TECHNICAL NOTE:

MEASURING GRAIN AND INSECT CHARACTERISTICS USING NIR LASER ARRAY TECHNOLOGY

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ABSTRACT. *The potential of using a compact eight-wavelength near-infrared (NIR) laser array spectrometer for measuring wheat characteristics (hardness index, moisture content, and waxy character) and determining tsetse fly pupae sex was investigated and compared to a commercial single kernel near infrared (SKNIR) system. Wheat hardness was predicted accurately by both NIR systems and results were in close agreement with reference values. The accuracy of predicting moisture content by either system was similar with predicted values within 0.5% moisture content of the reference values. Waxy character was predicted by the laser system with less accuracy than the SKNIR system, but tsetse fly pupae sex was predicted with similar accuracies for both systems. Prediction equations derived from the laser spectra show that wavelengths influencing classification models generally agree with published literature. Thus, this research shows that a NIR laser array system can be used to predict some grain and insect traits with accuracy similar to a commercial NIR system and some predictions may be improved if other wavelengths are used in the laser array system.*

Keywords. *NIR, Spectroscopy, Wheat, Insects, Near-infrared, Laser, Grading, Quality.*

Near-infrared spectroscopy (NIRS) is recognized as a rapid technique for measuring the characteristics of biological materials. NIRS is commonly used to measure proximates in bulk grain samples such as wheat protein content (AACC Method 39-25, Near-infrared reflectance method for protein content in whole-grain wheat, AACC 2000) and hardness (AACC Method 39-70A, Near-infrared reflectance method for hardness determination in wheat, AACC 2000). More recently, NIRS has been used to measure the traits of single kernels such as hardness (Delwiche, 1993) and waxy character (Dowell et al., 2009), and *fusarium* damage (Wegulo and Dowell, 2008). The techniques used to measure single kernel traits have also been applied to measuring insect characteristics such as honey bee fertility (Webster et al., 2009), insect age (Reeves et al., 2010), and tsetse fly sex (Dowell et al., 2005). Since the first simple and low-cost systems were developed in the 1960's (Norris, 1964), NIR technology has significantly impacted the ability to measure traits of biological materials.

Many commercial NIR instruments use a white light source in a pre-dispersive or post-dispersive configuration. In a post-dispersive system the sample is illuminated with white light, and then the reflected or transmitted light is dispersed into its component wavelengths before reaching a sensor. In a pre-dispersive system, white light is filtered into its component wavelengths before it illuminates a sample, and it then is reflected or transmitted to a sensor. With both of these technologies the system is limited by the energy that can be produced by the white light source. To overcome this energy limitation, lasers may be tuned to specific wavelengths to illuminate a sample. This method has the benefit of illuminating a sample with a very high energy source. The high energy source may result in more energy reaching the sensor and thus improve the system's signal-to-noise ratio. The potential advantages of this technology are a high signal-to-noise ratio, low power dissipation, no dispersive element, the potential of incorporation into a hand-held probe, no moving parts, and a potential cost savings resulting from the elimination of the dispersive element and detector arrays. NIR lasers are available at a variety of wavelengths; thus it is possible to select wavelengths to measure specific traits of interest.

Since laser technology offers several potential benefits over current NIR sensors, we investigated the application of laser technology to measuring various grain and insect traits. Grain quality parameters included in this study were wheat hardness index, moisture content, and waxy character. The insect trait studied was determination of tsetse fly pupae sex, which is important for effective implementation of the sterile insect technique for eradicating this pest insect. The objective of this research was to compare the performance of

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a laser-array NIR spectrometer to a commercial single kernel NIR (SKNIR) spectrometer.

Materials and Methods

Laser-Array Spectrometer

The multi-wavelength laser array spectrometer (fig. 1) developed by Praevium Research Inc. (Santa Barbara, Calif.) was supplied to the Engineering and Wind Erosion Research Unit at the USDA-ARS Grain Marketing and Production Research Center (Manhattan, Kans.) through a Cooperative Research and Development Agreement. The spectrometer had eight semiconductor lasers at 706, 907, 958, 1086, 1234, 1310, 1465, and 1615 nm. Lasers at other wavelengths are available, but these eight were selected to measure specific grain traits based on wavelengths identified in studies by Maghirang and Dowell (2003) and Dowell et al. (2006).

The lasers were powered with an LDC-3908 eight-channel laser diode controller (ILX Lightwave, Bozeman, Mont.). A BFL48-400 optical fiber (0.48 NA, low OH, 400um core, Thorlabs, Newton, N.J.) was used to transmit energy from the lasers to the sample. The sample, such as a single wheat kernel, was manually placed directly over the end of the fiber. A PM132 germanium sensor (Thorlabs, Newton, N.J.) was then placed directly over the sample to detect the energy transmitted from each laser. The eight lasers were sequentially turned on and off (from lowest to highest wavelength) during data collection over a period of about 2 minutes. The kernel completely covered the optical fiber, thus the sensor measured only transmitted light. Stray light was excluded by shielding any direct external light from the sensor. No attempt was made to correct for kernel size, but classification algorithms showed predictions were based on unique absorbance at specific wavelengths, not on an overall increase or decrease in absorbance that might be caused by difference in path length due to kernel size.

Perten SKNIR Spectrometer

The accuracy of the laser array was compared to the automated single kernel near-infrared system (SKNIR, Perten Instruments, Stockholm, Sweden). The SKNIR system nondestructively measures the quality characteristics of individual wheat kernels (Dowell et al., 2006) and has also been used to sex tsetse fly pupae for sterile insect technique eradication programs (Dowell et al., 2005). The SKNIR system measures absorbance from 900 to 1700 nm.

Hardness Index Samples

Three commercial hard red winter wheat samples that represent three hardness index (HI) levels were selected from a 2004 sample collection obtained from the Grain Inspection, Packers and Stockyards Administration, Kansas City, Mo. The predominant variety for each sample were (1) Tam 101 with HI = 64, (2) blend of Pioneer 2137 and 2174 with HI = 72 and (3) blend of Tam 107 and Jagger with HI = 80.

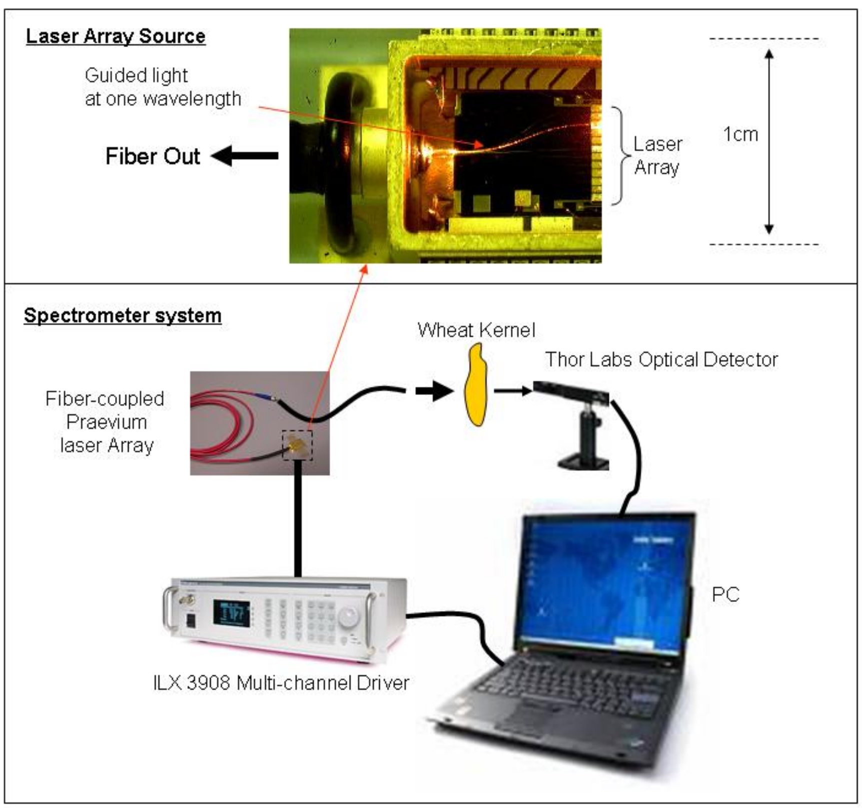

Figure 1. Schematic of a NIR laser array system for measuring grain and insect traits. The critical component of this system is the laser array source, consisting of 8 to 12 semiconductor lasers mounted on a common substrate, and guided by a polymer waveguide array to a common optical fiber. This assembly is mounted inside a 1- × 2- × 0.5-cm butterfly package, shown inside the dashed line and expanded at the top of the figure.

Using a hardness calibration developed for the SKNIR system (Maghirang and Dowell, 2003), each sample was sorted into three approximately equal portions of low, medium, and high hardness index values. From each sample and hardness category, spectral data for 25 kernels were obtained using the SKNIR and laser array systems, for a total of 225 kernels. Kernels were scanned individually and kept uniquely identified according to their placement in pill boxes. After scanning, the actual hardness index of each kernel was obtained using the Single Kernel Characterization System (SKCS 4100, Perten Industries, Stockholm, Sweden). The reject criterion of the SKCS 4100 automatically deleted some data that it determined to be invalid. Kernels that are too small or too dry may have been rejected. Only the 185 kernels with valid data, and corresponding spectra, were used for subsequent data analysis.

Moisture Content Samples

A 400-g hard white winter wheat sample (8% mc), was divided into four 100-g sub-samples and placed in plastic jars. Misted water was added to each of the three sub-samples, and the samples were intermittently shaken to obtain moisture content levels of about 11%, 14%, and 17%. The jars containing the wheat samples were sealed and allowed to equilibrate at room temperature for ~2 days. The spectral data of 50 kernels for each moisture level were collected using both the SKNIR and laser array systems. The SKCS 4100 was used to measure single kernel moisture content. Kernels were individually scanned and kept uniquely identified by their location in pill boxes.

Waxy Samples

A wheat line containing mixtures of waxy (amylose-free) and wild-type (amylose-bearing) kernels was obtained from Dr. Robert Graybosch, USDA ARS (Plant Breeding and Genetics, Lincoln, Nebr.). The amylose content calibration created for the SKNIR (Dowell et al., 2009) was used to obtain 50 waxy kernels and 50 wild-type kernels. Kernels classed as wild-type had a predicted amylose content of greater than 11.5%. Those predicted as waxy had a predicted amylose content less than 11.5%. The spectra of each kernel were collected using the Perten SKNIR and laser array systems. Kernels were individually scanned and kept uniquely identified by their placement in pill boxes. The waxy character of each kernel was confirmed by iodine staining (Pedersen et al., 2004).

Tsetse Fly Pupae Samples

Tsetse fly pupae were provided by the International Atomic Energy Agency, Seibersdorf, Austria. Two sets of 120 tsetse fly pupae were collected, and one set was scanned on each system. The same pupae could not be scanned on each system due to the time required to scan pupae in relation to their rapid development. Spectra were collected from each pupa at approximately 8, 6, 3, and 1 day(s) before the pupae emerged as adults. The pupae were placed in a controlled chamber (27°C, 72% RH) when not being scanned and until all live flies had emerged. The sex of each fly was determined by examining the eyes and genitalia. Some flies did not emerge; in the end, 70 valid pupae were scanned on the laser array system, and 77 valid pupae were scanned on the SKNIR system.

Data Analysis

Spectral data were analyzed by partial least squares (PLS) regression using Grams 32 (Thermo Scientific, Waltham, Mass.) and by multi-linear regression (MLR) using MAXR selection model in SAS (Cary, N.C.) Version 9.1. Cross-validation was used for the PLS predictions, and self-predictions were used for the MLR predictions. Grain quality and insect trait predictions based on the MAXR models were then compared to the PLS prediction results of the SKNIR and to the actual or reference data using Duncan's multiple range test. Only the wavelength range of 950 to 1640 nm was used from the SKNIR system due to noise outside of this range. Generally, the accuracy of predicting traits into high, medium, and low categories was examined since this is the most common application of single kernel analysis technology (Dowell et al., 2006).

Results and Discussion

No hardness index values predicted by the laser array were significantly different ($P<0.05$) from the SKNIR predicted values or from the reference values (table 1). Table 2 shows that absorption at 1234, 1465, and 1615 nm were wavelengths that contributed significantly to the laser array regression model. These regions agree with those reported by Maghirang and Dowell (2003), as having the most influence in their PLS models.

Moisture content predicted by the laser array system was not statistically different from the moisture content predicted by the SKNIR system ($P<0.05$) (table 1). All wavelengths, except 1465 nm, contributed significantly to the prediction equation. This was unexpected since 1465 nm is a strong water absorption band (Williams, 2001). However, water absorbs in many different broad regions including around 718, 894, 964, 1116, 1154, 1410, and 1778 nm, as reported by Williams (2001). These other wavelengths may account for the water absorbance, and the additional contribution at 1465 nm is not needed. The moisture content predicted by the SKNIR and laser array systems was significantly different from the reference values for most comparisons, but all of the predicted values were within 0.5% moisture content of single kernel reference values.

Waxy kernels can be separated from wild-type kernels with about 90% accuracy when using all wavelengths of the laser array system (table 1). All of the laser wavelengths contributed significantly to the prediction model. Most of these wavelengths correspond very closely to those reported by Dowell et al. (2009). The classification accuracy of the laser array system is lower than the SKNIR system, which can separate waxy kernels with 96% accuracy when using the wavelength range of 950 to 1640 nm. Amylose appears to absorb strongly at 1400 nm; given that this wavelength is not included in the current laser-array, it may account for the slightly poorer results.

Tsetse flies were classified by both systems with >94% accuracy at 3 days before emergence. Table 3 shows the results for the laser array sensor and the SKNIR system for 8, 6, 3, and 1 day(s) to emergence. The results, that the larvae are easiest to differentiate as male or females at about three

Table 1. Comparison of reference grain quality characteristics to those predicted by a single kernel near-infrared (SKNIR) system and a NIR laser array system.

Characteristics/Samples	n	Reference	Prediction SKNIR	Prediction NIR Laser Array
Wheat hardness index				
Tam 101 Low	22	58.9 (3.40) a[a]	62.5 (2.02) a	61.1 (1.71) a
Tam 101 Medium	22	75.9 (3.17) a	76.6 (1.32) a	76.2 (1.32) a
Tam 101 High	24	83.4 (1.84) a	80.7 (1.29) a	83.6 (1.26) a
2137 + 2174 Low	23	69.5 (3.12) a	71.9 (1.49) a	73.3 (1.60) a
2137 + 2174 Medium	19	75.7 (2.74) a	77.4 (1.21) a	76.7 (1.41) a
2137 + 2174 High	18	78.7 (2.66) a	79.2 (1.26) a	78.9 (1.23) a
Tam 107 + Jagger Low	22	62.5 (2.79) a	59.7 (1.83) a	59.3 (1.75) a
Tam 107 + Jagger Medium	17	81.1 (2.61) a	78.7 (1.71) a	77.7 (1.80) a
Tam 107 + Jagger High	18	84.7 (1.27) a	83.5 (0.77) a	82.4 (0.89) a
Moisture content (%)				
Original	150	8.4 (0.12) a	9.0 (0.13) b	8.9 (0.12) b
Low	50	11.7 (0.04) a	11.4(0.07) b	11.5 (0.04) b
Medium	50	14.4 (0.04) a	14.0(0.06) b	14.0 (0.04) b
High	50	16.5 (0.05) a	16.6 (0.06) a	16.7 (0.05) a
Waxy trait[b] (% correct)				
Waxy	50	100.0[c]	96.0	90.0
Wild-type	50	100.0	96.0	90.0

[a] Values in each row followed by different letters are significantly different ($P < 0.05$); Values in parentheses refer to standard error.
[b] Kernels were predicted as waxy (no amylose), or wild-type (amylose-containing).
[c] Waxy trait percentages are the averages of the 50 kernels with no replicates. Thus, there are no statistical comparisons.

days before emergence, were consistent with those reported by Dowell et al. (2005) where male and female spectra showed distinct changes in their absorbance in the 1100- to 1400-nm region about 3 to 5 days before emergence due to differences in their rate of development. That is, females start maturing very rapidly about 5 days before emergence, whereas males do not undergo this change until about 3 days before emergence. These changes may include hardening of the cuticle which will affect the NIR absorbance characteristics. The wavelengths that contributed significantly to the laser array classification model were at 1086, 1234, and 1319 nm, which agree with those reported in the literature (Dowell et al., 2005).

Table 3. Comparison of tsetse fly pupae sex predictions based on a single-kernel NIR system (SKNIR) and NIR laser array spectrometer. The same pupae were scanned each day.

Parameter	Prediction (% correct classification) SKNIR (n=77)	Prediction (% correct classification) NIR Laser Array (n=70)
8 days to emergence		
Female	15.7	8.8
Male	71.1	83.7
6 days to emergence		
Female	15.7	32.4
Male	76.3	74.4
3 days to emergence		
Female	96.9	94.1
Male	94.7	95.3
1 day to emergence		
Female	75.0	61.8
Male	81.6	76.7

Conclusions

The laser array spectrometer generally predicted wheat hardness index, moisture content, and fly pupae sex with accuracies similar to the SKNIR system and to reference values. Waxy character was predicted with lower accuracy by the laser array system than the SKNIR system. Selecting other wavelengths may improve the performance of the laser array system for these traits. Overall, the laser spectrometer performed well and could be a replacement for existing spectrometers for measuring NIR absorbance of small samples. The laser array contains no moving parts and could be more compact than conventional NIR technology. Further, the higher energy produced by the lasers results in a high signal-to-noise ratio.

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Table 2. Regression equations when predicting various grain and insect characteristics using eight wavelengths of the NIR laser-array spectrometer.

Characteristic	Constant	706 nm[a]	907 nm	958 nm	1086 nm	1234 nm	1310 nm	1465 nm	1615 nm
Wheat hardness index	82.5	-67.8	1521.4	-2817.4	-301.3	3772.6*	-839.2	6173.1*	-5028.0*
Wheat moisture content	12.3	-34.0*	515.7*	-960.5*	565.0*	876.3*	-825.8*	163.7	-313.0*
Wheat waxy character	1.4	4.3	-363.8*	593.1*	-212.4*	264.6*	-212.0*	234.4*	-272.4*
Gender: tsetse fly pupae									
8 days to emergence	0.96	0.0	-67.7	80.1	-14.6	60.5	-28.0	38.8	-90.5
6 days to emergence	3.15	0.0	-17.0	-40.8	108.1*	-64.0	-92.4	-155.2	201.4
3 days to emergence	1.48	-6.7	-39.1	-38.0	89.8*	119.8*	-171.0*	-88.7	97.3
1 day to emergence	-1.11	0.0	471.5*	-566.9*	-38.8	151.4	145.2	577.2*	-377.4*

Regression Coefficient columns: 706 nm through 1615 nm.

[a] Values followed by an asterisk refer to statistically significant wavelengths at $P < 0.05$.

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