783, 791, and 967 nm. The other one was a stepwise regression using wavelengths with a high correlation coefficient, where this method gave an R^2 of 0.77. All of the values for the parameters that were listed above are shown in table 3.

Table 3. the Prediction evaluation for two methods while testing Adult outside stage.

Analysis Method	Selected Wavelengths (nm)	SEC	RMSD	SEP	RPD	R^2
Stepwise	400, 783, 791, 967	13.54	10	9.41	2.41	0.87
Stepwise with high correlation	400	13.18	12.04	11.93	1.90	0.77

The relation between the prediction vs. actual degree of infestation for adult outside stage is shown in figure 4 below, where it used stepwise multiple linear regression method with average 10 wavelengths that gave the best R^2 value (0.87).

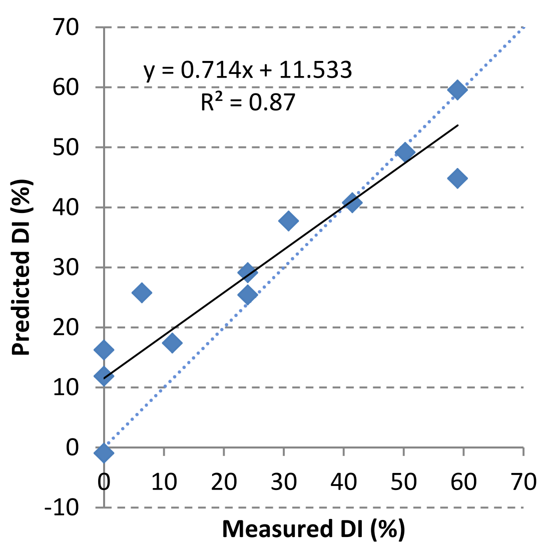

Figure 4. Measured vs. Predicted degree of infestation percentages for adult outside the seed growth stage using stepwise multiple liner regression with 0.01 significant level.

Conclusion

Spectral signature was used to determine the degree of insect infestation in triticale seed with eleven levels of infestation for two growth stages of early stage larvae second instar of rice weevils and end of development stage adult outside the seed. This test was done by using the visible and near infrared ranges in the spectrophotometer. The result showed that there was a high correlation coefficient between the infestation degree and the reflectance especially with adult stage and it was higher than the larvae 2nd stage.

In addition, the obtained result from this paper shows that the degree of infestation for the larvae 2nd instar stage can be detected using an average of 400 to 410 nm wavelengths, while for adult outside stages was a combination of these wavelengths of 400, 783, 791, and 967 nm. Both stages obtained a high coefficient of determination about 0.87. Moreover, the method that was used in both stages was stepwise mutable linear regression. The prediction of early growth stages was more challenging than for late growth stages, due to the smaller size of the larvae 2nd instar stage compared to the adult stage. Overall, NIR spectroscopy was proved to be useful to detect insect infestation. More results will be published soon using a combination of different stages and all the stages with many levels of infestation.

Acknowledgements

Thanks to my father, my wife and my big family for their continuing support and encouragements. Thanks to Dr. Blount for all of her help and for providing the seed for the experiment. Thanks to Betty A. Weaver in the USDA, ARS, CMAVE for all of her help. Thanks to Ce Yang, Han Li, Rebekah Combs, my lab mates, and my friends for their help in this project. Thanks to all the people who supported me and helped me to complete this project.

References

- Jones, C. D., J. B. Jones, and W. S. Lee. 2010. Diagnosis of bacterial spot of tomato using spectral signatures. *Computers and Electronics in Agriculture*. 74(2): 329-335.
- Min, M., and W. S. Lee. 2005. Determination of significant wavelengths and prediction of nitrogen content for citrus. *Transactions of the ASAE*. 48(2): 455−461.
- Paliwal, J., W. Wang, S. J. Symons, and Karunakaran, C. 2004. Insect species and infestation level determination in stored wheat using near-infrared spectroscopy. *Canadian Biosystems Engineering*, 46.
- Williams, P., and K. H. Norris. 2001. *Near-infrared technology in the agricultural and food industries.* 2nd ed. American Association of Cereal Chemists, Inc. St. Paul, Minnesota, USA.