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REMOTE SENSING

Detection of Phosphorus and Nitrogen Deficiencies in Corn Using Spectral Radiance Measurements

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ABSTRACT

Applications of remote sensing in crop production are becoming increasingly popular due in part to an increased concern with pollution of surface and ground waters due to over-fertilization of agricultural lands and the need to compensate for spatial variability in a field. Past research in this area has focused primarily on N stress in crops. Other stresses and the interactions have not been fully evaluated. A field experiment was conducted to determine wavelengths and/or combinations of wavelengths that are indicative of P and N deficiency and also the interaction between these in corn (*Zea mays* L.). The field experiment was a randomized complete block design with four replications using a factorial arrangement of treatments in an irrigated continuous corn system. The treatment included four N rates (0, 67, 134, and 269 kg N ha^{-1}) and four P rates (0, 22, 45, and 67 kg P ha^{-1}). Spectral radiance measurements were taken at various growth stages in increments from 350 to 1000 nm and correlated with plant N and P concentration, plant biomass, grain N and P concentration, and grain yield. Reflectance in the near-infrared (NIR) and blue regions was found to predict early season P stress between growth stages V6 and V8. Late season detection of P stress was not achieved. Plant N concentration was best predicted using reflectance in the red and green regions of the spectrum, while grain yield was estimated using reflectance in the NIR region, with the particular wavelengths of importance changing with growth stage.

Nitrogen is the most limiting nutrient in production of nonleguminous crops in central Nebraska and the Great Plains. As cropping practices become more intensive, other nutrients will likely become limiting as well. The second most limiting nutrient for corn production is often P. Current methods for estimating the amount of P available to growing crops include soil sampling or in-season plant sampling, both of which can be costly and labor intensive. The use of remote sensing techniques to estimate nutrient status could decrease the amount of labor needed for sampling, and could reduce the cost associated with sampling and analysis.

Destructive tissue testing is a common way to assess crop N and P status. Nondestructive methods have been developed to monitor crop N status. Blackmer and Schepers (1994) found that the chlorophyll meter was a useful method of monitoring corn N status, compared with measuring leaf N concentration, which requires destructive sampling. While the chlorophyll meter is a good indicator of in-season N status, the technique requires time and labor for data collection. The use of remote sensing could help eliminate the need for extensive field sampling while still providing a good detection of deficiencies.

Remote sensing is simply obtaining information about an object, area, or phenomenon by analyzing data acquired by a device that is not in contact with the object, area, or phenomenon (Lillesand and Kiefer, 1987). Recently, researchers have evaluated remote sensing techniques for estimating the N status of growing crops by determining the appropriate wavelength or combination of wavelengths to characterize crop N deficiency. Blackmer et al. (1994) stated that light reflectance near 550 nm (green) was best for separating N treatment differences, and could be used to detect N deficiencies in corn. Everitt et al. (1985) studied the relationship of plant leaf N concentration and leaf reflectance from 500 to 750 nm, concluding that buffalograss (*Buchloe engelm* Poaceae) receiving no fertilizer N had highest reflectance readings. Walburg et al. (1982) demonstrated that N treatments affected reflectance in both the red and near-infrared (NIR) regions of the spectrum, with red reflectance increasing and NIR reflectance decreasing for N-deficient corn canopies. Blackmer et al. (1996) reported that reflected radiation near 550 and 710 nm was better for detecting N deficiencies compared with reflectance at other wavelengths. They found that a ratio of the 550 to 600 nm band to the 800 to 900 nm band could distinguish between N treatments in irrigated corn canopies. Stone et al. (1996) demonstrated that total

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plant N could be estimated by using spectral radiance measurements at the red (671 nm) and NIR (780 nm) wavelengths. They calculated a plant-N-spectral-index for the amount of fertilizer N required to correct in-season N deficiency in winter wheat (*Triticum aestivum* L.). Yoder and Pettigrew-Crosby (1995) estimated total N and chlorophyll content using reflectance. They found that for fresh plant samples, the log transform of 1/reflectance in the short-wave infrared band was the best predictor of N content and that the visible bands were the best predictors of chlorophyll content.

There has been limited research investigating the potential of using remote sensing techniques to detect P and other nutrient deficiencies. Milton et al. (1991) grew soybean [*Glycine max* (L.) Merr.] plants in hydroponic solutions at three P concentrations and measured weekly changes in leaf spectral reflectance. They found that P-deficient plants had a higher reflectance in the green and yellow portions of the spectrum and did not show the normal shift of the red edge (chlorophyll absorption band at 680 nm). Al-Abbas et al. (1974) found that absorption at 830, 940, and 1100 nm was lower for P- and Ca-deficient corn leaves, whereas leaves deficient in S, Mg, K, and N had higher absorption in these wavelengths. Work by Masoni et al. (1996) found that Fe, S, Mg, and Mn deficiencies decreased absorption and increased reflectance and transmittance in corn, wheat, barley (*Hordeum vulgare* L.) and sunflower (*Helianthus annuus* L.) leaves. They also noted that mineral deficiency affected leaf concentration of other elements in addition to the deficient element, with nutrient concentrations varying according to species and deficiency level. Sembiring et al. (1998) found that by using a covariate of 435 nm, a 695/405 nm ratio was a good indication of P uptake by bermudagrass [*Cynodon dactylon* (L.) Pers.].

The objectives of this experiment were to determine wavelengths and/or combinations of wavelengths that are indicative of P and N stresses independently and the interaction between these using hyperspectral data in irrigated corn.

MATERIALS AND METHODS

A continuous irrigated corn experiment was conducted on a Hord silt loam (fine-silty, mixed, mesic Pachic Haplustolls) located at the Management Systems Evaluation Area (MSEA) project near Shelton, NE. The production system utilized conventional tillage with a linear drive irrigation system. Total irrigation amounts were 200.8 mm during 1997 and 115.2 mm during 1998. Growing conditions during 1997 and 1998 were similar with respect to average daily temperature and growing degree days, but rainfall amount was different between the 2 yr with 247 mm for 1997 compared with 457 mm in the 1998 season. The seeding rate was 74 000 plants ha^{-1} with Pioneer brand hybrid 3225 planted on 1 May 1997 and 4 May 1998. Initial surface soil test characteristics are reported in Table 1.

Table 1. Initial surface soil test characteristics.

Depth	pH	Organic matter	NO$_3$–N	P	Zn	K
		%		mg kg^{-1}		
Surface	6.5	1.8	21.5	7.0	1.44	493

The experimental design was a randomized complete block design with four replications. The treatment design was a four × four factorial arrangement. Nitrogen was applied as NH$_4$NO$_3$ at rates of 0, 67, 134, and 269 kg N ha^{-1}, and P was applied as triple superphosphate at rates of 0, 22, 45, and 67 kg P ha^{-1}. Phosphorus and a split application of N at one-half the N rate was applied preplant and incorporated. The second split N application at one-half the N rate was topdressed at V5 to V7. Treatments were applied to the same experimental areas both growing seasons. Plots were 9.14 by 15.24 m with 0.76 m row spacing. 'Guardsman' [dimethenamid (2-chloro-*N*-[(1-methyl-2-methoxy)ethyl]-*N*-(2,4-dimethyl-thien-3-yl)-acetamide) + atrazine (2-chloro-4-ethylamino-6-isopropyl-amino-*s*-trazine)] 53.2% a.i. were applied to all plots at a rate of 3.5 L ha^{-1} in early May. Phenology data according to Ritchie et al. (1997) were recorded weekly from 1 June until the end of August.

Hyperspectral reflectance measurements were collected from 350 to 1000 nm (1.4 nm intervals) with a Personal Spectrometer II manufactured by Analytical Spectral Devices[1] (Boulder, CO). A typical vegetation spectral curve with corresponding color reflected by at each wavelength is illustrated in Fig. 1. Six canopy measurements were taken randomly at a height of 3 m above the canopy with a 15° field of view throughout each plot. Spectral measurements were collected 19 June and 15 July in 1997; and 19, 24, and 29 June and 20 July in 1998. Sampling dates were 19 June and 15 July in 1997; and 19 June and 20 July in 1998. Spectral measurements collected 19 June 1997 were collected without consideration for row position within the field of view. Spectral measurement collected after 19 June 1997 were collected by centering the field of view over a row to minimize the presence of soil. All readings were averaged to obtain a representative reading for the entire plot. Canopy measurements were taken on cloud-free days at ±2 h from solar noon. All measurements were transformed into percent reflectance using a Spectralon[1] reference panel (Labsphere, Sutton, NH) for determining total reflected incoming radiation. Panel measurements were taken before initial canopy readings and repeated approximately every 15 min.

Integrating sphere measurements were collected on 17

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[1] Mention of trade name or proprietary products does not indicate endorsement of USDA and does not imply its approval to the exclusion of other products that may also be suitable.
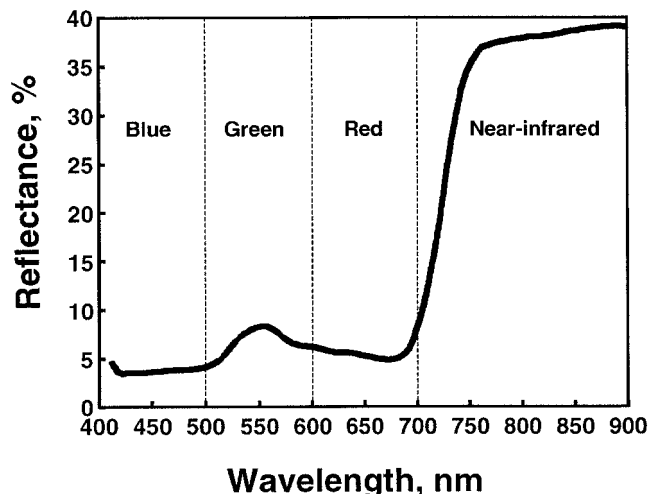

Fig. 1. Typical vegetation spectral reflectance curve and corresponding color reflected at each particular wavelength.

and 23 June and 29 July in 1997 on the 269 kg N ha^{-1} rate for the four different P treatments. Integrating sphere measurements were collected on 10 random uppermost collared leaves. Leaves were placed in a Ziploc bag and stored on ice until measurements were performed. Three spot readings per leaf were taken on the upper and underside of the leaf surface. Spectral measurements were taken by attaching the radiometer to an 1800-12 External Integrating Sphere manufactured by Li-Cor Inc.[1] (Lincoln, NE). External Integrating Sphere is a self-contained light source that collects spectral measurements on a 1-cm diameter circle of the leaf without interference from external light and/or environmental conditions. All readings taken from the plots were averaged and transformed to percent reflectance using a barium sulfate reference to obtain one representative reading per plot.

Spectral readings were collected throughout the growing season and averaged over 5-nm intervals to decrease the amount of data for analysis. Analysis of variance and single-degree-of-freedom contrasts were performed, using the GLM procedure in SAS (SAS Inst., 1988). Stepwise regression was performed, using the REG procedure in SAS (SAS Inst., 1988), on all data to develop multiple regression equations for predicting plant N and P concentration, biomass, grain yield, and grain N and P concentration.

Aboveground biomass sampling was performed throughout the growing season by taking 12 randomly selected plants from the east quarter of the plots. Whole plants were weighed and ears were separated once distinguishable. Sampling dates were 19 June, 15 July in 1997, and 19 June and 20 July in 1998. Whole plants were chopped with a chipper-shredder in the field to facilitate subsampling. Subsamples were weighed, oven-dried at 50°C, and then reweighed for water content. Leaf samples collected for integrating sphere measurements were saved for nutrient analysis. Leaf samples and ear samples were also oven-dried at 50°C before weighing. All samples were ground with a Wiley Mill to pass a 2-mm sieve. Nitrogen concentration was determined on all samples using dry combustion (Schepers et

al., 1989) and P concentration using energy dispersive x-ray fluorescence (Knudsen et al., 1981). Total dry matter per plot was calculated by combining the ear and total vegetative matter dry weights. Grain yield was estimated by hand harvesting 3.05-m row length from each of the four middle rows. Ears were shelled and water content determined. Grain samples were oven-dried at 50°C, ground, and analyzed as described above for biomass samples. Grain yield per plot was calculated and corrected to 155 g moisture kg^{-1}.

RESULTS AND DISCUSSION
In-Season Biomass and Grain Harvest

A significant linear and/or quadratic response to applied N was observed for all sampling dates for N content, grain yield, and biomass (Table 2–4). A significant biomass response to applied P in late June of 1997 and 1998 was observed (Tables 2 and 3). Later season biomass sampling in July did not exhibit a significant response to applied P. Biomass production increased with increasing N rate for the second growing season, while increasing N rate decreased P content (Table 3). Plots receiving no N had a higher P content compared with the N fertilized plots for all sampling dates. This could be due to the reduction in growth from an N deficiency. The response to applied N was greater for 1998 compared with 1997. There was a 26% increase in biomass production for the 269 kg N ha^{-1} rate compared with the 0 kg N ha^{-1} rate for the June 1997 sampling date, whereas the 1998 June sampling date had a 232% increase in biomass. Differences in biomass sampling for late July did not exhibit the same variation between the 2 yr. The 1997 sampling had an increase of 26% compared with 105% increase in 1998. This increase in

Table 2. Analysis of variance, N and P rates means, and single degree of freedom contrasts for biomass, N, and P concentration by sampling date, 1997.†

Source	df	Biomass		N		P	
		20 June	15 July	20 June	15 July	20 June	15 July
		——— Mg ha^{-1} ———		——————— g kg^{-1} ———————			
				Mean squares			
Rep	3	0.018	1.218	0.0811	0.0443	0.0001	0.0025
N rate	3	0.072**	5.740**	1.1195**	0.9220**	0.0008*	0.0022
Linear	1	0.203**	10.714**	2.7642**	2.1115**	0.0002	0.0050
Quadratic	1	0.001	6.261**	0.3585*	0.6540**	0.0013	0.0014
LOF	1	0.012	0.246	0.2358*	0.0004	0.0009	0.0002
P rate	3	0.078**	1.172	0.1151	0.0587	0.0007*	0.0025*
Linear	1	0.169**	3.513	0.2575	0.1691	0.0003	0.0017
Quadratic	1	0.054*	0.001	0.0826	0.0026	0.0010	0.0031
LOF	1	0.010	0.002	0.0052	0.0043	0.0008	0.0027
N rate × P rate	9	0.010	0.909	0.0193	0.0286	0.0001	0.0015
Error	45	0.009	0.757	0.0535	0.0227	0.0002	0.0009
SED		0.067	0.615	0.1636	0.1065	0.0100	0.0212
				Treatment means			
N rate, kg ha^{-1}							
0		0.61	4.02	2.83	1.45	0.27	0.23
67		0.63	4.72	3.29	1.75	0.25	0.24
134		0.71	5.37	3.33	1.94	0.26	0.25
269		0.77	5.08	3.56	1.97	0.26	0.25
P rate, kg ha^{-1}							
0		0.57	4.52	3.40	1.85	0.26	0.22
22		0.66	4.71	3.23	1.80	0.24	0.25
45		0.76	4.93	3.20	1.74	0.26	0.24
269		0.72	5.04	3.18	1.72	0.26	0.24

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
† df, degrees of freedom; LOF, lack of fit; SED, standard error of the difference between two equally replicated means.

Table 3. Analysis of variance, N and P rates means, and single degree of freedom contrasts for biomass, N, and P concentration by sampling date, 1998.†

Source	df	Biomass		N		P	
		19 June	20 July	19 June	20 July	19 June	20 July
		——— Mg ha^{-1} ———		——————— g kg^{-1} ———————			
				Mean squares			
Rep	3	0.007	8.173	0.4468	0.0245	0.0113	0.0047
N rate	3	1.149**	164.945**	2.1441**	0.9972**	0.0681**	0.0219**
Linear	1	3.208**	457.095**	5.5537**	2.9505**	0.1020**	0.0366**
Quadratic	1	0.166**	26.684*	0.8396**	0.0136	0.1003**	0.0290**
LOF	1	0.074	11.057	0.0392	0.0275	0.0021	0.0001
P rate	3	0.166**	7.061	0.3229**	0.0155	0.0069	0.0063**
Linear	1	0.349**	6.505	0.9497**	0.0410	0.0131	0.0178**
Quadratic	1	0.148*	13.828	0.0173	0.0049	0.0024	0.0007
LOF	1	0.002	0.852	0.0019	0.0004	0.0054	0.0006
N rate × P rate	9	0.042	5.436	0.1352	0.0401	0.0024	0.0008
Error	45	0.023	5.792	0.0621*	0.0226	0.0031	0.0015
SED		0.107	1.702	0.1762	0.1063	0.0394	0.0274
				Treatment means			
N rate, kg ha^{-1}							
0		0.28	7.38	1.99	0.84	0.40	0.27
67		0.59	11.19	1.94	1.05	0.29	0.22
134		0.68	12.31	2.12	1.16	0.25	0.20
269		0.93	15.13	2.73	1.44	0.27	0.21
P rate, kg ha^{-1}							
0		0.47	10.66	2.37	1.15	0.29	0.21
22		0.64	11.67	2.24	1.14	0.27	0.22
45		0.69	12.26	2.11	1.12	0.32	0.23
269		0.67	11.42	2.05	1.08	0.32	0.25

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
† df, degrees of freedom; LOF, lack of fit; SED, standard error of the difference between two equally replicated means.

growth corresponds to a decrease in plant P concentration with the plants having the higher biomass exhibiting the lower concentration due to dilution.

Grain yield increased by 29% in 1997 and 92% in 1998 for the 67 kg N ha^{-1} rate over the 0 kg N ha^{-1} rate (Table 4). There was no significant difference in grain yield between the 134 and 269 kg N ha^{-1} rates in 1997; in 1998 the 269 kg N ha^{-1} rate had a significantly higher yield. Small yield differences in 1997 could be attributed to high residual soil nitrate levels before initiating the experiment. Response to applied P was only observed between the 0 and 22 kg P ha^{-1} rates, with the three

Table 4. Analysis of variance, N and P rates means, and single degree of freedom contrasts for grain yield, N, and P concentration, 1997 and 1998.†

Source	df	Grain yield		N		P	
		1997	1998	1997	1998	1997	1998
		——— Mg ha^{-1} ———		——————— g kg^{-1} ———————			
				Mean squares			
Rep	3	1.34	0.85	0.0165	0.0040	0.0143	0.0002
N rate	3	29.96**	143.40**	0.1084**	0.3161**	0.0033	0.0012*
Linear	1	44.68**	391.64**	0.2594**	0.8212**	0.0040	0.0018*
Quadratic	1	39.49**	38.55**	0.0360	0.0860**	0.0040	0.0018*
LOF	1	5.71**	0.02	0.0299	0.0246*	0.0021	0.0001
P rate	3	5.51**	0.34	0.0052	0.0036	0.0035	0.0044**
Linear	1	11.59**	0.96	0.0001	0.0088	0.0069	0.0059**
Quadratic	1	4.48*	0.05	0.0153	0.0016	0.0033	0.0048**
LOF	1	0.44	0.10	0.0001	0.0003	0.0001	0.0027**
N rate × P rate	9	0.66	1.64	0.0110	0.0014	0.0043	0.0056
Error	45	0.73	0.69	0.0175	0.0035	0.0027	0.0029
SED		0.60	0.59	0.0935	0.0418	0.0367	0.0381
				Treatment means			
N rate, kg ha^{-1}							
0		9.12	3.30	1.05	0.91	0.41	0.18
67		11.73	6.33	1.08	0.88	0.40	0.18
134		11.98	8.55	1.20	0.99	0.37	0.18
269		11.88	10.25	1.21	1.19	0.39	0.20
P rate, kg ha^{-1}							
0		10.30	6.93	1.15	0.97	0.38	0.16
22		11.38	7.05	1.12	0.99	0.37	0.20
45		11.52	7.20	1.12	1.00	0.39	0.19
269		11.50	7.25	1.15	1.00	0.41	0.20

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
† df, degrees of freedom; LOF, lack of fit; SED, standard error of the difference between two equally replicated means.

higher rates having a similar response in both years (Table 4).

Hyperspectral Readings

Integrating sphere measurements were collected only to distinguish between differenced P treatments; therefore, readings were limited to the 269 kg N ha^{-1} treatment for the 1997 growing season. Regression analysis for the first two samplings (17 and 23 June) resulted in significant multiple regression equations for predicting P concentration, while the third sampling date (28 July) did not provide a significant equation for predicting total P (Table 5). At this time early season visual P deficiency symptoms were no longer present. Reflectance wavelengths used for predicting P concentration were in the NIR (730 and 930 nm) region of the spectrum for the 17 June sampling with an $R^2 = 0.68$ (Table 5). According to Lillesand and Kiefer (1987), reflectance in the NIR region of the spectrum is due primarily to the internal structure of the plant leaves. Jacob and Lawlor (1991) found that the initial effect of P stress on corn, wheat, and sunflower was an increase in the number of smaller cells per unit of leaf area compared with a nonstressed plant. Such an increase in the number of cells would suggest that NIR reflectance might be important for predicting P content of plants. Analysis of data from the second sampling date identified blue reflectance as an important segment for predicting P content in the leaves. Reflectance at 440 and 445 nm was significant in predicting P with an $R^2 = 0.61$ (Table 5). Under P stress, increases anthocyanin production causing a purple discoloration in the leave margins (Marchner, 1995). At V6 growth stage, purpling at the leave margins in the P stressed plots was observed. Salisbury and Ross (1978) stated that anthocyanin strongly absorbs in the green region while reflecting in the blue or red region of the spectrum, coinciding with the greater reflectance at 440 and 445 nm. Biolley and Jay (1993) characterized the colorimetric features of modern roses in relation to anthocyanin content, finding that petals containing large amounts of anthocyanin exhibited an increased reflectance between 400 to 580 nm compared with petals containing less anthocyanin. Further analysis of reflectance at these two wavelengths showed that reflectance was significantly higher at 440 nm for the 67 kg P ha^{-1} compared with the 22 and 45 kg P ha^{-1} rates at the 0.05 probability level (Fig. 2). Reflectance at 445 nm decreased with increasing P rates, which explains the difference in the sign of the coefficient for the 440 and 445 nm segments (Fig. 2 and Table 5). Reflectance for the 0 kg P ha^{-1} rate had a higher reflectance from 435 to 460 nm.
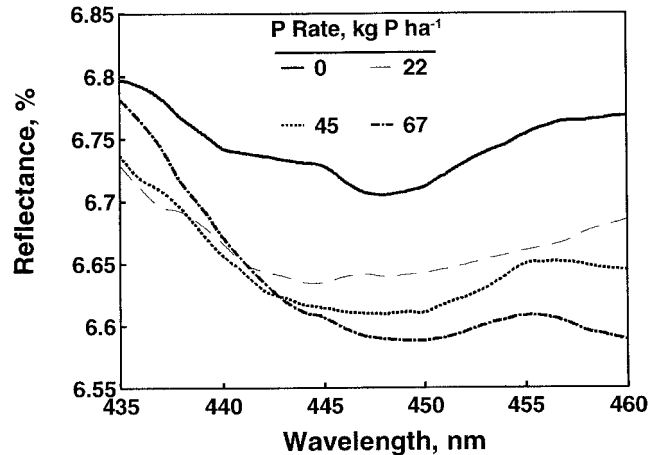

Fig. 2. Segment of spectral curve for integrating sphere measurement at the 269 kg N ha^{-1} rate, 23 June 1997.

Canopy measurements taken during 1997 and 1998 included both plant and soil reflectance. Therefore, during the growing season as the canopy closed, there was progressively less soil visible to interact with the canopy reflectance. Unlike the integrating sphere measurements, analysis of canopy measurements included all treatments. Therefore, prediction of N concentration was with and without the presence of P stress, and prediction of P concentration was with and without the presence of N stress. Stepwise regression for estimating plant N and P and total biomass was performed on those dates corresponding to a plant sampling dates. During the 1997 growing season, there were no significant multiple regression equations developed for predicting P or N content for the June sampling (Table 6). The equation for predicting total biomass was statistically significant ($R^2 = 0.43$), but was not considered to be a very good indicator of total biomass. Data from the 15 July sampling provided a better prediction of total biomass with an $R^2 = 0.68$ (Table 6). One of the factors that could have contributed to the inability to predict P and N content or biomass for the June 1997 sampling date was the method by which the readings were collected. The field of view for the fore-optic was not strictly centered over the row at all times; therefore, the readings could have contained a greater proportion of soil background compared with vegetation. After the 19 June 1997 sampling, the fore-optic was centered over the row to minimize the amount of soil present. The July 1997 sampling resulted in an equation for predicting N concentration in the plant using reflectance in the red and NIR region of the spectrum with an $R^2 = 0.81$ (Table 6). Different researchers have used reflectance or developed indices using reflectance at these wavelengths to identify N stress (Walburg et al., 1982; Blackmer et al., 1996; and Stone et al., 1996).

Overall, the 1998 growing season resulted in a greater ability to predict N and P content and biomass due to the larger response to applied fertilizers. Prediction of plant P was best for the June sampling date using reflectance in the blue and NIR regions with an $R^2 = 0.61$ (Table 6). Reflectance in these regions is attributed to internal cell structure and the presence of anthocyanin in the P stressed plots compared with the nonstressed

Table 5. Regression equations by sampling date for predicting P concentration from integrating sphere hyperspectral data at the 269 kg N ha^{-1} N rate.

Date	Growth stage	R^2	P concentration, g kg^{-1}†
17 June 1997	V6	0.68	$y = 0.085 - 0.031 \times R_{730} + 0.027 \times R_{930}$
23 June 1997	V7–V8	0.61	$y = 0.304 + 0.148 \times R_{440} - 0.160 \times R_{445}$
29 July 1997	V9–V11		No significant equation

† R_x: reflectance data collected at the specified wavelengths, ±2.5 nm.

Table 6. Regression equations for predicting biomass, N, and P concentration from canopy hyperspectral data by sampling date.

Date	Growth stage	R^2	Biomass, kg ha^{-1}†
19 June 1997	V6	0.43	$y = 705 - 1030 \times R_{535} + 860 \times R_{565} + 37 \times R_{805}$
15 July 1997	V14–VT	0.63	$y = 5528 + 2461 \times R_{740} - 2316 \times R_{760} + 1341 \times R_{850} + 4447 \times R_{905} - 4987 \times R_{920} + 93 \times R_{980}$
19 June 1998	V5–V7	0.87	$y = 709 + 1122 \times R_{405} - 2536 \times R_{415} + 1612 \times R_{440} - 1877 \times R_{465} + 1836 \times R_5\%1\%0 + 364 \times R_{615} + 17 \times R_{975}$
20 July 1998	V14–R1	0.68	$y = -1038 + 14239 \times R_{435} + 35473 \times R_{450} + 15347 \times R_{475} + 25107 \times R_{700} + 20295 \times R_{710} + 4167 \times R_{950} - 3739 \times R_{960}$
			N concentration, g kg^{-1}
19 June 1997	V6		**No significant equation**
15 July 1997	V14–VT	0.81	$y = 0.764 - 4.844 \times R_{600} + 2.577 \times R_{610} + 1.698 \times R_{625} + 0.734 \times R_{700} + 0.427 \times R_{805} - 0.373 \times R_{875} + 0.039 \times R_{975} - 0.029 \times R_{980}$
19 June 1998	V5–V7	0.78	$y = 1.232 + 1.944 \times R_{420} - 1.347 \times R_{565} + 1.431 \times R_{615} - 5.802 \times R_{650} + 2.647 \times R_{655} + 1.529 \times R_{725} + 0.102 \times R_{945} - 0.035 \times R_{990}$
20 July 1998	V14–R1	0.64	$y = 0.822 + 0.232 \times R_{740} - 0.244 \times R_{745} + 0.0401 \times R_{975}$
			P concentration, g kg^{-1}
19 June 1997	V6		**No significant equation**
15 July 1997	V14–VT		**No significant equation**
19 June 1998	V5–V7	0.61	$y = -0.023 - 0.219 \times R_{405} + 0.305 \times R_{415} + 0.428 \times R_{900} - 0.184 \times R_{905} - 0.241 \times R_{920}$
20 July 1998	V14–R1	0.36	$y = 0.285 + 0.022 \times R_{570} - 0.06 \times R_{770}$

† R_x: reflectance data collected at the specified wavelengths, ±2.5 nm.

plots. As previously mentioned, the ability to predict P later in the season was not very effective. Difference in positive and negative regression equations coefficient for wavelengths can be explained by slope shifts in the reflectance spectra at each particular wavelength for the different P levels. The ability to predict biomass was better in 1998 than 1997 while predicting N content was best for the 15 July 1997 sampling. The July sampling date in 1997 was the only date with a significant equation; both sampling dates in 1998 had significant equations for predicting N content. Prediction of N concentration was accomplished using reflectance in a number of different regions of the spectrum including chlorophyll absorption, green, red, and NIR for the June sampling ($R^2 = 0.78$) (Table 6). The equation for the July sampling used primarily reflectance in the NIR region, which could be attributed to differences in biomass at

this growth stage. At this particular sampling date, the low N treatments were at a late vegetative growth stage (V13) while the high N treatments were in a reproductive stage (R1). Plants from the low N treatment were also visibly less vigorous compared with those from the high N treatments. Prediction of total biomass was best for the June sampling with an $R^2 = 0.87$ whereas the July sampling had an $R^2 = 0.68$. Reflectances for these equations were composed of the different reflectance wavelengths for predicting plant N and P content (Table 6).

Stepwise regression was performed to estimate grain yield, and grain N and P content using the hyperspectral data for all sampling dates (Table 7). Prediction of grain yield was best using the 1998 data with $R^2 > 0.84$ and the best date in 1998 was 20 July. Data collected on 19 June 1997 exhibited difficulties in predicting any of the

Table 7. Regression equations for predicting grain yield, and grain N, and P from canopy hyperspectral data by measurement collection date.

Date	Growth stage	R^2	Grain yield, Mg ha^{-1}†
19 June 1997	V6	0.34	$y = 14.836 + 7.985 R_{525}† - 7.416 \times R_{565}$
15 July 1997	V14–VT	0.78	$y = 6.823 + 1.770 \times R_{730} - 1.794 \times R_{760} + 7.812 \times R_{875} - 9.229 \times R_{880} + 5.061 \times R_{890} - 3.724 \times R_{920} + 1.232 \times R_{965} - 0.211 \times R_{995}$
19 June 1998	V5–V7	0.88	$y = 9.765 - 7.326 \times R_{580} + 7.770 \times R_{625} - 8.180 \times R_{725} + 6.418 \times R_{730} + 0.184 \times R_{980}$
24 June 1998	V6–V8	0.84	$y = 5.933 - 8.750 \times R_{555} + 4.487 \times R_{740}$
29 June 1998	V8–V11	0.89	$y = 4.823 - 13.804 \times R_{670} + 11.790 \times R_{710} - 0.893 \times R_{995} + 1.721 \times R_{960} - 0.480 \times R_{965} - 0.114 \times R_{995}$
20 July 1998	V14–R1	0.90	$y = 4.410 + 0.713 \times R_{760} + 1.408 \times R_{920} + 1.107 \times R_{935} - 1.972 \times R_{945}$
			N concentration, g kg^{-1}
19 June 1997	V6		**No significant equation**
15 July 1997	V14–VT	0.37	$y = 0.974 - 0.425 \times R_{565} + 0.521 \times R_{600} + 0.016 \times R_{950}$
19 June 1998	V5–V7	0.84	$y = 0.699 + 0.286 \times R_{420} + 0.573 \times R_{465} - 1.701 \times R_{505} + 1.216 \times R_{510} - 1.438 \times R_{580} + 1.138 \times R_{585} - 0.699 \times R~7_{10} + 0.680 \times R_{715} + 0.048 \times R_{950} - 0.008 \times R_{980}$
24 June 1998	V6–V8	0.85	$y = 0.758 + 0.524 \times R_{420} + 1.147 \times R_{525} - 1.010 \times R_{555} - 0.862 \times R_{635} + 0.601 \times R_{640} - 0.674 \times R_{700} + 0.927 \times R_{730} - 0.352 \times R_{745} + 0.168 \times R_{935} - 0.844 \times R_{965}$
29 June 1998	V8–V11	0.78	$y = 1.034 + 0.287 \times R_{520} - 0.513 \times R_{555} + 0.462 \times R_{745} - 0.477 \times R_{760} + 0.311 \times R_{785} + 0.075 \times R_{915} - 0.159 \times R_{935} - 0.010 \times R_{980}$
20 July 1998	V14–R1	0.82	$y = 0.884 + 0.760 \times R_{430} - 0.973 \times R_{435} + 0.277 \times R_{535} - 0.099 \times R_{760} + 0.054 \times R_{965}$
			P concentration, g kg^{-1}
19 June 1997	V6		**No significant equation**
15 July 1997	V14–VT		**No significant equation**
19 June 1998	V5–V7	0.20	$y = 0.203 - 0.005 \times R_{745} + 0.003 \times R_{975}$
24 June 1998	V6–V8	0.41	$y = 0.240 - 0.408 \times R_{460} + 0.287 \times R_{495} + 0.573 \times R_{540} - 0.399 \times R_{550} - 0.067 \times R_{730} + 0.002 \times R_{990}$
29 June 1998	V8–V11		**No significant equation**
20 July 1998	V14–R1		**No significant equation**

† R_x: reflectance data collected at the specified wavelengths, ±2.5 nm.

three variables due probably to the collection method mentioned previously. Only the first two sampling dates in 1998 resulted in a statistically significant equation for predicting grain P. The ability to predict grain N was better for 1998 than 1997 in part due to the greater response to applied N in 1998. The wavelengths of reflectance important for predicting N occurred throughout the spectrum and changed throughout the growing season. These differences could be due to differences in the amount of soil background present, difference in growth stages, or a number of other factors. The wavelengths used to predict total biomass and grain yield were a combination of the wavelengths important for predicting N and P content of the plant.

CONCLUSIONS

The study demonstrated hyperspectral data can be used for estimating N and P concentration, biomass, and grain yield under the presence of a combination of nutrient stresses. Prediction of plant P was best in the early growth stages (before V8) using reflectance in the blue (440 and 445 nm) and NIR (730 and 930 nm) regions, while N concentration could be predicted throughout the growing season. Important reflectance wavelengths for predicting N content, biomass, and grain yield changed with sampling date, possibly due to the differences in percentage ground cover and growth stage. There was a greater response to applied N in the second year of the study with greater differences in yield and nutrient concentration between N rates. Effect of applied P was only between the 0 kg P ha^{-1} and the 22 kg P ha^{-1}, with no differences between the three highest P rates. Estimation of grain yield was best accomplished by using spectral data from the late July sampling date. Reflectance wavelengths used to estimate grain yield were a combination of those used to estimate N and P content for each particular growth stage. Reflectance in the near-infrared (NIR) and blue regions was found to predict early season P stress between growth stages V6 and V8. Late-season detection of P stress was not achieved. Plant N concentration was best predicted using reflectance in the red and green regions of the spectrum, while grain yield was estimated using reflectance in the NIR region, with the particular wavelengths of importance changing with growth stage.

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