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BARD

FINAL REPORT

PROJECT NO. IS-2767-96

Prediction of Nitrogen Stress Using Reflectance Techniques

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2001

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BARD Final Scientific Report
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Date of Submission of the report: November 4, 2001

BARD Project Number: IS-2767-96

Project Title: PREDICTION OF NITROGEN STRESS USING REFLECTANCE TECHNIQUES

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Keywords *not* appearing in the title and in order of importance. Avoid abbreviations.
Spectroscopy, Near Infra Red, Corn,

Abbreviations commonly used in the report, in alphabetical order:

N – Nitrogen, **NIR** - Near Infra Red, **SEP** – Standard Error of Prediction,

Budget: IS: $207.000 US: $153.000 Total: $360.000

Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted)				
Submitted, in review, in preparation			1	1
Invited review papers				
Book chapters				
Books				
Master theses				
Ph.D. theses				
Abstracts				
Not refereed (proceedings, reports, etc.)		2	1	3

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

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Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings	1	1		2
Longer Visits (Sabbaticals)				

Description of Cooperation:

The two research teams worked towards the common goal to develop a sensor for nitrogen in-field nitrogen assessment. Each team researched a different aspect of the sensor's technology, the sensor's capabilities and the interaction between the sensor and the crop. The two research teams cooperated in defining the spectral requirements of the sensor, exchanged experimental results and evaluated the field tests of the mobile sensors.

Patent Summary (numbers)

	Israeli inventor (s) only	US inventor (s) only	Joint IS/US inventors	Total
Submitted				
Issued (allowed)				
Licensed				

Abstract

Commercial agriculture has come under increasing pressure to reduce nitrogen fertilizer inputs in order to minimize potential nonpoint source pollution of ground and surface waters. This has resulted in increased interest in site specific fertilizer management. One way to solve pollution problems would be to determine crop nutrient needs in real time, using remote detection, and regulating fertilizer dispensed by an applicator. By detecting actual plant needs, only the additional nitrogen necessary to optimize production would be supplied. 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 first attempted to determine the system parameters necessary to optimize reflectance spectra of corn plants as a function of growth stage, chlorophyll and nitrogen status. In addition to that, an adaptable, multispectral sensor and the signal processing algorithm to provide real time, in-field assessment of corn nitrogen status was developed.

Spectral characteristics of corn leaves reflectance were investigated in order to estimate the nitrogen status of the plants, using a commercial laboratory spectrometer. Statistical models relating leaf N and reflectance spectra were developed for both greenhouse and field plots. A basis was established for assessing nitrogen status using spectral reflectance from plant canopies. The combined effect of variety and N treatment was studied by measuring the reflectance of three varieties of different leaf characteristic color and five different N treatments. The variety effect on the reflectance at 552 nm was not significant (α = 0.01), while canonical discriminant analysis showed promising results for distinguishing different variety and N treatment, using spectral reflectance.

Ambient illumination was found inappropriate for reliable, one-beam spectral reflectance measurement of the plants canopy due to the strong spectral lines of sunlight. Therefore, artificial light was consequently used. For in-field N status measurement, a dark chamber was constructed, to include the sensor, along with artificial illumination. Two different approaches were tested (i) use of spatially scattered artificial light, and (ii) use of collimated artificial light beam. It was found that the collimated beam along with a proper design of the sensor-beam geometry yielded the best results in terms of reducing the noise due to variable background, and maintaining the same distance from the sensor to the sample point of the canopy.

A multispectral sensor assembly, based on a linear variable filter was designed, constructed and tested. The sensor assembly combined two sensors to cover the range of 400 to 1100 nm, a mounting frame, and a field data acquisition system. Using the mobile dark chamber and the developed sensor, as well as an off-the-shelf sensor, in-field nitrogen status of the plants canopy was measured. Statistical analysis of the aquired in-field data showed that the nitrogen status of the corn leaves can be predicted with a SEP (Standard Error of Prediction) of 0.27%. The stage of maturity of the crop affected the relationship between the reflectance spectrum and the nitrogen status of the leaves. Specifically, the best prediction results were obtained when a separate model was used for each maturity stage.

In-field assessment of the nitrogen status of corn leaves was successfully carried out by non contact measurement of the reflectance spectrum. This technology is now mature to be incorporated in field implements for on-line control of fertilizer application.

Achievements

The need to reduce nitrogen fertilizer inputs has resulted in increased interest in site specific fertilizer management. One way to achieve that would be to determine crop nutrient needs in real time, using remote detection, and regulating fertilizer dispensed by an applicator. By detecting actual plant needs, only the additional nitrogen necessary to optimize production would be supplied. 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 first attempted to determine the system parameters necessary to optimize reflectance spectra of corn plants as a function of growth stage, chlorophyll and nitrogen status. In addition to that, an adaptable, multispectral sensor and the signal processing algorithms to provide real time, in-field assessment of corn nitrogen status were developed.

The first stage of the research project showed that N status can be reliably assessed by non contact reflectance measurements. Chemometric techniques were introduced, in order to compensate for the variability caused by leaf orientation and crop variety. This is contrast to previous works where N status was correlated with vegetation indices that use a limited number of wavelengths. The use of the whole spectrum, pre-processing the spectrum before applying statistical analysis and employment of chemometric methods for N status prediction yielded to satisfactory results in field test.

For in-field N status measurement, a mobile multispectral sensor, covering the visible and NIR region was developed. In contrast to laboratory instruments, the developed sensor is rugged and can be carried on field vehicles, has high speed scanning and on-line data acquisition and processing algorithms, Application of the developed chemometrics processing methods for N status prediction, together with the mobile sensor provide the potential of applying variable N fertilizer rate according to the crop status and need.

The robustness of the sensor was tested by exploring the feasibility of using spectral reflectance to predict nitrogen content in corn plant varieties with varying color. A problem identified by previous researchers is the variability of spectral responses from varieties with different canopy colors. Reflectance was measured for samples from subplots of three different corn varieties (selected for light, normal and dark green leaf color) and 5 N treatments. It was found that variety did not have a significant effect on the shape of the leaf spectral reflectance curves, thus making the spectral sensing technique applicable for assessing nitrogen status suitable for many corn varieties.

Interference of soil reflectance and sunlight spectral lines introduce significant noise to the crop's canopy reflectance spectra. A significant achievement of this research was the design of an artificial light assembly that solved this acute problem, typical for many field tests. The optical configuration of the sensor's system included illumination requirements and geometrical alignment of the sensor with respect to the illumination. This optical design enabled acquisition of spectral reflectance data, invariant to external light conditions and invariant to canopy structure and leaf orientation. This is particularly important at early stages of the crop, when a large portion of the soil is not covered with the crop and it is exposed to the sensor's field of view.

Although there are no known agricultural impacts yet of the research findings, it is expected that they will attract the interest of machine manufacturers to incorporate such sensor in their system. The application of micro-spectrometers in different industrial and other applications received a significant acceleration during the past three years. Many commercial companies world wide have identified the potential and devoted a lot of efforts to develop small, rugged, cheap and reliable spectrometers. During the course of this research, the status of the sensor used advanced dramatically. In the first year of the project, research was initiated using an experimental device provided by the manufacturer (OCLI) upon special request by the research team. Currently, OCLI is producing a commercial sensor that incorporates all signal processing and digitization into a single module with a serial output of the spectral data. The development of the multispectral sensor has occurred independently in parallel to the activities of this project. However, this development makes the potential commercial adoption of the nitrogen sensor envisioned here more feasible.

It still remains to be proven that the application of such a system is profitable and efficient use of fertilizers is achieved. The current research has shown that the nitrogen status of the nitrogen in the crop's canopy can be monitored on-line in the field, The fertilization level affects the nitrogen content. Additional nitrogen application is given where the nitrogen status is below the level that gives maximum yield.

The two research teams worked on the common goal to develop a sensor for nitrogen in-field nitrogen assessment. Each team researched a different aspect of the sensor's technology, the sensor's capabilities and the interaction between the sensor and the crop. Eventually. there was some overlap between the work of the two teams.

The two research teams cooperated in defining the spectral requirements of the sensor, exchanged experimental results and evaluated the field tests of the mobile sensors. During the visit of the Israeli scientist to the US he contributed his experience in data acquisition systems and promoted the development of the sensor.

Both teams used a laboratory spectrometer and performed experiments in order to identifying the spectral requirements for the sensor.

The Israeli team focused on defining the optical setup of the sensing system in order to eliminate background noise and create reliable readings using an off-the-shelf portable sensor and testing the effect of maturity stage on the nitrogen status prediction.

The US team focused its work on the development of the mobile multispectral sensor, development of signal processing algorithms for field operation and tested the effect of different varieties on the performance of the nitrogen sensor. They conducted studies with the multispectral sensor both in a light box with artificial lighting and under lighting ambient conditions with a range of sensor orientation relative to the corn plants. Spectral curves from the corn plants were found to be varying with sensor orientation relative to the corn leaves and sun position. This variation was sufficient to prevent accurate assessment of the leaf nitrogen content. While the potential for sensing under ambient lighting remains, additional development is required to minimize the sources of variability.

Data of leaf spectra was exchanged, and used to establish the spectral regions that are important for the developed sensor. Data of field experiments was exchanged in order to find a common model to predict N status. Such a model could not be derived, primarily due to the fact that the data was acquired using different instruments with different optical and electronic components.

There are no reviewed publications at the moment.

TECHNICAL

APPENDIX

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1. INTRODUCTION

Current heavy reliance on agricultural chemicals in US agriculture has raised many environmental and economic concerns. For an effective use of agricultural chemicals to get maximum yield without raising any environmental concern, farmers need to find a way of applying optimum amount of agricultural chemicals. Among agricultural chemicals, nitrogen (N) is the most important and essential for growing crops and is also most concerning nutrient element for maintaining healthy environment. Its accurate assessment in plants is a key to nutrient management.

N is an integral part of chlorophyll, which is the primary absorber of light energy needed for photosynthesis. If N is used properly in conjunction with other necessary soil fertility nutrients, it can speed the crop growth such as corn and small grains. Plants normally contain between 1 and 5% N by weight (Tisdale et al., 1993). If N is deficient in crop plants, the leaf color changes to light green or yellow-green and the leaf dies starting at the tip.

From the last few years, precision agriculture has been used widely as a management tool to maximize yield and to minimize cost. The GPS and GIS technology has been a basis for precision farming and site-specific application of agricultural chemicals and nutrients has been one of the major concerns in precision farming. By applying nitrogen only where it is needed, farmers could optimize yield while minimizing the cost.

The ultimate goal of this research is to develop a real-time multispectral sensor that can detect nitrogen deficiency in corn plant using spectral response from plant canopies. In this research, a nitrogen sensor for corn was developed. With the nitrogen sensor developed, fertilizers could be applied only where it is needed with accurate amount. A lot of expenses would be saved and environmental concerns would be greatly reduced.

One of the problems in developing a nutrient sensor would be variability of spectral response resulted from different canopy colors. Even when two different corn plants have same amount of nitrogen in their canopies, their spectral response would be different if their canopy colors are different. In this project, as a preliminary step for developing a nitrogen sensor, the leaf characteristic greenness was studied for three different varieties.

The overall objective of this research is to develop a real-time multispectral sensor that could detect nitrogen deficiency in corn plant using spectral response from plant canopies. Eventually the Nitrogen sensor could be used for variable rate fertilizer application. The specific objectives were

to construct an in-field sensor system for spectral measurement,

to build data acquisition system for real-time field use,

to build an algorithm to assess nitrogen status for corn, and

to test the N-Sensor in commercial cornfield.

2. DETERMINATION OF SPECTRAL REQUIREMENTS FOR NITROGEN STATUS PREDICTION

One way to save labor and time is using remote detection. That means that a system measures light reflected from the plant canopy instead of transmitted light, because light reflectance measurement could be made without attaching a meter or probe to a specific leaf. Walburg et al. (1982) indicated that spectral measurements of corn canopies could be used to detect N treatment differences. Thomas and Gausman (1977) showed that leaf reflectance at 550 nm was a good indicator of chlorophyll and carotenoid concentrations for eight different crop, including corn. Recently, Blackmer et al. (1994) found that reflectance near 550 nm measured on detached maize leaves from a field N fertilization experiment was able to separate N treatments. The near infrared/ red reflectance ratio (760 to 900 nm /630 to 690 nm) was reported to differentiate N treatments better than single band reflectance measures (Walburg et al., 1982).

Research to develop application for crop canopy reflectance has focused on wide bandwidths. High spectral resolution devices recently have improved in sensitivity, decreased in cost, and increased in availability (Blackmer et al., 1994). Improved technology and increased interest in site-specific management encourage close examination of relation between reflectance and leaf N concentration and designs of appropriate sensors to detect crop N stress.

Our objectives were: 1) to determine the system parameters necessary to optimize reflectance spectra of corn plants as a function of growth stage and nitrogen status; 2) to determine the relationship between spectral reflectance, plant maturity and nitrogen status.

MATERIALS AND METHODS

Experiment plot design

The experiments were conducted between September, 1998 and February, 1999 at the Institute of Agricultural Engineering, Volcani Center, Israel. Corn (Zea mays L. hybrid 8460) was grown outside greenhouse between 23, Sep. and 1, Nov. 1998, then moved into an unheated greenhouse at 2, Nov. 1998 because of low temperature.

Corn was planted in pots of 12 rows (100-cm spacing) in a south-north direction and 18 pots with two plantings in one row (10-cm spacing between plantings in rows). The density of plantings is 10 plants/m^2 . The volume of pot (r=12 cm, h=26 cm) was 10 liter with growth substrate named Touf M8 which contained no nutrients. The experiment plot was divided into three equal areas consisting of single nitrogen treatments designated as heavy N, middle N and no N . A complete set of plot was planted sequentially at weekly intervals for two weeks, Corn for three planting groups were sown on 23 Sep. , 7 Oct. and 21 Oct. 1998 respectively. There are 24 pots with 48 plants in one treatment per planting group.

Fertigation system

An automatic drip irrigation system with fertilizer injectors was installed for fertigation. There are three submain pipes in the irrigation system. Each submain controls one treatment.

Three fertilizer injectors were installed on three submain pipes which supply water for high N and medium N and no N treatments respectively. The dosing rate of the injector measured is approximately 2 L/m^3 (500:1). A pressure regulator was installed on every submain pipe for uniform fertigation rate. Fertigation took place via 2 L/h compensating emitter, with one dripper per pot (two plants). A simple controller controls the whole system through solenoid valves mounted on the submain pipes and main pipe.

Leaching irrigation was adopted. The leaching fraction (drainage/inflow) was approximately 0.75 L during 23 Sep. to 24 Nov.. Daily water application was 4 L/pot/day during the above periods. The number of irrigation per day was 2 in whole growth periods. Because the difference of the leaf tissue nitrogen measured in the lab between medium N and high N treatment was very small irrigation time was reduced to 20 minutes per time for all treatments from 24, Nov. That means the daily water application was reduced to 2.4 L/pot/day.

The three different fertilizer solutions from liquid fertilizer supplier for the three treatments were:

0:3:12 ($N:P_2O_5:K_2O$) for no N treatment

3:3:12 for middle N treatment

7:3:12 for heavy N treatment

Through a 500:1 fertilizer injector the concentration of N, P and K in the irrigation water were regulated to 0 mmol N, 4 mmol N (56 ppm) and 10 mmol (140 ppm) N for no N, middle N and heavy N treatments with same ratio of 5 mmol K (195.5 ppm) and 0.8 mmol P (24.8 ppm).

Measurements

Measurements were conducted at V6 stage and VT stage, including leaf area, leaf weight, leaf tissue nitrogen and leaf reflectance. Corn parameters, crop phenology and sampling date are shown in table 2.1. Measurements for VT stage in group one and group three were delayed by cloudy day and high humidity.

Table 2.1. Cultural parameters, crop phenology and sampling date

Group	Group 1	group 2	Group 3
Planting date	Sep.23	Oct. 7	Oct.21
Emergence date	Sep. 28	Oct. 13	Oct. 27
V6 date/days from planting date	Oct.21-25/28-32	Nov.7-10/30-33	Nov.21-24/30-33
Reading for V6 stage	Oct. 25,27	Nov.10	Nov.24
VT date/days from planting date	Nov.22-Dec.2/60-70	Dec.15-27/68-80	Jan.12-19/ 81-88
Reading for VT	Dec.6,7,13	Dec. 20, 21,28	Jan.25,28,31

Leaf area samples were taken on the newest fully expanded leaf (exposed leaf collar, called flag leaf) at V6 and VT stage during vegetative growth. The measurements were performed around solar noon ±1 h. Flag leaves were cut from collar. Leaves were placed immediately in plastic bags and were returned to the laboratory for leaf area and reflectance measurement. Twenty flag leaves were randomly collected per treatment and per sown group. Leaf weight was measured by a high precision balance. Leaf area was measured by scanning the sampled leaves with a bed-scanner. Simple image processing analysis was used to estimate leaf area from the scanned images. Leaf weight combined with leaf area was used to estimate leaf specific weight and leaf thickness.

Spectral reflectance of the leaves was measured in the range of 600-1200 nm at 0.5 nm intervals using a Quantum 1200 scanning spectrophotometer with the SpectraMetrix V1.70 software and LighTcal Plus V1.0 multivariate regression package (L.T. Industries Inc.Rockville, MD 20852). 1-inch-diameter leaf punches were taken on the leaf (same leaf used for leaf area measurement) at a point approximately half the distance from the leaf tip to the collar. Leaf disk was weighed. Spectral measurements were made on the leaf punches. Background reflectance was firstly measured at each measurement. Relative reflectance (leaf reflectance divided by background reflectance) data of leaf samples were collected and stored on the harddisk of a computer.

N concentration (g N/g DM) of leaf (same leaf used for leaf area and spectral reflectance measurements) was measured using a Technicon autonalyzer. These samples were dried at 63 °C for 48 h and then ground in a dental mill.

RESULTS AND DISCUSSION

Corn canopy development

Growth rate

All three N treatments developed uniformly before V6 stage. Corn growth rate reduced down from December due to lower temperature. The growth rate difference until V6 stage was only 3-4 days between no N and the other two N treatments. The numbers of days from planting to VT vary among repetitions. First repetition group reached VT 60-70 days after planting (depending on treatment), while the last repetition group reached VT 81-88 days after planting. This difference in growth rate is due to changes in temperature and day-light period during the season. Temperature was higher and day-light period was larger during first repetition and they decreased for second and third repetitions. Growth rate was also affected by the nitrogen treatment. Corn growth for no nitrogen treatment reached VT about 10 days later than the other two treatments (see table 2.1)

Leaf area

Nitrogen deficiency leads to reduced leaf area. Average leaf area in high nitrogen treatment in all three planting groups was increased 300% and 190% comparing to no nitrogen treatment at V6 and VT stage respectively. An average leaf area in middle nitrogen treatment in three planting groups was increased 300% and 170% comparing to no nitrogen treatment at V6 and VT stage respectively. At V6 stage the difference in leaf area between high nitrogen treatment and middle nitrogen treatments not significant in all three planting group. At VT stage the difference is significant, except in the third planting group. Table 2.2 shows the results and the statistical analysis for leaf area at V6 and VT stage.

Table 2.2. Mean value for leaf area in cm^2

Treatment	Stage	Mean	SD	SDD	Range	n
High nitrogen	V6	160.43	52.83	35.85	44.18-265.45	60
	VT	312.79	70.40	26.88	187.64-471.18	59
Middle nitrogen	V6	164.26	64.66	47.74	58.16-386.01	60
	VT	281.99	71.28	56.63	163.78-487.81	60
No nitrogen	V6	54.462	25.96	12.78	15.10-125.91	60
	VT	165.25	35.77	9.77	99.11-259.95	60

SD: standard deviation of total samples
SDD: standard deviation of differences between three planting data groups.
n: number of samples

Table 2.3. Mean value for specific leaf weight in g /cm^2 (on a fresh weight basis)

Treatment	Stage	Mean	SD	SDD	n
high nitrogen	V6	0.0335	0.0041	0.0025	60
	VT	0.0185	0.0013	0.0004	60
middle nitrogen	V6	0.0330	0.0040	0.0030	60
	VT	0.0179	0.0012	0.0008	60
no nitrogen	V6	0.0240	0.0025	0.0010	60
	VT	0.0159	0.0011	0.0003	60

SD: standard deviation of total samples
SDD: standard deviation of differences between three planting data groups.
n: number of samples

Specific Leaf weight

Specific leaf weight is defined as the mass of leaf per unit surface. Although specific leaf weight is not a direct measurement of thickness, it can be used to estimate leaf thickness if one assumes a universal leaf tissue density across treatments. Both light absorption and reflectance are affected by leaf thickness (Woolley, 1971). Table 2.3 shows the results and statistical analysis for specific leaf weight at V6 and VT stage. Not only N fertilizer affected leaf thickness, but also corn growth stage gives more influence on leaf thickness. We can see in table 2.3 that specific leaf weight at V6 stage in high and middle N treatment is almost two times of specific leaf weight at VT stage. Specific leaf weight did not change as fertilizer N rate increased from 0 to 56 ppm at VT stage. Nevertheless, figure 2.2 shows that leaf reflectance at 650 nm changed at least 20% across these two N rates. On the other side, specific leaf weight increased 50% between V6 and VT stage in no N treatment, but by comparing Fig. 2.4 and Fig.2.5 it can be seen that leaf reflectance at 650 nm maintained almost same. Therefore, specific leaf weight or leaf thickness could not be an important factor which affect the leaf reflectance.

Leaf nitrogen concentration

Table 2.4 shows the results of the statistical analysis for leaf nitrogen at V6 and VT stage. N concentration in high and middle N treatment was not significantly different, both in V6 and VT stage. The treatment with no N fertilizer resulted to significantly lower leaf N concentration, as expected.

Table 2.4. Mean value for leaf nitrogen concentration (g N/g DM) %

Treatment	Stage	Mean	SD	SDD	Range	n
high nitrogen	V6	4.82	0.57	0.44	3.97-6.90	60
	VT	3.32	0.61	0.69	2.5-4.64	60
middle nitrogen	V6	4.16	0.78	0.74	2.16-5.45	60
	VT	3.22	0.41	0.43	2.49-4.08	60
no nitrogen	V6	2.76	0.86	0.38	1.12-5.00	60
	VT	2.67	0.35	0.33	1.81-3.28	60

SD: standard deviation of total samples
SDD: standard deviation of differences between three planting data groups.
n: number of samples

The above results for leaf area, specific leaf weight and leaf N concentration show that the difference in all aspects between high N and middle N treatment is very small This is contributed to the fact that irrigation volume was too high and plants received almost the same amount of nitrogen from medium although N concentration in the irrigation water was different.

Leaf reflectance

Raw sample data pretreatment

A total of 360 leaf samples were measured for three treatments in V6 and VT stage of corn growth, however, only 344 samples were used for spectral analysis, 172 samples for each growth stage. The reflectance data of 8 samples in each stage were deleted due to big measurement error of spectral reflectance based on initial analysis of raw data.

The data for wavelengths from 600 nm to 650 nm and from 1150 nm to 1200 nm were deleted due to low sensitivity of the spectrophotometer, only data in the region of wavelength from 650 nm to 1150 nm were used to analysis spectral.

Spectral characterization

Mean relative reflectance for different N treatments at V6 and VT stage are shown in Fig.2.1 and Fig.2.2. The relative reflectance of leaf in the region of 730 nm to 1150 nm in the high N treatment is higher than that in the middle and no N treatment at V6 stage, however, the difference of relative reflectance between high N treatment and middle N treatment is very small. This is due to similar N concentration of leaf in both treatments which was caused by too much irrigation volume during corn growth. Fig. 2.2 shows that the reflectance in the high N treatment is lower than middle N treatment at VT stage because the average N concentration of leaf in high N treatment (2.93%) is lower than average N concentration in middle N treatment (3.22%). A common characteristic for all leaf is that relative reflectance of corn leaf is lower in the visible region (wavelength <690 nm) than in near infrared region. The relative reflectance increases sharply from 700 nm to 750 nm and keep stable after 750 nm. There are three low peak centered at about 675 nm, 970 nm and 1150 nm on reflectance curve.

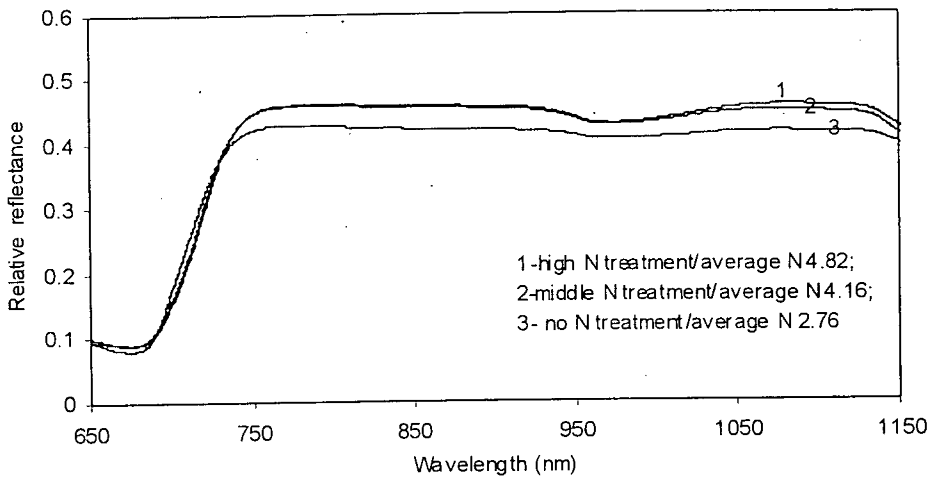

Fig.2.1 Mean relative reflectance of leaf in three N treanment at V6 stage

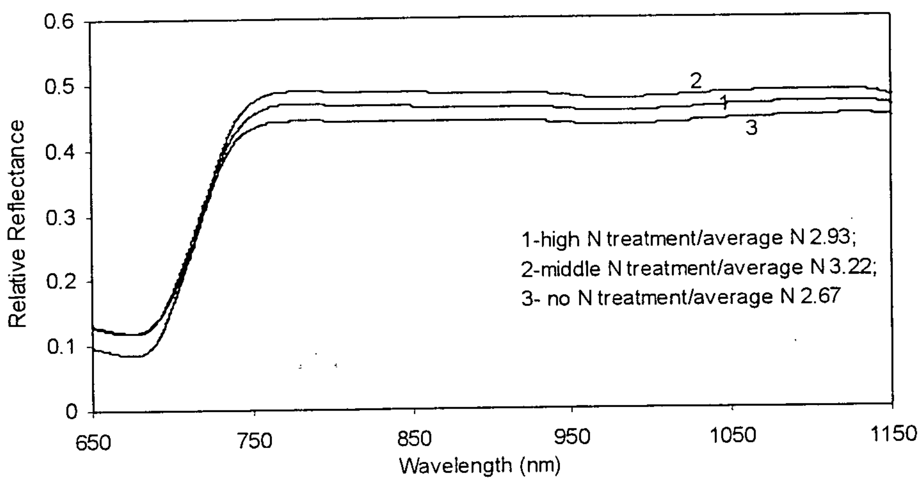

Fig.2.2 Mean relative reflectance of leaf in three N treanment at VT stage

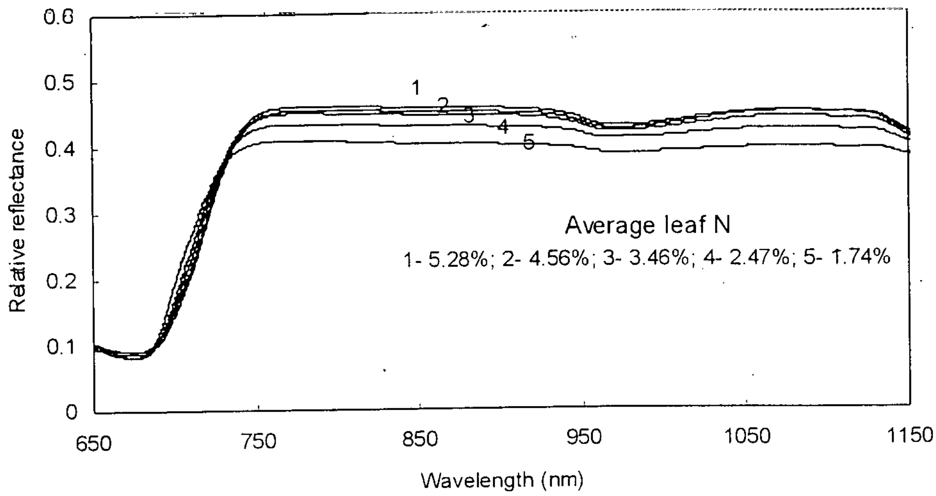

Fig.2.3 Mean relative reflectance of leaf based on sorting leaf N at V6 stage

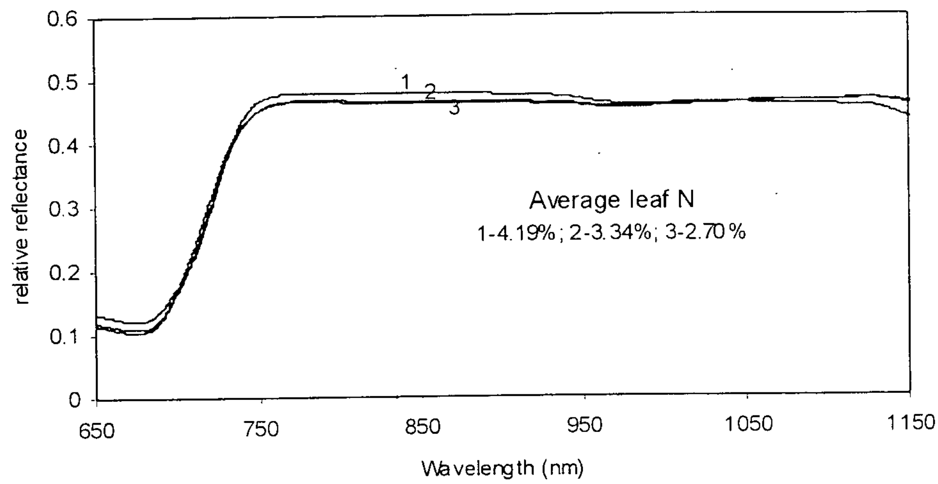

Fig.2.4 Mean relative reflectance of leaf based on sorting leaf N at VT stage

We found there is an overlap of N concentrations of leaf samples exists in different N treatment. Therefore the samples were sorted by nitrogen concentration of leaf, and then divided to four groups based on N concentration (6%-5%, 5%-4%, 4%-3%, 3%-2%). The reflectance curves for different N concentration groups for V6 and VT stage are shown in Fig.2.3 and Fig.2.4. We can see the relative reflectance of leaf increases with N concentration in the region from 730 nm to 1150 nm and decreases with N concentration in the region from about 700 nm to 730 nm.

Calibration and prediction

We used PLSR (partial least squares regression) and PCR (principle component regression) model with different data pretreatment (first derivative, second derivative, logarithms, Kubelka-munk) for calibration to seek best model configuration. The samples data was split randomly to two third as a calibration set and one third as a prediction set . Table 2.5 shows the corresponding calibration statistics for the samples of (V6+VT), V6 and VT stage. The regression coefficients are 0.7, 0.86, 0.81, respectively. The PCR model (no listed in table 2.5) for the above three groups are not better than PLSR model. The samples of (V6+VT) give much poorer calibration statistics than separated V6 samples or VT samples. This may be because the leaf thickness at V6 stage is different from VT stage

Table 2.5. Calibration results

Data	Data pretreatment	Model	Factor	R^2	SEP	TVD %	n
V6+VT	Second derivative	PLSR	6	0.70	0.47	76.42	344
V6	Second derivative	PLSR	10	0.86	0.39	87.84	172
VT	First derivative	PLSR	6	0.81	0.22	83.70	172

R^2: regression coefficient
SEP: standard error of prediction in calibration
TVD: total variance described in calibration
n: sample number

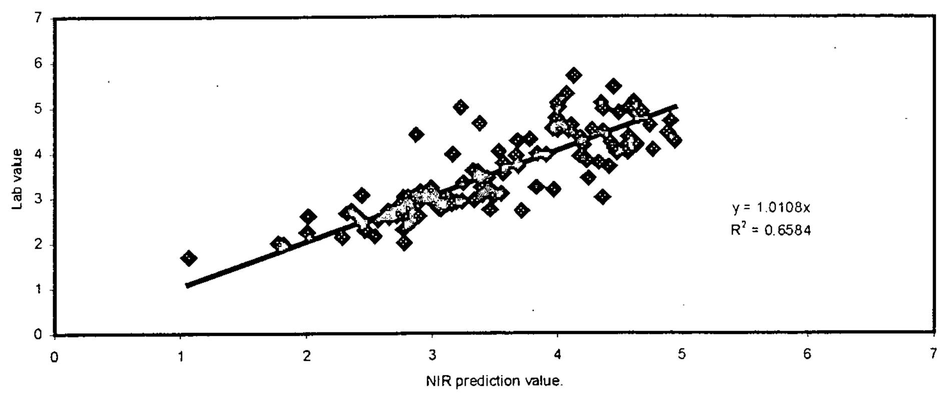

Fig.2.5 Prediction results fo (V6+VT) (PLS, six factors)

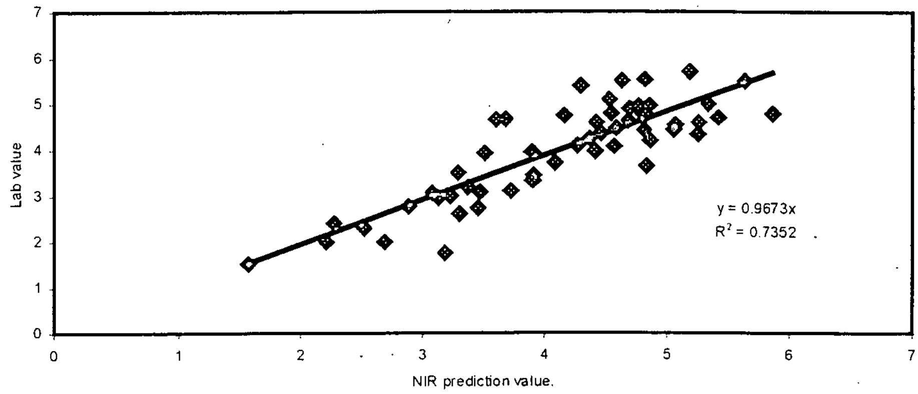

Fig.2.6 Prediction results fo V6 (PLS, ten factors)

Prediction results for data of (V6+VT), V6 and VT are shown in Fig.2.5, Fig.2.6 and Fig.2.7. The regression coefficient between prediction values and Laboratory values is 0.66, 0.74 and 0.84 for (V6+VT), V6 and VT respectively. The results suggest that calibration should be done separately for different growth stages of corn. But what factor cause the result is not clear.

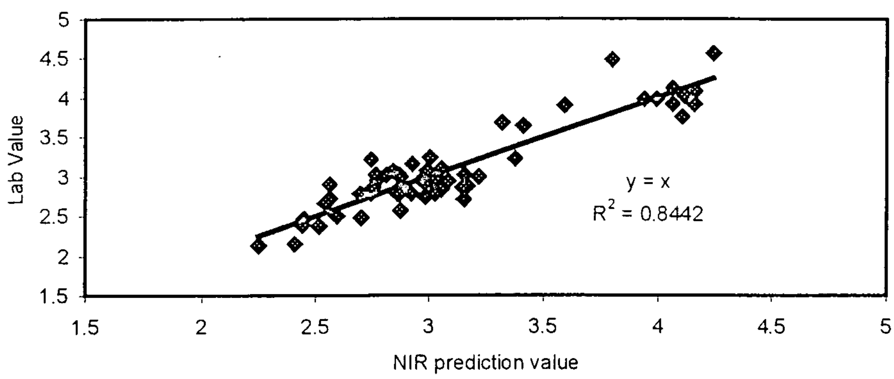

Fig.2.7 Prediction results fo VT (PLS, six factors)

CONCLUSIONS

A relatively strong correlation between leaf relative reflectance and leaf N was found. This indicates that reflectance techniques are promising for detection of corn N status. PLSR models with corresponding data pretreatment and factors can be used to predict leaf N concentration. The results of multivariate calibration for V6 and VT stage separately were better than for the mixture data of V6 and VT stage. The results in this experiment show also that the 10 factors and 6 factors in the PLSR calibration model for V6 and VT stage are optimum respectively.

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3. ASSESSING NITROGEN STRESS IN CORN VARIETIES OF VARYING COLOR

The ultimate goal of this research is to develop a real-time multispectral sensor that can detect nitrogen deficiency in corn plant using spectral response from plant canopies. One of the problems in developing a nutrient sensor would be variability of spectral response resulted from different canopy colors. Even when two different corn plants have same amount of nitrogen in their canopies, their spectral response would be different if their canopy colors are different. In this project, as a preliminary step for developing a nitrogen sensor, the leaf characteristic greenness was studied for three different varieties.

The objectives of this research were to determine the effect of leaf greenness in assessing nitrogen status using the spectral reflectance of corn leaves, and to develop a model that can predict nitrogen content from leaf reflectance measurement. This information could form the basis for development of a plant nitrogen sensor. With the development of the nitrogen sensor system, fertilizer could be accurately applied to specific locations only where needed.

BACKGROUND

Many researchers have tried to utilize visible and near infrared spectral response from plant canopies to detect plant status such as moisture content (Rigney and Brusewitz, 1997) and nutrient stress (Thomas and Oerther (1972), Al-Abbas et al. (1974), Walberg et al. (1982), Blackmer et al. (1994), Yoder and Pettigrew-Crosby (1995), Filella et al. (1995), Blackmer et al. (1996), Ma et al. (1996), Masoni et al. (1996), and Bausch et al. (1998)). The most common fact found by those studies is that reflectance near 550 nm shows good separation of leaf nitrogen concentration, thus can be used to detect N deficiency of crop plants. Reflectance ratios at other wavelengths were also studied for their capability to distinguish N status such as [550 nm and 675 nm] (Thomas and Oerther (1972)), [630 nm, 690 nm, 760 nm and 900 nm] (Walberg et al. (1982)), [450 nm, 630 nm, 690 nm, 710 nm, 760 nm, 800 nm, and 900 nm] (Blackmer et al. (1996)), and [702 nm] (Bausch et al. (1998)).

While these studies relied on laboratory work to assess nutrient status of the plants, some researchers have tried to implement a system for a real-time field use. Stone et al. (1996) developed a sensor for nitrogen detection and weed detection with photodiode sensing elements. They tested the sensor with winter wheat and found that reasonable correlations were obtained between N uptake and NDVI (Normalized Difference Vegetation Index). They reported that the interference of solar angle needed to be evaluated. Sui et al. (1998) developed a spectral reflectance sensor to detect nitrogen status of cotton plants with 4 spectral bands of blue, green, red and near infrared light. They tested the sensor in 2 situations: with an artificial illumination and with natural illumination. They reported the preliminary test results for diagnosing nitrogen status in cotton was promising.

A new commercial nitrogen sensor has just started being marketed (model: Hydro N-Sensor, Miles Opti-Crop division, Owensboro, KY). It measures crop reflectance with 4 light sensors (@gInnovator, 1999). A fifth sensor was used for compensating the sunlight change. From the field trials for wheat, the sensor reportedly increased the yield by 471 kg/ha (7 bu/ac).

Another way of assessing nitrogen stress is to use a chlorophyll meter since nitrogen is closely related to chlorophyll in the leaf. One widely used chlorophyll meter is The SPAD-502 (Minolta, Co.). The SPAD meter measures light transmittance at 650 nm as a source of chlorophyll concentration and at 950 nm as a reference to compensate such effect as leaf moisture content and thickness (Blackmer and Schepers (1995)).

Most of the studies related to the chlorophyll meter was to evaluate its feasibility for assessing nitrogen status of crop plants (Piekielek and Fox (1992), Schepers et al. (1992), Tracy et al. (1992), Blackmer and Schepers (1994), Smeal and Zhang (1994), and Piekielek et al. (1995)). Blackmer and Schepers (1995) tried to evaluate the ability of SPAD meter as a nitrogen assessment tool for corn plants. They found that a statistically significant linear correlation existed between grain yield and the chlorophyll meter reading at R4 (dough stage) and R5 (dent stage), however also reported that this trend was questionable since it was largely related to differences between sites instead of differences due to N applied within sites. Wood et al. (1992) conducted an experiment for feasibility of using field chlorophyll measurements for evaluation of corn nitrogen status. They found that significant curvilinear relationships between SPAD readings and tissue N concentrations at the V10 and midsilk stages (R^2 = 0.89 ~ 0.91) and between SPAD readings and grain yield (R^2 = 0.82 ~ 0.88).

Utilization of reflectance has also been used as a basis for remote sensing technology with the fact that different object has different reflectance at a given wavelength. Menges et al. (1985) studied a feasibility of using plant canopy reflectance as a means of distinguishing weeds from crops. Using color infrared (CIR) aerial photography, they successfully classified some crop plants and weed species with efficiency of 68% ~ 100%. GopalaPillai et al. (1998) used high resolution color infrared aerial images for detecting nitrogen stress in corn. They found that the canopy reflectance was well correlated with the applied nitrogen and that the yield could be predicted well from the canopy reflectance in the red channel. Thai et al. (1998) used spectral video images of bush bean plants taken with two bandpass filters to assess nitrogen status of the plants. They used neural network to distinguish different nitrogen treatments and reported that the two selected bands could be used to distinguish different nitrogen treatments.

MATERIALS AND METHODS

Field planting of corns

The main objectives of this research were to examine the effect of leaf characteristic greenness on the spectral reflectance of plant leaves with similar nitrogen status, and to determine if leaf reflectance can predict nitrogen status. To accomplish these objectives, three corn varieties with a wide range of characteristic color were chosen with the assistance of the local Asgrow Seed company representative. In order to observe the nitrogen effect to corn growth, five different nitrogen treatment levels were selected, Table 3.1. Prior to planting, soil samples were collected for examining soil fertility from 6 sampling locations at three different depths (0.22 m, 0.45 m and 0.90 m). All soil samples showed very low in nitrogen (1 ~ 19 ppm).

Table 3.1. Corn varieties and nitrogen treatment used in the experiment.

Variety (Asgrow)	N treatment (kg/ha)
	0 (N1)
RX897 (V1, lighter color)	67.0 (N2)
RX901W (V2, medium color)	134.1 (N3)
RX938 (V3, darker color)	201.1 (N4)
	268.1 (N5)

The corn was planted at the seeding rate of 59,300 seeds/ha on March 16, 1999 in the Texas A&M University experimental farm located in Brazos River bottom near College Station. The field was composed of 48 rows of 0.76 m wide and 213.4 m long and was arranged as a split-plot design with 4 replications, Figure 3.1. The split-plot design was selected since it was more practical for a field experiment and it was difficult to plant different variety in a small area. The field was composed of 60 subplots. Each subplot contained 4 0.76-m wide rows and 30.5 m long and had one variety and one nitrogen treatment. Nitrogen was applied in the form of UAN (Urea Ammonium Nitrate, 32% N) on March 26. All other cultural practices were managed conventionally.

Reflectance measurement

The reflectance measurement system (a monochromator (model: CM110, CVI Laser Corp.), a detector (model: AD120, CVI Laser, Corp.), an integrating sphere (model: IS040SL, Labsphere Inc.), and lighting system (model: AS220, CVI Laser Inc.)) was set up in a laboratory. Prior to measuring reflectance, the system was turned on for an hour prior to testing.

Five plants were randomly selected from the middle two rows in each subplot for sampling from Field B1 and B4, avoiding any boundary effects from the adjacent rows of different nitrogen treatment. A 2.5-cm diameter leaf disk was obtained from the middle portion of an ear leaf and a fully developed younger leaf from each plant using a specially designed circular knife. They were quickly put in a plastic bag and transported from the field to the laboratory (15 min. driving distance). The plants were at R3 (milk stage) to R5 (dent stage) growth stages (Ritchie and Hanway (1982)).

The soil contained enough moisture for the plants to maintain their vigor with 4 days' rain in the previous week of leaf sampling. Each sample was measured its reflectance from 400 nm to 1100 nm with 4 nm increment. Reflectance of a white reference was measured for every 5 samples using a reference cap painted with barium sulfate ($BaSO_4$).

There were 4 data sets of reflectance measurement, Table 3.2. The EarB4 and EarB1 are reflectance measured from ear leaves in Field B4 and B1, respectively. The

YoungerB1 and YoungerB4 were obtained as the same manner. Each data set from Field B1 and B4 consisted of 150 reflectance spectra (75 spectra for ear leaf and 75 spectra for younger leaf) with 2 dêpendent variables (variety and nitrogen treatment) and 176 independent variables (spectral bands).

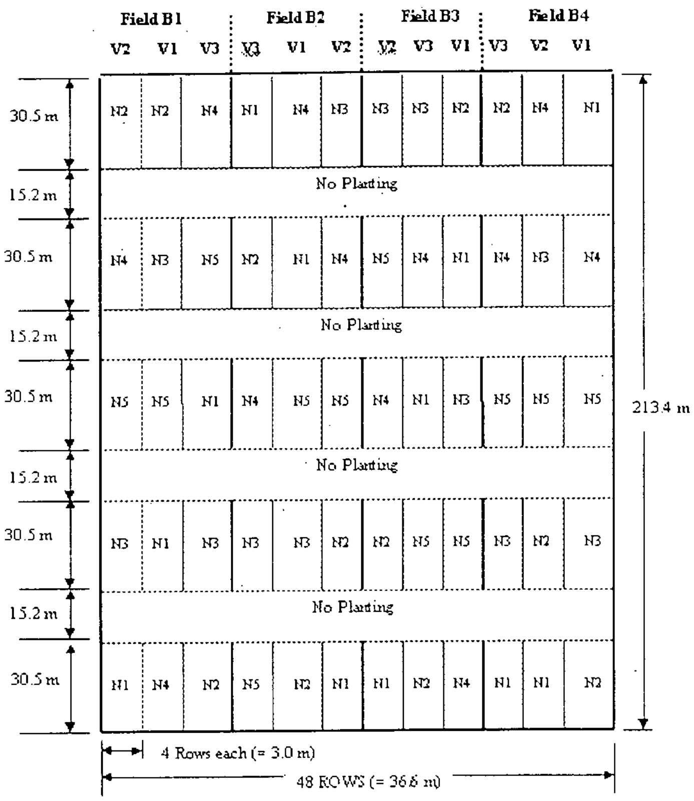


Figure 3.1. Field corn planting and N treatment layout.

Table 3.2. Reflectance measurement data set.

Data set	Description
EarB4	Ear leaf reflectance from Field B4, June 17-18, 1999
YoungerB4	Younger leaf reflectance from Field B4, June 17-18, 1999
EarB1	Ear leaf reflectance from Field B1, June 29-30, 1999
YoungerB1	Younger leaf reflectance from Field B1, June 29-30, 1999

Nitrogen analysis and chlorophyll measurement

The nitrogen content was analyzed for the leaf samples using LECO method (Sheldrick, 1986). A set of five leaf disks from a subplot was submitted as one sample for nitrogen analysis. Additionally, 4 sample sets of 5 leaf disks were selected such that reflectance in one set had uniform reflectance and the other set had varying reflectance for ear leaf and younger leaf. These samples were analyzed for individual nitrogen concentration to see whether the samples of uniform reflectance had actually homogeneous nitrogen concentration and whether the samples of varying reflectance had different nitrogen concentration in each sample. For each sample (2.5-cm leaf disk), its chlorophyll content was measured from five different locations with a chlorophyll meter (model: Spad-502, Minolta Co.). A regression analysis was conducted to correlate the SPAD measurements with actual nitrogen content of samples.

Multivariate statistical analysis

The objective of the statistical analysis was to find the wavelengths which best discriminate nitrogen treatment and variety. A series of multivariate statistical analysis was conducted.

First, a correlation analysis was conducted between nitrogen amount and reflectance at each wavelength to look at how much correlation there would be. The SAS procedure CORR (SAS, 1990) was used for this step. Then, in order to reduce the number of variables, a stepwise discriminant analysis was conducted for each data set. Since the stepwise discriminant analysis does not select the best subset of wavelengths due to multi-colinearity, the result from this analysis was used for further analysis. The SAS procedure STEPDISC was used for this process with stepwise selection method and significance level of 0.15.

With the wavelength bands selected by the STEPDISC, a canonical discriminant analysis was conducted. Canonical discriminant analysis is a dimension-reduction technique related to principal component analysis and canonical correlation. Given a classification variable and several quantitative variables, this analysis derives canonical variables (linear combination of the quantitative variables) that summarize between-class variation in much the same way that principal components summarize total variation. For canonical analysis, the SAS procedure CANDISC was used.

Additionally, principal component analysis was executed with the selected wavelengths by the STEPDISC procedure. Principal component analysis is generally used to maximize the variance of a linear combination of the variables and is used when highly correlated independent variables may produce unstable estimates. For this step, the SAS procedure PRINCOMP was used.

Prediction of nitrogen content

Another commonly used method to analyze spectral data is Partial Least Square (PLS) regression. PLS is a quantitative spectral decomposition technique that is closely related to Principal Component Regression (PCR). PLS finds factors that capture the greatest amount of variance in the predictor variables. The PCR method combines the Principal Component Analysis (PCA) spectral decomposition with an Inverse Least Squares (ILS) regression to crate a quantitative model for complex samples. In this research, PLS, PCR and MLR (multiple linear regression) were conducted to build a calibration model and compare their performance. The MATLAB (The Mathworks, Inc., Ver 5.0) PLS_Toolbox (Eigenvector Research, Inc., Ver 2.0, Manson, WA) was used for the analysis. The EarB1 and YoungerB1 were used for building calibration model and the EarB4 and YoungerB4 were used to validate the model. The standard error of prediction for calibration data (SEC) and the standard error of prediction for validation data (SEP) are calculated as follows (Williams and Norris, 1987).

$$\text{SEC} = \left\{\frac{\sum e^2}{n-2}\right\}^{1/2}$$

$$\text{SEP} = \left\{\frac{(\sum e^2 - (\sum e)^2/n)}{n-1}\right\}^{1/2}$$

where,

$e = y - y'$,

y = actual nitrogen content (%),

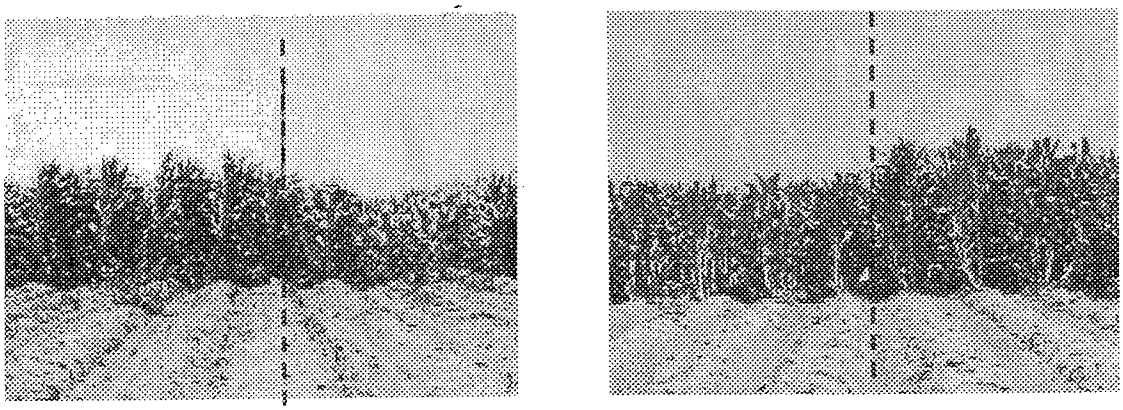
y' = predicted nitrogen content (%),

n = number of samples.

RESULTS AND DSCUSSION

N fertilization effect

Figure 3.2 shows some examples of the nitrogen treatment and varietal effects on their growth. These pictures were taken on May 14, 1999, 63 days after their planting. The line in the middle indicates an intersection of the two different varieties or two different nitrogen treatments. These figures show clear effect of different nitrogen treatment. The more nitrogen was applied to the plants, the taller the plants were. The leaf color of three different varieties was also examined with the plant leaves of no N applied. The order of the greenness from lighter to darker green was RX897 (V1), RX901W (V2) and RX938 (V3). This order confirmed the leaf characteristics originally obtained from the seed company.



(a) V3N4 & V3N1
(1.8 m tall) (1.5 m tall)

(b) V2N2 & V3N5
(1.2 m tall) (2.0 m tall)

Figure 3.2. Examples of nitrogen treatment effects on corn growth.

Leaf reflectance measurement

Figure 3.3 – 3.6 show typical reflectance spectra of the samples. Figure 3.3 shows N treatment effect on one variety for the ear leaf and the younger leaf. The first noticeable characteristic of the reflectance spectra is that the samples from the lower N treatment subplots (0 and 67.0 kg/ha N) showed higher reflectance near 550 nm than those from high N treatment subplots (134.1, 201.1 and 268.1 kg/ha N). At 550 nm, the more nitrogen was applied to the plants, the lower reflectance they had. This is because the samples from the high N treatment subplots had more nitrogen and chlorophyll in their leaves, which absorbed more light around 550 nm. The samples from the low N treatment subplots had also high reflectance in near infrared range.

Another important feature is that the reflectance between 400 nm and 500 nm was nearly identical for most of the samples regardless of their variety and N treatment levels. This fact is especially useful when a ratio would be used to distinguish different nitrogen amount from the leaves (e.g. ratio of 450 nm to 550 nm). All spectra had a dip around 980 nm, corresponding to a water absorption band.

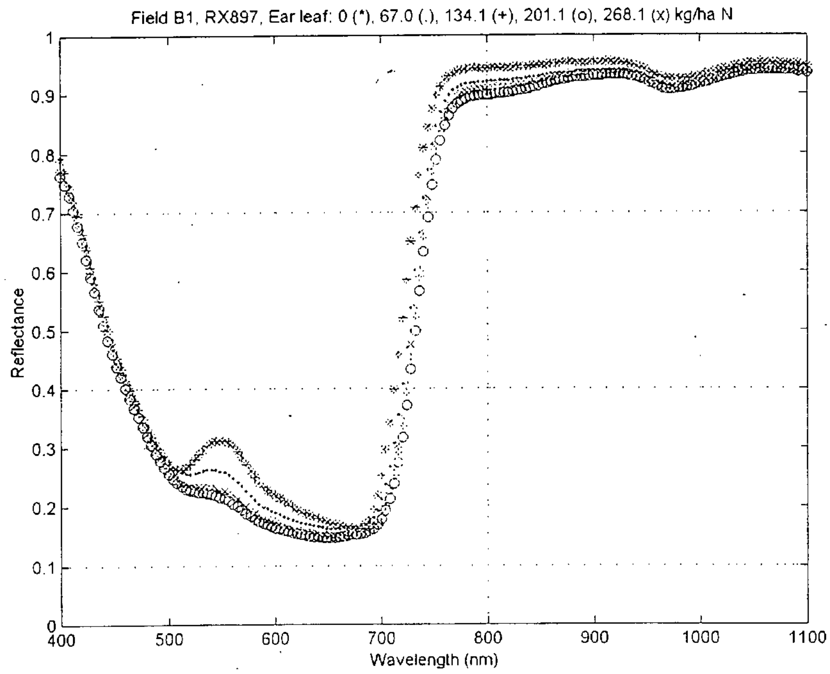
Figures 3.4 – 3.6 show the reflectance spectra of the three varieties for the zero, medium and highest nitrogen applications. In Figure 3.4, differences appear to relate to varietal differences, while figures 3.5 and 3.6 show little difference in reflectance. However, closer examination of the nitrogen analyses results show that the differences in the reflectance curves for the zero nitrogen treatment may be explained by nitrogen status variations. For the ear leaf, the nitrogen concentrations were 1.95, 1.25 and 2.37 percent for the RX897, RX901W and RX938, respectively. The stronger impact of nitrogen content is suspected since the highest reflectance was the medium colored variety and the lowest N percent.

To statistically evaluate the impact of variety and N treatment on the reflectance difference, a means test was conducted with a reflectance value at 552 nm using the SAS procedure GLM. The significance level was 0.01. The ear leaf data was taken from EarB1 and EarB4 and the younger leaf data was from YoungerB1 and YoungerB4. The result is shown in Table 3.3. Underlined treatments are not significantly different at $\alpha = 0.01$.

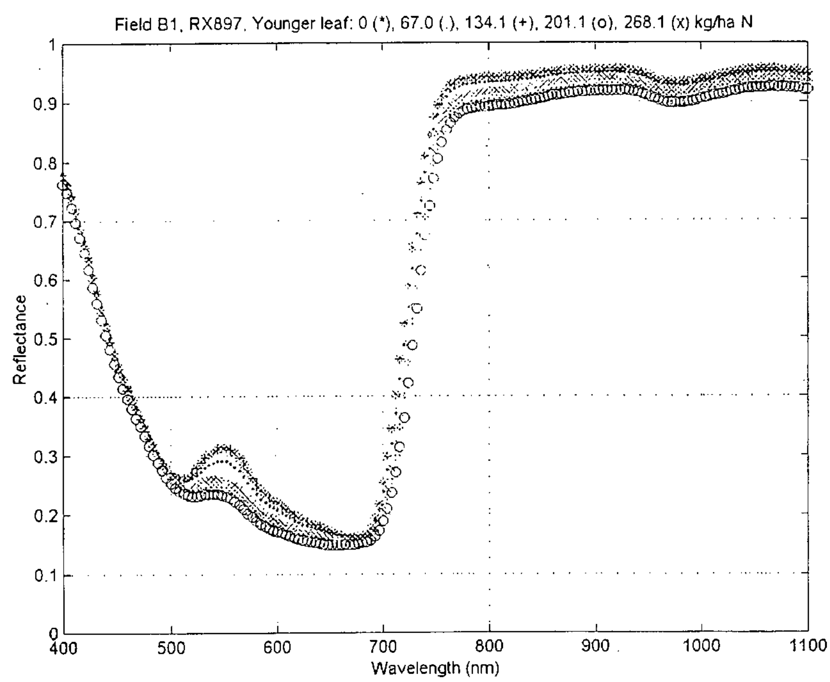
The variety effect to the reflectance at 552 nm was not significant at significance level 0.01 except the Waller test for both the ear leaf and younger leaf samples.

Table 3.3. Means test with reflectance at 552 nm ($\alpha = 0.01$).

Test method	Waller	Duncan	Tukey
Ear leaf by variety	V2 V3 V1	V2 V3 V1	V2 V3 V1
Ear leaf by N treatment	N1 N2 N3 N4 N5	N1 N2 N3 N4 N5	N1 N2 N3 N4 N5
Younger leaf by variety	V2 V1 V3	V2 V1 V3	V2 V1 V3
Younger leaf by N treatment	N1 N2 N3 N4 N5	N1 N2 N3 N4 N5	N1 N2 N3 N4 N5

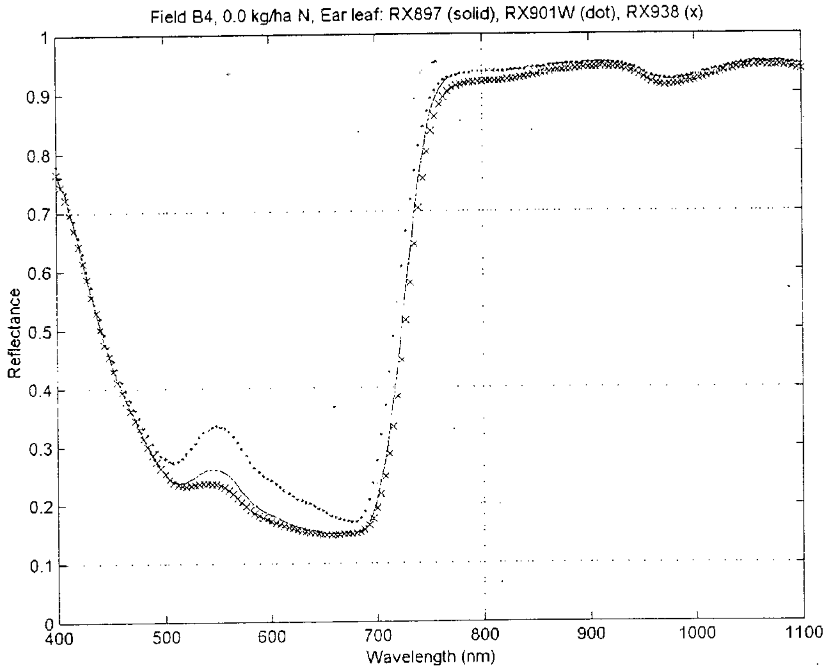


(a) Ear leaf.

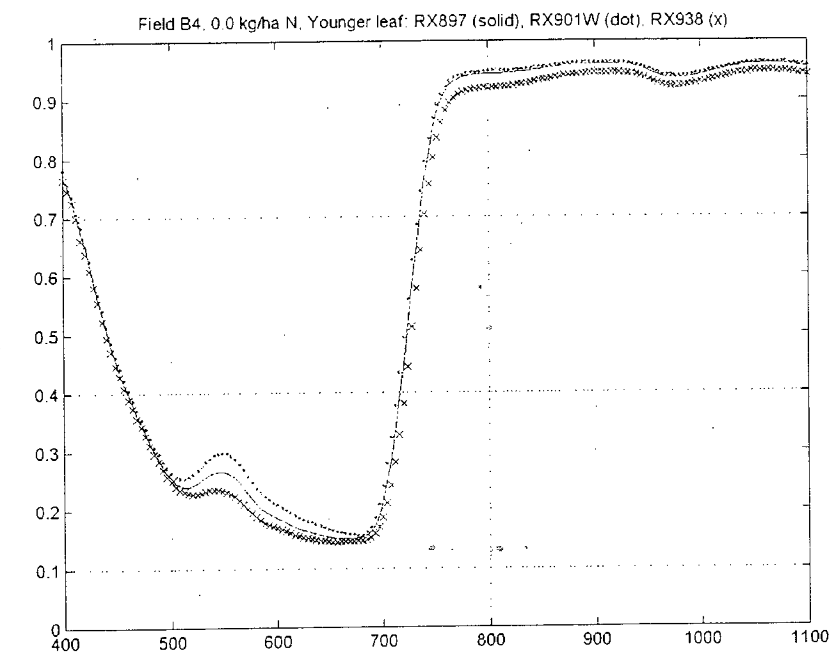


(b) Younger leaf.

Figure 3.3. Reflectance of ear leaf and fully developed younger leaf of V1 (RX897) with 5 different nitrogen treatments.

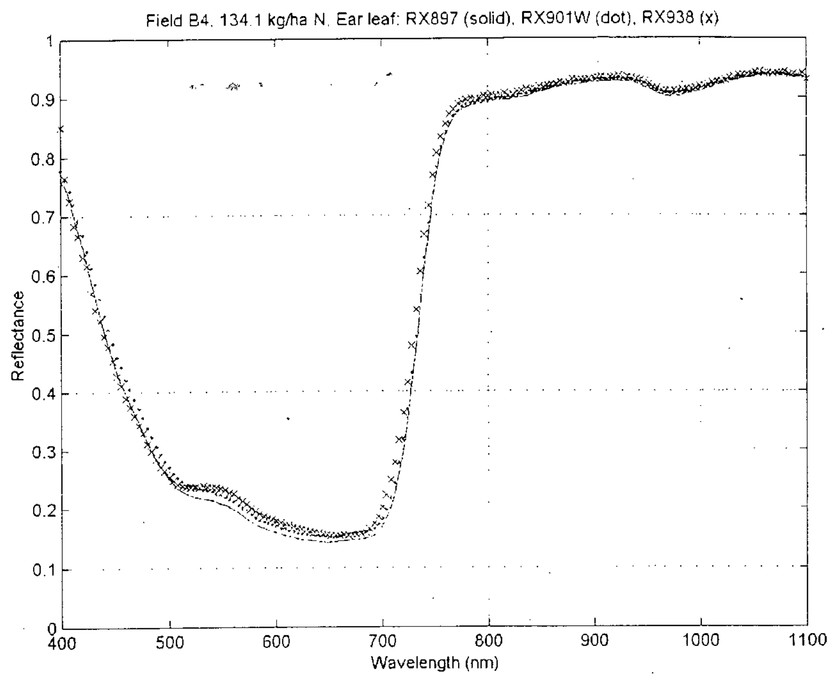


(a) Ear leaf. N percents were RX897-1.95%, RX901W-1.25%, RX938-2.37%.

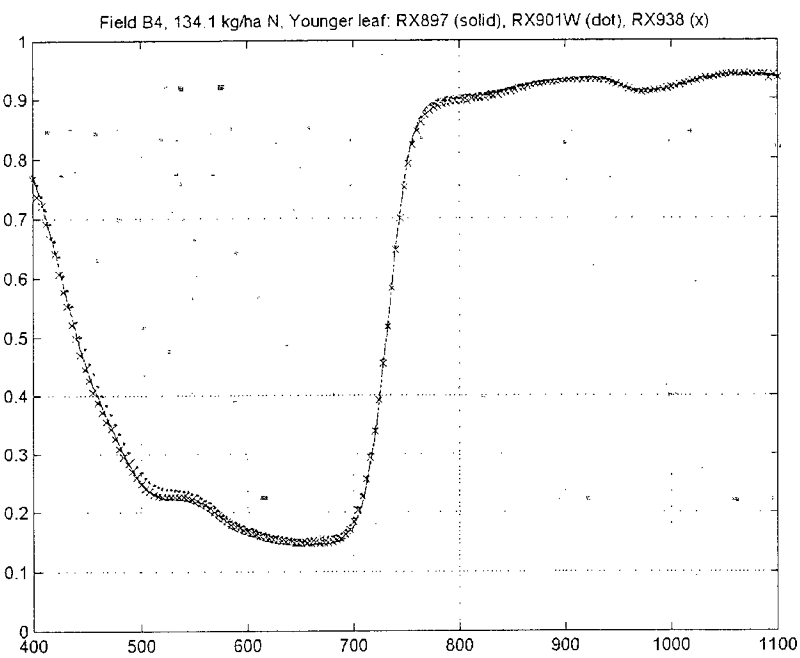


(b) Younger leaf. N percents were RX897-2.11%, RX901W-1.42%, RX938-2.36%.

Figure 3.4. Reflectance of ear leaf and fully developed younger leaf of V1 (solid), V2 (dot), and V3 (x) with 0.0 kg/ha N treatment.

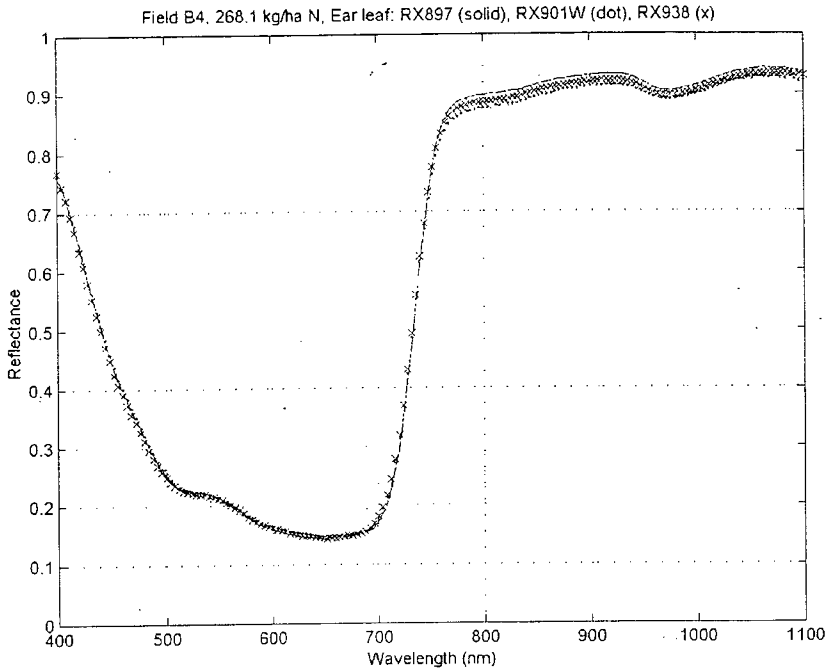


(a) Ear leaf.

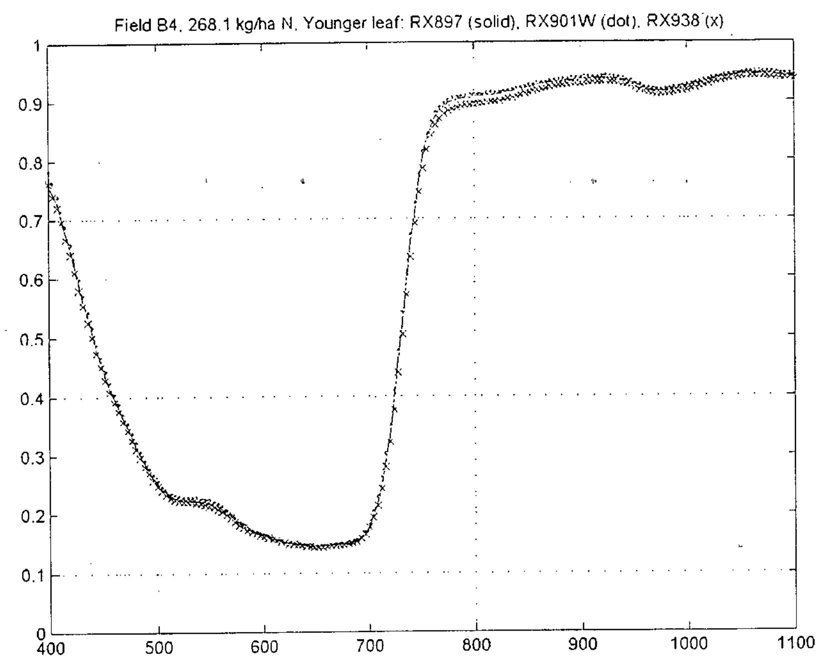


(b) Younger leaf.

Figure 3.5. Reflectance of ear leaf and fully developed younger leaf of V1 (solid), V2 (dot), and V3 (x) with 134.1 kg/ha N treatment.



(a) Ear leaf.



(b) Younger leaf.

Figure 3.6. Reflectance of ear leaf and fully developed younger leaf of V1 (solid), V2 (dot), and V3 (x) with 268.1 kg/ha N treatment.

All the N treatments showed that the reflectance of the samples from the subplots of [N1], [N2], and [N3, N4, and N5] were significantly different at $\alpha = 0.01$. This result was supported by the sample nitrogen content analysis shown below. Therefore, it can be concluded that the reflectance difference at 550 nm was not affected by variety, but by N treatment and that the excessive amount of N treatment over N3 (134.1 kg/ha) was not consumed by corn plants. If the N was more than or equal to the N3 treatment level, it was adequate for the corn plants in this growing season.

Lab analyzed nitrogen content

The samples were submitted for their nitrogen content analysis and these results are shown in Table 3.4. The general trend is that the actual N content for samples from N1 subplots were very low and that samples from N3, N4, and N5 subplots showed higher N content, with little difference from each other. In fact, according to the established critical N levels in the corn ear leaf at anthesis of 2.75 to 2.80% (Schepers et al., 1992), no nitrogen deficiency was experienced in any of the three larger application treatments. In fact, the corn plants in the subplots with N1 and N2 treatments showed severe N deficiency symptoms on their mature growth stages (R1 stage and beyond), especially those on N1 subplots.

Table 3.4. Lab analyzed nitrogen content (%dry matter basis).

Variety & N treatment	EarB4	YoungerB4	EarB1	YoungerB1
V1N1	1.95	2.11	1.50	1.50
V1N2	2.73	2.73	2.00	1.94
V1N3	3.52	3.32	2.92	2.36
V1N4	3.36	3.10	3.31	2.55
V1N5	3.66	3.01	2.73	2.41
V2N1	1.25	1.42	1.31	1.40
V2N2	2.14	2.28	1.55	1.67
V2N3	3.38	3.08	2.50	2.19
V2N4	3.22	2.72	2.59	2.67
V2N5	3.50	3.15	2.79	2.46
V3N1	2.37	2.36	1.35	1.54
V3N2	2.47	2.36	1.82	1.93
V3N3	3.05	2.96	2.48	2.34
V3N4	3.46	3.09	2.97	2.37
V3N5	3.51	3.03	3.03	2.51

For most of the samples, the N content of the ear leaf samples was higher than that of the younger leaf samples. Table 3.5 shows the N content analysis result of the samples of uniform and varying reflectance spectra. The samples of uniform reflectance showed lower standard deviations than those of varying spectra.

Table 3.5. N content (%) of the samples of uniform and varying spectra.

	Samples of uniform reflectance		Samples of varying reflectance	
	Sample 1	Sample 2	Sample 1	Sample 2
	3.37	2.17	1.17	1.59
	3.18	2.33	1.20	1.76
	3.20	2.20	1.48	1.50
	3.40	2.15	1.55	1.75
	3.39	2.09	1.16	1.74
Average	3.308	2.188	1.312	1.668
Std. dev.	0.108	0.089	0.188	0.117

SPAD meter measurements

The chlorophyll contents were measured for the same leaf samples using a chlorophyll meter (model: Spad-502, Minolta Co.), Table 3.6. Figure 3.7 shows Spadmeter readings of EarB4 and YoungerB4 sample set in bar graphs. Most of the time, the ear leaves had higher SPAD values than the younger leaves did. The result also showed that the SPAD readings increased as the nitrogen application rate increased. The result showed basically the same trend as the N content result. Note that the measurement result showed high variation since it measures very small area (2 mm x 3mm), which indicates that many measurements would be required in order to extract a proper management decision.

Table 3.6. Chlorophyll measurement results in SPAD units

(Avg = average of 5 replicates, Stdev = standard deviation of 5 replicates).

Variety &	EarB4		YoungerB4		EarB1		YoungerB1	
N-treatment	Avg	Stdev	Avg	Stdev	Avg	Stdev	Avg	Stdev
V1N1	43.7	1.32	42.4	2.05	35.0	1.99	34.2	2.66
V1N2	53.6	3.18	51.8	2.96	49.6	2.16	36.7	2.08
V1N3	64.3	4.70	55.3	2.30	59.0	3.82	48.4	2.80

V1N4	61.5	3.62	57.9	3.32	62.0	4.48	51.6	3.81
V1N5	67.2	4.35	57.5	2.85	56.9	2.85	43.2	2.84
V2N1	34.3	1.39	36.6	2.46	30.3	1.41	32.6	1.57
V2N2	51.9	2.04	49.1	1.53	37.3	1.74	38.0	1.67
V2N3	62.9	4.10	56.3	2.95	53.9	2.62	43.2	3.11
V2N4	61.8	3.34	53.2	2.06	54.2	2.22	45.1	4.28
V2N5	61.5	4.01	56.4	3.92	54.2	2.77	45.9	2.67
V3N1	51.5	2.24	49.4	1.54	28.5	1.27	30.3	1.50
V3N2	51.6	2.82	47.5	2.33	44.4	2.36	43.2	2.62
V3N3	59.3	2.99	55.8	3.82	53.6	2.40	48.6	3.10
V3N4	63.3	3.41	57.4	3.07	59.1	4.77	50.1	1.86
V3N5	62.8	4.03	57.3	4.41	59.1	3.83	49.9	3.67

A regression analysis was conducted between SPAD reading and actual N content of the samples, Table 3.7. Generally the SPAD reading shows good relationship with the actual N content with high R^2 values except YoungerB1 data. The YoungerB1 samples had many small degraded spots on their leaves, which could be considered as source of errors.

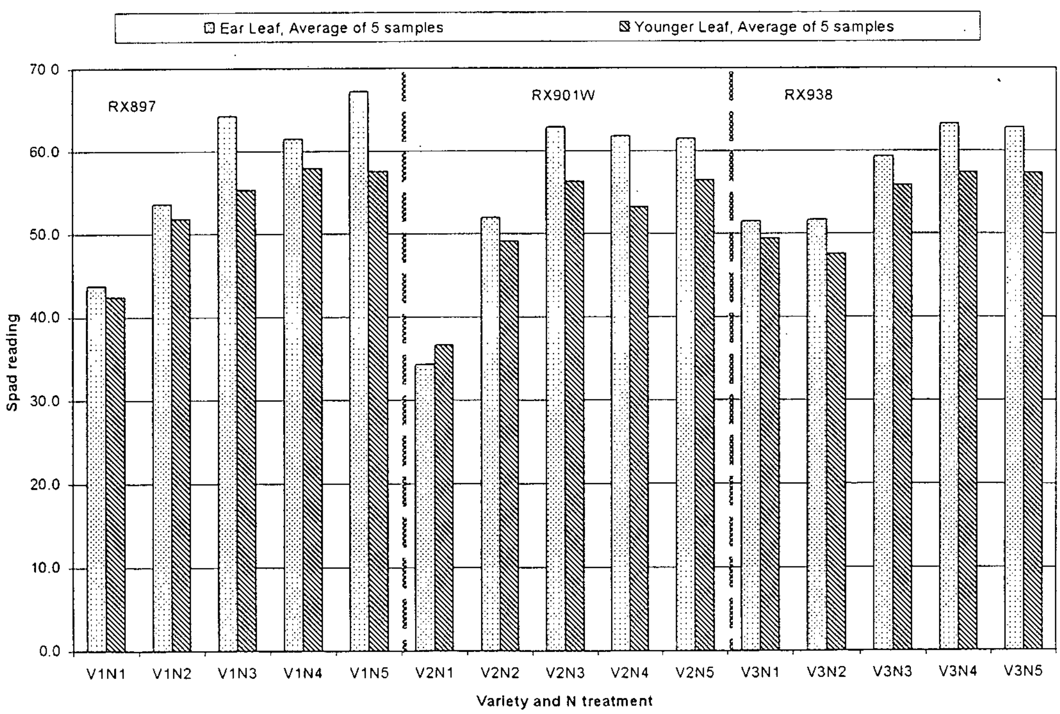


Figure 3.7. SPAD meter measurement for EarB4 and YoungerB4 samples.

Table 3.7. Regression result between SPAD reading (X) and actual N content (Y, %).

Data set	Regression equation	R^2
EarB4	$Y = 0.0789X - 1.576$	0.962
YoungerB4	$Y = 0.0788X - 1.405$	0.928
EarB1	$Y = 0.0583X - 0.544$	0.942
YoungerB1	$Y = 0.0558X - 0.261$	0.808

Statistical analysis

To find out how well actual N content would relate to reflectance, a correlation analysis (SAS CORR) was conducted, Table 3.8. The commonly selected wavelength ranges were 540 – 556 nm in the visible range, and 708 – 760 nm in the NIR range. This result was used as supplementary information for further analysis.

Table 3.8. Correlation analysis result (r: correlation coefficient).

Data set	Selected wavelength range	Selection criteria
EarB4	520 – 604, 696 – 1100	$\|r\| > 0.5$
YoungerB4	528 – 620, 700 – 1100	$\|r\| > 0.5$
EarB1	540 – 556, 708 – 868	$\|r\| > 0.9$
YoungerB1	528 – 576, 704 – 760	$\|r\| > 0.9$

A stepwise discriminant analysis (SAS STEPDISC) was conducted to reduce the number of variables. The analysis yielded a total of 29, 47, 73 and 21 wavelength bands for EarB4, YoungerB4, EarB1, and YoungerB1, respectively. With these selected variables, a canonical discriminant analysis (SAS CANDISC) was executed. Table 3.9 shows one of the results for EarB4 data set, conducted with N as a class variable. The R^2, given by the squared canonical correlation for EarB4 data set, was 0.955 between CAN1 and treatment N and 0.877 between CAN2 and treatment N. With CAN1 and CAN2, 86.4% of the total variance could be explained.

Table 3.9. Canonical discriminant analysis for EarB4 with N as a class variable.

Canonical variable	Eigenvalues	Canonical correlation	Squared canonical correlation	Cumulative	Approx F	Pr > F
CAN1	21.2	0.977	0.955	0.646	8.166	0.0001
CAN2	7.1	0.936	0.877	0.864	5.151	0.0001
CAN3	2.9	0.863	0.745	0.953	3.509	0.0001
CAN4	1.5	0.778	0.605	1.000	2.652	0.0020

Figures 3.8 and 3.9 show canonical variables calculated with N as a class variable and with variety as a class variable, respectively. In both cases, variety and N treatment levels could be separated very well with the calculated canonical variables. The CANDISC analysis from the other sets showed also very good separation among varieties and among N treatment levels with the calculated canonical variables. Therefore, it could be concluded that the calculated canonical variables derived from the wavelengths, selected by the stepwise discriminant analysis, were able to identify variety and N treatment levels.

Another way to examine the data set is Principal Component Analysis (PCA). PCA was conducted using the SAS procedure PRINCOMP. Table 3.10 shows one of the results, with the EarB4 data set. With the PRIN1, 66.7% of the total variance of EarB4 data could be explained and 99.6% of the total variance could be explained with the first 10 PCs. Figure 3.10 shows the first two principal components of EarB4 data set. The separation was not as good as canonical variables.

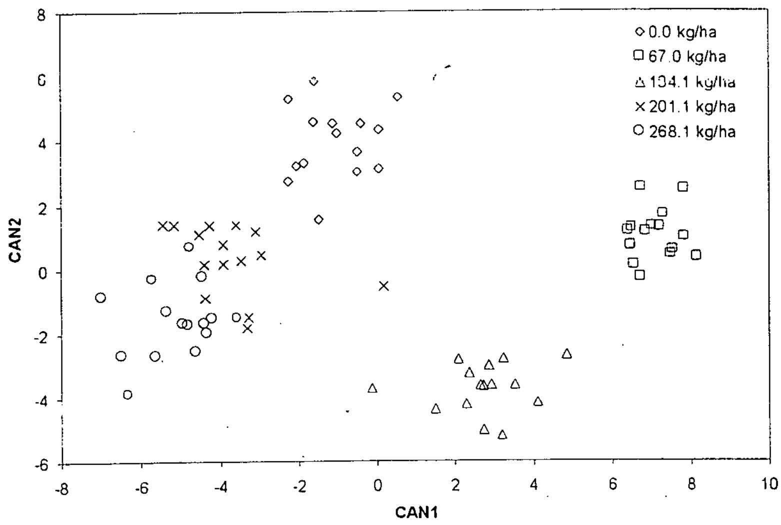


Figure 3.8. Canonical discriminant analysis of EarB4 data set with N as a class variable.

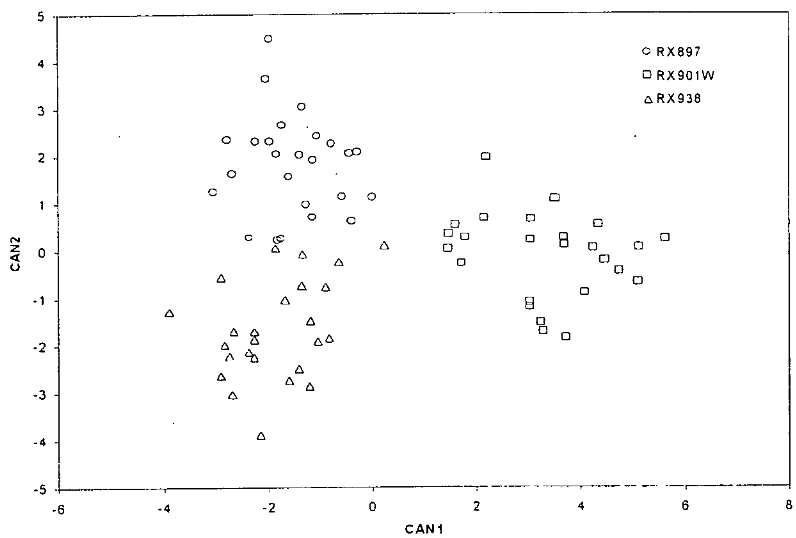


Figure 3.9. Canonical discriminant analysis of EarB4 data set with variety as a class variable.

Table 3.10. Principal component analysis result with EarB4 data.

Principal component	Eigenvalue	Difference	Proportion	Cumulative
PRIN1	19.3307	13.2300	0.667	0.667
PRIN2	6.1006	4.8439	0.210	0.877
PRIN3	1.2568	0.2859	0.043	0.920
PRIN4	0.9709	0.1352	0.033	0.954
PRIN5	0.8357	0.6847	0.029	0.983
PRIN10	0.0319	-	0.001	0.996

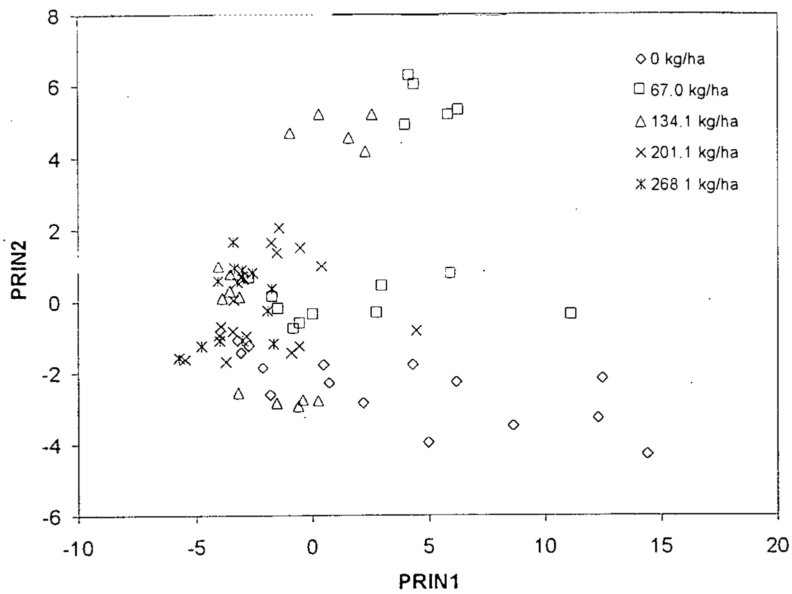


Figure 3.10. Principal component analysis for EarB4 data set.

N content prediction

The prediction results of the N content (%) by PLS, PCR and MLR are summarized in Tables 3.11, 3.12 and Figure 3.11. For this calculation, the MATLAB PLS_Toolbox was used.

The overall performance of the prediction model could be presented as SEP or R^2. The prediction models by PLS and PCR performed similar each other, however they were better than the MLR model. The MLR model produced an outlier (-0.435), which significantly decreased its performance. Either PLS or PCA could be the method to build a good prediction model in this analysis.

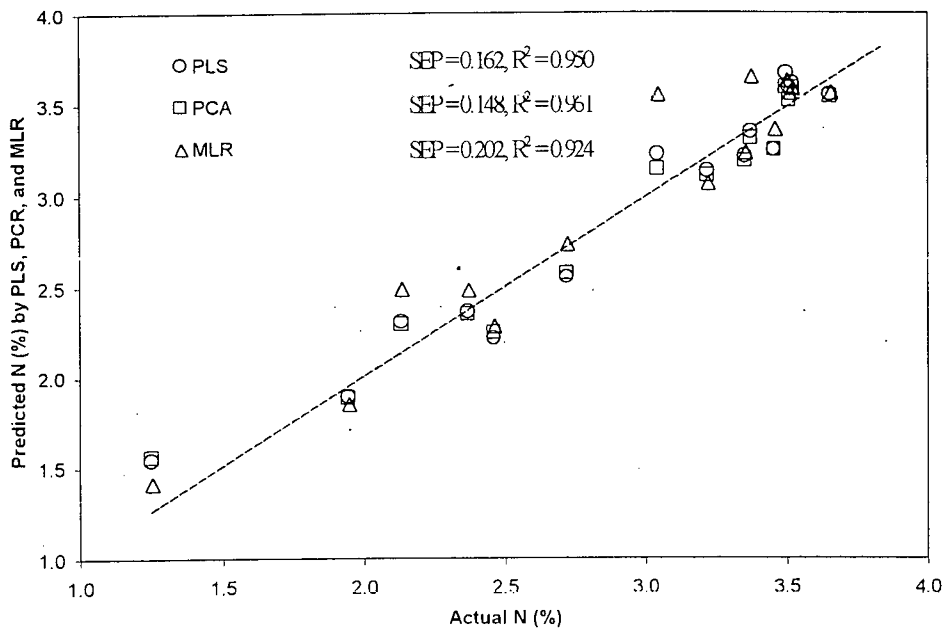
Another way of estimating the prediction error is to look at the predicted residual error sum of squares (PRESS), Figure 3.12. In the cross-validation process, it is important to find the optimum number of the parameters for the prediction model. If there were too few parameters, the prediction model would be insufficient. On the other hand, if there were too many parameters, the model would be over fitted, which would not work for other data sets. In this analysis, both PLS and PCR had the minimum PRESS at the 5th latent variable (LV) or principal component (PC) for EarB4 calibration (Figure 3.12) and 1 LV and 1PC for YoungerB4 calibration (data not shown).

Table 3.11. N prediction result (%) by PLS, PCR, and MLR for Ear leaf samples.

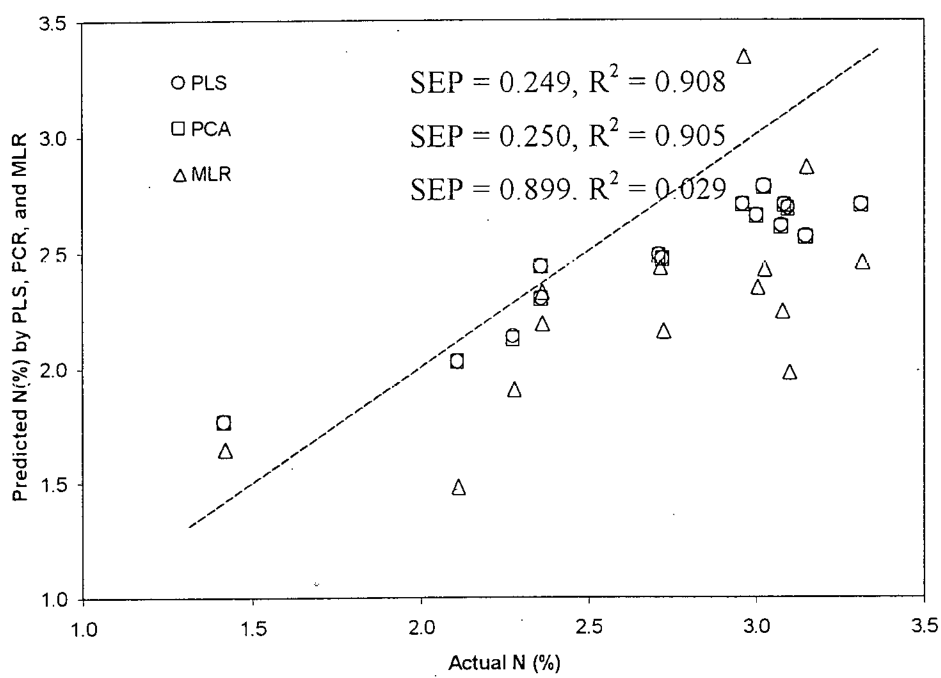
Calibration (EarB1)				Validation (EarB4)			
Actual	PLS	PCA	MLR	Actual	PLS	PCA	MLR
1.500	1.474	1.486	1.500	1.948	1.881	1.876	1.843
2.000	2.094	2.119	2.000	2.725	2.543	2.564	2.728
2.920	2.861	2.864	2.920	3.523	3.608	3.580	3.585
3.308	3.282	3.252	3.308	3.358	3.209	3.181	3.234
2.730	2.799	2.780	2.730	3.658	3.546	3.535	3.561
1.312	1.370	1.387	1.312	1.254	1.533	1.552	1.410
1.550	1.490	1.478	1.550	2.135	2.299	2.284	2.483
2.500	2.580	2.592	2.500	3.378	3.342	3.306	3.649
2.590	2.673	2.675	2.590	3.223	3.128	3.101	3.065
2.790	2.591	2.560	2.790	3.503	3.662	3.585	3.629
1.350	1.334	1.309	1.350	2.373	2.354	2.341	2.477
1.820	1.835	1.838	1.820	2.466	2.207	2.240	2.279
2.480	2.426	2.436	2.480	3.047	3.217	3.137	3.549
2.970	3.002	3.036	2.970	3.461	3.242	3.244	3.359
3.030	3.039	3.038	3.030	3.513	3.583	3.513	3.562
SEC	0.080	0.093	0.0	SEP	0.162	0.148	0.202
R^2 between Actual	0.987	0.983	1.000	R^2 between Actual	0.950	0.961	0.924

Table 3.12. N prediction result (%) by PLS, PCR, and MLR for Younger leaf samples.

Calibration (YoungerB1)				Validation (YoungerB4)			
Actual	PLS	PCA	MLR	Actual	PLS	PCA	MLR
1.500	1.660	1.661	1.500	2.114	2.023	2.022	1.479
1.940	1.864	1.862	1.940	2.725	2.467	2.465	2.159
2.360	2.402	2:400	2.360	3.316	2.702	2.697	2.455
2.550	2.633	2.633	2.550	3.098	2.688	2.682	1.978
2.410	2.202	2.199	2.410	3.005	2.655	2.650	2.346
1.400	1.661	1.667	1.400	1.420	1.760	1.759	1.645
1.670	1.721	1.720	1.670	2.280	2.131	2.120	1.905
2.190	2.268	2.269	2.190	3.078	2.611	2.603	2.243
2.670	2.331	2.330	2.670	2.715	2.484	2.479	2.434
2.460	2.325	2.324	2.460	3.150	2.565	2.558	2.867
1.540	1.320	1.321	1.540	2.363	2.434	2.433	2.328
1.930	2.141	2.144	1.930	2.363	2.296	2.292	2.190
2.340	2.252	2.250	2.340	2.964	2.703	2.700	3.341
2.370	2.538	2.538	2.370	3.088	2.703	2.698	-0.435
2.510	2.523	2.524	2.510	3.026	2.778	2.775	2.426
SEC	0.180	0.182	0.000	SEP	0.249	0.250	0.899
R^2 between Actual	0.832	0.830	1.000	R^2 between Actual	0.908	0.905	0.029



Ear leaf.



Younger leaf.

Figure 3.11. Actual N (%) and predicted N (%) by PLS, PCR, and MLR for corn leaf samples.

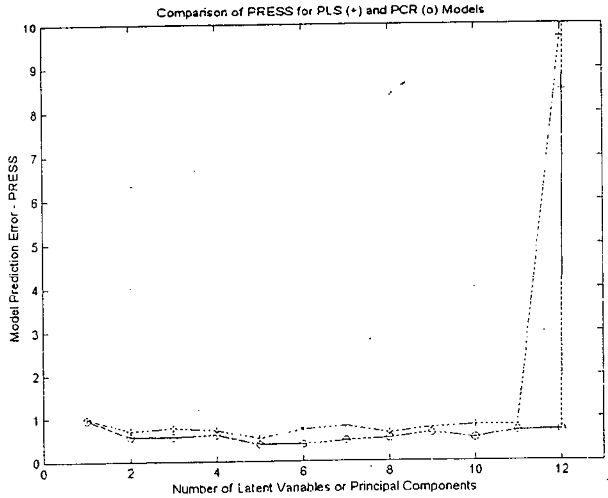


Figure 3.12. Predicted residual error sum of squares (PRESS) for N content (%) prediction for EarB4 data set.

CONCLUSIONS

This research was conducted as a preliminary step for developing a real-time multispectral nitrogen sensor for corn plants for site-specific variable rate application. One of the challenges in developing a multispectral sensor was expected to be the variability of spectral responses resulted from different canopy colors. This study was conducted to find effect of variety and nitrogen treatment to leaf spectral reflectance. Using three varieties of different leaf characteristic color and five different N treatments, reflectance was measured to assess nitrogen content. With the well known wavelength band for distinguishing nitrogen status of corn plants (i.e., 550 nm), a means test showed that the variety effect to the reflectance at 552 nm was not significantly different at $\alpha = 0.01$, however the N treatment effect to the reflectance at 552 nm was significantly different.

The leaf chlorophyll content was measured with SPAD-502 chlorophyll meter and the readings showed good relationship with the actual N content (%). However, the measurement result also showed high variation since it measures only small portion of leaf, which acquires many measurements for correct N assessment.

The canonical discriminant analysis (CANDISC) with the wavelengths selected by the stepwise discriminant analysis showed promising result to distinguish different variety and N treatment, using spectral reflectance. N prediction models built by PLS and PCR performed better than the one by MLR, while the performance of PLS and PCR was similar. The standard errors of calibration (SEC) for ear leaf were 0.08%, 0.09%, and 0.0% for PLS, PCR, and MLR, respectively and the standard errors of prediction (SEP) for ear leaf were 0.16%, 0.15%, and 0.20% for PLS, PCR, and MLR, respectively. For the younger leaf samples, the SEC were 0.18%, 0.18%, and 0.0% for PLS, PCR, and MLR, respectively, and the SEP were 0.25%, 0.25%, and 0.90% for PLS, PCR, and MLR, respectively.

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4. MULTISPECTRAL SENSOR FOR DETECTING NITROGEN IN CORN PLANTS

From the earlier results (Lee et al, 1999), it was found that the spectral difference resulted from different canopy colors was not affected by variety but by nitrogen content itself in the leaves. Continuing from the last year's effort, the nitrogen sensor was constructed and tested in a commercial corn field, along with laboratory analysis for N content in sample leaves.

The overall objective of this research is to develop a real-time multispectral sensor that could detect nitrogen deficiency in corn plant using spectral response from plant canopies. Eventually the Nitrogen sensor could be used for variable rate fertilizer application. The specific objectives were

to construct an in-field sensor system for spectral measurement,

to build data acquisition system for real-time field use,

to build an algorithm to assess nitrogen status for corn, and

to test the N-Sensor in commercial cornfield.

BACKGROUND

Numerous studies have been conducted to assess moisture and nutrient status by measuring spectral responses (Thomas and Oerther (1972), Al-Abbas et al. (1974), Walberg et al. (1982), Blackmer et al. (1994a), Yoder and Pettigrew-Crosby (1995), Filella et al. (1995), Blackmer et al. (1996), Ma et al. (1996), Masoni et al. (1996), Rigney and Brusewitz (1997), and Bausch et al. (1998)). These studies found that reflectance near 550 nm showed good separation of leaf nitrogen content. A chlorophyll meter has been used as another means to assess nitrogen stress in plants (Piekielek and Fox (1992), Schepers et al. (1992), Tracy et al. (1992), Blackmer and Schepers (1994), Smeal and Zhang (1994), and Piekielek et al. (1995)). These studies found reasonable relationship among yield, nitrogen content, and chlorophyll meter reading, however the chlorophyll meter required many measurements to assess plant status accurately. Utilizing aerial photograph or satellite imagery has been another way to assess plant status and yield (Blackmer et al. (1994b), Tomer et al. (1997), GopalaPillai et al. (1998), Deguise et al. (1999), Willis et al. (1999), and Wooten et al. (1999)).

Another way of assessing nitrogen stress is to implement a real-time nitrogen sensor system. Stone et al. (1996) developed a sensor system for nitrogen 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 a NDVI (Normalized Difference Vegetation Index). Sui et al. (1998) developed a spectral reflectance sensor to detect nitrogen status of cotton plants with 4 spectral bands of blue, green, red and near infrared light. They tested the sensor in 2 situations: with an artificial illumination and with natural illumination. They used a neural network to determine nitrogen deficient and non-nitrogen deficient plant. They reported the preliminary test results for diagnosing nitrogen status in cotton was promising. The 'Hydro N-Sensor' was developed by Hydro Agri GmbH, Deutschland as a commercial nitrogen sensor. It measures crop reflectance from 4 different angles, two in front pointing forward and two behind sensing backwards (@gInnovator, 1999). A fifth light sensor was mounted on top of the housing unit and used for compensating the sunlight change. Its performance in the United States was unknown at the time of this publication.

MATERIALS AND METHODS

Nitrogen sensor design

Two rugged portable spectrometers (model: MicroPac™, OCLI, Inc., Santa Rosa, CA) were used as sensors: MicorPac400™ for 400 – 700 nm and MicroPac600™ for 600 – 1100 nm. The resolutions were 5 nm for the visible sensor and 7 nm for the NIR sensor. The dimensions of the MicroPac were 1.42" x 1.42" x 0.63". The MicroPac utilized a photodiode array (model: S3901, Hamamatsu, Corp.) as a detector. The following equations show calibration result supplied by the manufacturer.

MicroPac400: Y = 1.5325X + 361.69

MicroPac600: Y = 1.9567X + 598.18

where X = pixel number and Y = wavelength (nm)

A driver/amplifier circuit board (model: C4070, Hamamatsu, Corp.) was used to control the detector and to generate necessary signals to operate the sensor system. A custom-designed interface board was used to relay master clock signal and to reduce the signal from the evaluation board to fit the range for digitization. An A/D board (model: Lab-PC+, National Instrument) was used to provide master clock signal and digitize output from the sensor. A housing for the MicroPacs was built using ¼" thick black Plexiglas. Its inside dimension was 3.5" x 4" x 12". The outside was painted in non-glossy black paint.

Focus point of the N-sensor

The focus point of the MicroPac was calculated as a function of the slope (α) of the C4070 driver/amplifier circuit board relative to the horizontal surface, Figure 4.1. Any additional focusing or collimating lenses were used. The distance to the focal point is denoted as f and could be calculated as

$$\tan\alpha = \frac{d}{(f+l)} = \frac{x}{d} = \frac{y}{H}$$

Thus,

$$f = \frac{d}{\tan\alpha} - l$$

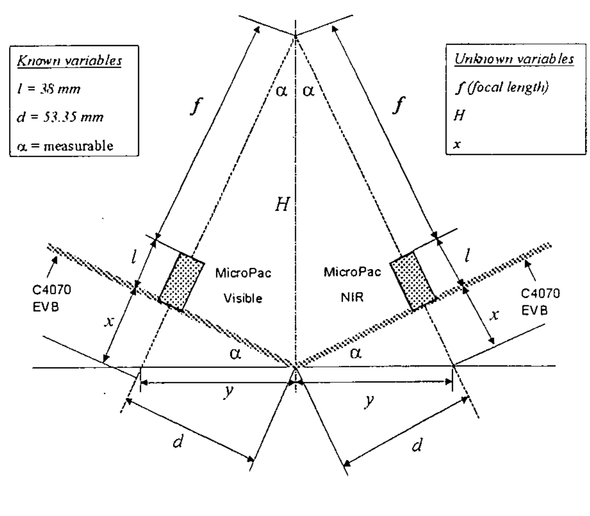


Figure 4.1. N-Sensor geometry.

Signal to noise ratio (SNR) of the Nitrogen sensor

A white Teflon sheet (12" x 12" x 1/8") was used as an object to calculate SNR using a halogen-tungsten lamp (model: AS220, CVI Laser, Inc.) as an illumination source. The Teflon sheet was placed on a table and the sensor was positioned 6" above the Teflon sheet, facing straight down. The light source was focused on the area that corresponded to the field of view of the sensor. For each sensor, 6 single spectra were acquired as well as dark spectra. The magnitude of the dark spectrum was all zero at all pixel locations for both sensors. One of the 6 spectra was used as a reference to calculate SNR.

Cornfield for the nitrogen sensor testing

Experimental corn plots of varying nitrogen treatments were established on the Texas A&M University experimental farm. Unfortunately, a hailstorm on May 1 destroyed the plots. As an alternative, nitrogen response plots established in another study were used. A commercial field near Temple, Texas (Figure 4.2) was being used for a variable rate application study. Rate response plots with 4 different nitrogen rate (0, 50, 100, and 150 N lb/ac) had previously been established. The field had nine of these nitrogen rate plots with 8 adjacent 50 ft long rows. Unfortunately in the commercial field, no information was available on the depletion of the residual nitrogen in the plots. Table 4.1 shows field testing schedule of the nitrogen sensor.

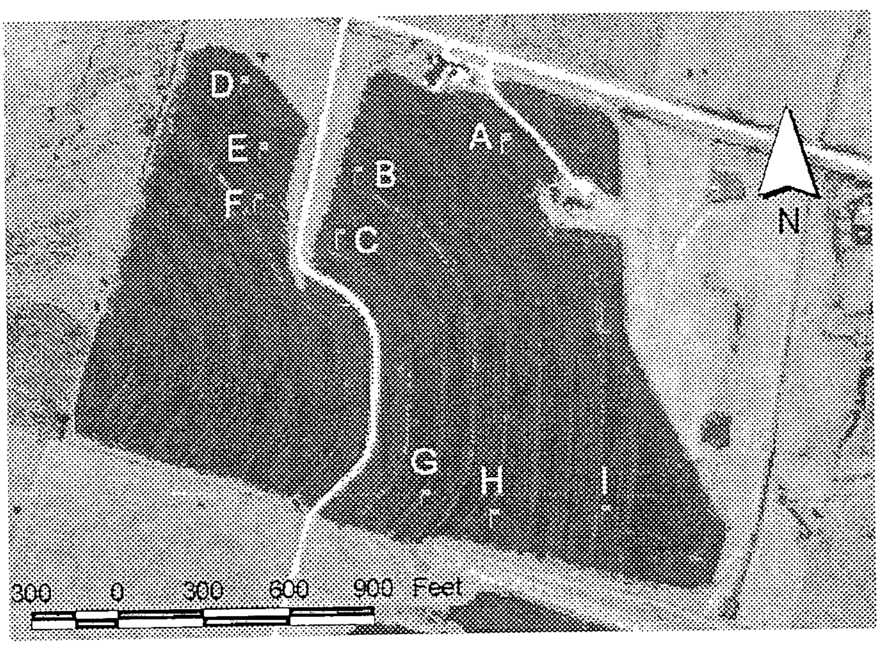


Figure 4.2. Sub field names used for nitrogen sensor testing at Coufal farm in Temple, TX.

Table 4.1. Field testing schedule of the nitrogen sensor.

Date	May 9	May 11	May 16	May 17	May 22	May 23
Sub field	A	C, D	H, A, B	C, D	F, G	B, C

Field data acquisition system

The field data acquisition system, Figure 4.3, consisted of the Nitrogen sensor, uniform illumination system, lighting chamber, a computer, a generator, and a high clearance sprayer. A lighting chamber for in-field data acquisition was constructed to measure reflected light energy from corn leaves as well as to keep the sunlight from coming in. Square tubing (1.25") and angle iron were used for constructing the frame and a 20-gage sheet metal (0.0299" thick) was used for the side of the chamber. The size of the chamber was 7' high, 4' long, and 3' wide.

To provide homogeneous illumination inside the chamber, a track lighting system (USA Light & Electric, Patton, CA) was used. Four 50W halogen lamps, two on each side of the sensor housing, were used for illumination. The direction of the lamps could be adjusted in every direction.

In order to cover the front and rear sides of the lighting chamber as well as for the plants to pass through in the middle of the chamber without being damaged, a lexane strip (2" x 20" x 1/8") was used to hold thin rubber sheets (8" x 8" x 1/16") in overlapping manner as well as to provide plasticity without being broken when corn plants passes. A set of lexane strip with 3 rubber sheets was attached from top to bottom of the right and left sides of the light chamber on front and rear sides, allowing overlapped area to ensure complete blocking of the sunlight, Figure 4.4 (b). Since the lighting chamber was 3' wide, a 20" long lexane strip left 4" overlapped region in the middle. The light chamber was attached on the toolbar of the sprayer (John Deere 6500 Self-propelled sprayer), Figure 4.4 (b).

A desktop computer with 66 MHz CPU was used inside the cab of the sprayer to control the sensor system. A generator was mounted on a platform behind the cab to provide power to the in-field data acquisition system. The plants were varied from V8 – V9 growth stages on May 9 to tasseling stages on May 23.

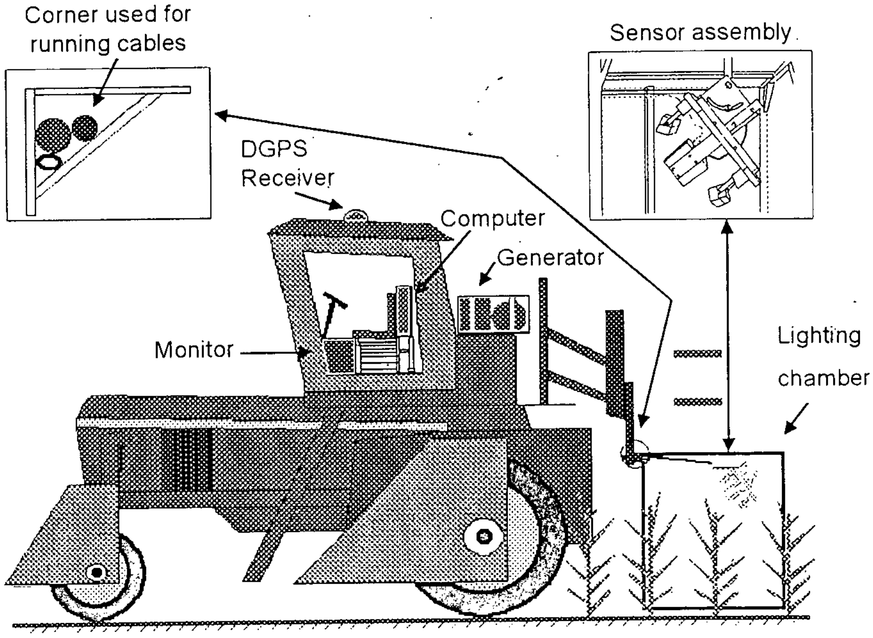
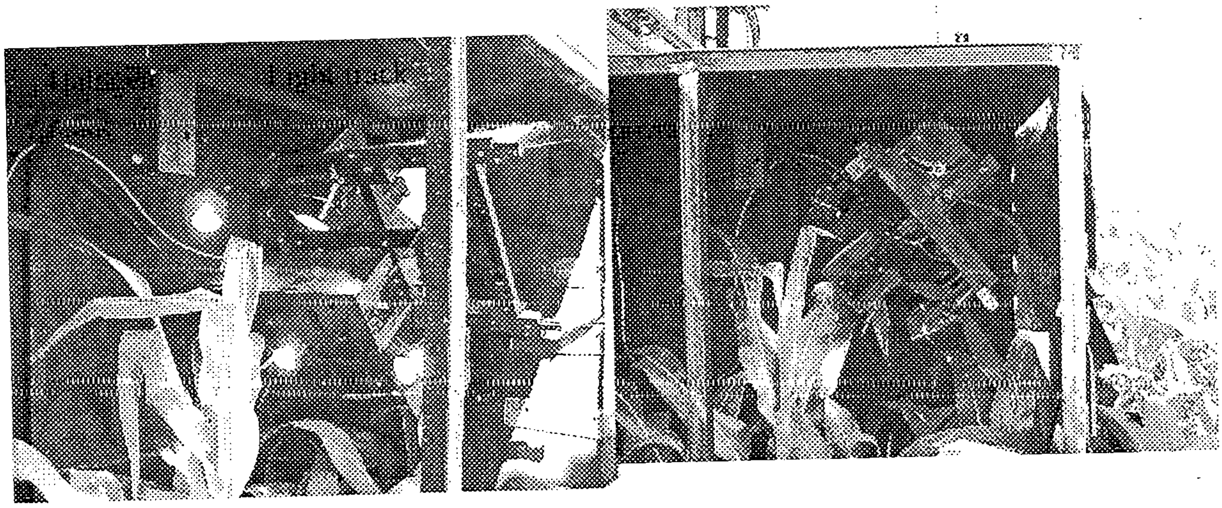


Figure 4.3. Complete assembly of the nitrogen sensor system on the sprayer.

Field testing of the nitrogen sensor

The sensor was tested in two different modes: stationary and moving modes. For a stationary mode measurement, a middle row from 8 rows was chosen to test the sensor to minimize any effect from adjacent rows of different rate of nitrogen treatment. While driving down the row, the sprayer was stopped at a location where the sensor generated higher output. At each location, the reflected light energy from corn leaf was measured. After the sensor measurement, a chlorophyll meter (model: SPAD-502, Minolta Co.) was used to measure chlorophyll content of the plant. Five measurements were made for each plant from top to bottom, and an average of the five measurements was used for later analysis. A GPS point was recorded at each measurement location with a hand-held GPS receiver (model: March II, CMT Inc.). The points were later post-processed. A corn leaf was obtained from the same plant as measured by the nitrogen sensor for laboratory analysis for actual nitrogen content.

(a) Back view of the sprayer. (b) Closer view of the lighting chamber.



(c) Inside of the lighting chamber: front view. (d) Inside of the lighting chamber: side view.

Figure 4.4. Field testing of the nitrogen sensor system.

For each measurement, integration time of the sensor (duration between two start pulses) was adjusted according to the sensor output in order to obtain higher output form the sensor. After the measurements in stationary mode, another set of measurements was made for the same row while driving the sprayer continuously without stopping (moving mode).

Analysis of leaf spectra

From previous results (Lee et al. (1999)), it was found that higher leaf nitrogen content resulted in lower reflectance near 550 nm. Differences were also found on the red edge near 700 nm between different N treatment samples. Based on this information, a 1^{st} derivative (1D) of reflectance spectra was utilized to predict nitrogen content from their reflectance spectra.

A multiple linear regression analysis was conducted between actual N content and 1D value near 525 nm and 715 nm. Four data sets (two from ear leaf, and two from younger leaf) from the spectrophotometer measurements in 1999 were initially used to test the feasibility of the 1D analysis. The same procedures were followed for the analysis of the measurements by the nitrogen sensor system this year.

Since the output of the nitrogen sensor was directly proportional to the amount of light energy received by the photodiodes, distance between the detectors and leaves, leaf geometry at the time of measurements relative to light beam angle, and the angle of the sensor relative to vertical surface would play very important role. Unfortunately, all of these factors would vary from plant to plant, thus normalizing by the reference spectra would not be plausible. Another way of normalizing the output was needed to compare spectra between different nitrogen treatments.

In an effort to normalize the measurement spectra against the variation of distance between leaf sample and the sensor, leaf orientation to the sensor, and leaf orientation to the lighting system, the spectra was normalized by an average raw values in 670-690 nm. It was decided to use an average of 670-690 nm rather than getting reference for every measurement, since this range didn't seem to be varying with different nitrogen content on the leaves from 1999 study (Lee, et al., 1999). Then, the spectrum of 400-700 nm from the visible sensor and the one of 700-1100 nm from the NIR sensor were combined to complete one measurement from 400-1100 nm. Since the raw output from the sensor had some noise, the raw spectrum was processed with a 20 point moving average. Then, the 1^{st} derivative (1D) of the spectrum was calculated by Savitzky-Golay algorithm to exclude any effect from different environment during measurements as well as smoothing spectra.

Matlab (ver. 5.3, The MathWorks, Inc.) was used for calculating 1D data. Then, relationship between actual N (%) content and peak 1D values near 525 nm and 715 nm was studied. The SAS system for windows (ver. 8.0, SAS Institute Inc., Cary, NC) was used for multivariate statistical analysis and prediction of N (%) content: correlation, stepwise discriminant analysis, canonical discriminant analysis, multiple linear regression and partial least square (PLS) regression. The data set was divided into 3 subsets for a PLS analysis. Two subsets were used as a calibration set and the other subset was used as a validation set. This process was repeated three times until each subset was used as a validation set. A regression analysis was conducted between actual N (%) and predicted N (%) after the PLS regression.

RESULTS AND DISCUSSION

Focal length of the nitrogen sensor

Figure 4.5 shows focal length of the nitrogen sensor versus an angle of the C4070 evaluation board from horizontal surface. Since the MicroPac detectors had an angle of 10° from horizontal surface, the focal length was found to be 13.8" (350 mm). With the angle over 55°, the focal length would be negative, which means that the focal length would extend over the centerline between two sensors.

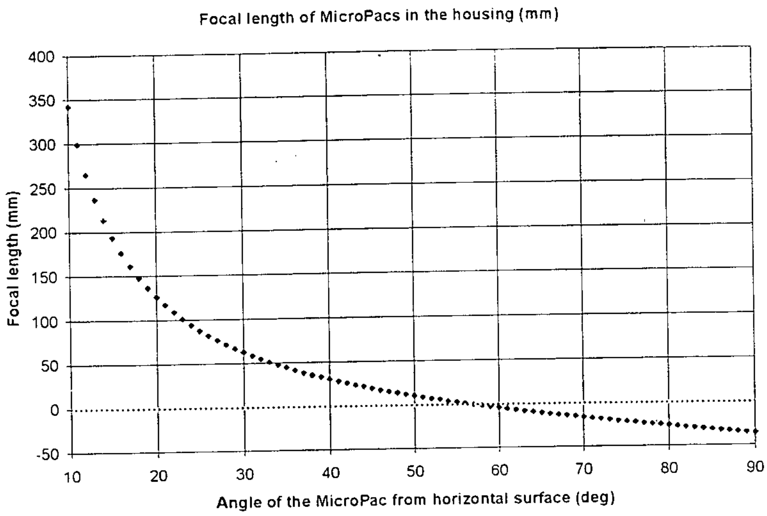


Figure 4.5. Focal length of the nitrogen sensor in the housing.

SNR of the MicroPac

Figure 4.6 shows signal to noise ratio (SNR) of the MicroPac400 detector. There was much variation in the first part of the pixels as well as some last pixels. The high variation in these ranges was due to low energy from the light source as well as low sensitivity of the sensor itself. Besides those pixels, the rest of them produced very good SNR, which mostly varied within ±1%. The MicroPac600 also had similar SNR, which varied within ±1% except some last pixels (170 < pixel number < 256).

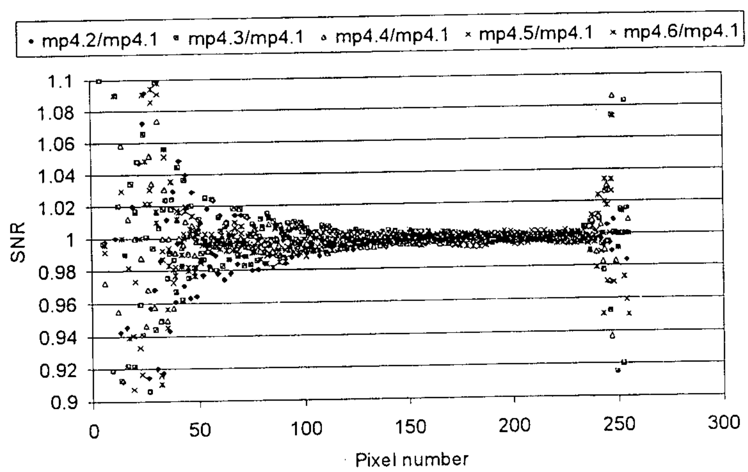


Figure 4.6. SNR of MicroPac400.

Calibration of the Nitrogen sensor with Didymium oxide filter

Figure 4.7 shows a result of transmittance measurement of the Didymium oxide filter (DOF) by the nitrogen sensor. The DOF is usually used for calibrating a spectrophotometer. The sensor output generally followed the certified spectrum, but did not match exactly with the certified. The sensor showed poor resolution in some areas. The numbers in the figure indicated certified absorption bands of the filter.

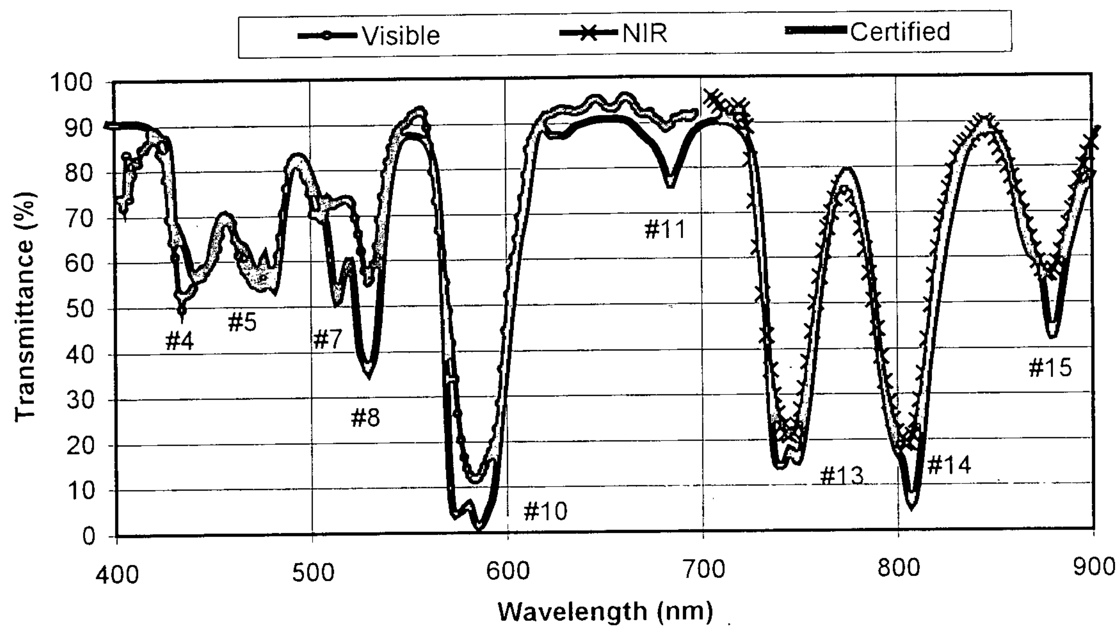


Figure 4.7. Measurements of the DOF by the nitrogen sensor.

1D analysis of the 1999 data set

The 1st derivatives (1D) of the reflectance spectra were calculated and examined closely to distinguish different nitrogen content from 1999 experiments (Lee, et al, 1999). In order to select leaf samples well separated by N (%) content, a histogram was drawn for the lab analyzed N (%) content with the increment of 0.25% N for the leaf samples, Figure 4.8.

Figure 4.9 shows example 1D spectra and their actual N (%) content. The peak 1D value near 525 nm varied inverse proportionally with actual N (%) content. A multiple linear regression analysis was conducted between actual N (%) and 1D peak values near 525 nm and 715 nm. For the 1999 leaf samples, the following relationship was found:

For ear leaf: $Y = 7.33 - 704.0X_1 - 230.9X_2$ ($R^2 = 0.798$)

For younger leaf: $Y = 1.99 - 586.5X_1 - 71.1X_2$ ($R^2 = 0.872$)

where X_1 = peak 1D value near 525 nm

X_2 = peak 1D value near 715 nm

Y = predicted N (%)

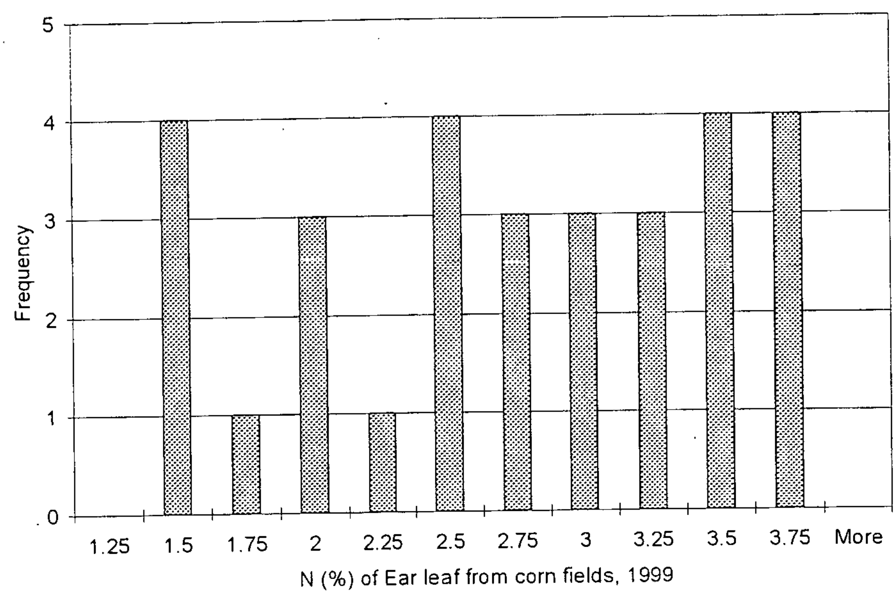
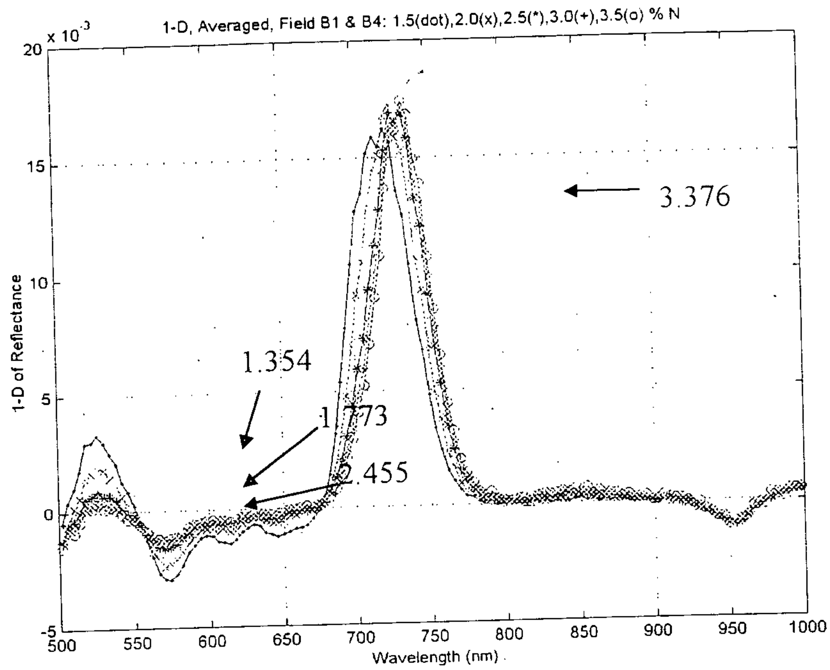


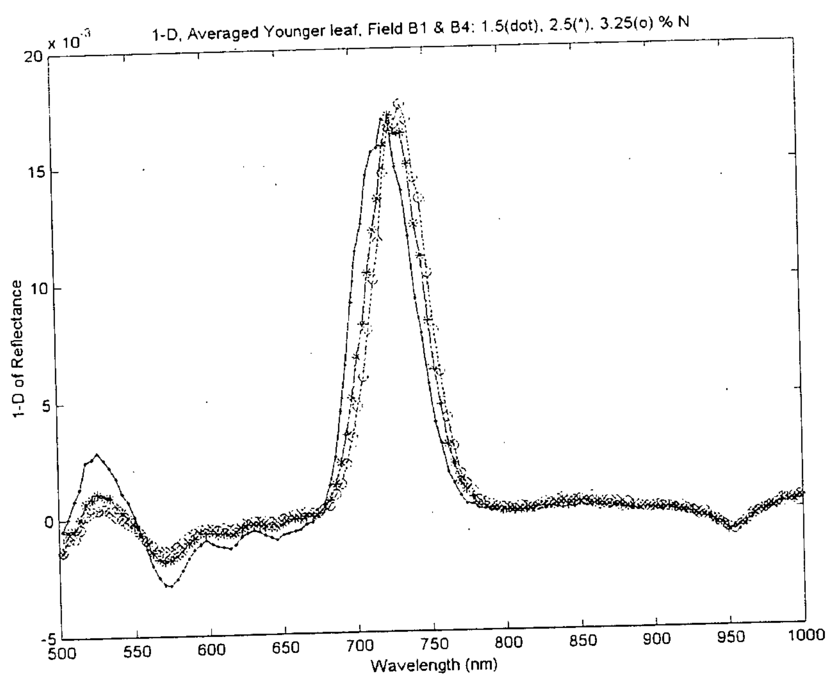
Figure 4.8. Histogram of N content (%) for ear leaf samples from 1999 experiment.

The root mean square errors (MSE) were 0.35% for the ear leaf and 0.20% for the younger leaf.

A PLS regression was conducted with one set of ear and younger leaf data as a calibration set and the other set of ear and younger leaf data set as a validation set. Then, a linear regression analysis was conducted between actual and predicted N (%) content. For the ear leaf data set, the R^2 between actual and predicted N (%) was 0.904 and root MSE was 0.23%. For the younger leaf, the R^2 between actual and predicted N (%) was 0.941 and root MSE was 0.13%. Note that for the N (%) content beyond 2.8%, the difference near 525 nm was small, so even the spectrophotometer was not able to identify luxury N consumption.



(a) Ear leaf.



(b) Younger leaf.

Figure 4.9. 1st derivative spectra for selected ear leaf and younger leaf samples from 5 different ranges of 1.25-1.50, 1.75-2.00, 2.25-2.50, 2.75-3.00 and 3.25-3.50% N from cornfields in 1999.

1D analysis of the nitrogen sensor measurements in 2000

The same 1D analysis procedures were executed with the nitrogen sensor measurement data set. Figure 4.10 shows a distribution of actual N (%) content of the samples. Note that there is not much availability for low N. Figure 4.11 shows a typical raw nitrogen sensor measurement ((a)), its normalized output ((b)), and its 1D spectra ((c)). Normalization seemed to reduce variation from different measurements in a same N treatment plot.

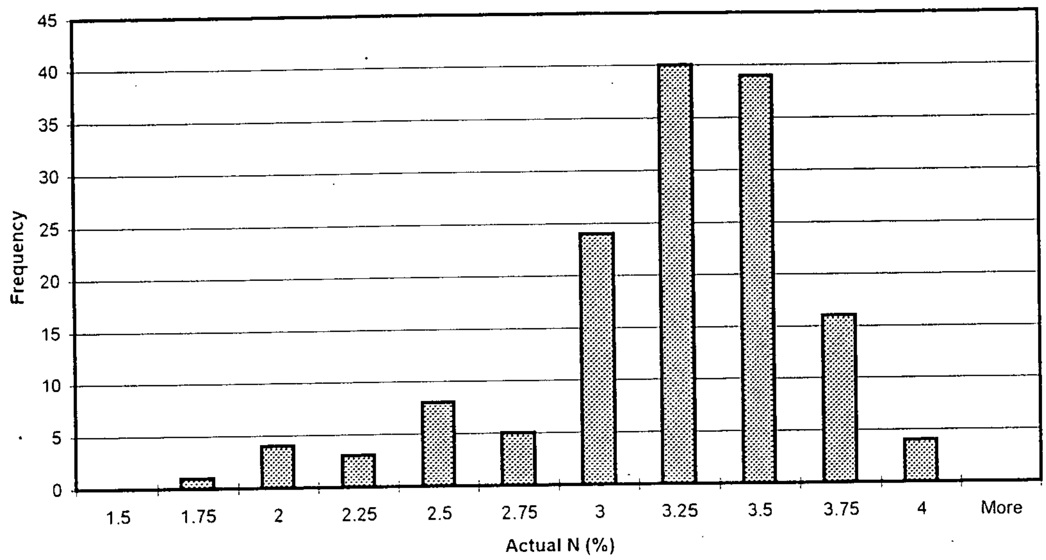


Figure 4.10. Actual N (%) distribution of all samples from Coufal farm, TX.

A multiple linear regression (MLR) analysis was conducted between actual N(%) and 1D peak values near 525 nm and 715 nm. For the 2000 leaf samples, the following relationship was found.

$Y = 3.43 - 9.495X_1 + 1.178X_2$ ($R^2 = 0.104$)

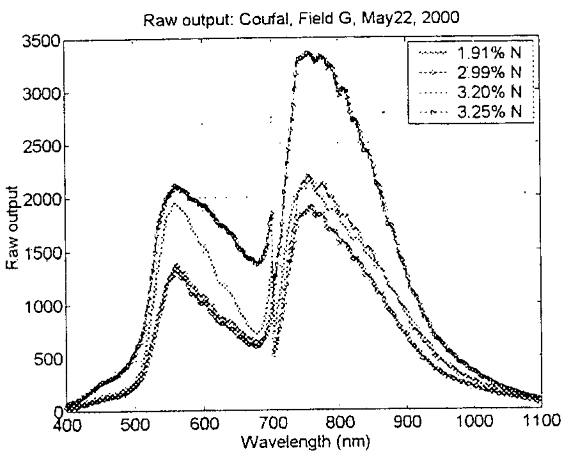
where X_1 = peak 1D value near 525 nm

X_2 = peak 1D value near 715 nm

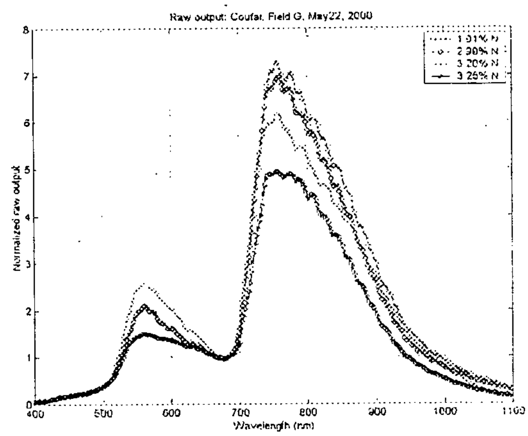
Y = predicted N (%)

The root mean square error (MSE) was 0.42%.

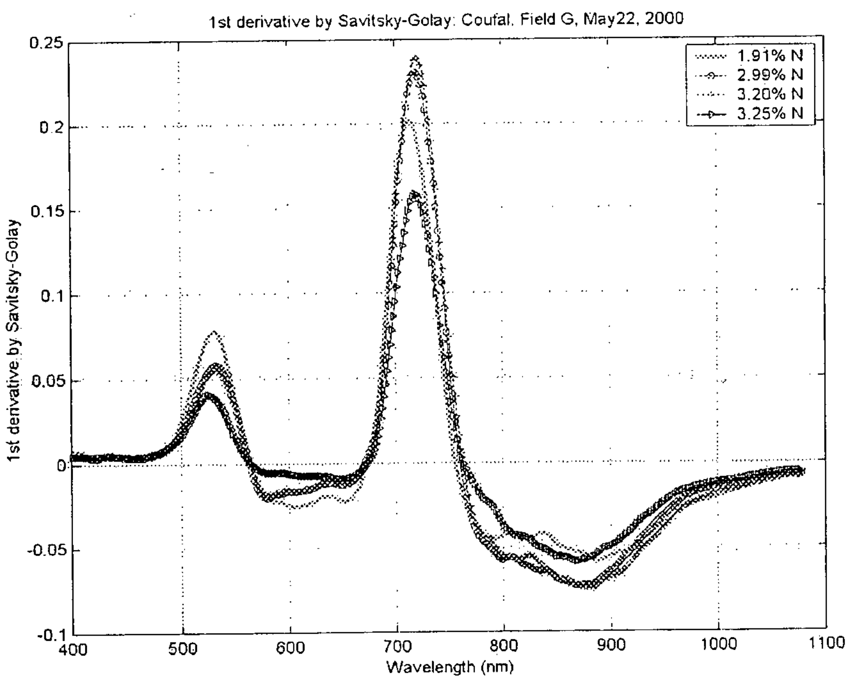
Even though the same 1D analysis procedures were followed with the Nitrogen sensor data this year, the predictability of N (%) content was not good. There might be several reasons for this poor predictability, primarily related to the lack of consistent illumination of the leaves and orientation of the leaves relative to the sensors. The variation in distance between the sensor and the leaves, and leaf orientation to the sensor appeared to have more effect on the sensor measurements than actual N changes in the leaves. The N content might have been varied in a plant, so the measured portion of the leaf had different N content than other part of the leaf used for N analysis. According to Jones and Eck (1973), nutrient elements are not evenly distributed within the corn leaf. Nor does the nutrient element concentration remain constant in any one plant part or section during the life cycle.



(a) Raw measurement.



(b) Normalized raw measurement.



(c) 1st derivative.

Figure 4.11. Sample measurement and its 1st derivative.

After difficulties with obtaining a consistent spectra were determined, a simple experiment was conducted using a single leaf to find an effect of varying orientation of a sample to the sensor and distance from the sample to the sensor. The leaf was moved in front of the sensor with different orientation and distance, and the spectra were recorded at each different orientation and distance. Then, their 1D spectra were calculated, Figure 4.12. This simple experiment showed that a single leaf produced different 1D spectra at different orientation and distance, even though the nitrogen content was same. Thus, it could be concluded that the lighting system had more impact on the sensor measurement than the nitrogen variation of the leaf itself. Further research would be needed to modify current sensing system.

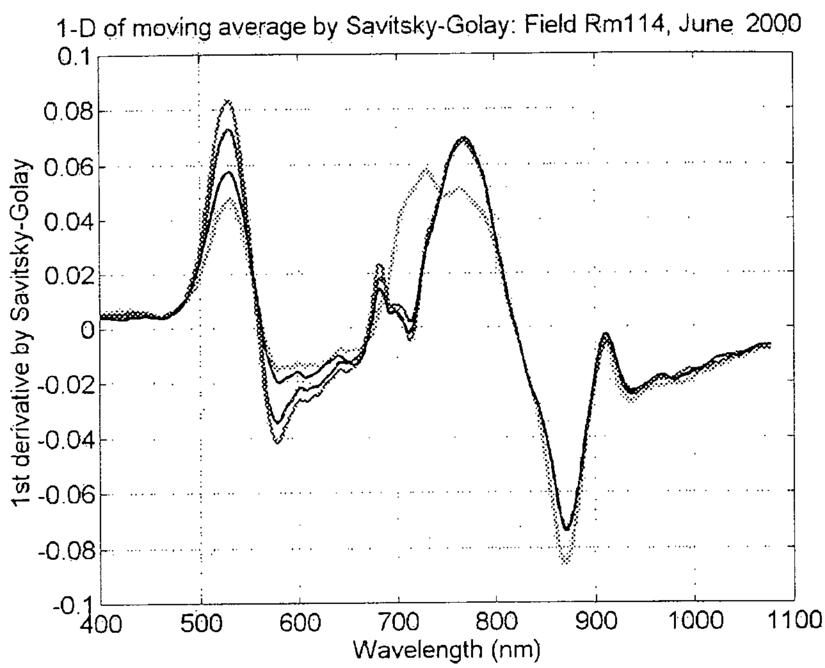


Figure 4.12. Variation of the 1st derivative spectra using a single leaf with varying distance and orientation to the sensor.

Multivariate statistical analyses (correlation analysis, stepwise discriminant analysis, and canonical discriminant analysis) were conducted to identify significant wavelengths to predict N (%) content using SAS, however none of them produced reliable result. This confirmed that this year's data set was clearly confound with the effect of the lighting system.

Each of 3 subsets was used as a validation set and the other two were used as a calibration set for a PLS analysis. After the PLS regression, a regression analysis was conducted between actual and predicted N (%). The R^2 between actual and predicted N (%) were 0.647, 0.441, and 0.367 for three different validation sets. The RMSE were 0.25%, 0.32%, and 0.38%, respectively.

CONCLUSIONS

The following observations resulted from this research.

The 1st derivative analysis algorithm for produced good predictability to N content (%) when spectra were formed in a consistent manner.

The hyperspectral sensor system with wide area flood lighting was not successful in assessing N status of corn plants.

An alternative design of the sensor and illumination system is necessary to generate consistent spectra.

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5. IN-FIELD MEASUREMENT OF LEAF N STATUS

A commercial mobile spectrometer, as well as the developed sensor were tested to acquire leaf spectra in experimental fields.

Commercial spectrometer characteristics

A spectrometer system was purchased from Ocean Optics, which included the following components:

Spectrometer model S200, with diffraction grating No 4 (covering the range from 530 to 1100 nm), slit width 50 microns. The spectrometer has a diode array of 2048 pixels and is connected to a 500KHz A/D board. A detector collection lens is mounted after the grating, which focuses the light on the detector.

Halogen light source, model LS-1. Halogen lamp 50W with convergence lens and SMA connector for fiber optic reflectance probe.

Reflection probe (R400-7-VIS/NIR) for VIS and NIR range. Illumination by 6 optic fibers arranged in a circle around the sensing fiber. Sensing fiber is 400 micron in diameter.

Separate optic fiber probe for sensing without artificial illumination. Optimized for VIS and NIR range. Fiber diameter 600 microns.

The spectrometer was calibrated from the factory and the regression curve between the pixel number and the wavelength was supplied.

The optical spectral resolution of the spectrometer is determined by the slit width and the grating range:

Dispersion (nm/pixel)=(Spectral range of grating)/(number of detector elements)=
=570/2048=0.278 nm/pixel

Resolution (pixels)= value from slit size. For 50 micron slit the value is 6.5 pixels

Optical resolution (in nm)=Dispersion *Resolution=0.278*6.5=1.8 nm (FWHM-Full Width Half Magnitude).

Corn plots were established at MIGAL experimental farm at KIRYAT SHMONA (170 Km North of Tel Aviv). Corn variety Sweet corn cv *Jubilee* was used.

During summer 1999 and 2000, six treatments of nitrogen were applied, in 6 replications, in a random block experiment. Each replication consisted of 6 rows (6m wide), Each row was 20m long.

Treatments

Leached: Prior to seeding, excess irrigation was applied to wash out the remainders of Nitrogen in the soil. No Nitrogen was applied in these treatments

NoN: Soil was not washed before seeding, but no fertilizer was applied

N12: 125 Kg/hectare Nitrogen fertilizer was applied at V6 stage

N25: Prior to seeding, 125 Kg/hectare Nitrogen fertilizer was applied, and an additional of 125 Kg/Hectare was applied at V6 stage

N50: Prior to seeding, 250 Kg/hectare Nitrogen fertilizer was applied. An additional 250 Kg/hectare was applied at V6 stage

N100: Prior to seeding, 500 Kg/hectare Nitrogen fertilizer was applied. An additional 500 Kg/hectare was applied at V6 stage

The in-field nitrogen assessment was performed in to stages. First in stationary mode with controlled sampling device and then, simulating motion of the system above the canopy, with a dark camber and especially designed optical configuration.

A. Stationary measurements

Leaf reflectance was measured while stationary, using a special sampling device that was designed and built for that purpose.

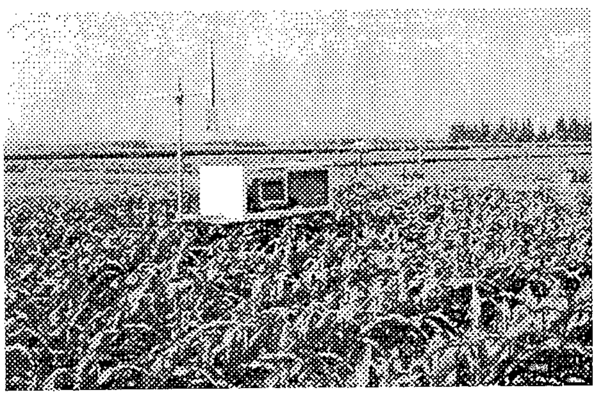
Reflectance sampling and laboratory analysis

Reflectance of the leaves was measured in three stages of growth on 7-7-1999, 18-7-1999 and 21-7-1999. Leaf samples were taken from the two central lines (out of 6) of each replication and several reflectance tests were performed:

Reflectance using 600 nm fiber optic probe and sunlight illumination (stationary, at different angles) : 7-7-1999, 18-7-1999

Reflectance using 400 nm reflection probe, halogen illumination and sampling cell: 18-7-1999, 21-7-1999

Each sampled leaf was put on a labeled bag and sent to the laboratory for N content analysis. On 18-7-1999, the basis of the stem was also harvested and sent to the lab for N analysis.



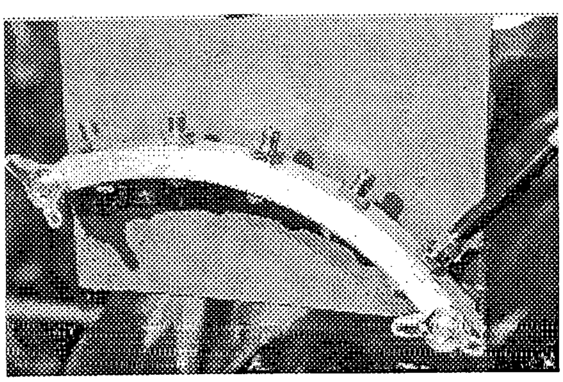
All measurements were performed in the field. The system was carried on a cart as shown in figure 5.1. The computer, the monitor and the spectrometer were placed in a white wooden box to protect from direct sunlight. Power was supplied by a generator to a UPS unit, which in turn, supplied 220VAC to all instruments.

Figure 5.1 Cart to carry on the data acquisition system

1. Reflectance measurements under sunlight

A special device was built to hold the fiber. The fiber could be installed in vertical direction, or in a 15, 30 or 45 degrees angle. SMA connectors were used to connect the fiber to the holding device. The fiber was then directed towards the crop row and the reflectance spectrum was acquired.

The height of the holding device above the crop and its orientation towards the crop



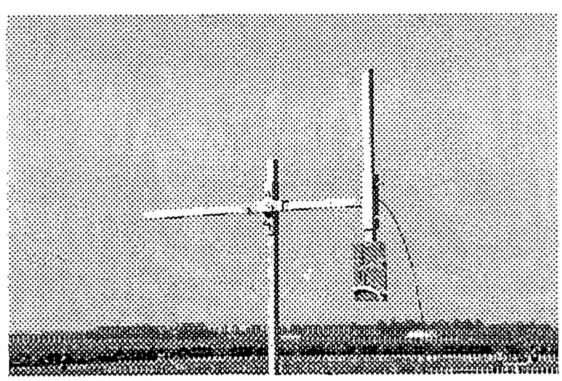


Figure 5.2 Special device to hold the fiber

lines could be adjusted through three axes, as shown in figure 5.2.

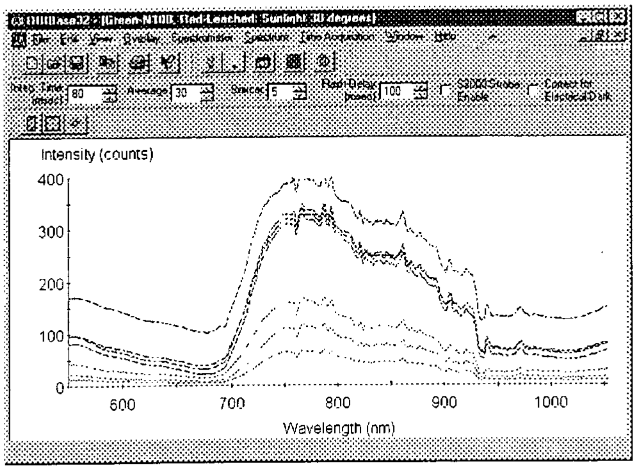


Figure 5.3 Sample results of leaf reflectance

Figure 5.3 shows sample results of leaf reflectance of N100 and leached treatments (7-7-1999). One can see that the non-regular spectrum of sunlight, combined with the non-linearity of the diode array (non-linearity in the intensity axis) causes the irregularities of the reflectance spectra. In this example, the two treatments have a big difference in the intensity of the reflected light. In order to get a more robust model, we should not rely only on intensity deference, but on the shape of the spectral curve (slope etc.). The local shape of the curve is greatly affected by the irregular nature of the sunlight.

In an effort to avoid these irregularities in the spectra, a sampling cell was designed and constructed, for measuring under artificial illumination. The sampling cell had to be opaque, easy to attach to a leaf and accommodate the reflection probe.

Reflectance using, halogen illumination and sampling cell

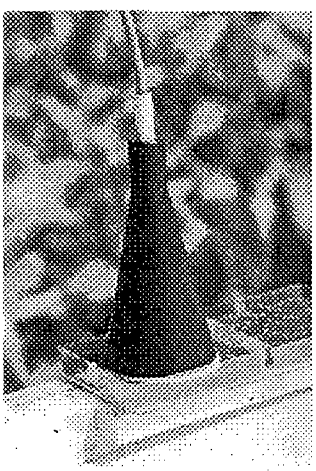


Figure 5.4 Sampling cell for the reflection probe

After the first sampling day using direct sun illumination, a sampling cell for the reflection probe was designed and built (see figure 5.4). The sampling cell was constructed from black Delrin (polymer material), in a cone shape (12 degr. Slope of the cone). The reflection probe (combined illumination and read fiber) were inserted from the top of the cone and the sample was placed at the open basis of the cone.

A white ceramic disk was used as a reference signal. The diameter of the ceramic disk matched the diameter of the cone basis. The sampling cell was placed on top of the reference disk in order to obtain the white reference signal. This procedure was repeated every time, before a leaf was sampled. The dark reference signal was sampled by turning illumination off and covering the read fiber with a dark cloth. Dark reference signal was sampled every 30 leaves.

A black hard surface was used to attach the leaf to the basis of the sampling cell, such that the area illuminated by beam (and exposed to the read fiber) was either the leaf or black background. Alternatively, a matching opposite cone was constructed, and was also used for attaching the leaf to the basis of the sampling cell. In addition to that, the matching cone could accommodate a separate read fiber, for transmittance measurements (which were not performed at this time).

During the experiments, the sampling cell was manually attached to a leaf, and the reflectance was measured. Although the sampling cell was used for most of the measurements, an additional effort has to be made to try to find out if there is a possibility of using direct sunlight as the illumination source. Very preliminary analysis shows that the spectral range of 550 to 650 nm has significant information of N content. In this spectral range the irregularities due to sunlight are minor. Another interesting range is from 950 to 1050 nm, which is also quite smooth.

The leaves are analyzed in the lab and we expect the results in the middle of August.

Results, Models and spectral analysis

First, we assumed that the fertilizer treatment is an indication of the nitrogen content in the leaf. Based on that assumption, we tried to find parameters that cluster the data to their fertilizer treatments groups.

Close examination of the spectral curves, revealed a difference in the slope in two different spectral ranges (Figure 5.5): 595-670 nm and 1040-1080 nm. The slope of the curves was then calculated at this spectral range by fitting a linear curve to the

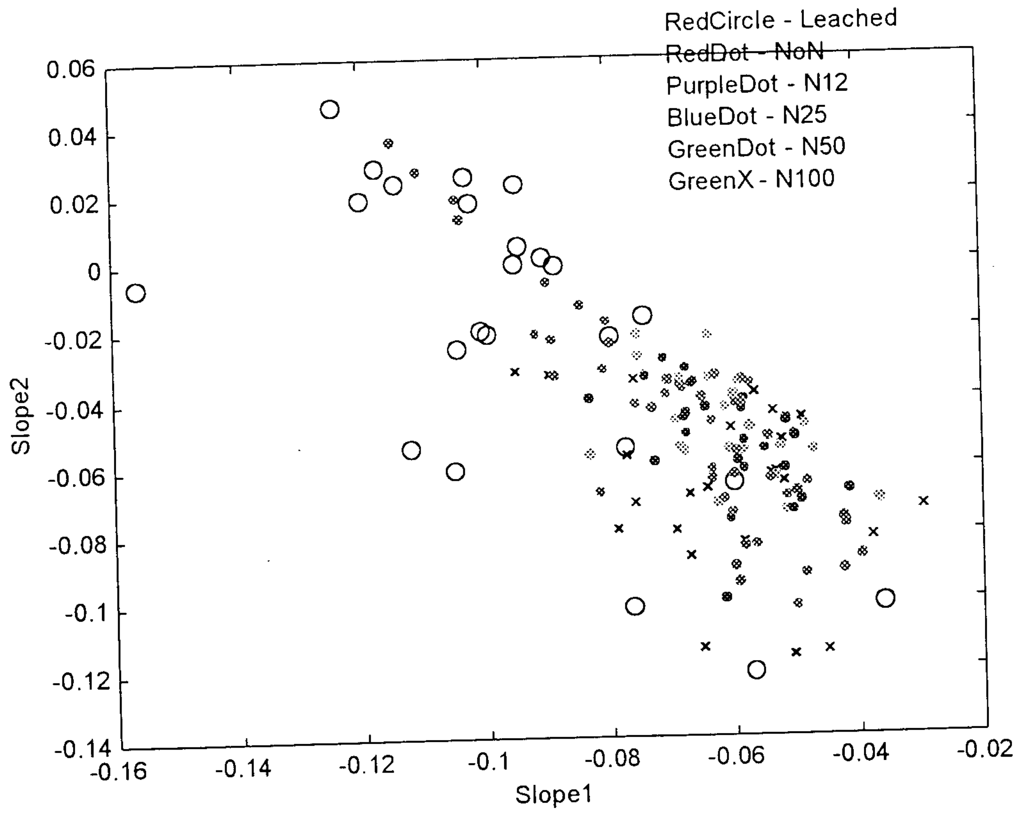


Figure 5.6 Clusters of different N treatments

spectral data. This was done with linear regression tools, using Matlab. Based on the two features, slope1 and slope2, (the two approximated slopes of the spectral curve) the leaves were plotted on the 2-D feature space. Figure 5.6 shows the clusters of the treatments: 1. The two extreme treatments are almost completely separable, N100 and leached, although the exact concentration of N in the leaf is not known and certainly not uniform. Part of the observed variability is due to the natural variability that exists in the data set (different levels of leaf N in the same fertilizer treatment) and part due to the model error. 2. The intermediate treatments are spread along the whole range and this may suggest that there is a great variability in the leaf N in the data.

Leaf N content as measured in the laboratory was then used as the independent variable in a model that links spectral reflectance to leaf nitrogen status. The reflectance spectra was preprocessed with first derivative operator and PLS models as well as single wavelength correlation models were built.

Single wavelength correlation revealed that there are two main spectral regions with highly correlated to leaf N: from 530 to 780 and from 1000 to 1070. Wavelengths between 780 to 1000 nm where poorly correlated to leaf N. The maximum single wavelength correlation was for 760 nm and its value was close to 0.68.

PLS models, using the first derivative of the spectrum were built. Part of the data set (2/3 of 174 samples) was used for calibrating the model and another part (the remaining 1/3) was used for prediction. LTCAL software (L.T.Industries) was used to analyze the data. A four factors PLS model was found to yield minimum standard error of prediction (SEP=0.303 % N). Figures 5.7 and 5.8 show the results of the calibration and prediction using the above model. The regression coefficient of the calibration set was 0.97, while for the prediction set was 0.76.

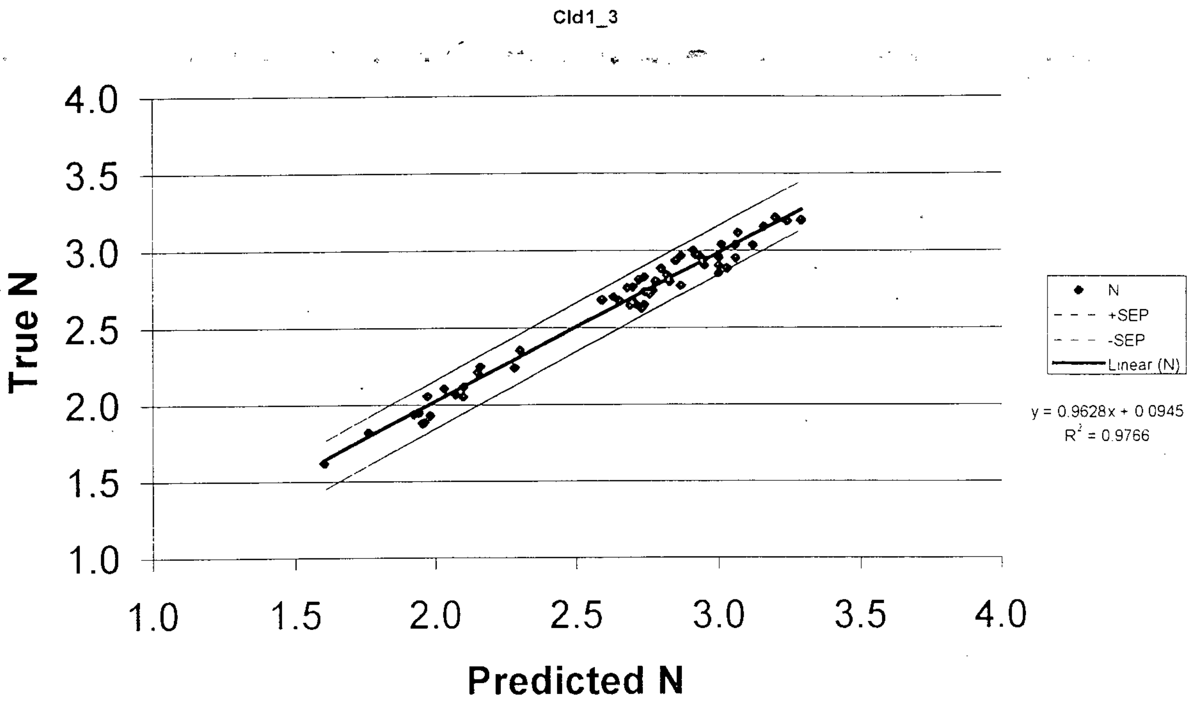


Figure 5.7 Calibration results

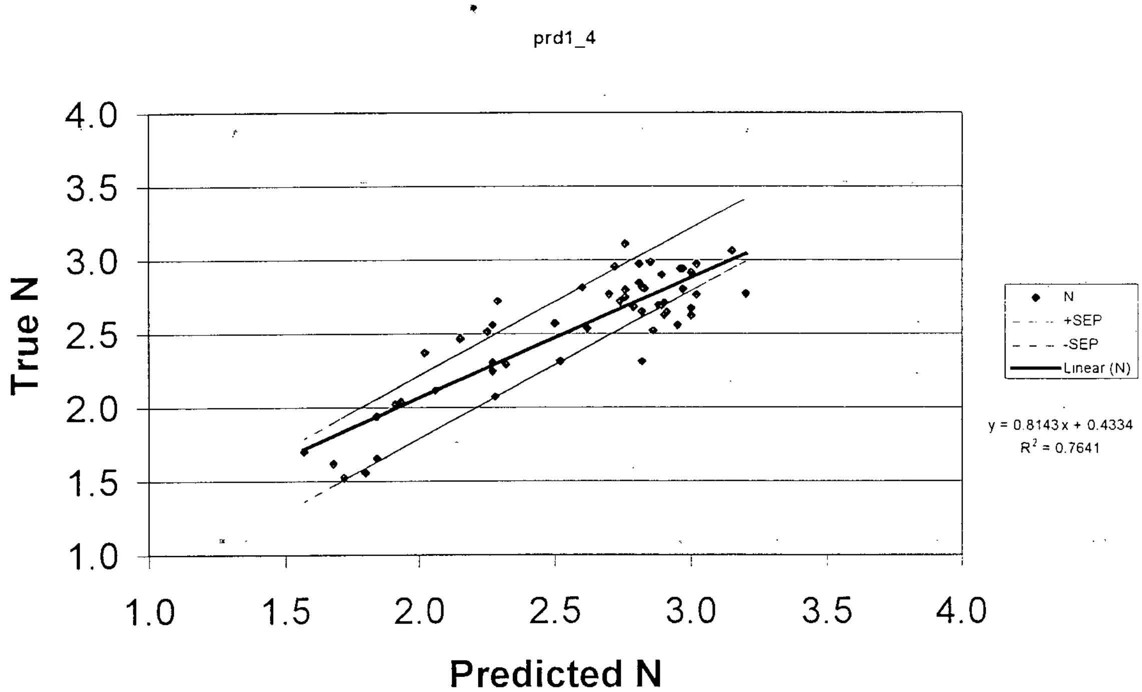


Figure 5.8 Prediction results

B. In-field moving measurements

Optical configuration of illumination and fiber

A mobile dark chamber was constructed for moving the sensing system above the crop canopy, as depicted in figure 5.9. The sensor assembly in the dark chamber included a collecting optical fiber for transferring the reflected light to the spectrometer and a collimated 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 degrees to the optical fiber. This optical configuration provided significant signals (signals with acceptable intensity) only when the sampled leaf intercepted the common path of the illumination beam and the optical fiber. Therefore, the signals that were acquired by the sensing system were only from leaves at a given distance from the illumination source and the optical fiber. Leaves that were not on 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.

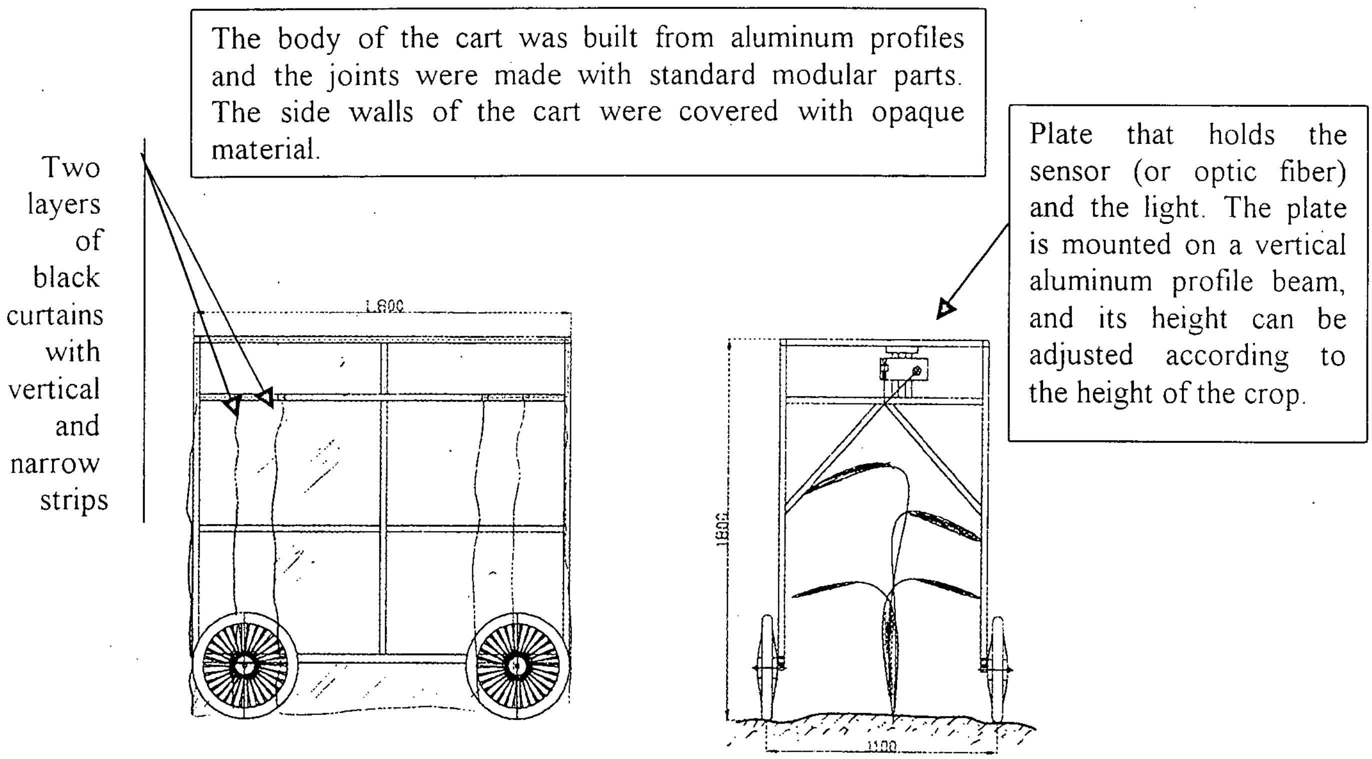


Figure 5.9 Mobile dark chamber

In order to demonstrate the optical configuration principle, a video camera was placed in the dark chamber and images of the sensor assembly along with the crop were acquired. Figure 5.10 shows a sample image of the sensor assembly in the dark chamber, during data acquisition. The leaf that is illuminated by the light beam is inside the sensor's field of view and therefore the spectrum is acquired and processed. Soil background as well as the rest of the crop's leaves are not illuminated and therefore do not contribute to the reflectance signal.

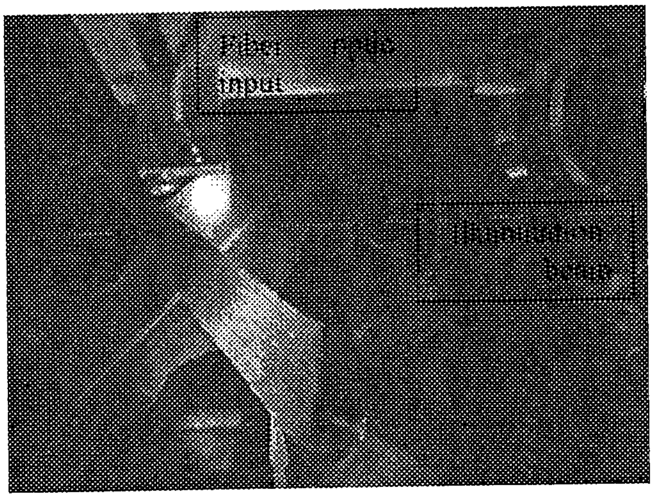


Figure 5.1 Sample image of the sensor assembly in the dark chamber, during data acquisition.

Data processing – graphs from PLS

Leaf N content as measured in the laboratory was used as the independent variable in a model that links spectral reflectance to leaf nitrogen status. The reflectance spectra was preprocessed with first derivative operator and PLS models were built

Matlab was used to analyze the data. The PLS models were validated using the Venetian Blinds validation scheme with 10 splits. Two maturity stages were measured: V6 and VT stage. When separate models were built for each maturity stage, SEP was 0.33%N and 0.21%N for V6 and VT respectively. When all data was compiled in one data set, a seven factors PLS model was found to yield minimum standard error of prediction: SEP=0.27%N. Figures 5.11 and 5.12 show the results of the calibration and prediction using the later model.

Partial Least Squares Regression Model

```
X-block: ddt 360 by 1832
Y-block: Nt  360 by 1
No. LVs: 7
RMSEC:   0.250221
RMSECV:  0.27453
Scaling: auto scaling
Cross-validation: venetian blinds
 with 10 splits

Percent Variance Captured by Regression Model
```

Figure 5.11 Results of the calibration and prediction

LV #	X-Block This LV	Total	Y-Block This LV	Total
41.92	41.92	52.22	52.22	1
70.13	28.21	78.94	26.72	2
72.92	2.79	90.33	11.39	3
78.84	5.92	92.26	1.93	4
80.57	1.72	92.99	0.73	5
81.25	0.69	94.88	1.89	6
82.11	0.86	96.08	1.20	7

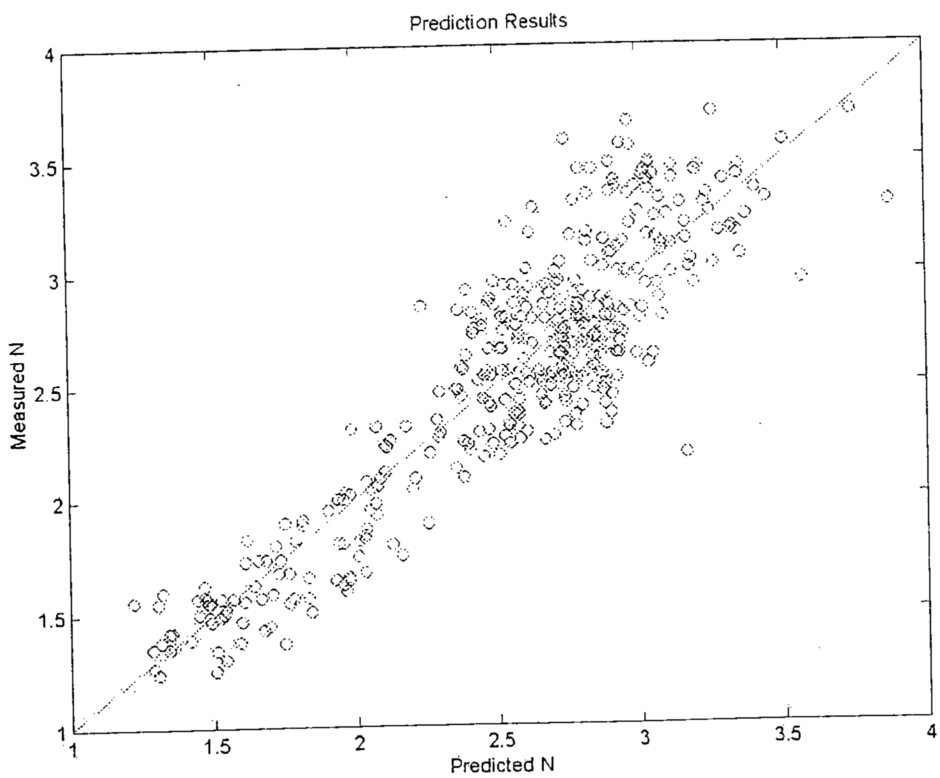


Figure 5.12. Prediction results

The above results show that nitrogen status can be predicted in-field, using non contact optical sensor based on spectral reflectance.

For efficient application of fertilizers, the nitrogen status of the crop has to be related to the required fertilization for achieving the potential yield. Figures 5.11 and 5.12 depict the relationship between fertilization regime and yield, as obtained from the measurements in the field experiments. It can be clearly seen that there is an optimum fertilization policy in order to achieve maximum yield. Statistical analysis showed that the yield obtained by applying 125 Kg/ha N was not significantly lower from higher nitrogen applications and was significantly higher than lower nitrogen application. Therefore 125 Kg/ha N can be considered the optimum fertilization regime for obtaining the potential yield. Figure 5.13 depicts the relationship between the fertilization regime and the nitrogen level in the crop's leaves, as measured in the laboratory. It can be seen that the nitrogen level in the leaves is not directly proportional to the fertilization regime. For example, leaves of N250 treatment have significantly lower nitrogen content than N125, in spite fact that N250 received more fertilizer. More than that, treatment N0 has significantly more N than N125. Nevertheless, with respect to yields, the higher the fertilizer treatment, the higher the yield.

From the above experiment one can conclude that the nitrogen level of the leaves is not an indication of the need for fertilizer! Nevertheless, spectral reflectance can be used to predict directly the fertilizer treatment, which is directly proportional to the potential yield. Therefore, spectral reflectance is indirectly related with the need for additional nitrogen application.

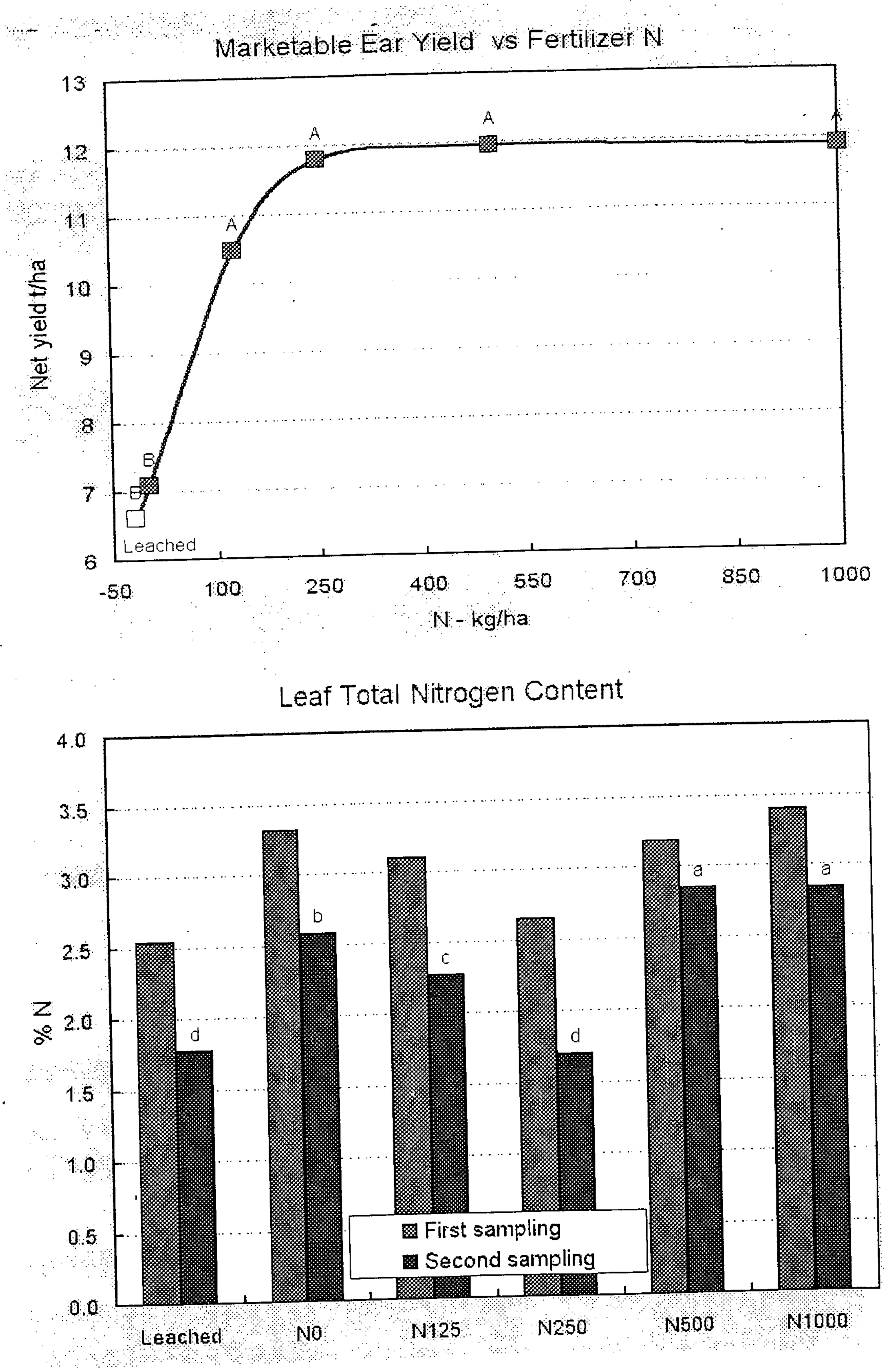


Figure 5.13 Relationship between the fertilization regime and the nitrogen level in the crop's leaves