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In-Field Assessment of Single Leaf Nitrogen Status by Spectral Reflectance Measurements

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Abstract. Commercial agriculture has come under increasing pressure to reduce nitrogen fertilizer inputs in order to minimize potential non-point source pollution of ground and surface waters. This has resulted in increased interest in site-specific fertilizer management. This research aimed to develop techniques for real time assessment of nitrogen status of corn using a mobile sensor with the potential to regulate nitrogen application based on data from that sensor. Specifically, the research attempted to determine the system parameters necessary to optimize reflectance spectra of corn plants as a function of growth stage and nitrogen status. An adaptable, multi-spectral sensor and the signal processing algorithm to provide real time, in-field assessment of corn nitrogen status were developed.

Keywords: variable rate application, spectroscopy, optical sensor, fertilization, NIR, minispectrometer

Introduction

Precision agriculture is based on using the inherent spatial and temporal variability in a field as a basis to manage farm operations. This is a site-specific approach and can reduce input costs, result in higher crop productivity and decrease environmental pollution.

There are two basic methods of implementing site-specific management (SSM) for the variable-rate application (VRA) of crop production inputs: map-based and sensor-based. The map-based SSM method is based on the use of maps to represent crop yields, soil properties, pest infestations, and VRA plans. The sensor-based SSM method provides the capability to vary the application rate of crop production inputs with no mapping involved.

The sensor-based method utilizes sensors to measure the desired properties, soil properties or crop characteristics, on the go. Measurements made by such a system are then processed and used immediately to control a variable-rate applicator. At this point, the major challenge is to develop sensors that will work accurately in field conditions at realistic working speeds. Sensor-based application systems must be capable of accomplishing the sensing, data processing, and application rate adjustment steps in one machine pass.

The present work describes the development of an optical sensing system for in-field detection of leaf nitrogen status in corn.

Background

Numerous studies have been conducted to assess nutrient status by measuring spectral responses. (Bausch *et al*., 1998; Li *et al*., 1999; Read *et al.,* 2002). Most of the studies indicated that leaf or canopy reflectance near the 550 nm has good correlation with leaf nitrogen content.

An indirect means to assess nitrogen stress in plants is by measuring the chlorophyll content of the leaves, using a chlorophyll meter, e.g. SPAD 502 (Piekielek *et al*., 1995; Smeal and Zhang, 1994). In these studies, reasonable relationships among yield, nitrogen content, and chlorophyll meter reading were found. However, the chlorophyll meter required many measurements to assess plant status accurately. Investigation of the relationship between chlorophyll content and narrow band hyperspectral reflectance data revealed models that can predict chlorophyll content both in the laboratory and in the field. (Tumbo *et al.,* 2002a, b).

Utilizing aerial photographs or satellite imagery has been another way to assess plant status and yield (Haboudane *et al*., 2002; Wooten *et al*., 1999). Nevertheless, data available from satellite sources, both for commercial or research use, provide wide spectral bands (50–100 nm) and low spatial resolution (10–30 m), and cannot be used instead of close range narrow band (1–2 nm) remote sensing systems. A number of airborne systems can provide narrow band (1.8–2.2 nm) hyperspectral data (e.g. CASI, ITRES Research Ltd., Calgary, Alberta, Canada), but they are less widely available and are beyond the scope of this work. Nonetheless, the results of the work reported in this paper can be applied to data collected by satellite or airborne, narrow band hyperspectral sensors when they become commercially available.

In order to apply nitrogen at a variable rate, the nitrogen stress of the crop can be either mapped off-line to generate application maps, or measured with an on-line sensor that can assess the nitrogen stress in real time in the field. Stone *et al*. (1996) developed a sensor system for crop nitrogen status and weed detection using photodiode detectors and interference filters. They tested the sensor with winter wheat and reported that total forage N uptake was highly correlated with normalized difference vegetation index (NDVI). Sui *et al*. (1998) developed a spectral reflectance sensor to detect nitrogen status of cotton plants with four spectral bands of blue, green, red and near infrared light. They tested the sensor in two situations: with artificial and with natural illumination. They used a neural network to determine nitrogen deficient and non-nitrogen deficient plants. They reported that preliminary test results for diagnosing nitrogen status in cotton were promising. A commercially available nitrogen sensor has also been developed ('Hydro N Sensor', by Hydro Agri GmbH, Deutschland). It measures crop canopy reflectance spectra from 3–4 m above the ground (Norsk-Hydro, 2002). Such sensors are sensitive to crop coverage and more suitable for fields with full soil coverage.

Lee *et al*. (1999) studied the effect of different corn varieties on spectral models for leaf nitrogen content prediction. They found that under laboratory conditions, leaf nitrogen content can be modelled by spectral reflectance data in spite of phenological differences between the different varieties that were tested.

In a recent work (Read *et al.*, 2002), it was shown that narrow band spectral reflectance data can be used to evaluate nitrogen status in cotton leaves. The work was performed on single leaves under laboratory conditions. This last work increases the demand for research in developing technology for in-field, single leaf narrow band sensing. Such technology will enable transfer of results produced in the lab to field conditions.

Objective

The objective of this work was to develop a sensing system for in-field assessment of plants (corn) leaf nitrogen status, based on single leaf reflectance. The system parameters necessary to optimize in-field measurement of reflectance spectra of corn plants were determined as a function of growth stage. The optical and geometrical configuration of the measuring device was designed. A hyperspectral mobile sensing system and processing algorithms to provide real time, in-field assessment of leaf nitrogen status were developed.

Materials and methods

Illumination

In-field leaf nitrogen assessment was performed in a stationary, controlled environment, as well as in non-stationary conditions. In stationary mode, a custom sampling device was used. In non-stationary mode, non-contact leaf reflectance measurement was performed inside a mobile dark chamber. The dark chamber included a custom optical configuration that was specifically designed and constructed for that purpose.

In both stationary and non-stationary measurements, a commercial mobile mini spectrometer (S2000, Ocean Optics Inc., USA) was used in order to acquire leaf reflectance spectra in the field. The mini spectrometer covered the range from 530 to 1100 nm, and had a 50 μm wide slit. It was equipped with a 2048 pixels CCD array with signal to noise ratio of 250 : 1 and was connected to an A/D data acquisition board. The optical spectral resolution of the system, which is determined by the slit width and the diffraction grating, was 1.8 nm (full width half maximum, FWHM). Each spectral scan took 25 ms.

Natural illumination is not always available (night, clouds) and varies in intensity and spectral characteristics through the day (Harvey, 1997; Tumbo *et al.*, 2002c). For narrow band applications such as the present one, it exhibits an irregular non-smooth spectral curve due to absorption by gases in the atmosphere. In addition, inexpensive mini-spectrometric systems, which can potentially be used in a commercial sensing system, exhibit non-linear response across the available dynamic range. As a result, in spectral ranges that are particularly irregular in the solar irradiance spectrum, unwanted noise appears in the calculated leaf reflectance spectra. In order to avoid the potential noise and to eliminate the need for additional reference signals to compensate for the temporal variations of the solar irradiance, both in stationary and in non-stationary systems, artificial illumination was used.

Stationary measurements

For stationary measurements, a halogen light source (LS-1, Ocean Optics Inc., USA) was used, in combination with a fiber optic reflectance probe. The reflectance probe included six 400 μm diameter optical fibers arranged in a circle which illuminated the sample and a sensing fiber which transferred the reflectance signal to the sensor. A sampling cell was designed and constructed for leaf reflectance measurements in the field, under artificial illumination. The sampling cell maintained a constant distance between the leaf sample and the reflection probe (illumination and signal collection). It was constructed from black opaque mat material, and was shaped as a cone (12° slope). The reflection probe was inserted in the top of the cone and the sample was placed at the open base of the cone (Figure 1). A white ceramic disk, as seen in Figure 1, was used as a reference signal. White reference signal was sampled before each leaf was measured. Dark reference signal was sampled by turning illumination off and covering the sensing fiber. Dark reference signal was sampled every 30 leaves. The spectrometer was operated with 25 ms integration time and averaged 3 spectra per acquisition.

Non-stationary measurements

In non-stationary measurements, the same mini-spectrometric system was moved above the canopy. In this case, leaves could not be manually attached to any sampling cell, as in stationary measurements. Therefore, a different sampling device was designed in order to imitate the optical conditions that were used in the stationary

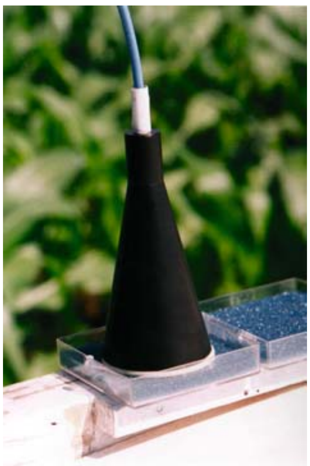

Figure 1. Sampling cell for the reflection probe.

measurements. The objective of the sampling device was to create controlled and constant artificial illumination to maintain as constant as possible the distance between leaf, sensing optical fiber and illumination source, and to restrict intrusion of solar radiation to the optical system. For that purpose, a mobile dark chamber, as depicted in Figure 2, was constructed for moving the mini-spectrometer system above the crop canopy. Two layers of black opaque curtains prevented sunlight from penetrating into the chamber. The sensor assembly inside the dark chamber was designed to provide the necessary geometrical and optical conditions for non-contact reflectance measurements of leaves, (Figure 2c). It included a 400 μm diameter collecting optical fiber with a 15° field of view, for transferring the reflected light to the spectrometer; a 40 W collimated tungsten light beam, for illuminating the leaves. The collecting fiber was placed in a vertical position (aiming downwards) and the illumination beam was placed at an angle of 45° to the optical fiber. This optical configuration provided signals with acceptable intensity only when an object (leaf) was in the effective sampling area, i.e. intercepted the common path between the illumination beam and the optical fiber. The range of distances between canopy and sensor for effective spectra acquisition was 170–250 mm. Leaves that were not in the path of the illumination beam did not reflect any light. Leaves that intercepted the illumination beam but were closer or further away from the designed sampling distance were outside the field of view of the optical fiber and therefore not seen by the sensor. Reflectance from the soil background was also filtered out from the sensor's field of view since it was not illuminated by the light beam. Therefore, the signals acquired by the sensing system were only from leaves at the designed distance from the illumination source and the optical fiber.

Spectra acquisition was performed while the sensing system moved above the crop canopy. The dark chamber was manually pushed along the crop lines and the sensing unit acquired and analyzed spectra continuously. A video camera was also placed in

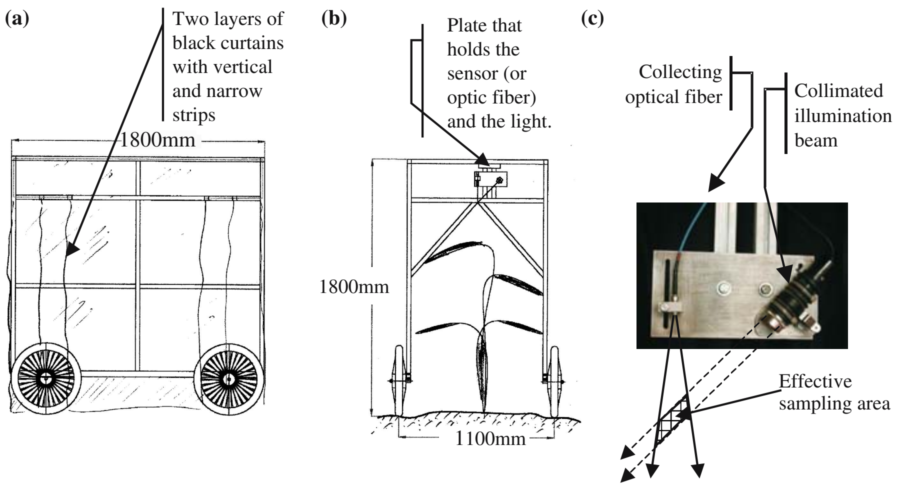

Figure 2. (a) Side and (b) front view of mobile dark chamber. (c) Detailed sensor assembly.

the dark chamber and top view images of the sensor assembly along with the crop were acquired. A spectrum that was within predefined intensity limits was interpreted as leaf spectrum and was saved. The corresponding leaf, identified on the video screen, was then harvested and sent to the laboratory for nitrogen content analysis.

Crop plots

Corn plots were established at MIGAL experimental farm near Kiryat Shmona (N33°9'15'', E35°37'10''). Corn variety Sweet corn *cv Jubilee* was grown during summer 1999 and 2000. Six nitrogen treatments (Table 1) were applied in six replications in a random block experiment. Each replication consisted of six rows (6 m wide). Each row was 20 m long. All the plots were irrigated by a drip irrigation system. The fertilizer was injected in the irrigation water and was computer controlled. The six nitrogen treatments were designed to provide plants with a wide range of nitrogen stress.

Spectral reflectance data was acquired from the last fully expanded leaf. Only plants from the two central lines (out of six) of each replication were sampled. Each sampled leaf was placed in a labeled bag and sent to the laboratory for nitrogen content analysis. In both years, the plots were sampled at tasseling (VT stage), while year 2000 plots were sampled at sixth leaf stage (V6 stage) as well (Hill, 1997). In the

Table 1. Nitrogen treatment plots

Treatment code	Description	Growth stage (number of samples)		
		1999	*2000*	
		VT	V6	VT
Leached	Prior to seeding, excess irrigation was applied to wash out the remainders of nitrogen from the soil. No additional nitrogen was applied	29	24	40
N0	Soil was not washed before seeding, but no additional fertilizer was applied	29	26	40
N12	125 kg hectare^{-1} nitrogen fertilizer was applied at V6 stage	29	14	40
N25	Prior to seeding, 125 kg hectare^{-1} nitrogen fertilizer was applied, and an additional of 125 kg hectare^{-1} was applied at V6 stage	29	18	40
N50	Prior to seeding, 250 kg hectare^{-1} nitrogen fertilizer was applied. An additional 250 kg hectare^{-1} was applied at V6 stage	29	15	40
N100	Prior to seeding, 500 kg hectare^{-1} nitrogen fertilizer was applied. An additional 500 kg hectare^{-1} was applied at V6 stage	29	23	40

non-stationary measurements, the mobile system was moved at a speed of approximately 1 km h^{-1} over the crop.

Leaf nitrogen content prediction models, based on regression equations of ground data and leaf spectral reflectance were developed both for stationary and non-stationary measurements. The prediction models were based on partial least squares regression (PLSR). Also, the effect of the applied nitrogen treatment on leaf nitrogen content is reported (analysis of variance). Using the fertilizer treatments that were found to have significantly different mean leaf nitrogen content, a descriptive presentation of the relationship between specific spectral bands and the fertilizer treatments are shown.

Results and discussion

Acquired spectra were pre-processed in order to normalize them and thus minimize the variations due to illumination intensity changes and distance between the leaf and the optical fiber input. Normalization was achieved by differentiating the spectra vectors along with signal smoothing. Since the optical FWHM bandpass of the system was 1.8 nm and each pixel on the photodiode array represented about 0.33 nm, the smoothing procedure was performed with a five pixels window (1.65 nm), which therefore did not reduce the narrow wavelength sensitivity of the spectrometer. Figure 3 shows a sample leaf reflectance spectrum, and its first derivative.

Leaf nitrogen content as measured in the laboratory was then used as the independent variable to build regression models that linked spectral reflectance to leaf nitrogen status. The first derivative of the reflectance spectra was used in single wavelength linear regression (SLR) models as well as in PLSR models.

Nitrogen content prediction in stationary measurements

A first attempt was made to use a single wavelength in order to predict the nitrogen content of the leaves. Single wavelength correlation between measured leaf nitrogen

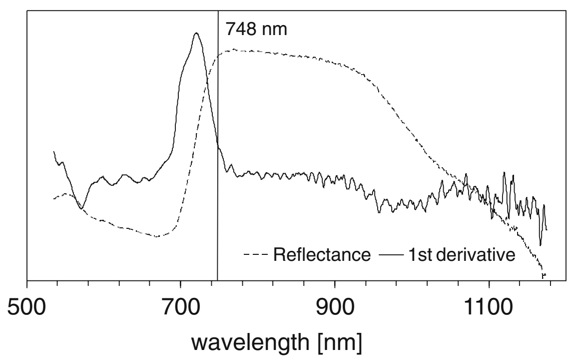

Figure 3. Sample leaf reflectance spectrum and its first derivative.

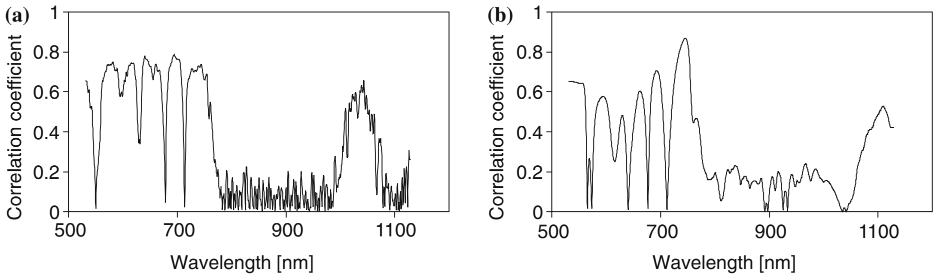

Figure 4. Single wavelength correlation coefficient for (a) stationary measurements (b) non-stationary measurements.

content and the slope of the reflectance curve (first derivative of reflectance) (Figure 4a) revealed that there are two main spectral regions which are highly correlated to leaf nitrogen content: from 530 to 780 nm (with six distinct peaks within) and from 1000 to 1070 nm (with one distinct peak). Wavelengths between 780–1000 nm where poorly correlated to leaf nitrogen. The highest single wavelength correlation coefficients and the corresponding wavelengths are summarized in Table 2. These results agree with results found in the literature for narrow band single wavelength correlation between first derivative of reflectance spectra and leaf nitrogen content (Read *et al.,* 2002).

PLSR models based on the first derivative of the spectra were built, using LTCAL software (L.T. Industries). Part of the data set (2/3 randomly selected of 174 samples) was used for calibrating the models and another part (the remaining 1/3) was used for validation. A four factor PLSR model was found to yield a minimum standard error of prediction (SEP). Table 3 summarizes the results of calibration and validation of the PLSR model with four factors. The standard error of calibration (SEC) was 2.1 g kg^{-1}, while the linear regression coefficient, r^2, between the measured and predicted values was 0.80. The respective coefficients for the validation set were SEP = 2.7 g kg^{-1} and $r^2 = 0.78$. Figure 5 shows graphically the relationship between the predicted and measured leaf nitrogen content in the calibration and the validation sets.

Table 2. Correlation coefficients of single wavelength leaf nitrogen prediction

Stationary measurements		Non-Stationary measurements	
Wavelength (nm)	Correlation coefficient	Wavelength (nm)	Correlation coefficient
640	0.78	748	0.86
692	0.78	695	0.70
740	0.74	540	0.65
1039	0.72	664	0.60
		596	0.57
		1111	0.52

Table 3. Results of leaf nitrogen prediction in stationary mode using a four factor PLSR model and spectra first derivative

Calibration		Validation	
SEC (g kg^{-1})	r^2	SEP (g kg^{-1})	r^2
2.1	0.80	2.7	0.78

Nitrogen content prediction in non-stationary measurements

The main goal of the dark chamber and the optical setup inside the chamber was to eliminate interference of sunlight and maintain as much as possible constant sampling, non-contact geometry. In order to demonstrate the physical setup of the optical configuration principle, a video camera was placed in the dark chamber and top view images of the sensor assembly along with the crop were acquired. Figure 6(a) shows a sample image of the sensor assembly in the dark chamber, during data acquisition, when a leaf is in the field of view of the sensing system. Figure 6(b) shows sample acquired reflectance spectra in two situations, demonstrating the results obtained using the designed system for achieving noise filtering and maintaining constant sampling geometry. When a leaf that is illuminated by the light beam is inside the sensor's field of view, it produces a spectrum signal of significant intensity and it can be acquired and processed (curve (i) in Figure 6(b)). Soil background as well as the rest of the crop leaves are not illuminated and make a small contribution to the reflectance signal (curve (ii) in Figure 6(b)). Therefore, in non-stationary measurements, the collimated illumination beam along with the optical design of the sensor-beam geometry yielded satisfactory results in terms of reducing the noise due to variable background and maintaining a constant distance between the sensor and the sample point on the canopy. White reference signal was acquired using the same white ceramic disc used with stationary measurements.

Single wavelength correlation was performed on the combined data set from stages V6 and VT. Results were similar to those obtained for stationary measurements. Figure 4(b) shows the correlation coefficient between leaf nitrogen content and first

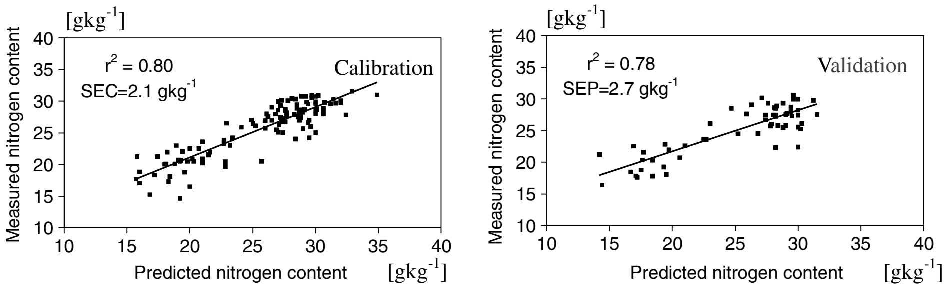

Figure 5. Graphical representation of PLSR models calibration and validation of stationary measurements.

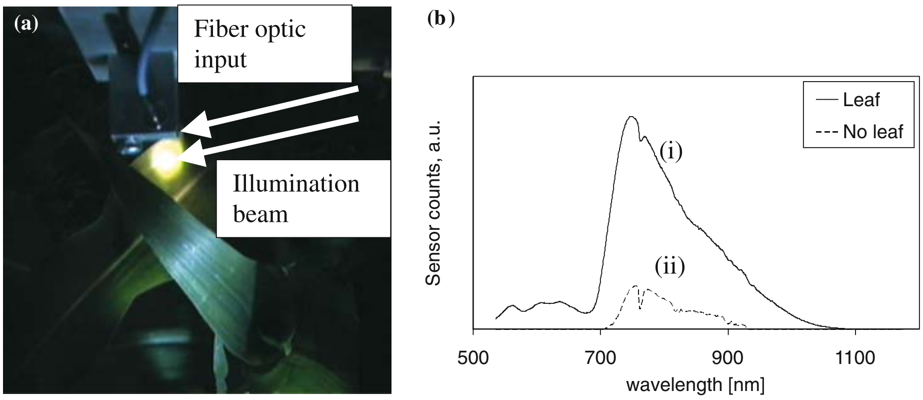

Figure 6. (a) Sample image of the sensor assembly in the dark chamber, during data acquisition. (b) Spectra acquired continuously while moving above the canopy with the dark chamber. (i) leaf, (ii) no leaf in field of view.

derivative of spectral reflectance, as a function of wavelength. Similar spectral regions (530–780 nm and 1000–1070 nm) exhibit high correlation with leaf nitrogen content, although the measurements were performed in non-contact field conditions and the optical configuration was less controlled than in the stationary measurements. Table 2 summarizes the highest correlation coefficients and their corresponding wavelengths. The highest single wavelength correlation coefficient was observed at 748 nm ($r = 0.86$). Figure 7 is a scattergram of the relationship between leaf spectra first derivative at 748 nm and leaf nitrogen content. A one variable least squares linear regression model was fitted to the data. The SEC of the linear regression model was 2.9 g kg^{-1} while the regression coefficient of the model was $r^2 = 0.75$.

Partial least squares regression models were then constructed, based on the first derivative of leaf reflectance spectra in order to predict leaf nitrogen content. The

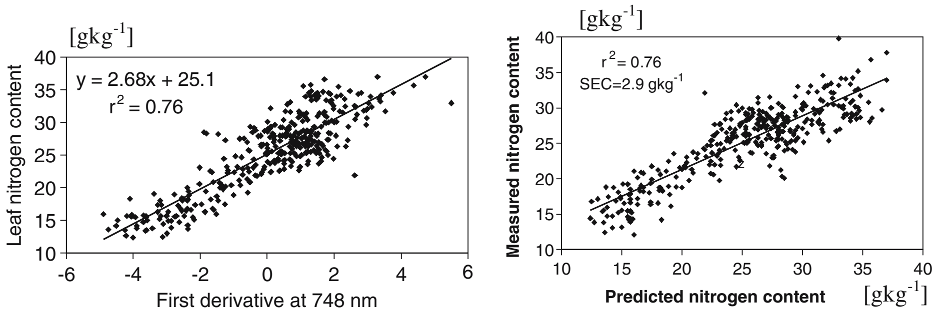

Figure 7. Single wavelength correlation between first derivative of reflectance spectra at 748 nm and nitrogen content.

Table 4. Calibration and validation results of PLSR and SLR models for non-stationary measurements

		Calibration		Validation	
Growth Stage	Model	SEC (g kg^{-1})	r^2	SEP (g kg^{-1})	r^2
V6	PLSR (7 factors)	2.5	0.46	3.3	0.46
VT	PLSR (4 factors)	1.9	0.87	2.1	0.83
V6 + VT	PLSR (7 factors)	2.5	0.81	2.7	0.81
V6 + VT	SLR	2.9	0.75	–	–

models were validated using the "leave one out" validation scheme. Two maturity stages were modelled: V6 and VT stage. Table 4 summarizes the results of the models' calibration and validation. When separate models were built for each maturity stage, SEP was 3.3 g kg^{-1} and 2.1 g kg^{-1} for V6 and VT respectively. When all data was compiled in one data set, a seven factor PLS model was found to yield a SEC of 2.5 g kg^{-1}. Validation of the model with in-field acquired data showed that the nitrogen status of the corn leaves can be predicted with a SEP of 2.7 g kg^{-1}. Figure 8 shows graphically the relationship between the predicted and measured leaf nitrogen content, in the calibration and the validation sets.

Prediction of nitrogen treatment

Analysis of variance of the effect of the applied nitrogen treatment on leaf nitrogen content showed that not all fertilizer treatments yielded to significant differences of leaf nitrogen content. Table 5 shows that in year 2000, the six applied fertilizer treatments form only four groups in which the means are significantly different from one another. Assuming that for significantly different fertilizer treatments this is an indication of the induced nitrogen stress, and that the latter is expressed as deficiency of nitrogen content in the leaf, spectral response was used in order to cluster the data according to their fertilizer treatment group.

Clusters of nitrogen treatment groups were plotted based on the first derivative of the spectra of the non-stationary measurements. The two wavelengths that exhibited the highest single wavelength correlation coefficient in each of the distinct high correlation regions (748 nm and 1040 nm) were chosen. Figure 9(a) shows the clusters of the statistically different treatment groups. The two extreme groups are almost completely separable ("N100", "N50" and "N0", "N25") although the actual concentration of nitrogen in each leaf is not known and certainly not uniform among all leaves in each treatment. Part of the observed variability is due to the natural variability that exists in the data set (different levels of leaf nitrogen content in the same fertilizer treatment) and part due to the model error. Figure 9(b) shows that the intermediate treatments are spread along the whole range and this may suggest that two wavelengths are not enough to describe the phenomenon or that it is affected by additional factors. Although for the extreme groups the clusters are separable, the present results are not conclusive for the intermediate treatments.

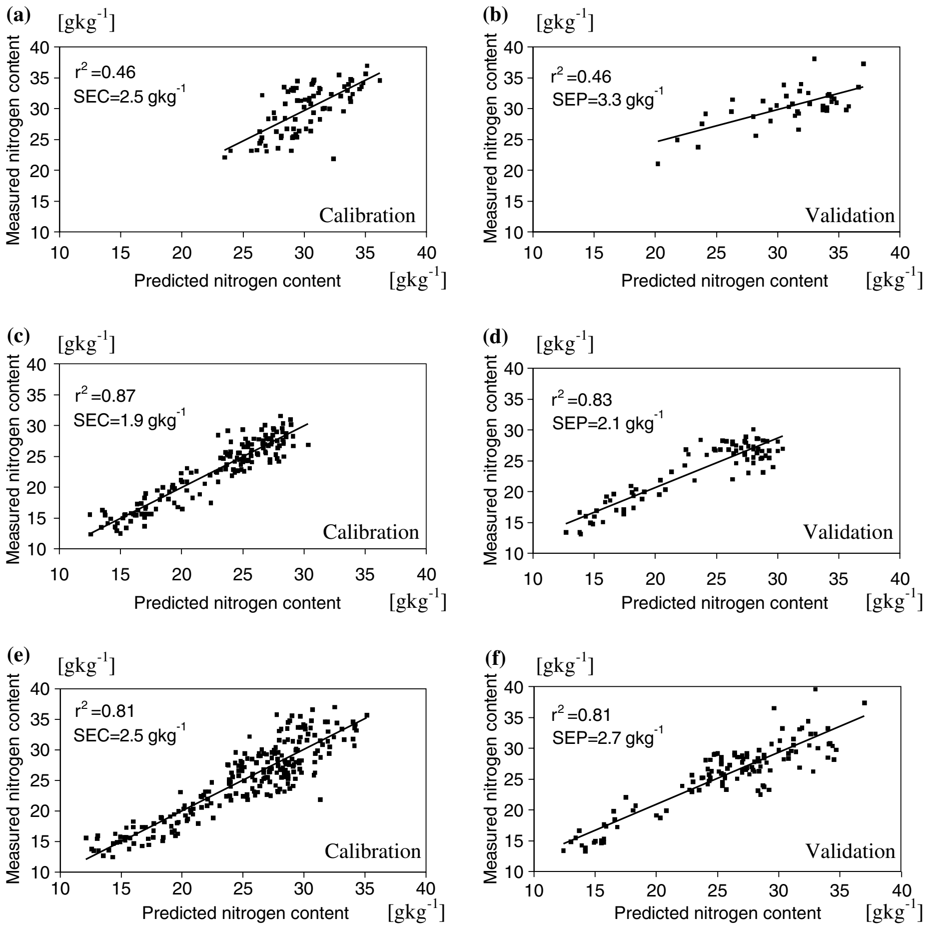

Figure 8. Graphical representation of calibration and validation results of PLSR models, using the first spectra derivative of non-stationary measurements at: (a), (b) V6 stage, (c), (d) VT stage, (e), (f) both growth stages together.

Further research should be conducted to incorporate additional information layers such as soil attributes.

The sensing principle of the system described in the present work can be combined with real-time VRT fertilizing systems. The optical head (illumination and optical fiber) can be positioned at the level of the average last fully developed leaf at the time of application, at an offset from the center of the row. Each row may be equipped with an individual optical head, all directed to one multi-channel spectrometer (multi-channel spectrometers with eight channels or more are commercially available at relatively low cost). Implementation of PLS spectral model involves calculation of linear regression equation which can be implemented in a microcontroller without significant computational complexity. The estimated value of nitrogen level in the

Table 5. Comparison of mean leaf nitrogen content between different fertilizer treatments

Treatment code	V6 Average (g kg^{-1})	V6 Standard deviation (g kg^{-1})	VT Average (g kg^{-1})	VT Standard deviation (g kg^{-1})
Leached	33.1^{c}	1.7	25.7^{c}	1.9
N0	25.3^{a}	2.3	16.8^{a}	2.6
N12	30.9^{b}	2.1	22.7^{b}	2.4
N25	26.4^{a}	2.8	16.5^{a}	2.5
N50	31.8^{b}	1.5	28.0^{d}	1.2
N100	34.0^{c}	1.5	27.8^{d}	1.5

Treatments with the same letter superscript have means that are not significantly different ($P = 0.05$).

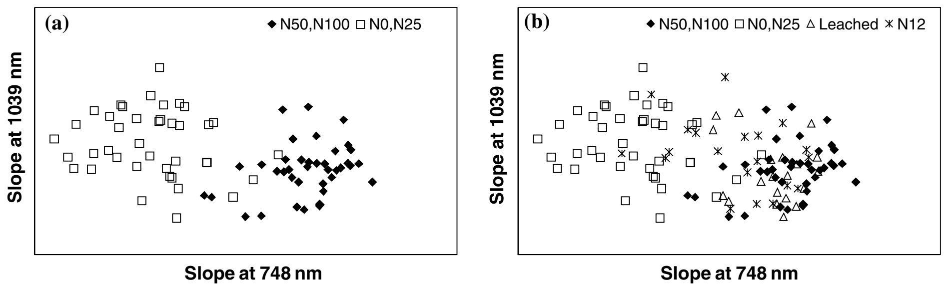

Figure 9. (a) Clusters of two extreme statistically different fertilizer treatments. (b) Clusters of all four statistically different fertilizer treatments.

leaves can then be passed to the management module of the VRT applicator which sets the local fertilizer application rate.

Conclusion

The results of this work showed that leaf nitrogen status can be predicted in-field, using a non-contact optical sensor based on single leaf spectral reflectance. The optical design of the sensor eliminated interference from soil background. The maturity stage of the crop affected the accuracy of the regression model that links between reflectance spectrum and nitrogen content of the leaves. Specifically, the best prediction results were obtained when a separate model was built for VT stage. Combination of all maturity stages together, yielded to a better prediction model than V6 alone.

Non-contact, single leaf spectra acquisition during motion did not reduce the quality of the signals or the accuracy of the prediction models. Similar results to the stationary measurements were obtained by the apparatus.

Further research should be conducted to incorporate direct prediction of nitrogen treatment based on leaf spectral reflectance combined with additional sources of information.

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