Journal of Terrestrial Observation

Volume 2, Issue 1 | *Winter 2010* | *Article 7*

Nitrogen and Water Stress Impacts Hard Red Spring Wheat (*Triticum aestivum*) Canopy Reflectance

C.L. Reese, D.S. Long, D.E. Clay, S.A. Clay, and D.L. Beck

 ISSN 1946-1143.

*Nitrogen and Water Stress Impacts Hard Red Spring Wheat (*Triticum aestivum*) Canopy Reflectance*

C.L. Reese*, D.S. Long†, D.E. Clay*, S.A. Clay*, and D.L. Beck*

*Plant Science Department, South Dakota State University

†USDA-ARS Columbia Plateau Conservation Research Center

ABSTRACT

Remote sensing has been proposed as a method for implementing an in-season spring wheat (*Triticum aestivum*) nitrogen (N) fertilization program. However, in fields where yields are influenced by both water and N stress, accurate N recommendations require that that the N and water stress signals be separated from each other. The objective of this study was to determine the impact of water and N stress on canopy reflectance and the ability of vegetation (NDVI, GNDVI, and BNDVI), and chlorophyll (CGreen and CRedEdge) indices to separate water and N stress. A split-plot experiment containing four blocks was conducted in 2002, 2003, and 2005 at Aurora South Dakota. The treatments were two soil moisture regimes and four N rates. Canopy reflectance was measured with a handheld multispectral radiometer at Haun 2, 4-4.5, 6, and 10-10.2. Canopy reflectance was measured in 16 different bands. Remote sensing-based prediction models for yield, yield loss due to N stress, yield loss for water stress, and protein were developed. Yield loss due to N stress decreased with increasing N, while yield loss to water stress had the opposite relationship. Protein concentration generally increased with N. The remote sensing models for protein and yield loss due to N stress explained more of the variability than the yield model at Haun 4-4.5 and Haun 6. These data suggest that canopy reflectance can be used to separate N and water stress signals in hard red spring wheat.

KEYWORDS

Hard Red Spring Wheat, Nitrogen, NDVI, GNDVI, CRedEdge

INTRODUCTION

In the Great Plains, wheat (*Triticum aestivum* L.) yields can be reduced by both too much and too little N and water that can vary extensively across landscapes. Because N requirements increase with water (Thomas and Oerther, 1972; Sinclair and Horie, 1989), misdiagnosing crop water stress as N deficiency can result in over-fertilization and reduced yields in summit areas (Reese et al., 2002). Remote sensing may provide the information needed for simultaneous management of water and N stress across highly variable production fields (Shanahan et al., 2007; Raun et al., 2002), although different wavebands and indices may be better suited for assessing one stress or the other.

One of the most common remote sensing-based approaches for assessing crop health is the Normalized Difference Vegetation Index (NDVI; Rouse et al., 1973). NDVI contrasts the reflectance in the chlorophyll-absorbing red wavelengths with reflectance in the near infrared (NIR) wave lengths. In North Carolina, Flowers et al. (2003) used the NDVI to estimate the early-season N status of wheat and direct in-season N application between tillering and stem elongation when biomass was > 1000 kg ha^{-1}. Raun et al. (2001 and 2002) used NDVI as a basis for in-season N recommendations for wheat grown in eastern Oklahoma. In spite of these successes, several problems have been reported. First, NDVI may be better at predicting water than N stress (Clay et al., 2006), and second, the index value can become saturated, reducing its sensitivity (Gitelson and Merzylak, 1996; Daughtry et al., 2000). Saturation occurs because as biomass increases, canopy reflectance in the red band decreases, resulting in the difference between NIR and red approaching the value of the canopy reflectance in the NIR band.

Chlorophyll indices were developed on the assumption that a greener crop (i.e., more chlorophyll) is a healthier crop and to overcome the problems described above (Gitelson and Merzlyak, 1994a, 1994b, 1996; Penuelas et al., 1994, 1995; Gitelson et al., 1996, 2003, 2005). For example, Penuelas et al. (1994) used the normalized pigment chlorophyll index (NPCI) to determine chlorophyll content of sunflower (*Helianthus annuus*). Gitelson et al. (2005) reported that the CRedEdge index was highly correlated to the chlorophyll content in both maize (*Zea mays*) and soybean (*Glycine max*), and Perry and Roberts (2008) reported better prediction of N stress using CRedEdge than indices that relied on visible and NIR canopy reflectance. Clay et al. (2006) reported that in corn, the green normalized difference vegetation index (GNDVI) was better at predicting N stress than NDVI, which was better at predicting water stress.

Combinations of indices have also been used to overcome problems associated with NDVI (Daughtry et al., 2000). For example, Rodriquez et al. (2006) reported that the Normalized Difference Red Edge Index (NDRE) divided by NDVI (Barnes et al., 2000) is correlated to the foliar N content in dryland wheat. Haboudane et al. (2004) reported that the Transformed Chlorophyll in Absorption Ratio Index (TCARI) divided by the Optimized Soil Adjusted Vegetation Index (OSAVI) can be used to enhance chlorophyll sensitivity and reduce soil background noise. Eitel et al. (2007) reported that the ratio between the Modified Chlorophyll in Absorption Ratio Index (MCARI; Daughtry et al., 2000) and second Modified Triangular Vegetation Index (MTV12; Haboudane et al., 2004) can be used to predict flag leaf N concentration of dryland wheat.

The objective of this study was to determine the impact of water and N stress in combination and alone on canopy reflectance. The ability of vegetation (NDVI, GNDVI, and BNDVI), and chlorophyll (CGreen and CRedEdge) indices were assessed to separate water and N stresses.

MATERIALS AND METHODS

Experimental Design and Cultural Practices

This study was conducted on a Brandt silty clay loam (fine-*silty*, mixed, superactive, frigid calcic Hapludoll) at a site located near Aurora, South Dakota (96°40'W, 44°18'N) in 2002, 2003, and 2005. Previous crops were soybean (*Glycine max*) in 2001 and 2002 and flax (*Linum usitatissimum*) in 2004. The field was no-tilled in 2002 and 2003, and chisel-plowed and disked in 2005. Composite soil samples (0-15 cm and 15-60 cm), consisting of 15 to 20 individual cores, were collected in spring prior to urea application. Samples were air dried and analyzed for NH_4-N and NO_3-N after extraction with 1.0 M KCl (Clay et al., 2006) (Table 1). Phosphorus and potassium fertilizers were applied in accordance with university fertilizer guidelines. Hard red spring wheat cultivars "Russ" and "NorPro" were planted at a rate of 100 kg ha^{-1} on 15 April 2002 and 2003 and 7 April 2005. Russ is an awned, medium-height variety that has early-midseason maturity whereas NorPro is an awned, short semi-dwarf wheat variety that has midseason maturity.

The two water treatments were rainfed (Table 1) or rainfed and supplemental irrigation and were split within plots. Irrigation was applied with a lateral-move irrigation system with drop nozzles. Randomization was achieved across the areas by plugging adjacent nozzles along different sections of the lateral-move irrigator. The four N rates were 0-, 56-, 140-, and 224-kg urea-N ha^{-1}, applied pre-emergence. A randomized split-plot design was used with water as the main treatment and N rates randomized within each plot. Blocks were replicated four times. Individual plot size was 12.2 m wide and 24.4 m long in 2002 and 12.2 m long in 2003 and 2005.

Growing degree days (GDD, base 4.4°C, Table 1) were calculated based on the equation:

$$GDD = \frac{(Temperature_{max} + Temperature_{min})}{2} - 4.4^{o}C \qquad (1)$$

Table 1. Water, preseason inorganic N, and growing degree days in 2002, 2003, and 2005

	Water		Inorganic N		GDD
	Rainfed	Rainfed and Irrigation	NH_4 and NO_3-N	NH_4-N	Base
Year	Cm		kg ha^{-1}		4.4°C
2002	59.4	65.7	51	ND[†]	1416
2003	43.6	49.4	70	26	1274
2005	61.2	63.7	73	10	1458

[†] ND, not determined

Canopy Reflectance Measurements

Canopy reflectance was measured at Haun 6 and 10-10.2 in 2002; Haun 2, 4, and 10-10.2 in 2003; and Haun 4.5 and 6.0 in 2005 (Haun, 1973). The Haun scale for wheat was used because the scale is sensitive to early leaf growth.

The Cropscan Multispectral radiometer (Cropscan Inc., Rochester, Minnesota), factory-calibrated each season, was held 2 m above the canopy with the sensor receptor oriented parallel to the canopy surface. Based on the height of the sensor, data were collected from a 1-m diameter circle. Irradiance and canopy reflectance readings were collected simultaneously between 11:00 a.m. and 3:00 p.m. Central Standard Time. Canopy reflectance values in 5 broad bands [blue, 485 ± 45 nm; green, 560± 40 nm; red, 660 ± 30 nm; NIR, 830 ± 70 nm; and mid infrared (MIR), 1650 ± 100 nm] and 11 narrow bands (510 ± 3.65, 566 ±5, 610 ± 5.15, 661 ± 5.8, 710 ±6.2, 760 ± 5.3, 810 ± 5.7, 840 ± 6, 870 ± 6 nm, 905 ± 5, and 1050 ± 5 nm) were measured.

Spectral Indices Calculations

The percentage canopy reflectance was calculated from readings taken by the radiometer using the equation:

$$\%\text{ Canopy Reflectance} = \frac{\text{Down sensor reading}}{\text{Up sensor reading}} \times 100 \quad (2)$$

Canopy reflectance values among years were compared by growth stage.

In addition to canopy reflectance, seven different spectral indices were calculated (Table 2) using various combinations of values from different bands. Normalized difference vegetation indices, $NDVI_w$ and $NDVI_n$, were calculated using either the wide (w) or narrow (n) red and NIR canopy reflectance, respectively. Green normalized difference vegetation indices, $GNDVI_w$ and $GNDVI_n$, were developed using the wide (w) or narrow (n) green band and NIR canopy reflectance, respectively. The narrowband indices ($NDVI_n$ or $GNDVI_n$) are based on canopy reflectance in similar regions of the electromagnetic spectrum as commercially available two-sensor systems that measure canopy reflectance values from a light emitting diode source. The wide bands of blue and NIR were used to create a blue normalized vegetation index, $BNDVI_w$. The $BNDVI_w$ index was selected due to overlap in the green area of the electromagnetic spectrum and due to previous successes by Yang et al. (2004) and Hancock and Dougherty (2007) to estimate biomass in cotton and alfalfa, respectively. CGreen and CRedEdge were calculated by dividing the narrow NIR by either the narrow green or narrow red reflectance bands, respectively, and subtracting 1. These indices were selected due to reported increased sensitivity to plant chlorophyll (Gitelson et al., 2005).

Table 2. Spectral indices computed from the actual Cropscan bands. Formulas to derive the index are provided in the column titled "Cropscan Bands Used to Calculate Indices"

Description	Index	Cropscan Bands Used to Calculate Indices	Reference
Normalized Difference Vegetation Index	$NDVI_w$	(830 - 660) / (830 + 660)	Rouse et al., 1973
	$NDVI_n$	(760 - 661) / (760 + 661)	
Green Normalized Difference Vegetation Index	$GNDVI_w$	(830 - 560) / (830 + 560)	Gitleson et al., 1996
	$GNDVI_n$	(870 - 566) / (870 + 566)	
Blue Normalized Difference Vegetation Index	$BNDVI_w$	(830 - 485) / (830 + 485)	Hancock and Dougherty, 2007; Yang et al., 2004
CGreen	CGreen	(810 / 566) - 1	Gitleson et al., 2005
CRedEdge	CRedEdge	(810 / 710) - 1	

Yield and Grain Measurements

Wheat was harvested with a Massey Ferguson model MF8 combine (AGCO, Bloomington, Minnesota) at physiological maturity in late July (2002 and 2005) or early August (2003). Yield was determined at 13.5% moisture. Grain protein (12% moisture) was determined using a Foss Tecator Infratec™ 1241 Grain Analyzer (Eden Prairie, Minnesota). Grain samples were dried at 60° C to constant weight, ground to flour, and analyzed on a Europa Ratio Mass Spectrometer (Europa Scientific Ltd., United Kingdom) for total N, carbon (C), ^{13}C, and ^{15}N. The ^{13}C natural abundance approach was used to calculate yield loss to N stress (YLNS) and yield loss to water stress (YLWS) (Clay et al., 2001, 2005, and 2006).

Methods and Calculations to Determine Yield Loss Due to Water or N Stress

Yield loss due to N and water stress was determined based on isotopic discrimination measured in grain samples and based on calculations described by Clay et al. (2001, 2005). The grain gives a season-long integrated look at these stresses in the plant environment as N and water stress have differential impacts on the relative amounts of ^{13}C and ^{12}C fixed during photosynthesis.

Wheat is a C3 plant where the enzyme RuBisCo takes carbon dioxide (CO_2) in the stomata to make the three carbon sugar phosphoglycerate (3-PGA). RuBisCo prefers $^{12}CO_2$, and therefore when the stomata are open the relative proportion of $^{12}CO_2$ fixed is high. When the plant experiences water stress, the stomata close, resulting in more $^{13}CO_2$ being fixed. N impacts the relative amount of ^{13}C that is fixed by influencing the amount of chlorophyll contained in the plant. Reducing chlorophyll reduces the potential of the plant to fix CO_2, which in turn impacts the relative amounts of ^{13}C and ^{12}C fixed by the plant. The relative proportions of ^{13}C and ^{12}C contained in the plant are reported as either $\delta^{13}C$ or Δ values. These values are defined with the equations,

$$\delta^{13}C = [R(sample)/R(standard)-1] \times 1000‰ \quad (3)$$

$$\Delta = (\delta^{13}C_a - \delta^{13}C_p)/(1+\delta^{13}C_p/1000) \quad (4)$$

where R(sample) is the $^{13}C/^{12}C$ ratio of the grain sample, and R(standard) is the $^{13}C/^{12}C$ ratio of PDB, a limestone from the Pee Dee formation in South Carolina (O'Leary, 1993; Farquhar and Lloyd, 1993), $\delta^{13}C_a$ is the $\delta^{13}C$ value of air (-8‰) and $\delta^{13}C_p$ is the measured value of plant material. Yield losses due to water and N stress as described by Clay et al. (2001, 2005) are calculated by solving the following equations,

$$Yield_{maximum} - Yield_{measured} = YLNS + YLWS \quad (5)$$

$$d\Delta_{N,\ water} = YLWS\ d\Delta_{water\ stress} + YLNS\ d\Delta_{N\ stress} \quad (6)$$

$$d\Delta_{water\ stress} = m_{water\ stress} * (dYield) + b_{water\ stress} \quad (7)$$

$$d\Delta_{nitrogen\ stress} = m_{nitrogen\ stress} * (dYield) + b_{nitrogen\ stress} \quad (8)$$

Solving these equations requires that values for $d\Delta_{water\ stress}$ and $d\Delta_{N\ stress}$ be calculated from the dataset. The boundary line technique is used to calculate both these values. The boundary line approach is graphically shown in Figure 1. These $d\Delta_{water\ stress}$ and $d\Delta_{N\ stress}$ values are the slopes for two boundary conditions. The $d\Delta_{water\ stress}$ value is the slope on the lower boundary line, while $d\Delta_{N\ stress}$ is the slope on the line that intersects the lower boundary line (Figure 1).

Statistical Analysis

Yield parameters were analyzed using PROC GLM of SAS ver. 9.1 (SAS Institute, Cary, NC) for main treatment effects of year, N, water, and interactions. Values from F tests were used to calculate *P* values. Pearson correlation coefficients (r) were computed using PROC CORR of SAS to determine strength of relationships between each of the seven spectral index and yield parameters. The SAS procedure MAXR was used to develop predictive equations for each yield parameter using

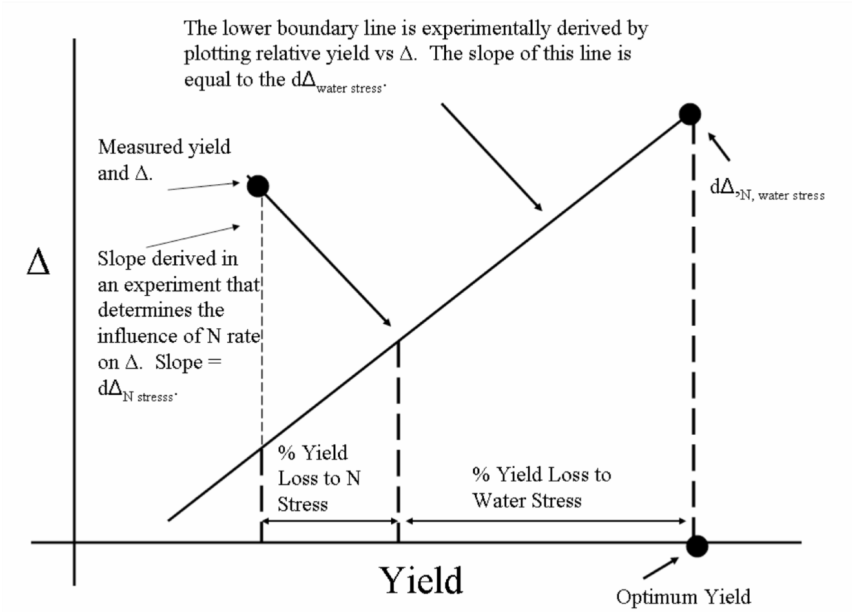

Figure 1. Boundary line technique for determining yield loss to nitrogen stress (YLNS) and yield loss to water stress (YLWS)

the spectral indices as regression estimators. The MAXR method selects regression models that provide the highest degree of correlation with the least number of variables and is an improvement over the STEPWISE method in that many more models are evaluated.

RESULTS AND DISCUSSION

Wheat Yield and Quality

Grain yields were highest in 2003 and lowest in 2002, whereas protein was lowest in 2003 and highest in 2002 (Table 3). The inverse relationship between yield and protein has been reported by others (Clay et al., 2001; Kim et al., 2008; Norword, 1995). The high yields in 2003 were attributed to consistently lower seasonal temperatures, as observed by 11% fewer GDD, as contrasted with lower yields in 2002 and 2005, when seasonal GDD was higher. (Table 1).

Protein contents were much more sensitive to N in 2003 than 2005 (Figure 2). The small response to N in 2005 was attributed to chisel plowing the field prior to planting that caused high soil N mineralization rates that could be observed in high protein content (14.8%) of grain from unfertilized plants. The lack of a response in 2005 was not attributed to high inorganic N levels at the beginning of the season. For example, in 2003 a protein response to N fertilizer was observed when spring soil test total N (NO_3-N and NH_4-N) was 96 kg-N ha^{-1}, which was slightly higher than the 2005 spring soil test total N (83 kg-N ha^{-1}) (Table 1).

As expected, YLNS was greater than YLWS under conditions of relatively infrequent water stress that are typical of eastern South Dakota. In all three years, YLNS decreased as N rate increased. For example, the highest N rate alleviated 78% of the calculated YLNS of the 0N rate in 2002, 48% in 2003, and 16% in 2005. The YLWS increased as N rate increased in 2002 and 2003 and did not vary with N in 2005 (Table 3). An N rate × water interaction also was observed for the YLWS

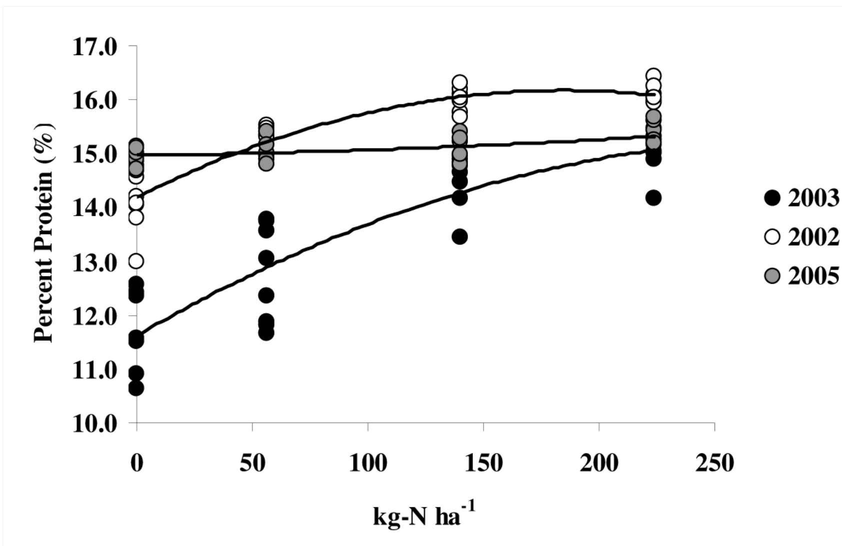

Figure 2. Hard red spring wheat protein response function to increasing N rates in 2002, 2003, and 2005

data and generally was lower under irrigated conditions and at higher N rates. In the 0N treatment, YLWS was reduced by 34% in the irrigated compared with the rainfed treatment. In corn, Kim et al. (2008) reported that water alleviated some N stress due to higher soil mineralization rates under irrigation and the subsequent replacement of fertilizer N (δ^{15}N value = -1 to 0) with soil-derived N (δ^{15}N value >1) (Bateman and Kelly, 2007). Calculated values for YLNS were not influenced by water in this study. However, the N rate × year and water main effect influenced δ^{15}N values. The δ^{15}N values were greatest in the 0N for all years and decreased from 1.23 in 0N to -0.825 in the highest N treatments, indicating more soil-derived N in the 0N treatment. Applying irrigation influenced the δ^{15}N value with values of -0.167‰ (more fertilizer-derived N used) in rainfed and 0.046‰ (more soil N used) in irrigated conditions.

Canopy Reflectance: Individual Bands

At Haun 2 (leaf 2 emerged), there were generally poor relationships among canopy reflectance and end of the season yield parameters (Table 4). The poor relationships were attributed to confounding of reflectance values by bare soil (Daughtry, 2001; Hong et al., 2006).

At Haun 4-4.5 (leaf 4 emerged, tillering), the correlation coefficients among end-of-season crop measurements and canopy reflectance improved. At this stage, canopy reflectance in the blue, red, NIR, and MIR bands was correlated with yield, YLNS, and YLWS. Yield and YLWS were correlated positively with blue and red bands and correlated negatively with NIR and MIR bands. YLNS was correlated negatively with blue and red, and correlated positively with NIR and MIR (YLWS only). Grain protein was correlated negatively to blue, green, red, and NIR canopy reflectance. Grain $\delta^{15}N$ values were correlated positively with canopy reflectance in the green and MIR bands.

At Haun 6 (leaf 6, tillering), yield and YLNS were correlated negatively to canopy reflectance in the blue, green, red, and MIR bands and correlated positively with NIR. YLWS also was correlated with all bands, but was correlated positively to blue, green, red, and MIR bands, and correlated negatively with NIR, which was just the opposite of yield and YLNS. Grain protein had mixed results. In 2002 protein was correlated negatively with blue, green, red, and MIR canopy reflectance and positively with NIR canopy reflectance, whereas in 2005 protein was not correlated with canopy reflectance.

At Haun 10-10.2, the correlation coefficients among canopy reflectance and yield parameters were lower than those observed for Haun 6. At this growth stage, the canopy reflectance surface was a mixed environment of flag leaf and emerging awns. The lower correlation coefficients at Haun 10-10.2 were attributed to wheat starting to head, which confounded canopy reflectance measurements.

Canopy Reflectance: Indices

Stronger relationships among canopy reflectance indices values and end of the season measurements were observed at Haun 4-4.5 and 6 than at Haun 2 (Table 5). These results were similar to those observed for the individual bands (Table 4). At Haun 4-4.5 and Haun 6, grain protein and YLNS were correlated positively to all indices whereas YLWS was correlated negatively to the indices. Degree of correlation was stronger for YLNS and YLWS at Haun 4-4.5 and stronger for protein at Haun 6. Yield was correlated negatively to all indices at Haun 4-4.5 but correlated positively at Haun 6. These differences were a consequence of averaging years with different response functions and datasets. For example, Haun 4 contained data from 2003 and 2005, whereas Haun 6 contained data from 2002 and 2005.

The chlorophyll indices of CGreen and CRedEdge at Haun 4-4.5 and Haun 6 generally had higher positive correlation to YLNS than most of the vegetation indices. These results suggest that the chlorophyll indices provided more reliable infor-

Table 3. The influence water regime, N rate, and year on wheat yield, protein content, yield from N stress (YLNS), yield loss from water stress (YLWS), and grain $\delta^{15}N$

Water Regime	N Rate	Year	Grain Yield	Protein	YLNS	YLWS	$\delta^{15}N$
	kg-N ha^{-1}		kg ha^{-1}	%	kg ha^{-1}		‰
Rainfed	0	2002	2964	14.1	980	385	1.07
	56		3564	15.4	446	319	0.36
	140		3087	16.2	555	687	-1.25
	224		3615	16.3	217	497	-1.61
Irrigated	0		3161	14.5	932	236	1.26
	56		3128	15.4	676	525	0.39
	140		3274	16.1	390	665	-1.06
	224		3644	16.1	206	479	-1.14
Rainfed	0	2003	3585	10.8	1506	297	1.11
	56		3963	12.2	1239	186	-0.09
	140		4030	13.8	857	501	-1.43
	224		4130	15.0	701	558	-0.76
Irrigated	0		3819	11.9	1419	149	1.66
	56		3740	12.7	1350	298	0.43
	140		3809	14.4	1053	527	-1.08
	224		4127	14.6	819	442	-1.22
Rainfed	0	2005	3422	14.8	1909	167	1.07
	56		3529	14.8	1751	217	-0.15
	140		3538	14.9	1689	269	-0.11
	224		3673	15.0	1605	219	-0.22
Irrigated	0		3424	14.6	1896	177	1.23
	56		3524	14.7	1767	206	0.17
	140		3606	14.6	1702	189	-0.08
	224		3752	15.0	1607	138	0.01

Interaction or Factor	DF[†]	P Value and LSD () at P = 0.50				
Water x N Rate X Year	6	0.051	0.183	0.020	0.235	0.468
Water x N Rate	3	0.004 (139)	0.056	0.043 (82)	0.009 (86)	0.854
Year x N Rate	6	0.126	<0.001 (0.4)	<0.001 (100)	<0.001 (105)	<0.001 (0.50)
Year x Water	2	0.781	0.006 (0.2)	0.262	0.849	0.957
Water	1	0.883	0.100	0.200	0.519	0.280
N Rate	3	<0.001 (98)	<0.001 (0.2)	<0.001 (58)	<0.001 (61)	<0.001 (0.29)
Year	2	<0.001 (87)	<0.001 (0.4)	<0.001 (47)	<0.001 (56)	0.015 (0.30)

[†] DF, degrees of freedom

Table 4. Correlation coefficient (r) values for relationships between canopy reflectance in individual wideband [blue (BW), green (GW), red (RW), near infrared (NIRW), and mid infrared (MIRW)] and yield, protein YLNS, YLWS, or $\delta^{15}N$ at different Haun growth stages. Data were aggregated (n = 64) for Haun 4-4.5, Haun 6, and Haun 10-10.2 between dates presented. Correlation coefficient values for Haun 2 are for 2003 only (n = 32). Values of (±) 0.250 and (±) 0.325 represent significance at *P* = 0.05 and 0.01, respectively.

	Canopy Reflectance				
	BW	GW	RW	NIRW	MIRW
	Nm				
Factor	**440-530**	**530-600**	**630-690**	**760-900**	**1550-1750**
Haun 2 (05/20/03)					
Yield	0.12	0.14	0.11	0.24	-0.13
Protein	-0.06	-0.04	-0.02	0.15	-0.05
YLNS	0.13	0.09	0.11	-0.14	0.14
YLWS	-0.36	-0.33	-0.31	-0.11	-0.05
$\delta^{15}N$	0.27	0.25	0.26	-0.01	0.25
Haun 4-4.5 (06/04/03 & 05/29/05)					
Yield	0.44	0.08	0.47	-0.56	-0.58
Protein	-0.73	-0.67	-0.73	0.66	0.11
YLNS	-0.59	-0.14	-0.63	0.75	0.73
YLWS	0.34	0.02	0.38	-0.49	-0.52
$\delta^{15}N$	0.05	0.40	-0.01	0.16	0.56
Haun 6 (06/14/02 & 06/06/05)					
Yield	-0.57	-0.54	-0.58	0.57	-0.59
Protein†	-0.78	-0.73	-0.81	0.82	-0.78
YLNS	-0.64	-0.62	-0.57	0.63	-0.60
YLWS	0.41	0.38	0.36	-0.41	0.40
$\delta^{15}N$	0.12	0.10	0.18	-0.17	0.14
Haun 10-10.2 (06/24/02 & 06/29/03)					
Yield	-0.67	-0.57	-0.44	0.26	-0.27
Protein	0.39	-0.04	-0.19	0.24	0.02
YLNS	-0.46	-0.06	0.09	-0.15	-0.05
YLWS	0.14	-0.07	-0.17	0.39	0.20
$\delta^{15}N$	0.16	0.37	0.45	-0.50	0.06

† At Haun 6, correlation between protein and indices represent only 2002 data. Protein was unresponsive to N in 2005.

Table 5. Correlation coefficient (r) values between yield, protein, yield loss due to N stress (YLNS), yield loss due to water stress (YLWS), $\delta^{15}N$, and 7 indices at hard red spring wheat growth stages Haun 2, Haun 4-4.5, Haun 6, and Haun 10-10.2. Data were aggregated (n = 64) for Haun 4-4.5, Haun 6, and Haun 10-10.2 between dates presented. Correlation coefficient values for Haun 2 are for 2003 only (n = 32). Values of (±) 0.250 and (±) 0.325 represent significance at $P = 0.05$ and 0.01, respectively.

	Vegetation Indices					Chlorophyll Indices	
Factor	**$NDVI_w$**	**$NDVI_n$**	**$GNDVI_w$**	**$GNDVI_n$**	**$BNDVI_w$**	**CGreen**	**CRedEdge**
Haun 2 (05/20/03)							
Yield	0.07	0.08	-0.01	0.12	0.02	0.12	0.15
Protein	0.14	0.11	0.21	0.28	0.15	0.21	0.30
YLNS	-0.26	-0.23	-0.27	-0.40	-0.25	-0.31	-0.39
YLWS	0.32	0.26	0.44	-0.48	-0.37	-0.33	-0.42
$\delta^{15}N$	-0.39	-0.36	-0.38	-0.41	-0.35	-0.43	-0.45
Haun 4-4.5 (06/04/03 & 05/29/05)							
Yield	-0.48	-0.47	-0.46	-0.48	-0.47	-0.54	-0.54
Protein	0.73	0.72	0.73	0.72	0.73	0.69	0.69
YLNS	0.65	0.65	0.64	0.66	0.64	0.72	0.72
YLWS	-0.41	-0.42	-0.41	-0.43	-0.41	-0.46	-0.46
$\delta^{15}N$	0.03	0.03	-0.01	-0.03	-0.02	0.09	0.10
Haun 6 (06/14/02 & 06/06/05)							
Yield	0.59	0.59	0.56	0.57	0.58	0.58	0.56
Protein†	0.83	0.84	0.86	0.86	0.82	0.86	0.85
YLNS	0.52	0.53	0.58	0.64	0.57	0.57	0.74
YLWS	-0.32	-0.32	-0.34	-0.39	-0.34	-0.34	-0.49
$\delta^{15}N$	-0.23	-0.22	-0.2	-0.15	-0.2	-0.23	-0.06
Haun 10-10.2 (06/24/02 & 06/29/03)							
Yield	0.35	0.22	0.43	0.29	0.54	0.38	0.37
Protein	0.28	0.37	0.19	0.39	-0.13	0.32	0.30
YLNS	-0.18	-0.26	-0.08	-0.26	0.22	-0.21	-0.18
YLWS	0.32	0.39	0.27	0.38	0.10	0.35	0.36
$\delta^{15}N$	-0.55	-0.54	-0.51	-0.57	-0.35	-0.57	-0.56

† At Haun 6, correlation between protein and indices represent data from 2002 only. Protein was unresponsive to N in 2005.

mation about N stress than vegetation indices. In addition, these two chlorophyll indices were correlated negatively to YLWS at these two growth stages, indicating that N stress and water stress differentially influenced canopy reflectance.

Predicting N and Water Stress

Regression models for predicting yield, grain protein, grain $\delta^{15}N$, YLNS, and YLWS were developed using spectral bands and selected indices as predictors (Table 6). Index $BNDVI_w$ was not significant in any model and is not discussed further. Overall, models explained from 26 to 93 % of the variance in the parameters. Models for Haun 2 explained 46% of the variance in yield, which was similar with yield models at other growth stages. However at Haun 2, less variance was explained for the YLNS, YLWS, protein, or $\delta^{15}N$ parameters compared with models at later growth stages. These results were expected at this growth stage because of a large percentage of soil that was still exposed.

At later growth stages (Haun 4-4.5, 6, and 10-10.2), regression modeling became more effective in predicting all variables except yield. Scatter plots of observed versus predicted values for grain yield (Figure 3), YLNS (Figure 4), and grain protein (Figure 5) reveal slopes near unity and similar intercepts across these three growth stages. At Haun 6, the models explained 93%, 70%, and 57% of the variability observed in the YLNS, protein, and YLWS data, respectively. The CGreen and CRedEdge were the only parameters used in the models for prediction of all three, although the coefficients for YLNS were positive values for CGreen and negative values for CRedEdge, compared with negative values of CGreen and positive values of CRedEdge for YLWS and protein. These results suggest that in-season remote sensing fairly early in wheat growth can provide reasonable estimates of N and water stress.

SUMMARY AND CONCLUSIONS

Yield losses due to N stress (YLNS) decreased with increased fertilizer application that also increased yield. Yield loss to water stress (YLWS) was alleviated but not eliminated with irrigation and generally increased as fertilizer rate increased. Multivariate regression prediction models explained more variance in protein and YLNS at Haun 6 than at Haun 4-4.5 and Haun 10-10.2. Wavebands most used often in developing the prediction models for YLNS and protein at Haun 4-4.5 were wide-band canopy reflectance in green (560 nm) and NIR (830 nm) and narrow band canopy reflectance in red edge (710 nm) and NIR (810 nm) for CRedEdge (grain protein only). At Haun 6, YLNS and grain protein were predicted using narrow band canopy reflectance at red edge (710 nm) and NIR (810 nm) for CRedEdge, and green (568) and NIR (810 nm) for CGreen. At Haun 10-10.2 (heading), canopy reflectance in the wide blue band (440-530 nm) was a component in prediction models for all end of season parameters.

The results from this study are sufficiently promising to suggest that spectral bands together with chlorophyll indices, which do not saturate as readily as vegetation indices, may be good regression estimators of YLNS and grain protein. This remote sensing information may be used for determination of wheat N status early in the growing season and applying in-season N topdress applications to improve yield and grain protein.

Table 6. Growth stage dependent remote sensing multiple regression models for predicting yield, yield loss due to N stress (YLNS), yield loss due to water stress (YLWS), grain protein, and grain $\delta^{15}N$

		Spectral Band					Spectral Index						
	Intercept	Blue	Green	Red	NIR	MIR	$NDVI_w$	$NDVI_n$	$GNDVI_w$	$GNDVI_n$	CGreen	CRedEdge	R^2
Growth Stage: Haun 2 (05/20/2003)													
Yield	-4555.8	2108.8			-576.6				-21501.0		13276.00		0.46**
YLNS	10193.0	-2644.7		1318.2				27056			-11565.0		0.42**
YLWS	-4300.4							-6070.1	15559.0				0.27*
Protein	4.6						-101.5				34.2		0.26*
$\delta^{15}N$	-4.6					0.2		90.2				-59.4	0.28*
Growth Stage: Haun 4-4.5 (06/04/2003 & 05/29/2005)													
Yield	-1939.5									11706.0		-995.4	0.48***
YLNS	4285.7				79.0				-8396.6				0.73***
YLWS	2936.9		-287.6					-1205.6					0.36***
Protein	-9.0								38.9			-1.9	0.60***
$\delta^{15}N$	-13.1		2.6		-0.5								0.40**
Growth Stage: Haun 6 (06/14/2002 and 06/06/2005)													
Yield	11846.0					-130.6		6983.3	-17988.0			501.6	0.43**
YLNS	1364.1										1128.8	-2073.7	0.93**
YLWS	112.8										-310.3	611.7	0.57**
Protein	12.6										-1.4	3.1	0.73***
$\delta^{15}N$	-6.5							18.2			1.7	-5.4	0.54***
Growth Stage: Haun 10-10.2 (06/24/2002 and 06/29/2003)													
Yield	6173.6	-1978.7				81.9							0.48**
YLNS	425.6	-4030.7	2029.1										0.64**
YLWS	-1524.3	612.4									85.4		0.30**
Protein	18.7	17.3	-9.4										0.70***
$\delta^{15}N$	8.48	-1.79										-1.06	0.36**

* Significant at P=0.01; ** Significant at P=0.001; *** Significant at P=0.0001.

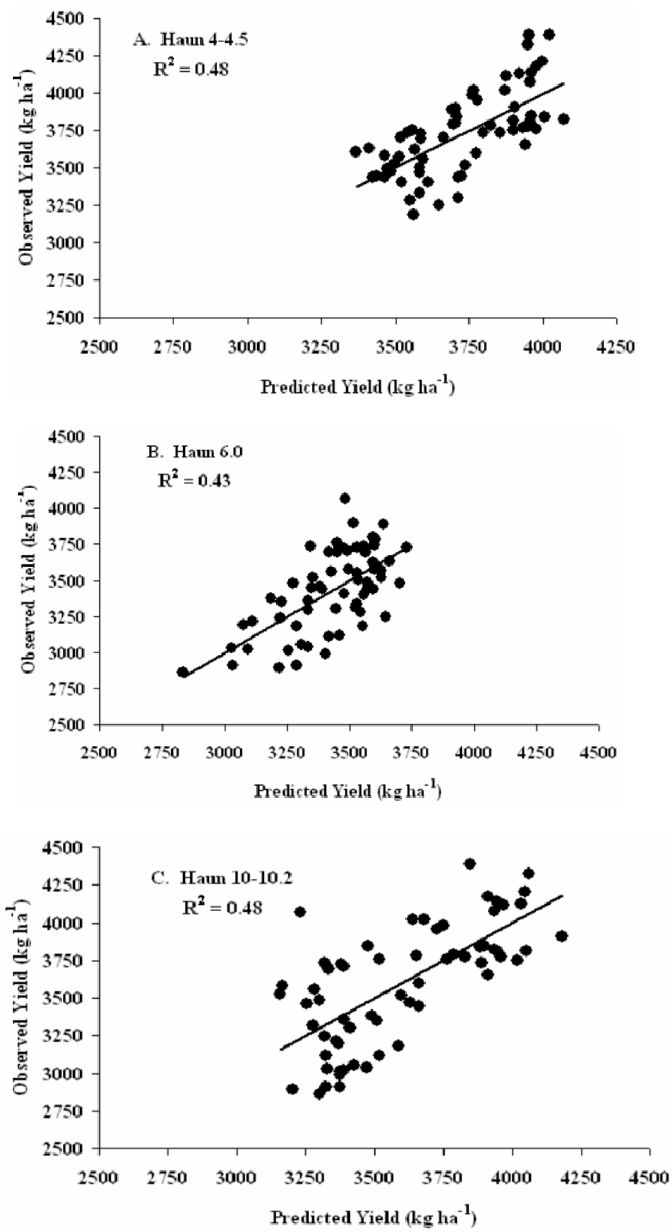

Figure 3. Relationship between the predicted and observed yield at Haun 4-4.5, Haun 6, and Haun 10-10.2 using the models presented in Table 6

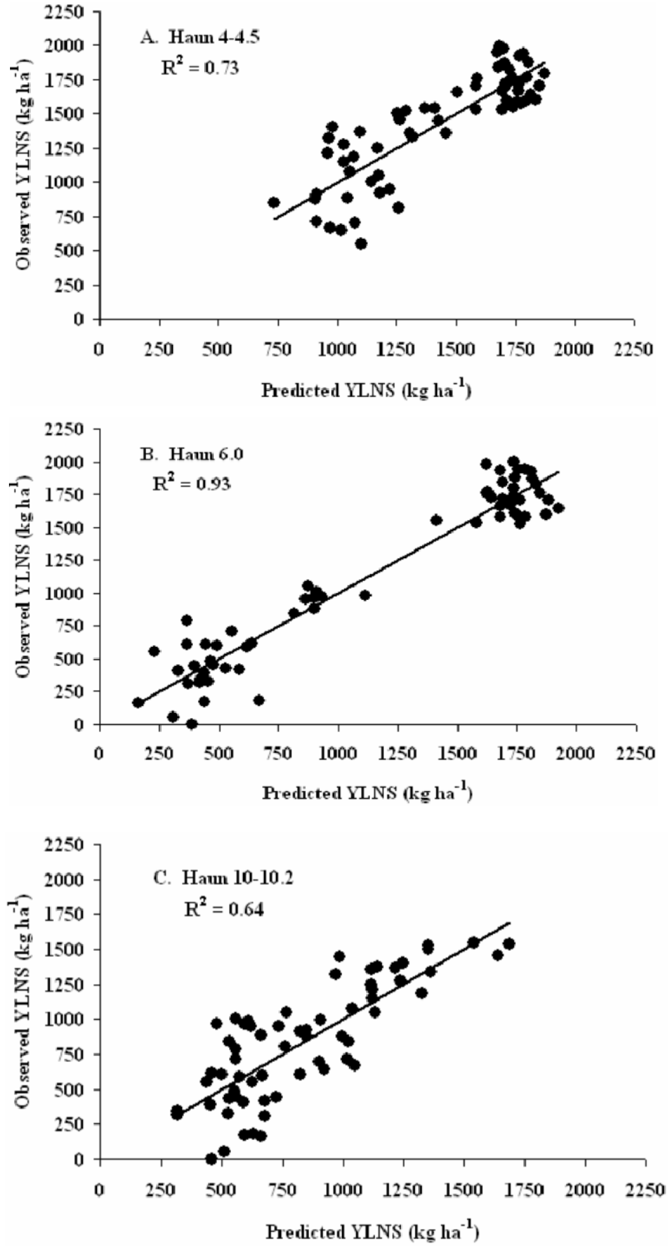

Figure 4. Relationship between the predicted and observed YLNS at Haun 4-4.5, Haun 6, and Haun 10-10.2 using the models presented in Table 6

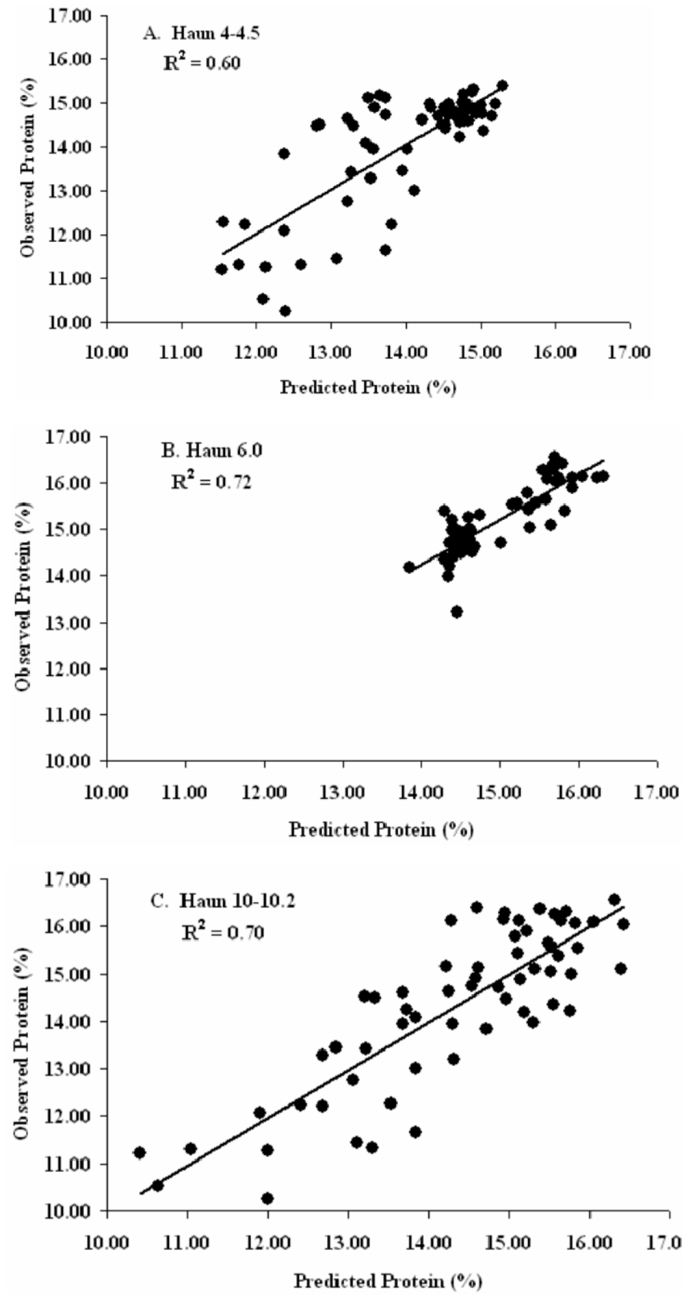

Figure 5. Relationship between the predicted and observed grain protein at Haun 4-4.5, Haun 6, and Haun 10-10.2 using the models presented in Table 6

REFERENCES

Barnes, E.M., Clarke, T.R., Richards, S.E., Colaizzi, P.D., Haberland, J., Kostrzewski, M., Waller, P., Choi, C., Riley, E., Thompson, T., Lascano, R.J., Li, H., and Moran, M.S. 2000. Coincident detection of crop water stress, nitrogen status and canopy density using ground-based multispectral data [CD ROM]. In Robert, P.C., et al. (eds.). *Proceedings of the 5th International Conference on Precision Agriculture*, Bloomington, MN. 16-19 July 2000. ASA, CSSA, SSSA, Madison, WI.

Bateman, A.S., and Kelly, S.D. 2007. Fertilizer nitrogen isotope signatures. *Isotopes in Environmental and Health Studies* 43:237-247.

Clay, D.E., Kim, K., Chang, J., Clay, S.A., and Dalsted, K. 2006. Characterizing water and nitrogen stress in corn using remote sensing. *Agronomy Journal* 98:579-587.

Clay, D.E., Clay, S.A., Lyon, D.J., and Blumenthal, J.M. 2005. ^{13}C discrimination in corn grain can be used to separate and quantify yield losses due to water and nitrogen stresses. *Weed Science* 53:23-29.

Clay, D.E., Engel, R.E., Long, D.S., and Liu, Z. 2001. Nitrogen and water stress interact to influence carbon-13 discrimination in wheat. *Soil Science Society of America Journal* 65:1823-1828.

Daughtry, C.S., Walthall, C.L., Kim, M.S., De Colstoun, E. Brown, and McMurtrey, J.E. 2000. Estimating corn leaf chlorophyll concentration from leaf and canopy reflectance. *Remote Sensing of Environment* 74:229-239.

Daughtry, C.S.T. 2001. Discriminating crop residues from soil by shortwave infrared reflectance. *Agronomy Journal* 93:125-131.

Eitel, J.U.H., Long, D.S., Gessler, P.E., and Smith, A.M.S. 2007. Using in-situ measurements to evaluate the new RapidEye satellite series for prediction of wheat nitrogen status. *International Journal of Remote Sensing* 28:4183-4190.

Farquhar, G.D., and Lloyd, L. 1993. Carbon and oxygen isotope effects in the exchange of carbon dioxide between terrestrial plants and the atmosphere. P. 47-70. In Ehleringer, J.R., Hall, A.E, and Farquhar, G.D. (eds.). *Stable Isotopes and Plant Carbon-Water Relations.* Academic Press, Inc.: New York, NY.

Flowers, M., Weisz, R., and Heiniger, R. 2003. Quantitative approaches for using color infrared photography for assessing in-season nitrogen status in winter wheat. *Agronomy Journal* 95:1189-1200.

Gitelson, A.A., and Merzlyak, M.N. 1994a. Quantitative estimation of chlorophyll-a using reflectance spectra: Experiments with autumn chestnut and maple leaves. *Journal of Photochemistry and Photobiology* 22:247-252.

Gitelson, A.A., and Merzlyak, M.N. 1994b. Spectral reflectance changes associated with autumn senescence of *Asculus hippocastanum* and *Acer platanoides* leaves. Spectral features and relation to chlorophyll estimation. *Journal of Plant Physiology* 143:286-292.

Gitelson, A.A., and Merzlyak, M.N. 1996. Signature analysis of leaf reflectance spectra: algorithm development for remote sensing of chlorophyll. *Journal of Plant Physiology* 148:494-500.

Gitelson, A.A., Kaufman, Y., and Merzlyak, M.N. 1996. Use of a green channel in remote sensing of global vegetation from EOS-MODIS. *Remote Sensing of Environment* 58:289-298.

Gitelson, A.A., Gritz, U., and Merzlyak, M.N. 2003. Relationships between leaf chlorophyll content and spectral reflectance and algorithms for non-destructive chlorophyll assessment in higher plant leaves. *Journal of Plant Physiology* 160:271-282.

Gitelson, A.A., Viña, A, Rundquist, D.C., Ciganda, V., and Arkebauer, T.J. 2005. Remote estimation of canopy chlorophyll content in crops. *Geophysical Research Letters* 32:L08403.

Haboudane, D., Miller, J.R., Pattey, E., Zarco-Tejada, P.J., and Strachan, I.B. 2004. Hyperspectral vegetation indices and novel algorithms for predicting green LAI of crop canopies: modeling and validation in the context of precision agriculture. *Remote Sensing of Environment* 90:337-352.

Hancock, D., and Dougherty, C.T. 2007. Relationships between blue- and red-based vegetation indices and leaf area and yield of alfalfa. *Crop Science* 47:2547-2556.

Haun, J.R. 1973. Visual quantification of wheat development. *Agronomy Journal* 65:116-119.

Hong, S., Rim, S., Sudduth, K.A., Kitchen, N.R., Kim, Y., Lee, J., and Kwak, H. 2006. *Radiometric estimation of soil properties using multiple image sensors in rice paddy and dryland fields.* World Congress of Soil Science, July 9-15, 2006, Philadelphia, PA. 56-13.

Kim, K., Clay, D.E., Carlson, C.G., Clay, S.A., and Trooien, T. 2008. Do synergistic relationships between nitrogen and water influence the ability of corn to use nitrogen derived from fertilizer and soil? *Agronomy Journal* 100:551-556.

Norwood, C.A. 1995. Comparison of limited irrigated vs. dryland cropping systems in the U.S. Great Plains. *Agronomy Journal* 87:737-743.

O'Leary, M.H., 1993. Biochemical basis of carbon isotope fractionation. P. 19-28 In Ehleringer, J.R., Hall, A.E., and Farquhar, G.D. (eds.). *Stable Isotopes and Plant Carbon-Water Relations.* Academic Press, Inc.: New York, NY.

Penuelas, J., Gamon, J., Freeden, A., Merino, J., and Field, C. 1994. Reflectance indices associated with physiological changes in nitrogen and water limited sunflower leaves. *Remote Sensing of Environment* 48:135-146.

Penuelas, J., Baret, F., and Filella, I. 1995. Semi-empirical indices to assess carotenoids/chlorophyll a ratio from leaf spectral reflectance. *Photosynthetica* 31:221-230.

Perry. E.M., and Roberts, D.A., 2008. Sensitivity of narrow-band and broad-band indices for assessing nitrogen availability and water stress in an annual crop. *Agronomy Journal* 100:1211-1219.

Raun, W.R., Solie, J.B., Johnson, G.V., Stone, M.L., Lukina, E.V., Thomason, W.E., and Schepers, J.S. 2001. In-season prediction of potential grain yield in winter wheat using canopy reflectance. *Agronomy Journal* 93:131-138.

Raun, W.R., Solie, J.B., Johnson, G.V., Stone, M.L., Mullen, R.W., Freeman, K.W., Thomason, W.E., and Lukina, E.V. 2002. Improving nitrogen use efficiency in cereal grain production with optical sensing and variable rate application. *Agronomy Journal* 94: 351-815.

Reese, C.L., Clay, D.E., Beck, D., Liu, Z., and Carlson, C.G. 2002. The influence of landscape position and nitrogen rate on hard red spring wheat (*Triticum aestivum L.*) quality and quantity [CD-ROM]. In Robert, P.C. et al. (eds.). Precision agriculture. *Proceedings of the 6th International Conference on Precision Agriculture*, Minneapolis, MN. 14-17 July 2002. ASA, CSSA, and SSSA, Madison, WI.

Rodriquez, D., Fitzgerald, G.J., Belford, R., and Christensen, L.K. 2006. Detection of nitrogen deficiency in wheat from spectral reflectance indices and basic crop eco-physiological concepts. *Australian Journal of Agricultural Research* 57:781-789.

Rouse, J.W., Haas, R.H., Schell, J.A., and Deering, D.W. 1973. Monitoring vegetation systems in the Great Plains with ERTS, *Third ERTS Symposium*, NASA SP-351 I, 309-317.

Shanahan, J.F., Kitchen, N.R., Raun, W.R., and Schepers, J.S. 2007. Responsive in-season nitrogen management for cereals. *Computers and Electronics in Agriculture* 61(1):51-62.

Sinclair, T.R., and Horie, T. 1989. Leaf nitrogen, photosynthesis, and crop radiation use efficiency: a review. *Crop Science* 29:90-98.

Thomas, J.R., and Oerther, G.F. 1972. Estimating nitrogen content of sweet pepper leaves by reflectance measurements. *Agronomy Journal* 64:11-13.

Yang, C., Everitt, J.H., Bradford, J.M., and Murden, D., 2004. Airborne hyperspectral imagery and yield monitor data for mapping cotton yield variability. *Precision Agriculture* 5:445-461.