USE OF GROUND-BASED CANOPY REFLECTANCE TO DETERMINE

RADIATION CAPTURE, NITROGEN AND WATER STATUS,

AND FINAL YIELD IN WHEAT

by

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ABSTRACT

USE OF GROUND-BASED CANOPY REFLECTANCE TO

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AND FINAL YIELD IN WHEAT

by

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Ground-based spectral imaging devices offer an important supplement to satellite imagery. Hand-held, ground-based sensors allow rapid, inexpensive measurements that are not affected by the earth's atmosphere. They also provide a basis for high altitude spectral indices.

We quantified the spectral reflectance characteristics of hard red spring wheat (*Triticum aestivum* cv. Westbred 936) in research plots subjected to either nitrogen or water stress in a two year study. Both types of stress reduced ground cover, which was evaluated by digital photography and compared with ten spectral reflectance indices. On plots with a similar soil background, simple indices such as the normalized difference vegetation index, ratio vegetation index, and difference vegetation index were equal to or superior to more complex vegetation indices for predicting ground cover. Yield was estimated by

integrating the normalized difference vegetation index over the growing season. The coefficient of determination (r^2) between integrated normalized difference vegetation index and final yield was 0.86.

Unfortunately, none of these indices were able to differentiate between the intensity of green leaf color and ground cover fraction, and thus could not distinguish nitrogen from water stress. We developed a reflective index that can differentiate nitrogen and water stress over a wide range of ground cover. The index is based on the ratio of the green and red variants of the normalized difference vegetation index. The new index was able to distinguish nitrogen and water stress from satellite data using wavelengths less than 1000 nm. This index should be broadly applicable over a wide range of plant types and environments.

(134 pages)

ACKNOWLEDGMENTS

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CHAPTER 1

INTRODUCTION

OVERVIEW

Remote sensing offers a viable solution to the costs associated with wide-range plant stress detection in fields. Solar radiation interacts with many of the chemicals important to plant growth and function, resulting in identifiable plant reflectance characteristics (Curran, 1989). Common reflective components include chlorophyll, water, proteins, and cell wall materials. Reflectance measurements have demonstrated the possibilities of using broadband and narrowband reflectance indices to determine plant health, but no widely used reflectance method determines wheat nitrogen and water deficiencies separate from ground cover. The goal of this research was to identify water-stressed and nitrogen-stressed wheat based on reflectance characteristics.

Water limitations limit plant growth at several levels. Mild water stress has a dramatic effect on leaf expansion rate, and photosynthesis decreases with moderate water-deficiency. However, translocation of assimilates through the phloem is unaffected until late in the stress period, after photosynthesis has already been strongly inhibited (Taiz and Zeiger, 1998). Nitrogen deficiency decreases crop yield and quality by limiting amino acid and chlorophyll synthesis. Visual symptoms of nitrogen stress include plant chlorosis and leaf senescence (Marschner, 1995).

Remote sensing has generally been used for monitoring the health of high-value crops, but is now practical for use on field crops. The estimate of plant ground cover in itself, however, is not an estimate of plant health. Although canopy reflectance indices have been correlated to nitrogen status (Fernández et al., 1994; Hinzman et al., 1986) and water status (Jackson et al., 1983), these parameters are estimated on a ground area basis, and the indices do not differentiate between leaf color and ground cover (Adams et al., 1999). Spectral indices that estimate plant water content directly usually use water absorption bands at 1200, 1450, and 1780 nm (Aldakheel and Danson, 1997; Gao, 1996; Shibayama et al., 1993). The drawback to using these bands is that detectors that can measure above 1000 nm are expensive. Peñuelas et al. (1997) reported the successful use of the small water band at 970 nm to detect water stress, but the 970 nm water band falls near the detection limit of low-cost spectrometers. A method to identify water stress at wavelengths below 950 nm would allow inexpensive monitoring of field crops.

OBJECTIVES

The overall objective is to measure canopy reflectance and compare it with tissue samples, ground cover measurements, SPAD chlorophyll measurements, and final yield to determine reflectance signatures for stress and yield. In particular, I seek to:

1. Refine techniques to determine radiation capture and ground cover fraction from spectral reflectance data.

2. Develop techniques to determine nitrogen and water stress using

wavelengths from 400 to 900 nm.

3. Refine techniques to predict final grain yield from measurements of spectral

reflectance during the growing season.

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CHAPTER 2

LITERATURE REVIEW

LEAF INTERACTIONS WITH VISIBLE AND NEAR-INFRARED RADIATION

The interaction of solar radiation with plant molecules controls visible and infrared reflectance. Biochemical and structural components influence the tendency of plants to absorb, transmit, and reflect different wavelengths of shortwave solar radiation (280 nm-2800 nm). Shortwave radiation absorption by plants is controlled by molecular interactions within the plant tissue, where molecular electrons absorb incoming solar radiation at wavelengths that are controlled by chemical bonds and structure (Gates, 1980; Jones, 1997). Therefore, changes in the concentrations of absorptive chemicals provide a basis for changes in plant absorbance, transmittance, and reflectance.

The two primary visible and infrared absorbing components of plant leaves are chlorophyll and water. Chlorophyll absorption is primarily affected by electron transitions between 430 to 460 nm and 640 to 660 nm (Curran, 1989; Taiz and Zeiger, 1998), while water absorption bands center at 970 nm, 1200 nm, 1450 nm, and 1780 nm (Curran, 1989). Other important absorbing biochemicals include proteins, lipids, starch, cellulose, nitrogen, and oils (Table 1). Identification of biochemical concentrations of these compounds through infrared reflectance is difficult because of the overlapping spectral absorption bands of several biochemicals.

Table 1. Visible and NIR absorption features that have been related to particular foliar chemical concentrations (from Curran, 1989).

λ (nm)	Electron Transition or Bond Vibration	Chemical(s)	Detection Considerations
430	Electron Transition	Chlorophyll a	Atmospheric Scattering
460	Electron Transition	Chlorophyll b	
640	Electron Transition	Chlorophyll b	
660	Electron Transition	Chlorophyll a	
910	C-H stretch, 3rd overtone	Protein	
930	C-H stretch, 3rd overtone	Oil	
970	O-H stretch, 1st overtone	Water, starch	
990	O-H stretch, 2nd overtone	Starch	
1020	N-H stretch	Protein	
1040	C-H stretch / C-H deformation	Oil	
1120	C-H stretch, 2nd overtone	Lignin	
1200	O-H bend, 1st overtone	Starch, sugar, lignin, water	
1400	O-H bend, 1st overtone	Water	
1420	C-H stretch / C-H deformation	Lignin	
1450	O-H stretch, 1st overtone / C-H stretch / C-H deformation	Starch, sugar, lignin, water	Atmospheric Absorption
1490	O-H stretch, 1st overtone	Cellulose, sugar	
1510	N-H stretch, 1st overtone	Protein, nitrogen	
1530	O-H stretch, 1st overtone	Starch	
1540	O-H stretch, 1st overtone	Starch, cellulose	
1580	O-H stretch, 1st overtone	Starch, sugar	
1690	C-H stretch, 1st overtone	Lignin, starch, protein, nitrogen	
1780	C-H stretch, 1st overtone / O-H stretch / H-O-H deformation	Cellulose, sugar, starch	
1820	O-H stretch / C-O stretch, 2nd overtone	Cellulose	
1900	O-H stretch / C-O stretch	Starch	
1940	O-H stretch / O-H deformation	Water, lignin, protein, nitrogen, starch, cellulose	Atmospheric absorption
1960	O-H stretch / O-H bend	Sugar, starch	
1980	N-H asymmetry	Protein	
2000	O-H deformation / C-O deformation	Starch	
2060	N=H bend, 2nd overtone / N=H bend / N-H stretch	Protein, nitrogen	
2080	O-H stretch / O-H bend	Sugar, starch	
2100	O=H bend / C-O stretch / C-O-C stretch, 3rd overtone	Starch, cellulose	
2130	N-H stretch	Protein	
2180	N-H bend, 2nd overtone / C-H stretch / C-O stretch / C=O stretch / C-N stretch	Protein, nitrogen	
2240	C-H stretch	Protein	Rapid signal-to-noise decrease of detectors
2250	O-H stretch / O-H deformation	Starch	
2270	C-H stretch / O-H stretch / CH_2 bend / CH_2 stretch	Cellulose, sugar, starch	
2280	C-H stretch / CH_2 deformation	Starch, cellulose	
2300	N-H stretch / C=O stretch / C-H bend	Protein, nitrogen	
2310	C-H bend, 2nd overtone	Oil	
2320	C-H stretch / CH_2 deformation	Starch	
2340	C-H stretch / O-H deformation / C-H deformation / O-H stretch	Cellulose	
2350	CH_2 bend, 2nd overtone / C-H deformation, 2nd overtone	Cellulose, protein, nitrogen	

MEASURING PLANT/RADIATION INTERACTIONS

Definitions of Reflectance, Transmittance, and Absorbance

Reflectance and transmittance are defined as the ratios of reflected or transmitted radiation to incident radiation. Incident radiation that is not reflected or transmitted by a leaf is presumed to be absorbed. Reflectance and transmittance are presented as either a percent or as a fraction of incident radiation. Absorption is characterized either as a ratio of incident radiation or as a function of optical density (Porra et al., 1989; Rabideau et al., 1946).

Instrumentation

Instruments that measure quantities of shortwave radiation use detectors made from photoexcitable materials such as silicon or Indium Gallium Arsenide (InGaAs). Silicon is a common photoexcitable material that produces an electrical current in response to visible and near-infrared (NIR) radiation (300-1100 nm). However, silicon does not respond to radiation above about 1100 nm, so more expensive materials, such as InGaAs detectors, are used for mid-infrared shortwave measurements (commonly 1000 nm to 2500 nm).

A spectrometer measures radiation at discrete wavelength intervals over a defined spectral region. Narrowband spectrometers characteristically have spectral resolutions of ten nanometers or less in the visible and NIR spectral regions, and 50 nm or less in the mid-infrared shortwave regions (ASD, 1999). A spectrometer can be calibrated to a standard light source to provide irradiance

measurements, or it can measure reflected or transmitted radiation as a ratio of incident radiation.

Incident solar radiation is generally measured as reflected radiation from a highly reflective plate oriented at a 90° angle from the receptor. This allows a standardized incident irradiance estimate without the need for a cosine-corrected attachment. Two examples of appropriate reflective materials are barium sulfate and pressed polytetrafluoroethylene (PTFE) (Weidner and Hsia, 1981). These materials exhibit greater than 97% reflectivity between 300 nm and 1600 nm. In addition to measuring incident and reflected radiation, a spectrometer must compensate for current that is transmitted from the sensors even in the absence of incoming radiation. This temperature-affected current is referred to as dark current or noise. Therefore, a complete reflectance measurement is described by Equation 1:

$$R = \frac{\Theta_{REF} - C_{DARK}}{\Theta_O - C_{DARK}} \qquad [1]$$

where 1_{REF} is the measured reflected radiation, 1_O is the measured incident radiation, and C_{DARK} is the dark current (Baret et al., 1987). The ratio of reflected to incident radiation is dimensionless, so ground level reflectance measurements do not generally require instrument calibration.

Single leaf transmittance and reflectance can be measured using an electric light instead of solar radiation as a radiation source. Transmittance measurements use either an integrating sphere (Carter and Spiering, 2002) or a

direct beam measurement (Monje and Bugbee, 1992) to determine transmittance

of a material. One commonly used transmittance measuring device is the Minolta

SPAD-502 chlorophyll meter (Minolta, Ramsey, NJ), a dual-wavelength meter

that emits light from a red LED and an infrared LED in sequence through a leaf to

measure leaf absorbance (Monje and Bugbee, 1992).

Leaf Reflectance and Plant Stress

Chlorophyll dominates leaf reflectance and transmittance of visible radiation.

Nitrogen is a principle component of chlorophyll (Taiz and Zeiger, 1998), and

chlorophyll concentration often correlates closely with nitrogen concentration in

plant leaves (Costa et al., 2001; Fernández et al., 1994; Filella et al., 1995;

Serrano et al., 2000). Chlorophyll absorbs red and blue radiation, resulting in

little red or blue reflectance by green vegetation (Figure 1). The blue absorbance

peak of chlorophyll overlaps with the absorbance of carotenoids, so blue

reflectance is not generally used to estimate chlorophyll concentration (Sims and

Gamon, 2002). Maximum red absorbance occurs between 660 and 680 nm

(Curran, 1989), but relatively low chlorophyll concentrations can saturate this

absorption region (Sims and Gamon, 2002). Therefore, chlorophyll concentration

is usually predicted from reflectance in the 550 nm or 700 nm ranges, because

these regions saturate at higher chlorophyll concentrations. Changes in the

shape of the reflectance spectra between 550 nm and 660 nm can also

sometimes be used to identify chlorosis (Adams et al., 1999; Carter and Spiering,

2002).
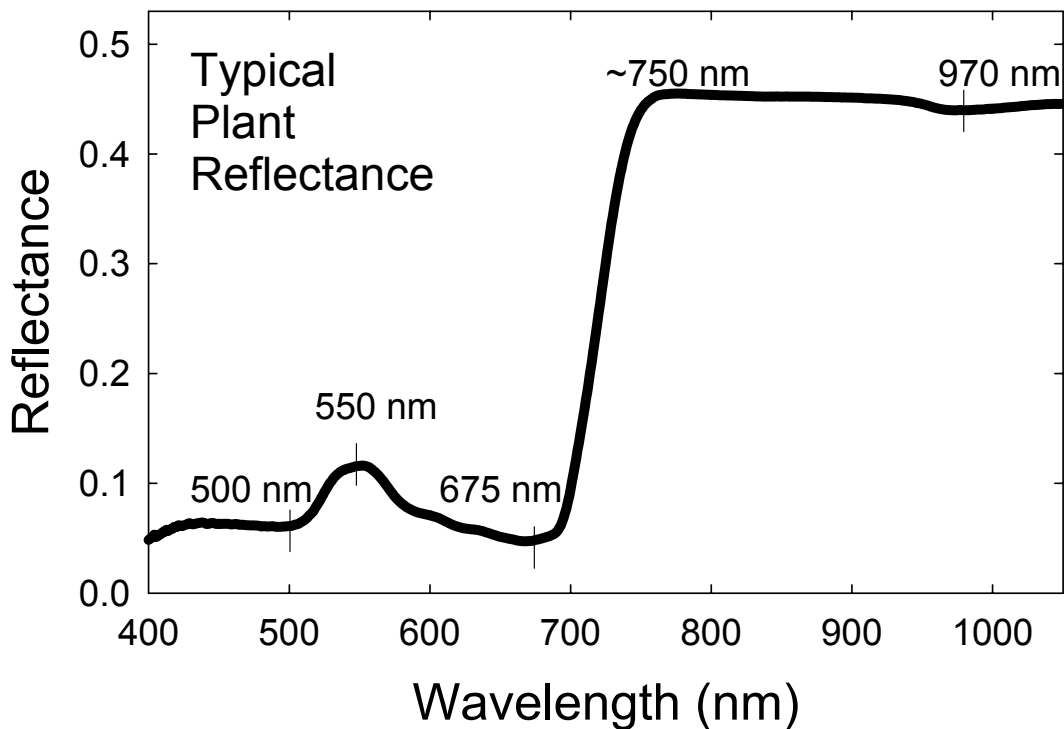
Figure 1. Typical visible and NIR plant reflectance. Spectral features at 500 nm, 550 nm, 675 nm, and the red edge (about 690 to 750 nm) are controlled by chlorophyll concentration, while reflectance at 970 nm is related to water concentration.

Leaf mesophyll reflects a large proportion of NIR radiation (Huete et al., 1984; Taiz and Zeiger, 1998). The region of rapid increase in reflectance between the red and infrared regions of the spectrum, called the red edge, is frequently used to indicate plant health (Dawson and Curran, 1998; Horler et al., 1983a; Horler et al., 1983b; Jago et al., 1999). Horler et al. (1983b) observed that chlorophyll concentration in leaves correlated with the maximum slope of reflectance at the boundary between the red and NIR spectral domains. The red

edge tends to be sensitive to a wide range of chlorophyll concentration, but is sensitive to plant type and changes in ground cover (Carter and Spiering, 2002).

Peñuelas et al. (1994) identified an increase in reflectance between 500 and 600 nm in nitrogen-stressed sunflower leaves from unstressed leaves and examined it using derivative spectra. Gamon et al. (1992) noted a similar pattern in canopy reflectance of sunflower canopies, and specifically noted changes between 8:00 a.m. and noon in reflectance between stressed and unstressed plants.

Water concentration is often estimated in remote sensing by examining shortwave infrared reflectance of plant leaves. The bulk of plant water concentration research has focused around the water bands, spectral water absorption features centered at 970 nm, 1240 nm, 1400 nm, and 1900 nm. As plant water concentration decreases, these bands become less dominant, a feature that is identified with water stress. Peñuelas et al. (1997) and Tian et al. (2001) point out that the strongest water absorption bands in plants occur in the 1400 nm and 1900 nm regions of the spectrum. Tian et al. (2001) used the 1650 to 1850 nm absorption features to detect water deficiency in wheat leaves. However, atmospheric water absorption is also high in these regions, making reflectance measurements of whole plants or canopies difficult. Earlier research suggested that the 970 nm absorption feature was inadequate for detection of water stress, but Peñuelas et al. (1993) showed that this region can be a useful water status indicator for complete canopies where LAI does not vary greatly. Therefore, Peñuelas et al. (1993) and Peñuelas et al. (1997) suggest the use of

the ratio of reflectance at 970 nm (Figure 1) to that of a non-water absorbing region to detect plant water status.

Growth Stage and Canopy Geometry

Reflectance is influenced by the geometric structure (leaf angle) of plant canopies (Ahlrichs and Bauer, 1982; Demetriades-Shah et al., 1990). Plant structure changes during the growing season, so growth stage is an important factor in plant reflectance. Baret et al. (1987) noted that the general behavior of wheat canopy spectra over a growing season was independent of planting date and cultivar, but strongly dependent on the growth stage of the plants. Ahlrichs and Bauer (1982) found the highest correlations of spectral data with plant parameters between the initiation of tillering and anthesis. They reported good correlations between reflectance and five plant parameters: percent soil cover, leaf area index, fresh biomass, dry biomass, and plant water content.

Thenkabail et al. (2002) studied broadband and narrowband spectral indices and reported that NIR crop reflectance between 750 and 950 nm changes from flat to upward sloping as plants senesce. They also found a steeper slope of reflectance between 750 and 950 nm for erectophile plants than for planophile plants. However, the authors did not elaborate on the cause of these slopes.

Sensor angle

The effect of sensor angle on reflectance has been recognized for many years. For instance, Woolley (1971) reported changes in leaf reflectance

between 400 and 2500 nm based on sensor angle. Woolley showed that absolute reflectance increased as sensor angle changed from straight-on, and that reflectance properties change based on whether they are performed on the abaxial or adaxial leaf surface. Pinter et al. (1987) also observed that spectral band ratios were significantly affected by off-nadir viewing, and that the NIR/Red ratio was highest when the sensor was pointed toward a canopy 'hotspot' (pointing west in the morning and east in the afternoon) and lowest when the sensor was pointed away. According to Otterman et al. (1995), vegetated terrain exhibits strong forward and backscattering.

Solar angle and time of day

Diurnal reflectance measurements of wheat canopies over the visible and NIR regions of the spectrum suggest that visible reflectance remains roughly constant throughout the day and infrared reflectance increases as angle from solar azimuth increases. Asrar et al. (1985) observed that increased solar zenith angle generally increased LAI estimates that used red and infrared spectral indices, due to the increase in the infrared. Pinter et al. (1987) reported that changes in solar angles significantly impacted the NIR/red ratio of winter wheat. They found that maxima in the NIR/red ratio were attained mid-morning and mid-afternoon, and minima coincided with the high solar position near midday. Rahman et al. (1999) also observed that reflectance amplitude varied with sensor angle in relation to solar angle. Spectral studies are often performed near the

solar zenith to decrease the effects of solar angle on canopy reflectance (Osborne et al., 2002; Otterman et al., 1995; Serrano et al., 2000).

Solar position may also influence plant reflectance by influencing the quantity of light that is incident to the plant. Gamon et al. (1992) suggested that the xanthophyll chemical changes due to changes in light intensity are partly responsible for changes in absorption efficiency and changes in leaf reflectance between morning and afternoon.

**Ground cover and the soil
background**

A significant issue in whole-canopy reflectance experiments is the variation between green plant cover and the soil background. During early stages of growth, the soil constitutes a large portion of canopy reflectance. The primary variable in soil reflectance is brightness, because nearly all spectral data for a soil falls along a line extending from the origin (Kauth and Thomas, 1976). High reflecting, light-colored soils influence indices more than do dark, low-reflecting soils (Jackson et al., 1983). Spectral differences between soils may be attributed to variations in surface moisture, particle size distribution, soil mineralogy, soil structure, surface roughness, crusting and presence of shadow (Huete et al., 1984; Huete et al., 1985). Many reflectance indices are sensitive to ground cover because ground cover affects red and NIR reflectance.

Jackson et al. (1983) stated that the change in soil reflectance ratios changes little due to wetting, following the fact that a change in soil reflectance due to water concentration is about the same in the visible and near-infrared

(NIR) regions of the spectrum. They also stated that since vegetation reflectance is very different from soil reflectance, the ratio of red and NIR reflectance is theoretically a good discriminator of vegetation. Jackson et al. (1983) concluded that the ratio is not a good discriminator for green vegetation covers less than 50%, but becomes a very sensitive indicator as the ground cover increases.

CURRENT SATELLITE CAPABILITIES

Several commercial and governmental groups sponsor satellite imaging, and recently launched satellites offer high spatial resolution, several bands, and rapid return time (Table 2). These characteristics allow a transition between ground-based imagery and satellite data.

Table 2. Current and planned satellites with features pertinent to reflectance measurements (adapted from Dyke, 2002).

Satellite	Launch	Pixel Size	Bands	Return time
ORBView-3	2000	1 m Pan 4 m MSS	MSS 4 bands Pan 1 band ±45° off nadir	<3 days
QuickBird	1999	1 m Pan 3.5 m MSS	Pan 450-900 nm MSS – VNIR	1-5 days
IKONOS	1999	1 m Pan 4 m MSS	MSS 4 bands Pan 1 band	
ALOS	2002	2.5m MSS	Channel 1: 0.42 - 0.50 µm Channel 2: 0.52 - 0.60 µm Channel 3: 0.61 - 0.69 µm Channel 4: 0.76 - 0.89 µm	
IRS-P5	1999	2.5m	Pan Stereo	
NEMO	2002	5m Pan 30 m HIS	210 bands 400-2500 nm @10 nm PAN 0.5-0.7µm ORASIS - real-time processing	7 day repeat 2.5 day global average
EO-1	1999	30 m	220 bands 0.4 to 2.5 µm @10nm Grating Imaging Spec	
Landsat 7	1999	15 m Pan 30 m MSS 60 mTIR	7 Bands MSS VNIR-TIR	16 days

SPECTRAL INDICES

Vegetation indices attempt to maximize the spectral contribution from green vegetation and minimize the effects of soil background and other factors (Huete et al., 1985; Major et al., 1990). Spectral reflectance has been correlated with plant health and several leaf biochemical concentrations (Curran, 1989; Curran et al., 2001).   Many reflectance studies use spectral vegetation indices to determine these parameters.

Spectral indices have been derived for both single-leaf and plant canopy reflectance measurements. Single-leaf measurements offer the advantage of higher signal-to-noise ratio and more control over the operating environment, while canopy measurements allow measurements over a broader scale. Leaf-scale experiments have ranged from in vivo reflectance of dry plant tissue to measure leaf biochemical concentrations (Curran et al., 2001) to in situ chlorophyll concentration determination of intact leaves (Carter and Spiering, 2002). Plant canopy reflectance is also analyzed for green cover and chlorophyll concentration from both ground and satellite level (Dawson, 2000; Demetriades-Shah et al., 1990). Studies of both single leaf data (Peñuelas et al., 1993; Peñuelas et al., 1994) and plant canopy data (Gao, 1996; Peñuelas et al., 1997) suggest the use of infrared water absorbing bands to detect water stress.

Spectral indicators of crop growth include individual band reflectance factors, linear combinations of bands by multiple regression, orthogonal "greenness," and ratios of infrared and red bands (Dusek et al., 1985). Sims and

Gamon (2002) suggest that multiple bands are useful because of changes in absorbance of confounding pigments, such as carotenoids. Best and Harlan (1985) reported that leaf area estimates using several bands correlated somewhat more closely with LAI than leaf area estimates using two bands (r^2 = 0.73 vs. r^2 = 0.69), although Fernández et al. (1994) concluded that NDVI appears to be the most powerful spectral index that correlates canopy reflectance with leaf area in winter wheat.

Narrowband spectroradiometers are commonly used for ground-based and aerial imaging platforms, while satellites with spatial imaging capabilities sufficient to measure cropland generally employ broadband spectroradiometers. Baret et al. (1987) noted that although high spectral resolution data correlate well with classical broadband information, the relationship appears to be dependent on the phenological stage of the crop. They also stated that a spectral resolution of about 5 nm appears to be sufficient to show the detail of narrow spectral properties. Narrowband spectral indices measure slope (Demetriades-Shah et al., 1990; Peñuelas et al., 1994), shape (Tian et al., 2001), and depth (Curran et al., 2001; Kokaly and Clark, 1999) of absorption bands, while broadband indices are limited to measuring the depth.

Ratio and Difference Vegetation Indices

Ratio indices of reflected and transmitted radiation have been used since the late 1960s to estimate plant growth. Jordan (1969) first published on the use of the simple ratio vegetation index (RVI), in which he used the ratio of

transmitted radiation at 800 nm to 675 nm to estimate the leaf area index of a forest.

Rouse et al. (1973) introduced a variation of the RVI, in which the authors normalized the reflectance ratio to account for solar angle. This index, later known as the normalized difference vegetation index (NDVI), includes the NIR and red reflectance in both the numerator and the denominator. Other researchers have used a variation of NDVI called green NDVI, or GNDVI to account for variations in green reflectance instead of red reflectance (Gitelson and Merzlyak, 1997). NDVI and RVI are the most common vegetation indices used in remote sensing today.

Soil-Adjusted Vegetation Indices

One approach to dealing with the soil background is to try to eliminate it using indices that correct for the "soil line." An early attempt to correct for the soil line was introduced by Kauth and Thomas (1976), and is referred to as the perpendicular vegetation index (PVI). The PVI estimates soil brightness by supplying a soil slope (a) and an offset (b) derived from the NIR vs. red soil baseline.

The soil-adjusted vegetation index (SAVI) simplifies the soil relationship to canopy reflectance by adding a simple brightness factor (L), which is typically set to 0.5, but can range from 0 to 1 (Elvidge and Chen, 1995; Huete, 1988). This allows a robust estimate of ground cover, although an exact brightness coefficient is difficult to determine.

Derivative Indices

One novel narrowband reflectance technique minimizes soil background effects on canopy spectral signatures by the use of high-resolution derivative spectra (Demetriades-Shah et al., 1990; Elvidge and Chen, 1995; Hall et al., 1990; Peñuelas et al., 1994). This allows the discrimination of plant spectra from the soil background (Figure 2).

The use of derivatives is not new; analytical chemists have used derivatives to remove background noise for decades. Martin (1957) addressed the use of both first order and second order derivatives to decrease background interference. Savitzky and Golay introduced a method for smoothing spectral data for derivative analysis in 1964 that is still commonly used. However, the usefulness of derivative spectra was not recognized in plant spectra was not fully realized until the 1980s (Demetriades-Shah et al., 1990; Hall et al., 1990).

Spectral derivatives can be used for both single-leaf and whole-plant spectral analysis. Demetriades-Shah et al. (1990) demonstrated that derivative spectra could yield spectral indices that were superior to conventional broad-band spectral indices for their studies of plant canopies, and Peñuelas et al. (1994) separated healthy, water-stressed, and nitrogen-stressed sunflower leaves based on spectral derivatives. Derivatives eliminate most of the soil background noise from plant canopy spectral data by normalizing the slope of the composite plant-soil reflectance spectrum.
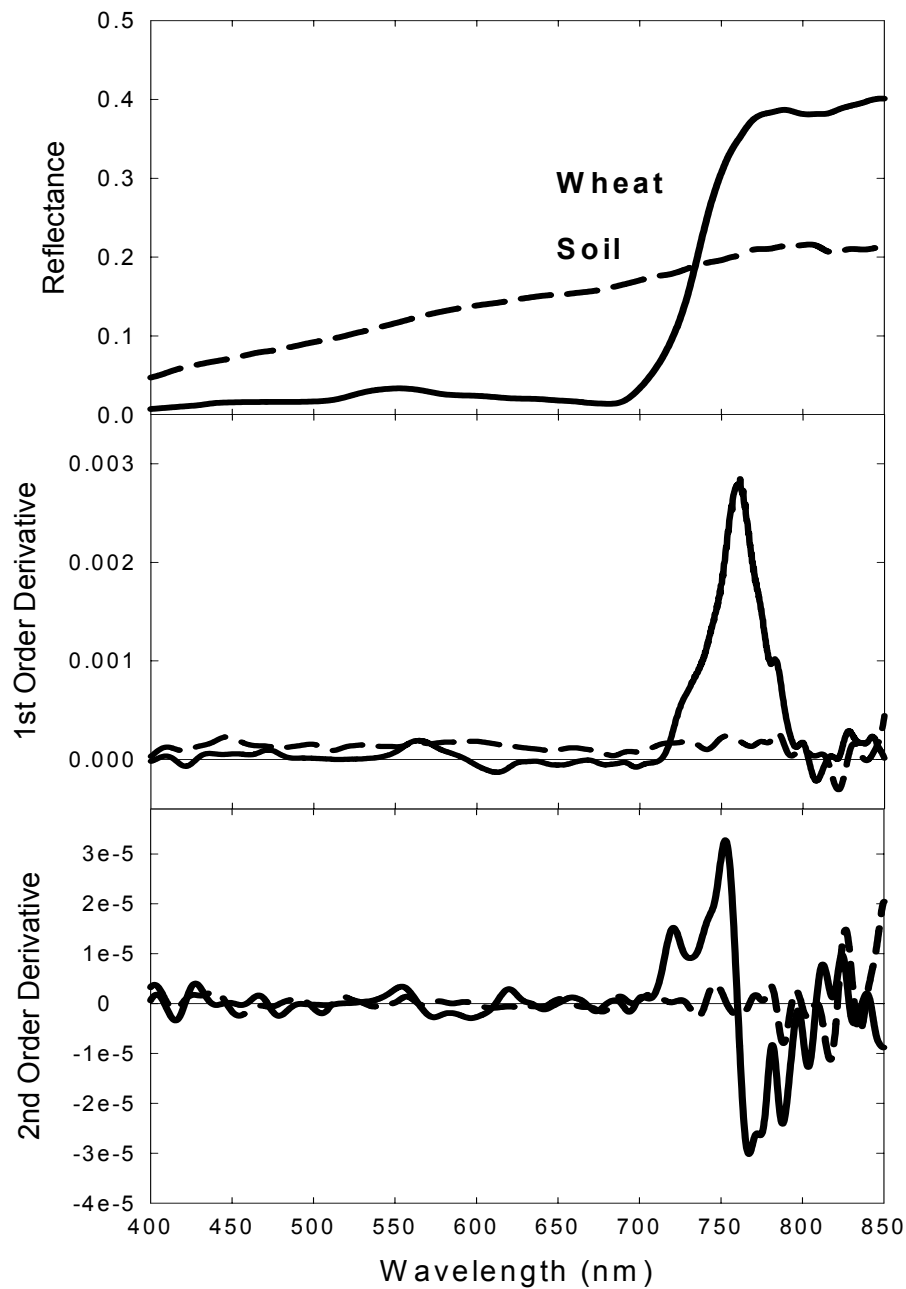
Figure 2. Using the linear nature of soil reflectance to eliminate soil background signal using derivatives of reflectance spectra (adapted from Demetriades-Shah et al., 1990).

Elvidge and Chen (1995) used integrated spectral derivative vegetation indices with a soil baseline to estimate leaf area index and percent green cover in a pinyon pine canopy with five different gravel backgrounds. Integrated derivatives, or antiderivatives, are related to derivative spectra by the fundamental theorem of calculus (Equation 2).

$$\int_{\lambda_1}^{\lambda_2} r(x)dx = R(\lambda_1) - R(\lambda_2) \quad \text{[2]}$$

This theorem states that the sum of the instantaneous changes is a quantity equal to the overall change of the quantity. Therefore, the integrated derivative between two wavelengths is the value at the second wavelength minus the value at the first wavelength, and the integrated derivative index is analogous to the difference vegetation index (DVI). A baseline correction improves ground cover estimates over a variety of soil backgrounds, because it dampens the effects of the soil slope. Derivatives can also be analyzed by the shape, placement, and height of their peaks to extract information about plant health (Peñuelas et al., 1994), although these characteristics can change between leaf and canopy level measurements.

Elvidge and Chen (1995) tested derivative green cover estimates using both first- and second-order derivative indices (Figure 3). The first-order derivative index was normalized to the spectral slope at 626 nm. The authors observed that a first-order derivative green vegetation index integrated between 626 and 795 nm with a local baseline provided the most linear relationships to LAI and percent green cover over several soil backgrounds (r^2 = 0.945), closely

followed by the second derivative green vegetation index. These derivative

indices were found to be superior to PVI and SAVI at estimating ground cover

and LAI (Figure 4). Without the local baseline, first derivative correlations were

substantially poorer than second-derivative correlations.

The first-order analysis is essentially an application of the simple DVI, with

a correction for the slope of the soil background added through the local

baseline. The second-order derivative analysis did not show substantial

improvement using this correction method because of its inherent ability to

eliminate the soil slope.
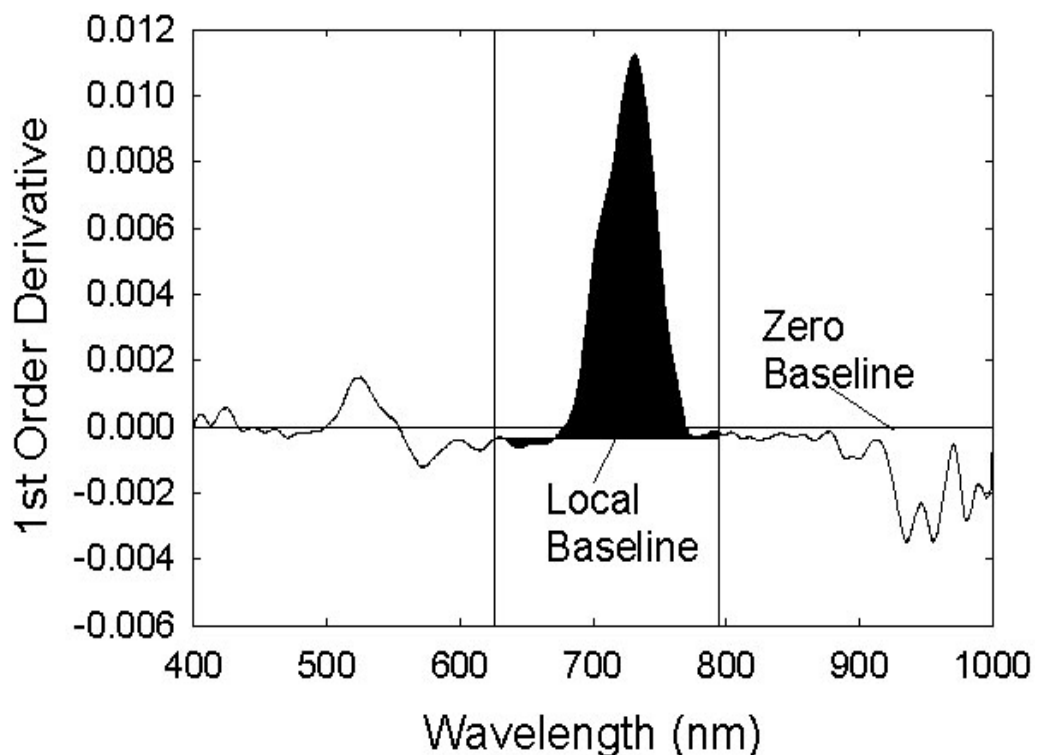
Figure 3. Use of a local baseline with 1st order derivative spectra to eliminate soil background signal. This idea was first published by Elvidge and Chen (1995).
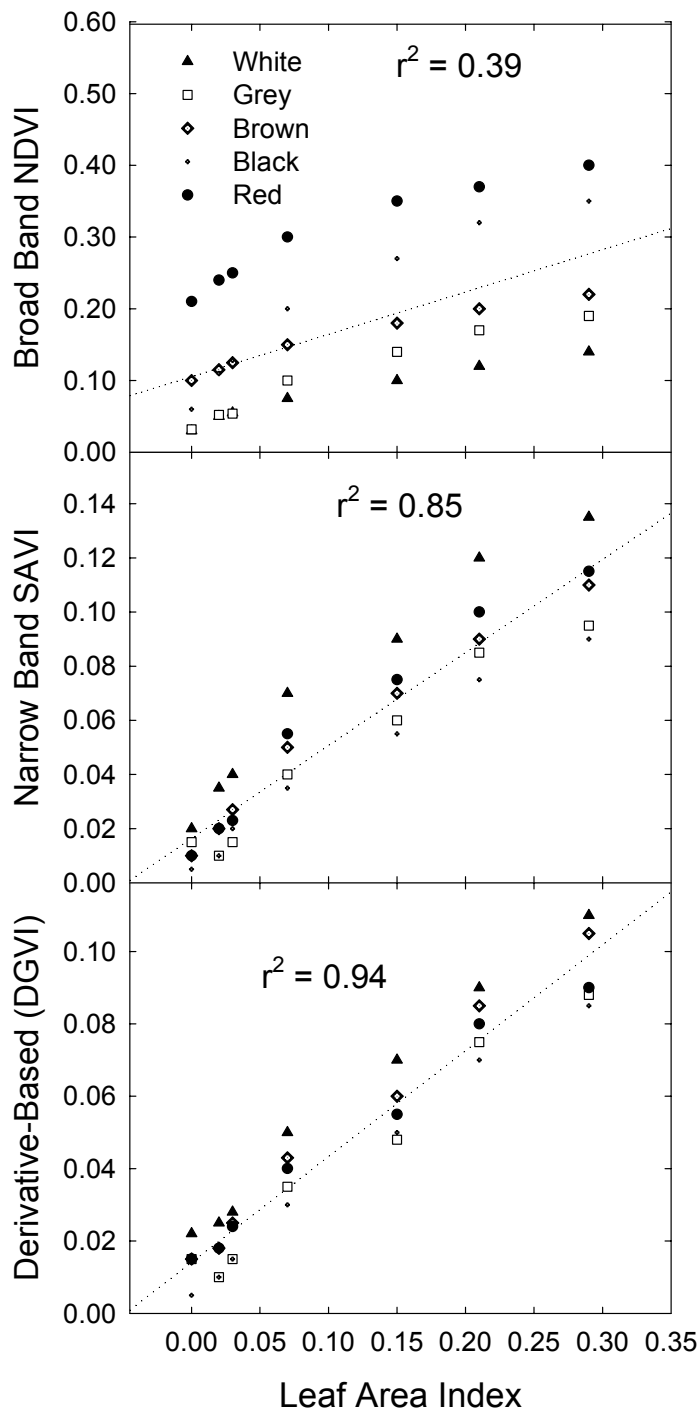
Figure 4. Comparison of derivative green cover estimates with common non-derivative vegetation indices to estimate LAI with different background colors (adapted from Elvidge and Chen, 1995).

Another application of derivative analysis has been proposed by Adams et al. (1999) as a method for determining chlorophyll concentration. The yellowness index is a three-point approximation of the second derivative between 550 and 650 nm and has shown positive results in identifying the chlorophyll concentration of manganese-deficient soybean plants.

The premise of the yellowness index is that the shape of the green reflectance spectra changes as plants become chlorotic. A second derivative analysis of this shape accentuates these changes in shape, allowing the identification of chlorotic plants based on this change in shape. This method assumes that all chlorotic plants exhibit this characteristic. The authors noted that although the 550 to 650 nm range was used for their study, other wavelengths might be appropriate, depending on the crop species and other physiological and environmental factors.

Band Depth Analysis

Another method for decreasing background effects is normalized band depth analysis. Because the absorptions of different plant materials are similar and overlapping (Table 1), single absorption bands cannot generally be isolated and directly related to the concentration of a single plant constituent. Therefore, Kokaly and Clark (1999) proposed the normalization of broad absorption bands to investigate plant stress. The reflectance signature is first processed through continuum removal (Clark and Roush, 1984).

A continuum line is approximated by a linear function that passes over an absorption feature of interest and connects two points of the reflectance spectrum that are unaffected by the absorption feature. The band depth is then calculated by dividing the absorption band reflectance by the continuum line. The continuum-removed spectrum is then normalized by measuring the depth at the center of the band and the area under the band depth curve. These measurements are termed band depth normalized to band depth at the center of the absorption feature (BNC) and band depth normalized to area of absorption feature (BNA). The result is an index that is insensitive to spectral contaminants. This technique was demonstrated to be effective in regions of the spectrum above 1000 nm.

Curran et al. (2001) found that reflectance analysis performed on dried and ground slash pine needles using the Kokaly and Clark methodologies compared favorably with laboratory biochemical assays, and that both BNC and BNA methods of band normalization resulted statistically more accurate differences in biochemical concentration estimates than derivative methods. Furthermore, Clark and Roush (1984) found moderate ($r^2 \approx 0.60$) to high ($r^2 >$ 0.95) levels of accuracy for estimation of twelve foliar biochemicals using band normalization.

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CHAPTER 3

DEVELOPMENT OF A NOVEL SPECTRAL REFLECTIVE INDEX TO

DIFFERENTIATE NITROGEN AND WATER STRESS

FROM EMERGENCE TO CANOPY CLOSURE

ABSTRACT

Spectral reflectance of plant canopies provides an accurate indication of ground cover fraction, which is highly correlated with radiation capture. If nutrients and water do not limit growth, radiation capture is highly correlated with daily growth rate and ultimate yield. However, if nutrients and water limit growth, none of the common spectral indices are able to separate a more developed, stressed canopy from a less-developed, rapidly growing canopy. We have long known that nitrogen stress is highly correlated with reduced chlorophyll concentration and increased reflectance of green radiation. Conversely, water stress inhibits leaf expansion, which increases chlorophyll concentration and decreases green reflectance. In a two-year study, we measured ground-based canopy reflectance of wheat plots in nitrogen and water stressed environments and found that all of the common spectral indices were highly correlated with ground cover fraction, but none of them could distinguish the intensity of leaf color from ground cover fraction. Here we report a reflective index that can differentiate nitrogen and water stress over a wide range of ground cover. The index is based on the ratio of the green and red variants of the normalized difference vegetation index ($NDVI_{green}/NDVI_{red}$). The new index was able to

distinguish N and water stress from satellite data using wavelengths less than 1000 nm. This index should be broadly applicable over a wide range of plant types and environments.

INTRODUCTION

Reflectance of red and green radiation by plants is heavily influenced by chlorophyll absorption. Past studies of reflective leaf biochemicals have emphasized that the photosynthetic pigment chlorophyll dominates visible and near-infrared leaf reflectance and forms the basis for most reflectance estimates of plant health (Curran, 1989). Chlorophyll concentration is closely correlated with changes in visible and near-infrared reflectance, so ratios of reflectance at chlorophyll-sensitive and chlorophyll-insensitive wavelengths are often used to determine chlorophyll concentration.

Red is the most widely used chlorophyll-sensitive spectral region. Red reflectance is sensitive to very low chlorophyll concentrations, making it ideal to estimate ground cover. However, red reflectance saturates at moderate chlorophyll levels, making it insensitive to higher levels of chlorophyll content.

The red edge is another chlorophyll-sensitive spectral region. The red edge, as described by Horler et al. (1983), is the area of sharp change in leaf reflectance between the red and near-infrared spectral domains. The red edge tends to be sensitive to a wide range of chlorophyll concentration, but is also affected by plant type and changes in ground cover (Carter and Spiering, 2002).

Green reflectance is also sensitive to a wider range of chlorophyll concentration than is red reflectance and is broader, flatter, and less sensitive than the red edge to variation of plant type and changes in ground cover. Therefore, at moderate to high chlorophyll levels, red reflectance remains generally constant, but green reflectance continues to decrease with increasing chlorophyll concentration (Gitelson and Merzlyak, 1997).

Nitrogen and water availability both affect the chlorophyll concentration of plant leaves. Nitrogen is an integral part of the chlorophyll molecule (Taiz and Zeiger, 2002), and nitrogen stress decreases chlorophyll concentration (Yoder and Pettigrew-Crosby, 1995). Water stress, on the other hand, does not directly affect chlorophyll synthesis, but rather decreases leaf area. Because the leaf area decreases and the amount of chlorophyll does not change, the net effect is an increase of leaf chlorophyll concentration (Peñuelas et al., 1994). Fernández et al. (1994) noted that unirrigated wheat plants were also more erectophile than irrigated plants in their study. They found that this characteristic affected plant reflectance in two ways. First, the vertical elements of an erectophile canopy would trap larger quantities of radiation than a planophile canopy, resulting in a decrease of reflected radiation from the canopy to the sensor. Second, the erectophile leaves would increase leaf area index for a given ground cover fraction, resulting in a higher leaf chlorophyll density per unit ground area. This would also result in an apparent increase in chlorophyll concentration as sensed by a spectrometer. These findings are consistent with the findings of Jackson and Pinter (1986).

**Methods for Estimating Ground
Cover**

Many studies have determined ground cover fraction and chlorophyll per

unit ground area using spectral indices. These indices maximize the spectral

contribution from green vegetation and minimize background effects, such as soil

reflectance (Huete et al., 1985; Major et al., 1990). Most of these indices can be

used to estimate ground cover (the percent of soil covered by plants in a given

area) or leaf area index (LAI; the ratio of leaf area to ground area).

Vegetation indices detect ground cover based on the sharp spectral

changes that occur as vegetation covers the soil. Soil reflectance slopes

gradually upward through the visible and near-infrared. Vegetation, however, is

heavily influenced by chlorophyll absorption, and reflects more green and near-

infrared radiation than red radiation. Vegetation fraction is identifiable through

changes in visible and near-infrared reflectance, and ratios of red and near-

infrared radiation typically correlate closely with ground cover.

Table 3 lists the common spectral indices that estimate ground cover and

correct for contaminating spectral influences. The simplest vegetation indices

use simple ratios or differences between two spectral regions to estimate growth.

The two simplest indices are called the ratio vegetation index (RVI) and the

difference vegetation index (DVI). The RVI is usually determined as the ratio of

near-infrared reflectance to red reflectance, although researchers have used

ratios of other bands as well to determine plant health (e.g. Peñuelas et al.,

1997).. The DVI is determined as the simple difference between two

wavelengths. Rouse et al. (1973) introduced a normalized variation of the RVI,

which was later called the normalized difference vegetation index (NDVI). The

NDVI is widely used to estimate fractional ground cover over a wide range of light

intensity.

Table 3. Broad-band and narrow-band vegetation indices that are widely used for
ground cover determination. These indices are based on the differential
absorption of green, red, and near-infrared radiation by plant chlorophyll. The
indices are arranged from oldest to newest.

Abbreviation	Name	Vegetation Index	Reference
RVI	Ratio Vegetation Index	$RVI = \dfrac{NIR}{RED}$	(Jordan, 1969)
NDVI$_{red}$	Normalized Difference Vegetation Index	$NDVI = \dfrac{(NIR - RED)}{(NIR + RED)}$	(Rouse et al., 1973)
PVI	Perpendicular Vegetation Index	$PVI = \dfrac{NIR - aRED - b}{\sqrt{1 + a^2}}$	(Richardson and Weigand, 1977)
DVI	Difference Vegetation Index	$DVI = NIR - RED$	(Tucker, 1979)
SAVI	Soil Adjusted Vegetation Index	$SAVI = \dfrac{(NIR - RED)}{(NIR + RED + L)}(1 + L)$	(Huete, 1988)
1DL_DGVI	First-order derivative green vegetation index using local baseline	$1DL\_DGVI = \sum_{\lambda_1}^{\lambda_n} \lvert \rho'(\lambda_i) - \rho'(\lambda_1) \rvert \Delta\lambda_i$	(Elvidge and Chen, 1995)
1DZ_DGVI	First-order derivative green vegetation index using zero baseline	$1DZ\_DGVI = \sum_{\lambda_1}^{\lambda_n} \lvert \rho'(\lambda_i) \rvert \Delta\lambda_i$	(Elvidge and Chen, 1995)
2DZ_DGVI	Second-order derivative green vegetation index	$2DZ\_DGVI = \sum_{\lambda_1}^{\lambda_n} \lvert \rho''(\lambda_i) \rvert \Delta\lambda_i$	(Elvidge and Chen, 1995)
NDVI$_{green}$	Green Normalized Difference Vegetation Index	$GNDVI = \dfrac{(NIR - GREEN)}{(NIR + GREEN)}$	(Gitelson and Merzlyak, 1998)
YI	Yellowness Index	$YI \propto \dfrac{R(\lambda_{-1}) - 2R(\lambda_0) + R(\lambda_{+1})}{\Delta\lambda^2}$	(Adams et al., 1999)

The green NDVI, or $NDVI_{green}$, is analogous to NDVI, except that it substitutes green reflectance for red reflectance (Gitelson and Merzlyak, 1997). Unless otherwise specified in this paper, NDVI will refer to the red variation of the NDVI and will be used to denote the estimate of ground cover by NDVI. The index will be referred to as $NDVI_{red}$ and $NDVI_{green}$ on occasions where the two variations of this index are compared with each other.

Although the oldest vegetation indices are still widely used, several newer indices have been developed in an attempt to increase the accuracy of ground cover estimation.

During early stages of vegetation development and growth, the soil constitutes a large portion of plant canopy reflectance. To overcome the effects of soil, some vegetation indices add a correction factor to the basic index that accounts for soil brightness. An early attempt to correct for soil brightness was introduced by Kauth and Thomas (1976), and is referred to as the perpendicular vegetation index (PVI). The PVI assumes that soil reflectance is essentially linear throughout the visible and near-infrared spectral regions. A slope (a) and an offset (b) of the soil reflectance are measured, and plant reflectance is computed as a perpendicular function from the original soil line. Later, Huete (1988) suggested the application of a soil-adjusted vegetation index (SAVI) to account for soil brightness. The L value in the SAVI equation is a brightness coefficient that accounts for soil color. The L value can be set between zero (black soil) and one (white soil). The estimate of a soil brightness coefficient is

difficult to ascertain, as alluded to by the authors, and is often arbitrarily set to 0.5.

Derivative vegetation indices, such as the first derivative green vegetation index and the second derivative green vegetation index (Elvidge and Chen, 1995), attempt to eliminate soil reflectance based on its linear nature. Although derivative indices are analogous in their most basic form to the DVI, Demetriades-Shah et al. (1990) pointed out that first- and second-derivative indices can essentially eliminate soil signal. Derivative indices were found by Elvidge and Chen (1995) to be superior to ratio indices in determining plant ground cover over a variety of backgrounds during their tests (see Appendix 3).

The estimate of plant ground cover in itself, however, is not an estimate of plant health. Although canopy reflectance indices have been correlated to nitrogen status (Fernández et al., 1994; Hinzman et al., 1986) and water status (Jackson et al., 1983), these parameters are estimated on a ground area basis, and the indices do not differentiate between leaf color and ground cover (Adams et al., 1999).

Spectral Indicators of Plant Color

As referenced earlier, the heavy influence of plant ground cover on canopy reflectance allows vegetation indices to estimate ground cover, but complicates the analysis of chlorophyll and nitrogen concentration, especially for incomplete crop canopies. Demetriades-Shah et al. (1990) pointed out that standard spectral vegetation indices are unable to differentiate between low plant

cover and decreases in plant health. In the past, several researchers have avoided the ground cover issue by correlating canopy reflectance with chlorophyll density on a soil area basis (e.g. Hinzman et al., 1986). Unfortunately, this method does not give any indication of leaf chlorophyll concentration separate from ground cover.

Demetriades-Shah and Court (1987) discussed the shortcoming of vegetation indices to estimate chlorophyll concentration and suggested the use of oblique (low angle) reflectance measurements to maximize the vegetation reflectance within the field-of-view and minimize the contribution of the soil reflectance to the overall reflectance measurement. Most spectral measurements are made with the sensor facing perpendicular to the soil surface, but oblique measurements are made with the sensor nonperpendicular to the surface. Demetriades-Shah and Court (1987) used this method to separate nitrogen-stressed from unstressed canopies using NDVI. However, Pinter et al. (1987) observed that oblique measurements significantly affect the ratios of red and near-infrared reflectance, and Otterman et al. (1995) noted that the reflectance of vegetated terrain is influenced heavily by the bidirectional scattering of solar radiation by plants. Changes in solar angle make oblique measurements difficult to replicate, and oblique readings do not allow the measurement of ground cover and plant color at the same time. Oblique measurements also cannot be scaled directly from ground-based to satellite measurements.

Other studies have attempted to identify stress by comparing spectral bands. Fernández et al. (1994) attempted to resolve plant nitrogen status by making a linear correlation between wheat nitrogen status and plant reflectance of red and green radiation. However, both red reflectance and green reflectance are affected by ground cover, and the authors did not explain how this linear correlation eliminates the ground cover issue from their estimate. Osborne et al. (2002) compared similar linear combinations of green, red, and near-infrared reflectance to determine nitrogen status in corn. However, they did not report a method to correct for ground cover, and the reported relationship between their coefficients of reflectance and plant nitrogen concentration was dependent on the sampling date.

Another suggested solution to the challenge of separating ground cover from plant greenness has been the use of derivative analysis of reflectance spectra to identify plant stress independently of ground cover. Demetriades-Shah et al. (1990) demonstrated the use of derivative spectra to suppress low-frequency background noise associated with soil reflectance, resulting in derivative spectral indices that identified plant chlorosis based on spectral shifts in the reflectance between healthy and chlorotic plants. Derivative analysis eliminates the effects of soil reflectance, which is characteristically linear throughout the visible and near-infrared spectral regions. However, derivatives introduce new complexity into spectral estimates, increase low-level noise, and may still need to be normalized to correct for soil characteristics (Elvidge and Chen, 1995). It is notable that integrated first-derivative indices are analogous to

the difference vegetation index over the wavelengths of interest. Derivative

indices are also difficult to use from broad band spectral data, although Adams et

al. (1999) suggested the use of broad band estimates of derivatives as a solution

to this challenge.

Based on this discussion, current vegetation indices are unable to

satisfactorily separate plant color from ground cover. Oblique measurements are

subject to scattering effects and do not provide a ground cover estimate. Linear

comparisons of green and red wavelengths do not address the ground cover

issue, because they do not take into account the changes of reflectance for each

color with changes in ground cover. Derivatives add complexity and high-level

noise.

The combination of the chlorophyll-saturated $NDVI_{red}$ as a ground cover

indicator and the chlorophyll-sensitive $NDVI_{green}$ as a plant color indicator has the

potential to separate plant color from the ground cover component of plant

health. Because $NDVI_{red}$ and $NDVI_{green}$ are normalized to the same near-infrared

wavelength, the influence of ground cover is minimized.

The focus of this research was to find a method to differentiate plant

chlorosis from ground cover. Therefore, this research consisted of two

objectives. The first objective was to test several vegetation indices to find the

most effective index for detecting vegetation cover so that nitrogen stress

determination could be based on the most robust index. The second was to

evaluate the extent to which nitrogen-stressed plant canopies can be

differentiated from unstressed and water-stressed canopies independent of

ground cover based on the varying sensitivities of green and red plant
reflectance to chlorophyll concentration.

MATERIALS AND METHODS

Data were collected at the Greenville Research farm in Logan, UT from
May 15 to July 1 during the 2001 and 2002 growing seasons. The predominant
soil in this area is the Millville silt loam, a well-drained, calcareous silt loam that is
dark grayish brown and has a Munsell color of 10 YR 4/2. The bare soil was
spectrally consistent throughout all of the plots during both growing seasons. N
and water stress treatments during both growing seasons were randomized and
divided in half by a line-source sprinkler system (Figure 5).
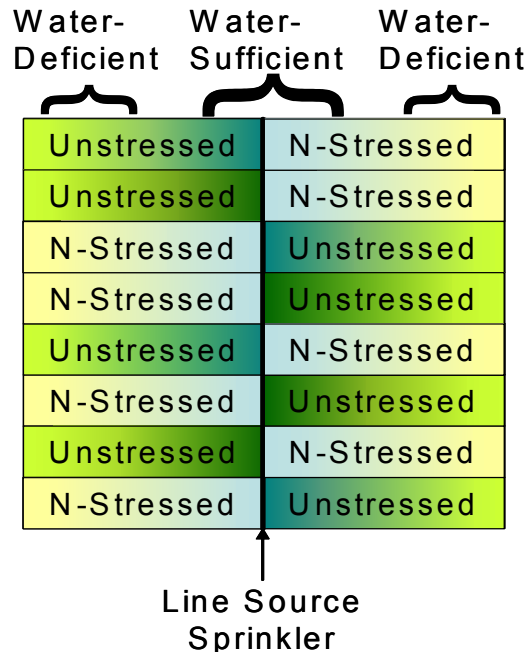
Figure 5. Experiment design of wheat test plots with line source sprinkler system.
The line source provided sufficient water to the area nearest the sprinkler system
and gradually less water with increasing distance from the line.

Control plots were initially fertilized with 100 kg ha^{-1} nitrogen, 110 kg ha^{-1}

phosphorus, and 45 kg ha^{-1} potassium. Nitrogen-deficient plots were fertilized

with the same phosphorus and potassium, but no nitrogen. A line source

sprinkler system provided a gradated water application from the center outward,

which introduced a gradual addition of water stress. Plots were divided into three

water treatment groups based on proximity to the line source. The half of each

plot closest to the line source received ample water during the growing season

and was used as the control. The outer regions of each plot received 50% and

10% of potential evapotranspiration (ET) based on the application to the inner

plots. Sample points were chosen randomly throughout the plots and provided

five to eight replicate sample points for each treatment. Plots were watered

every three to five days, depending on weather conditions. Irrigation was

measured with water gauges placed along the length of a test strip to verify water

deficit. All measurements were performed when the surface in all treatments

was dry to eliminate the effects of varying soil reflectance between treatments.

Reflectance measurements were made on sunny days from tillering through

anthesis with a field-portable fiber optic spectrometer (Model EPP2000,

StellarNet, Inc., Tampa, FL) with a 2-nm spectral resolution (FWHM) and a

spectral range of 400-950 nm. Each spectral measurement was made with the

sensor suspended 1.5 meters above the surface. Almost all measurements were

made within two hours of solar noon to minimize view angle effects. Spectral

data was smoothed using Savitzky-Golay least squares spectral smoothing over

a 5 nm range (Savitzky and Golay, 1964). Smoothed data was exported into a spreadsheet and analyzed. The spectral indices listed in Table 3 were calculated for each sample plot during the growing season. For the RVI, $NDVI_{red}$, PVI, DVI, and SAVI, 675 nm was used as the red reflectance. The $NDVI_{green}$ used 550 nm as the green reflectance. All of the indices used 840 nm as the NIR reflectance. The derivative vegetation indices were calculated between 626 nm and 795 nm, as described by Elvidge and Chen (1995). For the yellowness index, reflectance at 580 nm was used as 8_{-1}, reflectance at 624 nm was used as 8_0, reflectance at 668 nm was used as 8_{+1}, and $\Delta 8$ was 44 nm, as examined by Adams et al. (1999).

Plant ground cover was measured using a digital camera that was suspended over each sample point. A large white foam board was used as a baffle to block direct solar radiation, allowing pictures to be taken on sunny days without sharp shadows below the plants. This minimized the analysis time for each picture and increased the accuracy of the ground cover estimate.

Image analysis was performed in Adobe Photoshop (Adobe Systems, Inc., San Jose, CA), an off-the-shelf photo-editing program. The soil component was deleted using the magic wand tool and the magic eraser tool, both of which allow the selection of pixels with similar colors. Ground cover was then calculated for each image using the histogram function, with green pixels calculated as the fraction of total pixels. Previous studies in our laboratory using this technique have indicated that these digital images of ground cover were highly correlated

with radiation capture (r^2 = 0.995) and canopy photosynthesis (r^2 = 0.998) (Klassen et al., 2003).

Leaf chlorophyll concentration was measured with a chlorophyll meter (SPAD-502, Minolta, Ramsey, NJ), and the chlorophyll measurements were used as a basis for comparison with the spectrometer. The SPAD-502 chlorophyll meter is a dual-wavelength meter that emits light from a red LED and an infrared LED in sequence through a leaf to measure leaf absorbance. Peak chlorophyll absorbance is measured at 650 nm, and nonchlorophyll absorbance is measured at 940 nm by sensors inside the instrument (Monje and Bugbee, 1992). The meter output value is based on the ratio of transmittance at these wavelengths and ranges from about 15 (highly chlorotic) to 60 (dark green) for Westbred 936 spring wheat. Ten SPAD chlorophyll readings of separate plants were made on at each sample site and averaged to estimate the chlorophyll concentration of the plants at the site. All measurements were performed on the top unfolded leaf of each plant.

After the derivation of our method, a 2002 IKONOS satellite image of a production Westbred 936 spring what field at Minidoka, Idaho. The soil at this site was generally uniform and consisted of alluvial and loess deposits of silt loam. Most of the soil in the region is Minidoka silt loam, a coarse-silty, mixed superactive, mesic Xerollic Durorthid with a color of 10YR 6/3, 10YR 4/2 moist. The Idaho plots consisted two replicates of four treatments: 0, 60, 150, and 195 kg ha^{-1} applied nitrogen. The nitrogen application of the control study was 150

kg ha^{-1} pre-plant.   Plant cover at the time of the image ranged from 50 to almost 100%, depending upon the treatment.

RESULTS

Comparison of Indices to Measure
Ground Cover

All but one of the 10 common spectral indices accurately determined ground cover (as determined from digital images) throughout the growing season (Table 4). The ratio vegetation indices (RVI, NDVI$_{red}$, NDVI$_{green}$, PVI, and SAVI) had a higher coefficient of determination of ground cover than the difference or derivative indices (Appendix 6). The ratio indices were more consistent than the simple difference indices, because the ratio of reflectance between wavelengths is more constant than differences of reflectance between wavelengths.

Indices that corrected for soil brightness did not improve the prediction of ground cover for this study, because the soil was spectrally similar throughout the test site.

The yellowness index (YI) was poorly correlated with ground cover, probably because the YI was designed to derive plant yellowness and not ground cover. Digital ground cover estimates and NDVI were well correlated with plant growth during the growing season (Figure 6). Although the average plot NDVI varied among sampling dates during the growing season, it followed plant growth and allowed a full-season estimate of ground cover and final yield (Appendix 1).

Table 4. Comparison of ratio and difference vegetation indices with digital images of ground cover collected during the entire growing season. Ratio indices were better correlated with ground cover than difference indices. The yellowness index had a poor correlation with ground cover.

Index Class	Name	Coefficient of Determination (r^2) with Ground Cover
Ratio	Ratio Vegetation Index (RVI)	0.88
	Normalized Difference Vegetation Index (NDVI)	0.88
	Perpendicular Vegetation Index (PVI)	0.87
	Soil Adjusted Vegetation Index (SAVI)	0.90
	Green Normalized Difference Vegetation Index ($NDVI_{green}$)	0.90
Difference	Difference Vegetation Index (DVI)	0.82
Derivative	First-order derivative green vegetation index using local baseline (1DL_DGVI)	0.83
	First-order derivative green vegetation index using zero baseline (1DZ_DGVI)	0.82
	Second-order derivative green vegetation index (2DZ_DGVI)	0.73
	Yellowness Index (YI)	0.28
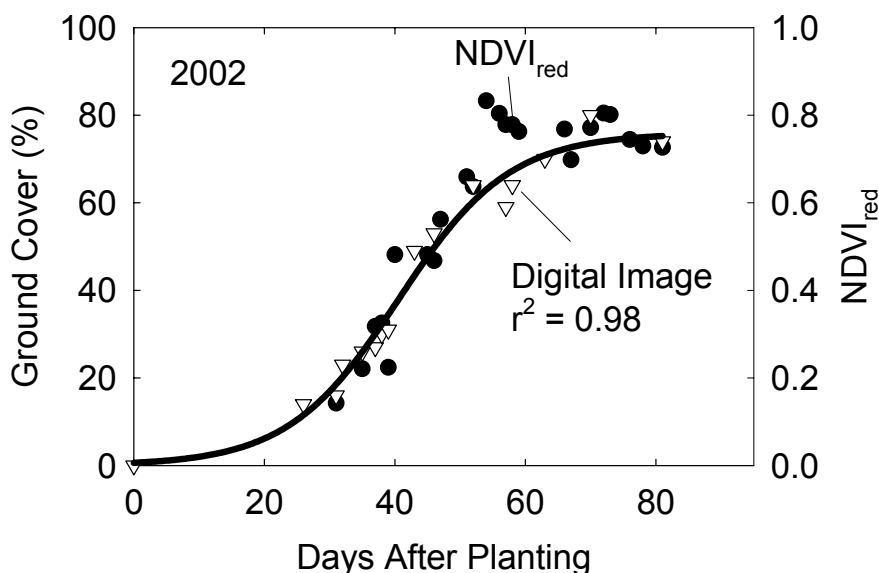
Figure 6. Comparison of NDVI with ground cover (measured by a digital camera) over time. Digital images and NDVI increased in a similar manner during the growing season based on plant growth.

Differentiating N and Water Stress

Although stressed treatments were easily differentiated from unstressed treatments during both growing seasons based on NDVI (Figure 7), the close relationship between NDVI and ground cover indicated that most of this separation was due to changes in ground cover. Water-stressed and nitrogen-stressed plots were almost identical during the 2001 growing season (Figure 7). This similarity emphasizes the confounding effects of ground cover, since water and nitrogen stress could not be separated by NDVI, even though the chlorophyll concentration in water-stressed and nitrogen-stressed leaves was quite different.
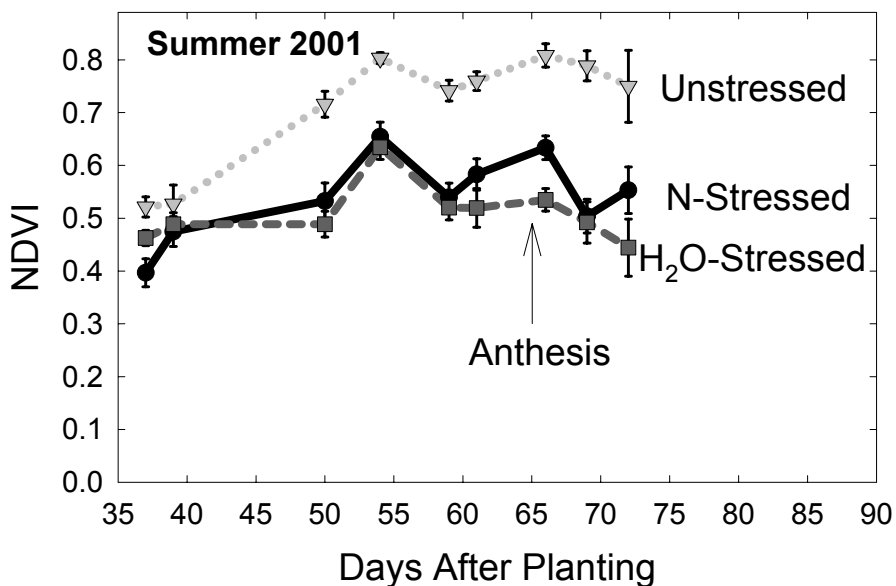
Figure 7. NDVI values of unstressed, nitrogen-stressed, and water-stressed plots during the growing season (error bars are standard error of the mean). Nitrogen and water-stressed canopies had similar NDVI values, so they could not be identified by NDVI alone.

**Identifying Plant Chlorosis Using
Normalized Green and Red
Reflectance**

The second objective of this study was to identify plant greenness and
stress independent of ground cover. The soil-adjusted vegetation indices were
not used to identify chlorosis, because their extra complexity did not increase
their accuracy in predicting ground cover for our studies. The RVI was initially
attractive because of its simplicity, but the relationship between RVI and ground
cover fraction was nonlinear for our studies, and this increased complexity
negated its simplicity. The red and green variants of the NDVI ($NDVI_{red}$ and
$NDVI_{green}$) were used to identify plant chlorosis because both indices were simple
and had a high linear coefficient of determination with the digital image ground
cover measurements.

To correct for ground cover effects, $NDVI_{green}$ was compared with $NDVI_{red}$.
This normalized both green and red reflectance to near-infrared reflectance and
minimized the effects of ground cover fraction on the direct comparison of green
and red reflectance. This $NDVI_{red}$ and $NDVI_{green}$ comparison is referred to as the
normalized green:red (NGR) relationship in this study.

Nitrogen-stressed plots had a lower NGR relationship than did unstressed
or water-stressed plots, meaning that $NDVI_{green}$ values for this treatment were
smaller compared to $NDVI_{red}$ values than in other treatments. This is due to the
higher green reflectance of nitrogen-stressed plots, which results in a lower
$NDVI_{green}$ value.

A comparison of $NDVI_{green}$ with $NDVI_{red}$ during the growing season indicated a general separation between the nitrogen, water, and unstressed treatments (Figure 8). This result was anticipated because green reflectance is sensitive to a wide range of chlorophyll concentration, while red reflectance is insensitive to all but low levels of chlorophyll concentration (Gitelson and Merzlyak, 1997). $NDVI_{red}$ is thus a chlorophyll-insensitive ground cover indicator, and $NDVI_{green}$ is a chlorophyll-sensitive indicator of chlorosis. Deviations from the relationship between these indices for an unstressed canopy can therefore signal changes in $NDVI_{green}$ due to changes in plant canopy chlorophyll concentration.

The best-fit linear regression of $NDVI_{green}$ versus $NDVI_{red}$ for the unstressed plots was used to define an unstressed ratio line ($r^2 = 0.97$; Figure 8). All unstressed plots had a close NGR relationship to this line (Figure 8). The water-stressed treatments had a similar NGR relationship to the unstressed plots, but many sample points were higher than the unstressed NGR line, suggesting interaction between water stress and an increased $NDVI_{green}$.

Nitrogen-stressed plants had a similar NGR relationship at low ground cover, but had a lower NGR relationship than unstressed plots at higher ground cover. Nitrogen-stressed plants had similar reflectance characteristics to unstressed plants at low levels of ground cover, because plants did not exhibit stress characteristics until the N in the soil was depleted. However, stress became more evident later in the season as the plants depleted the soil N and began to actively show chlorosis.
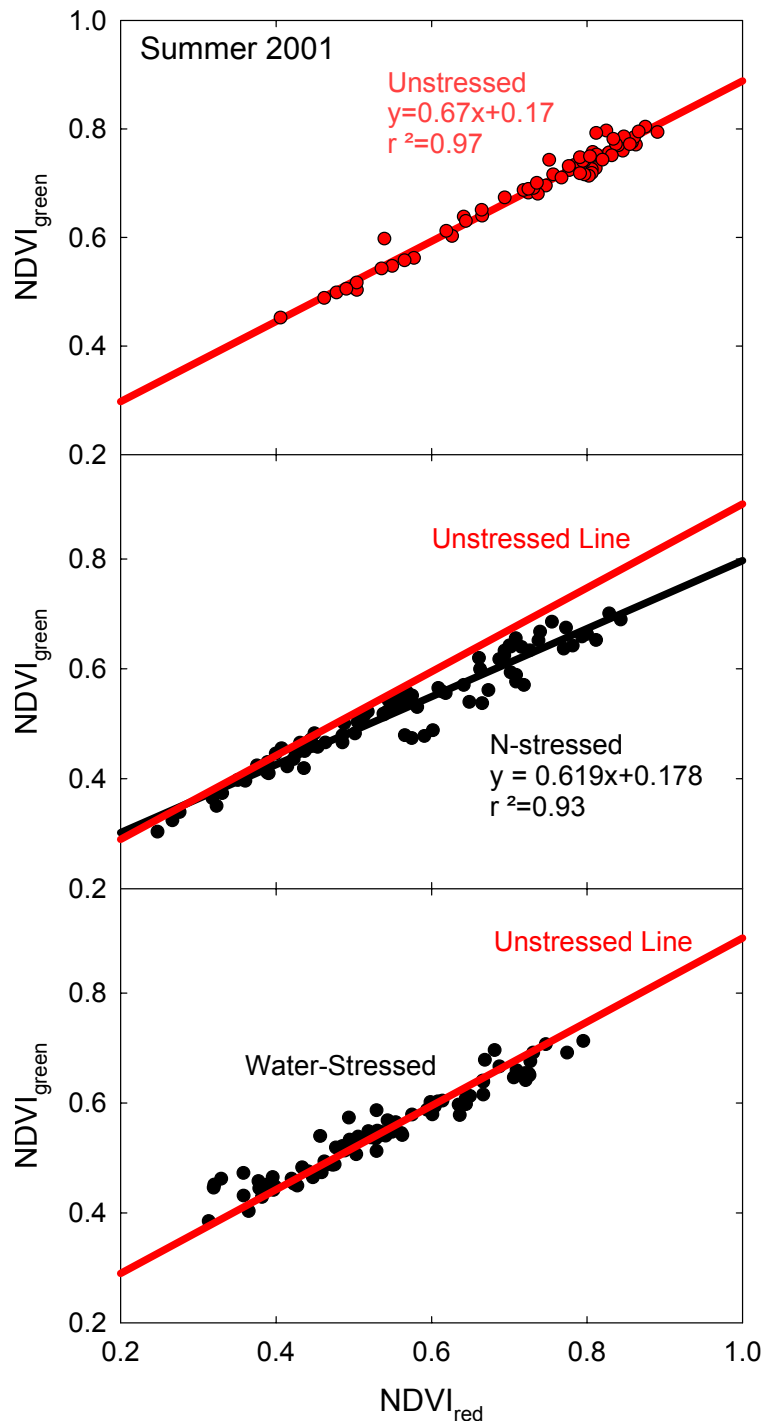
Figure 8. Comparison of NDVI$_{green}$ and NDVI$_{red}$ for unstressed plots, nitrogen-deficient plots, and water-deficient plots. The slope of NDVI$_{green}$ vs. NDVI$_{red}$ was lower for nitrogen-stressed plots than for unstressed plots or water-stressed plots, allowing the separation nitrogen-stress from ground cover.

The NGR relationship for water-stressed, unstressed, and nitrogen-stressed plots from day 35 to day 69 is shown in Figure 9. The residuals from the unstressed NGR line determined in Figure 8 were calculated, and the mean residual of each treatment from the unstressed line was plotted for each day (Figure 9). After day 35, nitrogen-stressed plots had consistently negative residuals, indicating chlorosis. Water-stressed plots, on the other hand, had residuals similar to the unstressed plots until day 60, when the residuals became more positive than those of the unstressed plants. This positive residual probably occurred because no rainfall was measured after day 45 and water stress increased during the growing season.
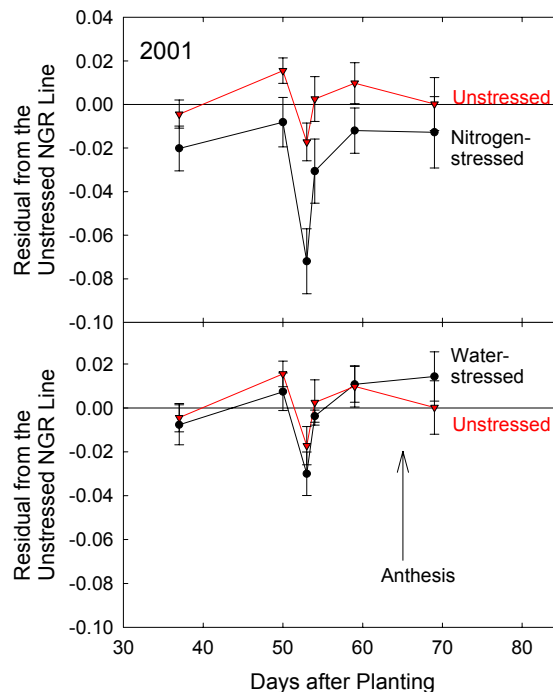
Figure 9. Comparison of deviations from the unstressed correlation line by treatment. Error bars represent ± one standard deviation.

Measurements were made more frequently in the second summer (2002). The NGR relationship for the unstressed plots during 2002 was best estimated by the linear regression of $NDVI_{green} = 0.704 \times NDVI_{red} + 0.14$ ($r^2 = 0.98$). Slopes of the unstressed NGR lines for the 2001 and 2002 seasons were compared using the test statistic: ((slope a - slope b) - 0)/(variance of slope a) = t $_{(degrees\ of\ freedom\ of\ slope\ a)}$ (Neter et al., 1996). The slopes were tested using both the data from 2001 and 2002 (Table 5). The slopes were not significantly different (smallest P > 0.30). This allowed the unstressed NGR lines for 2001 and 2002 to be pooled, with the following regression equation: $NDVI_{green} = 0.701 \times NDVI_{red} + 0.14$ ($r^2 = 0.98$).

The $NDVI_{green}/NDVI_{red}$ (NGR) relationship for each treatment in 2002 was plotted by day and compared to the NGR values of the unstressed treatment (Figure 10). Both nitrogen and water-stressed plots were identified by their deviation from the unstressed treatment (Appendix 5).

Table 5. Statistical parameters of the 2001 and 2002 regression equations used to test whether the slopes are different.

Year	Regression Line	Degrees of Freedom	Standard Error of Slope	t (slope)	P (slope)	Standard Error of Intercept	t (int)	P (int)
2001	$NDVI_{green} = 0.67 \times NDVI_{red} + 0.17$	57	0.613	0.075	P>0.4	0.435	0.069	>0.4
2002	$NDVI_{green} = 0.70 \times NDVI_{red} + 0.14$	513	0.095	0.486	P>0.3	0.054	0.556	>0.3
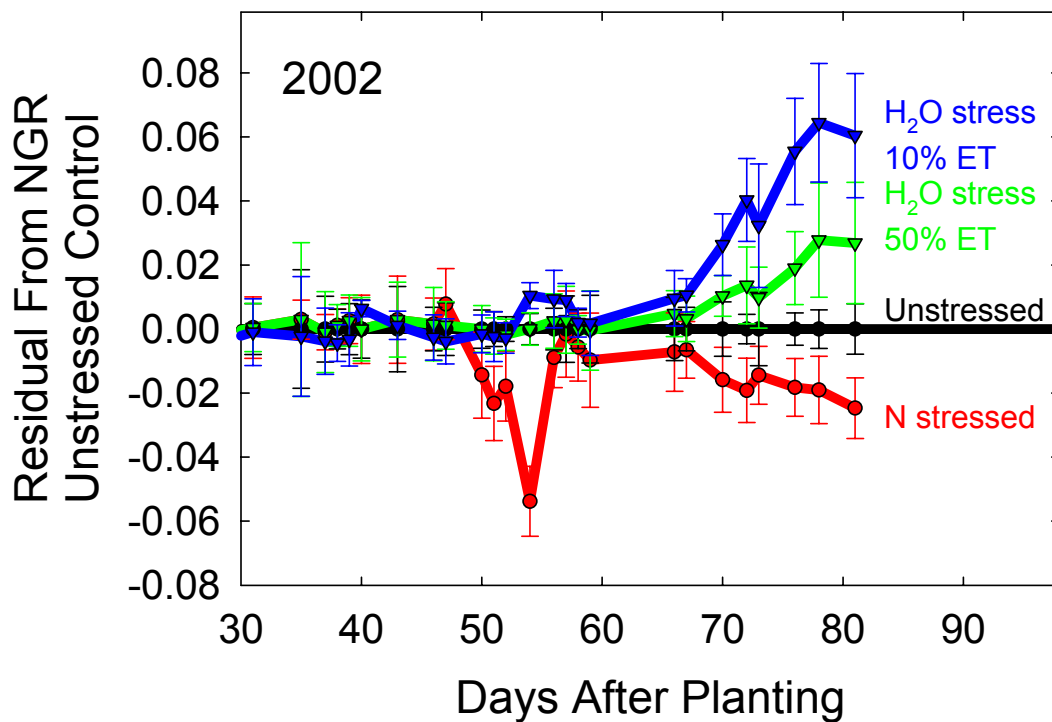
Figure 10. Comparison of water and nitrogen-stressed plot NGR values with unstressed plots during the 2002 growing season. Error bars represent ± one standard deviation.

The normalized green:red (NGR) relationship in this study used the ratio of $NDVI_{red}$ to $NDVI_{green}$. However, NDVI can be separated into even more basic indices. NDVI is equal to (RVI-1)/(RVI+1), so the green and red variants of RVI might yield a simpler index that can separate nitrogen and water stress. Another basic index within the NDVI is the DVI. Mathematical manipulation separates the NGR ratio into two component parts: a DVI ratio and a (NIR+G)/(NIR+R) ratio. The (NIR+G)/(NIR+R) ratio is related to ground cover and ranged from about 0.94 at low ground cover to 1.06 at high ground cover for our experiment (Figure

11). Because this ratio was close to one, we speculated that the NGR

relationship could be simplified by using the DVI with NIR reflectance acting as a

normalizing factor for both the red and green reflectance in a comparison of

DVI_{green} and DVI_{red} (Appendix 5).

The NDVI and DVI variations of the NGR relationship showed significant

differences between nitrogen, water, and unstressed plots, although the DVI

variation showed slightly less separation between treatments than the NDVI

variation (Figure 12). This is presumably due to the missing influence of the

(NIR+G)/(NIR+R) factor in the DVI comparison. However, the differences

between the NDVI and DVI were slight.
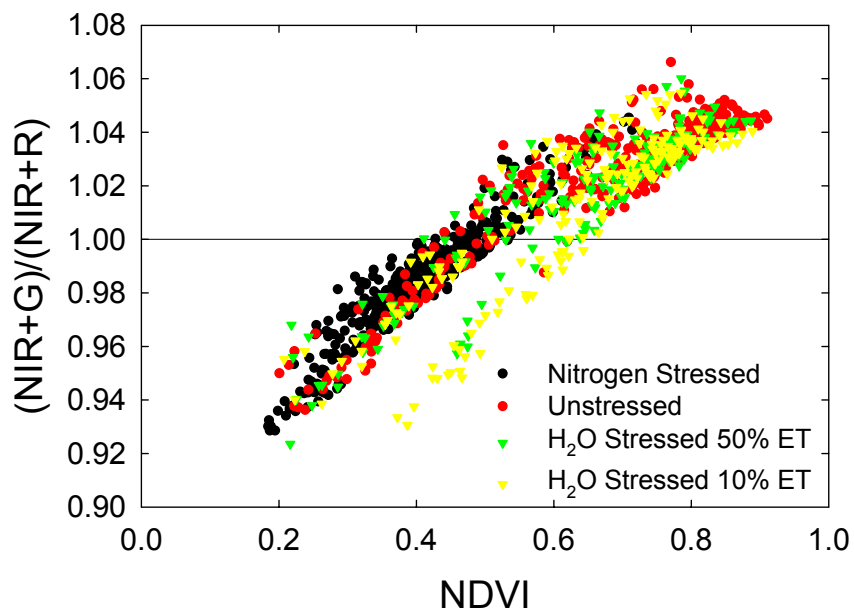
Figure 11. Comparison of the (NIR+G)/(NIR+R) ratio with NDVI. Although the
(NIR+G)/(NIR+R) ratio correlated with NDVI and was affected by treatment, 95%
of the points fell between 0.94 and 1.06, suggesting that the actual influence of
this ratio in the NGR relationship is small.
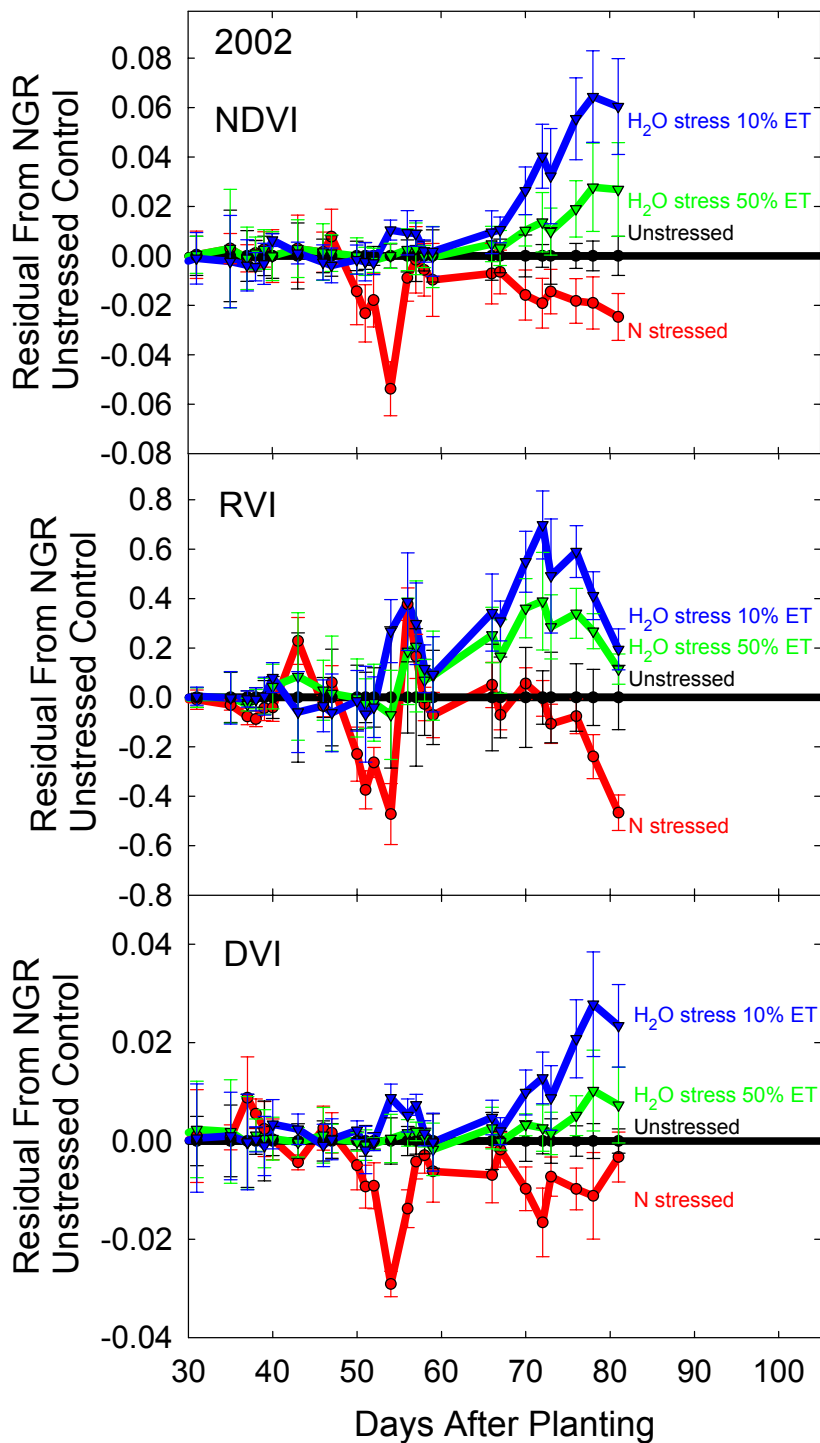
Figure 12. Separation of unstressed, nitrogen-stressed, and water-stressed plots using the NDVI, RVI, and DVI normalized green:red relationships.

The RVI NGR relationship showed wide within-treatment variations and less treatment separation than either the DVI or the NDVI. The RVI NGR relationship is nonlinear, and much of the variation within this test can be attributed to the inability of the nonlinear function to accurately reflect changes from low to high RVI (Figure 12).

Figure 13 shows that the NGR relationship correlated better with full-season SPAD chlorophyll readings than did either NDVI$_{red}$ or NDVI$_{green}$ alone. Figure 13 also alludes to the primary useful aspect of the unstressed line concept: positive and negative residuals from the unstressed line can indicate chlorophyll concentration. The relationship between NDVI$_{red}$ and NDVI$_{green}$ identified chlorophyll concentration, and thus nitrogen stress, throughout a wide range of ground cover.

The correlation of NDVI$_{green}$ and NDVI$_{red}$ with the SPAD readings can be misleading, since leaf greenness and ground cover both increase during the season, making it difficult to distinguish the effects of these factors from each other. Furthermore, a specific NDVI$_{green}$ or NDVI$_{red}$ value cannot determine whether the plant is stressed, while a specific NGR value can.

The NGR relationship also identified the effects of N and water stress on leaf chlorophyll concentration (Figure 14). Nitrogen-stressed plots had negative NGR and low SPAD values, while water-stressed plots had positive NGR values and SPAD values similar to the unstressed plots.
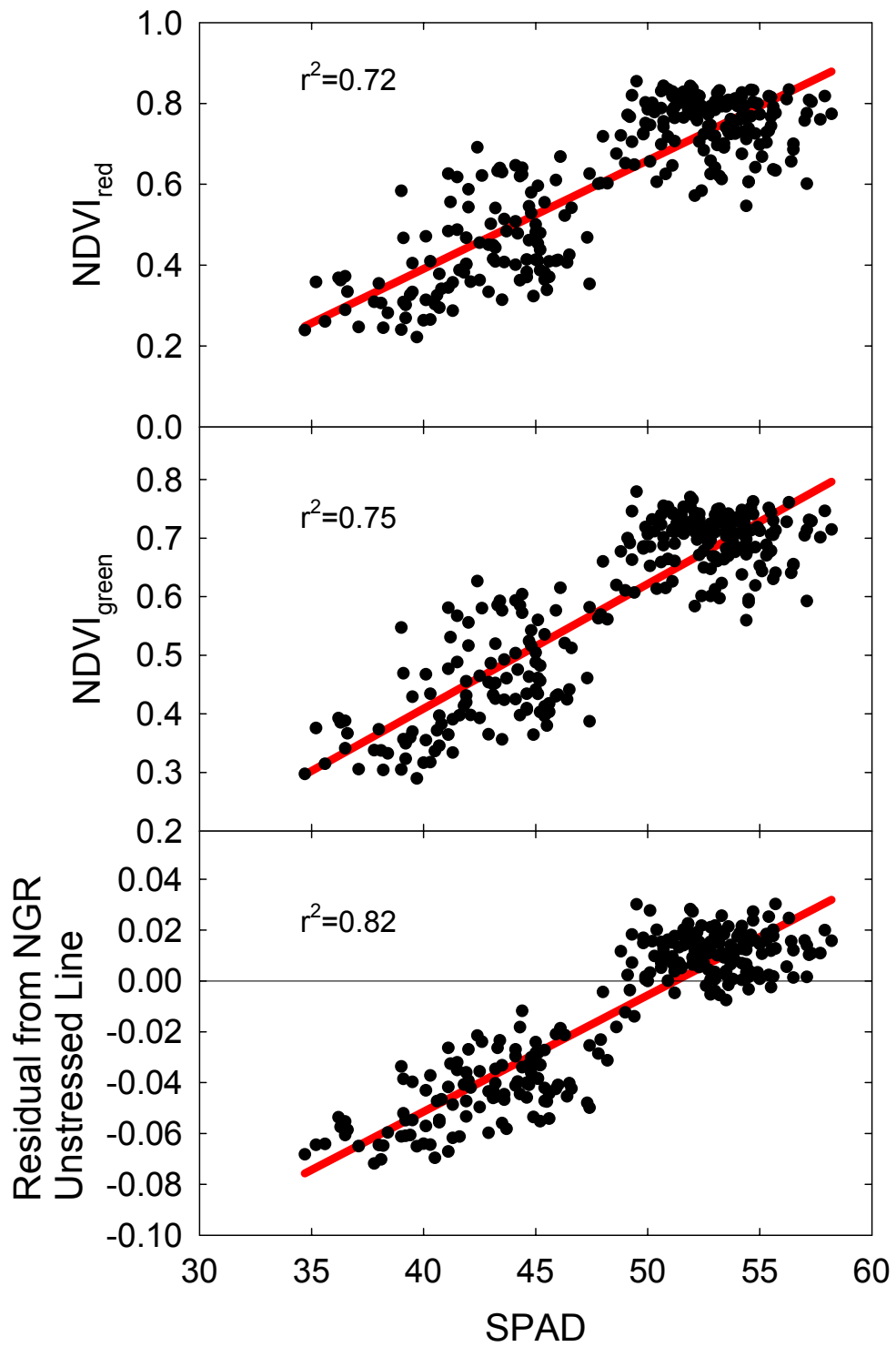
Figure 13. Comparison of NDVI$_{red}$, NDVI$_{green}$, and the unstressed line with SPAD readings during 2002. Data are for unstressed and N-stressed plots only.
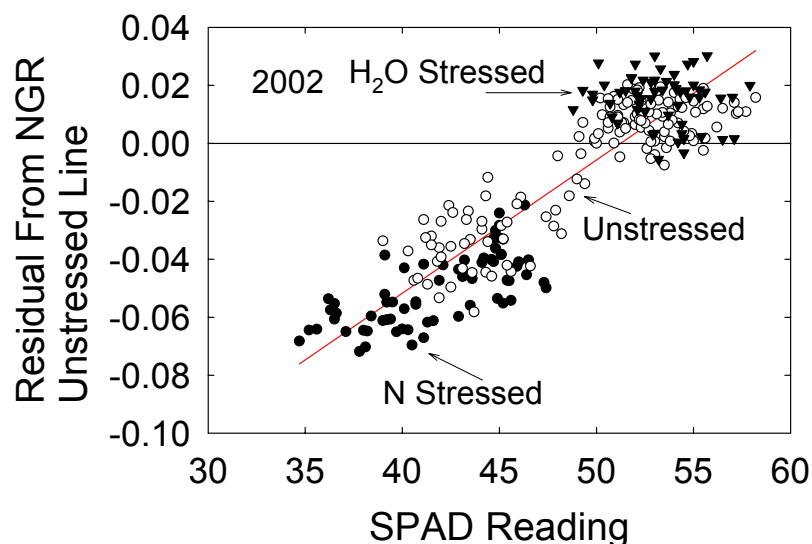
Figure 14. Comparison of the residuals from the 2001/2002 unstressed NGR line with SPAD values during the growing season. Nitrogen-stressed plots had low SPAD readings and negative residuals from the mean. Water-stressed plots had high SPAD readings and positive residuals from the unstressed line.

Using the NGR Relationship to
Identify Water Stress

Water stress decreases leaf expansion, so the ratio of $NDVI_{stressed}$ to

$NDVI_{control}$ provides a sensitive indication of water stress. The onset of water-

stress during the 2002 season was indicated by changes in ground cover as

estimated by NDVI (Figure 15). Figure 15 also shows that highly water-stressed

(10% ET) and moderately water-stressed plots (50% ET) had an increase in the

NGR relationship compared to the unstressed line. This change in the NGR

relationship was identifiable soon after the decrease in the NDVI of the water-

stressed plots. The close relationship between deviations from the unstressed

line and decreases in NDVI indicate that water stress causes a decrease in

reflectance of green radiation. As discussed earlier, water stress increases leaf

chlorophyll concentration indirectly by decreasing leaf area (Peñuelas et al.,

1994). Fernández et al. (1994) also noted that unirrigated wheat plants can be

more erectophile than irrigated plants under some circumstances. If erectophile

leaves increased leaf area index for a given ground cover fraction, it would cause

a higher leaf chlorophyll density per unit ground area, with a similar effect on

canopy reflectance as that of decreased leaf area. The decrease in leaf area

and the possible erectophile tendency of water-stressed plants might both affect

NDVI and increase the NGR relationship of water-stressed plants.
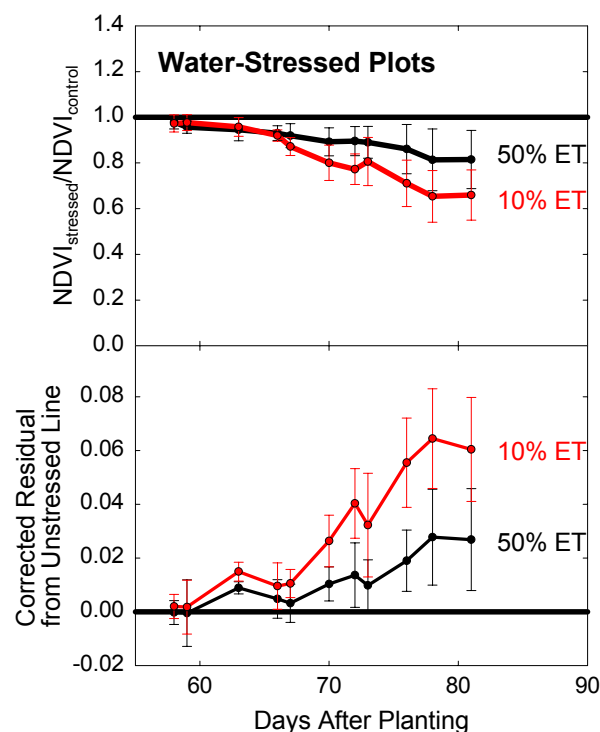
Figure 15. Deviations of water-stressed plots from the NDVI and the unstressed line of unstressed plots during 2002. The water-stressed plots that received the least irrigation had the highest levels of stress based on changes of NDVI and residuals from the unstressed plots. Error bars represent ± one standard deviation.

Figure 16 shows the correlation between decreases in ground cover (estimated by NDVI) due to water stress and the increase in NGR values. Decreases in ground cover correlated closely with changes in the NGR relationship with the onset of water stress. Conversely, the nitrogen-stressed plots in our studies did not show any significant correlation between NGR and changes in ground cover. This suggests that changes in plant color, not ground cover, are the primary reason for changes in the NGR of nitrogen-stressed plots.
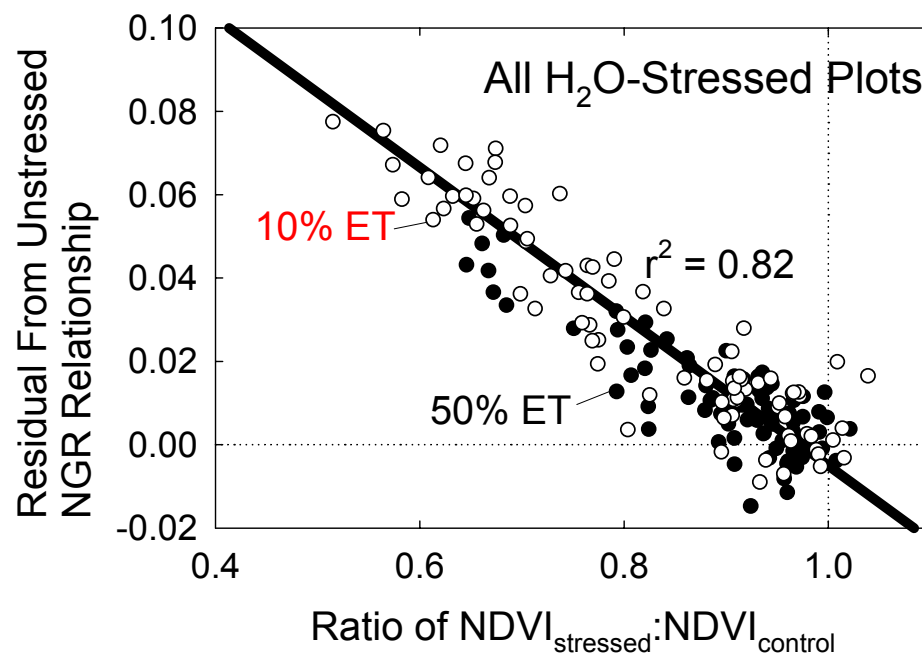
Figure 16. Comparison of residuals from the unstressed line with the decrease of NDVI for water-stressed plots in relationship to the unstressed plots during 2002. Changes in the $NDVI_{green}:NDVI_{red}$ relationship from the unstressed line correlated closely with decreases in NDVI for the water-stressed plots.

**Identification of N Stress from
Satellite Data**

Figure 17 emphasizes the potential of the unstressed line as a plant stress indicator. An IKONOS image collected on June 14, 2002 over a center pivot wheat field in Idaho showed a marked separation between treatments of 0 and 65 kg ha^{-1} of applied nitrogen, and the unstressed nitrogen treatments. Although many of the plots were approaching complete canopy cover, the deviations of the stressed treatments from the unstressed NGR line at lower levels of ground cover suggest that this method can be effective with partial ground cover as well as complete ground cover.
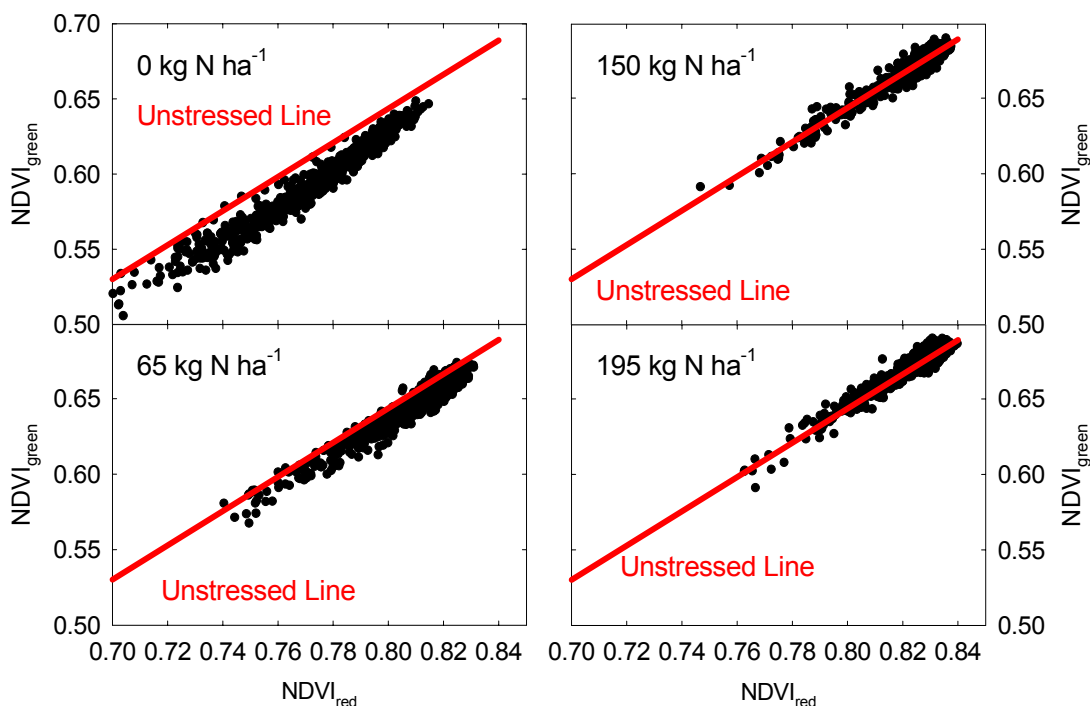
Figure 17. Comparison of the IKONOS NDVI$_{green}$:NDVI$_{red}$ relationship for four nitrogen treatments with an unstressed line derived from control fertilizer treatment line on June 14, 2002. The 0 and 65 kg ha^{-1} nitrogen treatments deviated significantly from the unstressed line, while the 195 kg ha^{-1} treatment was almost identical to the 150 kg ha^{-1} control treatment.

The use of an unstressed NGR relationship as an estimator of plant chlorosis can yield a greater treatment separation than NDVI alone (Figure 18). An NDVI estimate of ground cover did not yield significant differences between the 65 kg ha^{-1} and higher N treatments, but the NGR comparison of the treatments revealed a separation of these treatments.
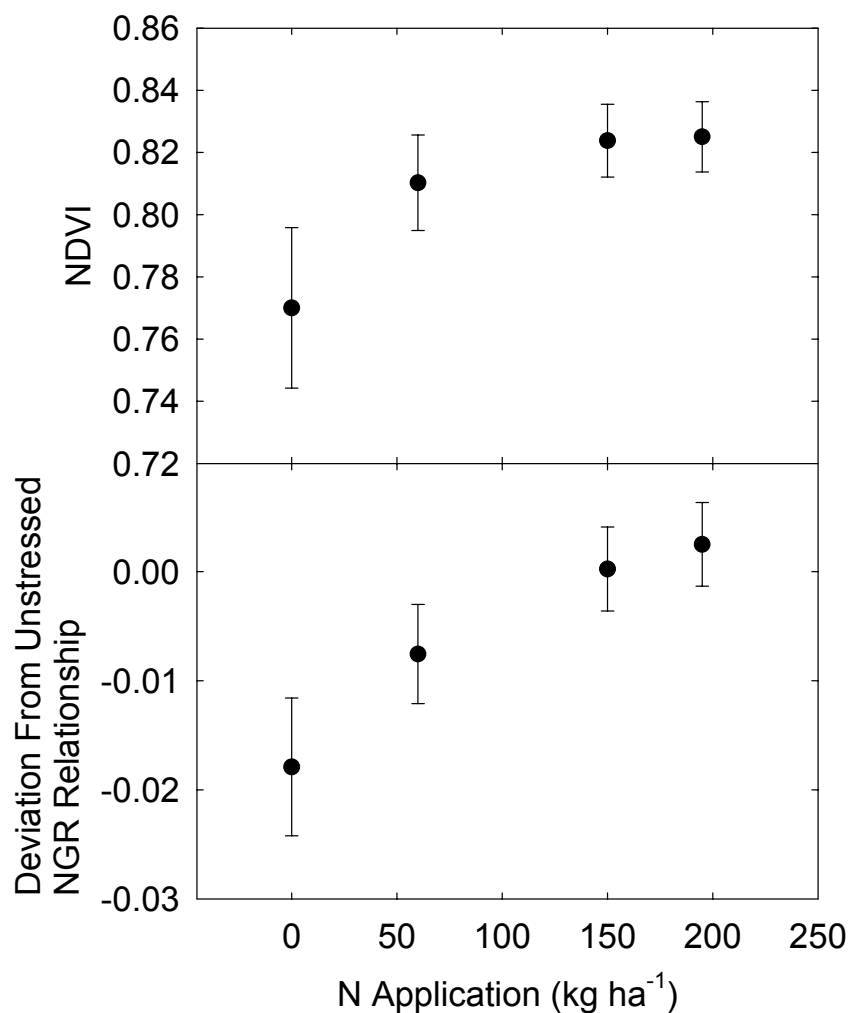
Figure 18. Comparison of NDVI and NGR with N application in Idaho field experiment. The NGR relationship was able to separate plots by treatment better than a simple NDVI comparison.

DISCUSSION

Advantages of the NGR Relationship

Separation of nitrogen-stressed wheat based on the relationship between $NDVI_{green}$ and $NDVI_{red}$ allows identification of nitrogen stress separate from ground cover. This method should allow identification of nitrogen stress without having to resort to oblique measurements or derivative estimates. Because the changes between the green and red regions of the spectrum are influenced by the same factors that affect derivative indices, this method can offer a comparable result without spectral smoothing and derivative calculations. This technique was more useful than simple difference measurements, because both the red and the green regions were normalized to the same point. Difference indices are subject to the influence of slight changes in the relationship between the reference reading and the crop reflectance reading, since even minor changes in incident radiation can influence the magnitude of the reflectance.

Effects of Solar Angle

It is notable that all treatments during the 2001 growing season had a more negative mean residual from the unstressed line on day 54 than on other days (Figure 9), and a similar trend on day 47 during 2002 (Figure 10). Measurements on these days were made early in the morning, when the solar elevation was lower. Based on our studies, $NDVI_{red}$ is typically more sensitive to solar angle than is $NDVI_{green}$, resulting in a higher $NDVI_{green}$:$NDVI_{red}$ relationship for this day (Appendix 4). The effect of solar angle on plant canopy reflectance is

an important issue in any type of plant health study. Middleton (1991) reported

that the response of vegetation indices to solar angle depends on canopy

structure, and that a general correction factor to remove sun angle effects is

inappropriate. Therefore, measurements should be made at similar solar angles.

Failure to do this can overshadow the small effects of changes in plant color.

Much of the variability due to solar angle can be eliminated on a day-to-day basis

if all of the plots are measured near the same time of day.

Comparison of Spectral Indices with
Chlorophyll Content

The comparison of the NGR relationship values for each plot over the

growing season with SPAD measurements showed a higher correlation

throughout the growing season than the comparison of SPAD readings with

either $NDVI_{red}$ or $NDVI_{green}$ alone (Figure 13). This suggests that the NGR

relationship can increase the accuracy of chlorophyll concentration prediction.

Because $NDVI_{red}$ or $NDVI_{green}$ are closely related to ground cover, the

relationship of $NDVI_{red}$ or $NDVI_{green}$ alone with SPAD chlorophyll measurements

seems anomalous. However, much of this positive relationship is explained by

the fact that nitrogen stress decreases both leaf area and chlorophyll content,

resulting in a positive correlation between ground cover and chlorophyll content

for nitrogen-stressed plants. This relationship does not exist in water-stressed

wheat canopies, as shown in Figure 14.

**Satellite Data and the NGR
Relationship**

This technique was tested on only a limited basis with satellite data, but

the data suggests that satellites will be able to identify nitrogen-stressed plots

independent of ground cover, despite atmospheric effects and the broad band

nature of satellites (Figure 17). Both $NDVI_{red}$ and $NDVI_{green}$ are robust indices

that are based on the high-signal near-infrared regions of the spectrum. One

challenge is the identification of relatively slight changes in green reflectance of

stressed plots and the separation of this characteristic from other optical noise.

Another challenge for long-term estimates will be the correction of satellite data

to allow the comparison of NGR values between sampling dates.

**Comparison of 2001 and 2002
unstressed lines**

Unstressed plots from the 2001 and 2002 experiments had similar

normalized green:red relationships. Because the unstressed lines for the two

seasons were not statistically different, this method should allow a robust

estimate of plant health that can be used during multiple growing seasons.

However, the precise relationship between these parameters should be

determined based on individual field characteristics, such as soil color and plant

type. The measurement platform (satellite vs. ground-based) should also be

considered when identifying a specific NGR relationship for a field. As

mentioned earlier, solar angle should also be considered in making all ground

cover and plant color measurements. However, extensive calibration for a single

set of measurements should not be necessary. Instead, an unstressed line can be drawn through the data that with the highest NDVI values at a specific sample time. Areas that have lower NDVI values than the unstressed plots can then be identified as nitrogen or water-stressed based on their deviations from the unstressed NGR relationship.

CONCLUSIONS

Simple ratio vegetation indices such as NDVI and RVI were found to have similar predictive ability of ground cover as more complex indices over a spectrally uniform soil background. However, NDVI was preferred over RVI for this study because of its linear relationship with ground cover fraction before canopy closure.

This research also identified a normalized method to compare green and red reflectance of wheat canopies. The normalization minimizes the effects of minor variations in incident radiation between reference and sample measurements and allows the separation of nitrogen stress from the similar spectral effects of ground cover fraction. This method may allow a broader application of current leaf reflectance research to canopy-scale and field-scale nutrient estimates.

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CHAPTER 4

SUMMARY

Reflectance of red and green radiation by plants is heavily influenced by chlorophyll absorption. Spectral reflectance of plant canopies provides an accurate indication of ground cover fraction, which is highly correlated with radiation capture. If nutrients and water do not limit growth, radiation capture is highly correlated with daily growth rate and ultimate yield. However, none of the common vegetation indices are able to separate a more developed, stressed canopy from a less-developed, rapidly growing canopy.

Nitrogen stress is highly correlated with reduced chlorophyll concentration and increased reflectance of green radiation, while water stress inhibits leaf expansion can increases chlorophyll concentration and decrease green reflectance. All but one of the 10 common spectral indices that we tested accurately determined ground cover, but we found that none of them could distinguish the intensity of leaf color from ground cover fraction. Here we report a reflective index that can differentiate nitrogen and water stress over a wide range of ground cover. The index is based on the ratio of the green and red variants of the normalized difference vegetation index ($NDVI_{green}$/$NDVI_{red}$). The normalization minimizes the effects of minor variations in incident radiation between reference and sample measurements and allows the separation of nitrogen stress from the similar spectral effects of ground cover fraction. This method may allow a broader application of current leaf reflectance research to canopy-scale and field-scale nutrient estimates.

The new index was able to distinguish N and water stress from satellite data using wavelengths less than 1000 nm. This index should be broadly applicable over a wide range of plant types and environments. The issues of solar angle and soil background color were also examined to determine their effects on plant reflectance measurements.

APPENDICES

APPENDIX 1: PREDICTING GROUND COVER AND YIELD

ABSTRACT

Crop yield estimates are useful for many aspects of crop production, including marketing, storage planning, and identification of stress. An accurate yield estimate can be a valuable production tool. Two promising methods of yield estimation are radiometric remote sensing and digital imaging. The dominant factors in crop yield are the quantity of incident radiation and the absorptive ability of the crop canopy. Vegetation indices based on the reflective properties of plants have been used for several years to estimate ground cover. These ground cover estimates can be used to calculate yield based on the close relationship between ground cover and a crop canopy's absorptive capacity during the growing season. Photographic images can also be used to determine plant ground cover and measure plant stress for GIS applications. Like spectral indices, photographs determine ground cover to estimate crop radiation absorption. Photographic images are less sensitive to soil color, provided that plants are visually separable from the background. Common vegetation indices (red NDVI, green NDVI, and RVI) and digital image estimates of plant ground cover were tested for their ability to predict crop yield for spring wheat test plots. Digital image estimates of ground cover were also compared with vegetation indices to ascertain which indices correlate most closely with imaged ground cover. All indices showed high correlation with final yield prior to anthesis, with RVI providing a slightly more linear prediction of final yield based on vegetation

cover than the other indices. Digital images and indices showed similar correlation with final yield until awn emergence (day 60), after which vegetation indices became a more accurate predictor of final yield. The vegetation indices that correlated most closely with digital images of ground cover included green and red NDVI, SAVI, RVI, and DVI.

INTRODUCTION

Plant radiation capture limits all other components of yield potential for an unstressed plant (Volk et al., 1995). Radiation absorption by plants is a function of canopy cover, plant architecture, and leaf absorption efficiency. Plant phenology and leaf absorption efficiency remain relatively constant within a species at a given growth stage (Baret et al., 1987), so canopy cover should be an accurate estimator of radiation absorption. Canopy cover can also be used as a predictor of final yield, because the majority of plant growth components are controlled by plant cover. The primary plant stresses decrease yield by inhibiting canopy growth. Water limitations limit plant growth at several levels. Mild water stress has a dramatic effect on leaf expansion rate prior to any effects on leaf photosynthetic rate or translocation rate through the phloem (Taiz and Zeiger, 1998). Nitrogen deficiency decreases crop yield and quality by limiting amino acid and chlorophyll synthesis, resulting in growth inhibition and leaf senescence (Marschner, 1995).

Plant canopy imaging provides a quick, nondestructive method for determining plant growth. Because photographic imaging is relatively simple, it

has been used in many remote sensing applications as a method for determining ground cover, and as a standard upon which to base the success of other imaging types, such as satellite imagery and radiometric applications. Overhead photography is useful as a total ground cover estimator, but also as an indicator of spatial variability in ground cover (Blazquez et al., 1981; Stone et al., 1988). Beverly (1996) suggested that plant vigor estimates provide a more direct and integrative indication of plant response to soil properties and management than does soil testing.

Past experimental methods for determining ground cover include line-transect analysis, meterstick measurements, and photographic grid testing (Hayes and Han, 1993). Ground cover has also been estimated in the laboratory by comparison of photos with photos of known ground cover or by superimposing a grid with a photograph or a projected slide of an area. Each of these methods requires an observer to visually determine whether each specified point in the picture is soil or plant, a process that is time consuming and impractical for most applications. These methods are also subject to human perspective and error. Machine vision methods have been used to differentiate between plants and soil via thresholding (Hayes and Han, 1993; Olthof and King, 2000; Stone et al., 1988), but they can be prone to error as soil and plant colors change (Hayes and Han, 1993).

Recent advances in image manipulation software allow the union of visual discrimination and computer thresholding. This speeds up the separation

process between leaf and background and allows a visual cross-check of accuracy.

Potential Errors in Ground Cover Estimation

One potential ground cover measurement error in near-remote imaging (<5 m) involves vertical plant growth. The apparent size of an object increases as it approaches the camera proportional to the distance formula, d_1^2/d_2^2, where d_1 is the original distance from the camera to the object, and d_2 is the new distance to the object. Plants growing toward a set camera increase in apparent size and cause the overestimation of ground cover. This problem is negligible if plant growth is small in comparison to the distance from the camera to the plant, but can result in large errors if the camera is close to the plants.

Another difficulty that may arise in image analysis is shadowing (Hayes and Han, 1993). Shadowing makes discrimination between leaf and ground difficult. Several methods can be used in small plots to minimize problems from shadowing. Pictures can be taken under low light with a camera flash on to align the camera line of sight with the primary light incident to the plant leaves. Alternatively, pictures can be taken on cloudy days, a barrier may be used to block direct lighting, or the camera can be placed in line with the light source. These methods are most effective if the camera lens remains perpendicular to the ground.

Spectral estimates of ground cover are also influenced by outside factors. One common confounding factor in ground cover estimation using spectral

indices is the influence of soil color (Huete, 1988; Huete et al., 1985). During early stages of growth, the soil constitutes a large portion of canopy reflectance. The primary variable in soil reflectance is brightness, because nearly all spectral data for a soil falls along a line extending from the origin (Kauth and Thomas, 1976). High reflecting, light-colored soils influence indices more than do dark, low-reflecting soils (Jackson et al., 1983). Spectral differences between soils may be attributed to variations in surface moisture, particle size distribution, soil mineralogy, soil structure, surface roughness, crusting and presence of shadow (Huete et al., 1984; Huete et al., 1985).

MATERIALS AND METHODS

Spring wheat (Triticum aestivum cv. Westbred 936) was planted April 8, 2002 at the Greenville Farm research plots in Logan, Utah, at a density of 112 kg per ha^{-1}. Treatment transects consisted of four randomized replicates of each fertilizer treatment listed in Table 6.

Table 6 Summer 2002 treatments

Treatment	Fertilizer
Control	No nitrogen or phosphorus added
Phosphorus	67 kg phosphorus ha^{-1}
Nitrogen	67 kg nitrogen ha^{-1}
Nitrogen/Phosphorus	67 kg nitrogen 28 kg phosphorus ha^{-1}
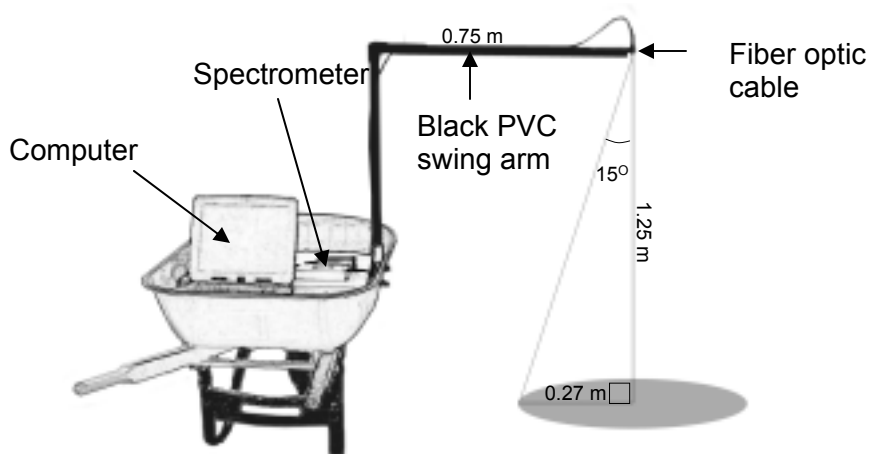
Figure 19. Sensor mounting design for summer 2002 tests. A rigid swing arm positioned the sensor over the canopy away from the wheelbarrow.

The line-source irrigation method was used to introduce varying levels of water stress in the plots. Measurements were performed on 64 sample points – four in each replicate treatment. The sample points included both water-sufficient and water-deficient plots.

On seventeen dates during the growing season, each plot was photographed from above (height = 1.5 m), and the pictures were analyzed for ground cover. Ground cover analysis was performed in Adobe Photoshop 6.0® (Adobe Systems, Inc., San Jose, CA). Soil was selectively erased using the magic eraser tool, the magic wand tool, or the box selection tool. Selection tolerances generally ranged from 10 to 35, depending on the soil homogeneity and the soil/plant contrast. After soil elimination, ground cover was estimated using the histogram function. In addition, growth stage was recorded and based on the Zadoks growth staging parameters (Zadoks et al., 1974).

Reflectance measurements were made using a StellarNet EPP2000 visible/NIR spectrometer with fiber optic cable (StellarNet, Inc., Tampa, FL). The spectrometer was mounted in a wheelbarrow, and the cable was attached to the end of a rigid metal support arm that was 1.25 m high and extended 0.75 m over the plots (Figure 19). A reference standard made of pressed polytetrafluoroethylene (PTFE) was used to measure incident radiation, and reflectance ratio was calculated as the ratio of reflected to incident radiation. Reflectance data was smoothed using a five nanometer Savitzky-Golay filter at the time of collection (Savitzky and Golay, 1964). Spectral indices were calculated in Microsoft Excel® (Microsoft Corp., Redmond, WA), and broadband variants of narrowband indices were calculated from integrated reflectance over the broadband intervals. The broadband intervals were based on Landsat band intervals (Kauth and Thomas, 1976).

At harvest, 1 meter square plots were harvested at each sample point. The wheat was dried and threshed, and the seed mass for each plot was recorded. Protein analysis was performed on a sub sample of each plot sample. Final plot yield was compared to the in-season NDVI and ground cover measurements.

Yield and Ground Cover

Final yield was compared with spectral indices and ground cover estimates of each measurement date by determining the linear correlation (r^2) of

final yield with NDVI. The slope and intercept of the correlation line were also compared by date and growth stage.

In addition, yield was estimated from the integrated NDVI of each plot over the growing season. A light bar was used to measure canopy reflectance and transmittance of photosynthetically active radiation (PAR) for wheat plots on four days, and a spectrometer was used to calculate NDVI. The absorbance of PAR was calculated by the following formula:

$$PAR_{abs} = PAR_{inc} - PAR_{ref} - PAR_{trans} + PAR_{ref-ground} \quad [4]$$

PAR absorbance was correlated with NDVI, and the slope of the correlation line was used to estimate PAR absorbance at all NDVI values throughout the growing season. Integrated PAR absorbance was calculated by integrating the values at the midpoint between each measurement over the course of the growing season.

RESULTS AND DISCUSSION

2001

The linear correlation of NDVI with final yield increased with time until it reached a maximum of 0.70 on June 20 (Zadoks 71), after which the correlation decreased (Table 7). The slope of correlation between yield and NDVI increased until June 14 (Zadoks 62), then decreased until the end of the study. Intercepts for this study were negative until late in the study.

Table 7 Comparison of final yield with in-season NDVI values during 2001.

Date (2001)	Stage (Zadoks)	Linear r^2 (Yield vs. NDVI)	Slope	Intercept
May 29	30	0.35	390	-34
June 11	50	0.67	341	-52
June 14	62	0.67	435	-165
June 15	65	0.61	373	-112
June 20	71	0.70	366	-69
June 30	83	0.62	247	1
July 3	87	0.41	230	33

The negative y intercept suggests that plants underwent severe stress near the end of their growth, resulting in low yield compared to mid-range midseason NDVI values. Two potential causes of stress during this time period were the ever depleting water and nitrogen resources, and a hard frost that occurred at anthesis, resulting in decreased yield due to infertile heads (white heads). The increasing intercept and decreasing slope of the yield vs. NDVI correlation line after June 15 were caused primarily by a decrease in NDVI of the stressed plots, suggesting that the main factor in the slope change was the increased nitrogen and water deficiency at the end of the growing season.

2002

The 2001 yield vs. NDVI plots exhibited a higher slope than those for the 2002 study (Tables Table 7 and Table 8). The intercept was positive for each date, and the intercepts ranged from 10% to 20% of the slope for each plot. NDVI was higher compared to yield for water-stressed plots compared to nitrogen-deficient and control plots until late in the season, when ground cover

decreased due to water stress. This feature decreased the slope of the correlation graphs, because water-stressed plots appeared had high NDVI and low final yield. The correlation values of unstressed plots were highest on and after June 13 (Zadoks 60). Vegetation indices and digital image ground cover estimates showed similar correlation with final yield until approximately anthesis. After anthesis, the spectrometer vegetation indices correlated more closely with final yield than did digital images (Figure 23).
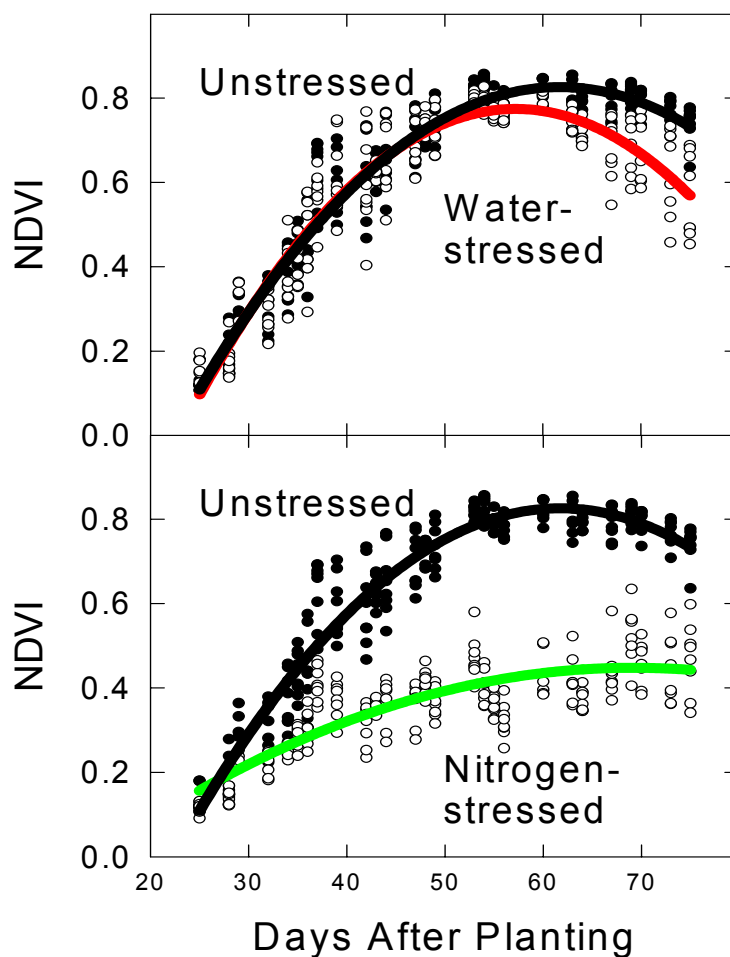

Figure 20. Comparison of NDVI during 2002 growing season for unstressed, nitrogen-stressed, and water-stressed plots.
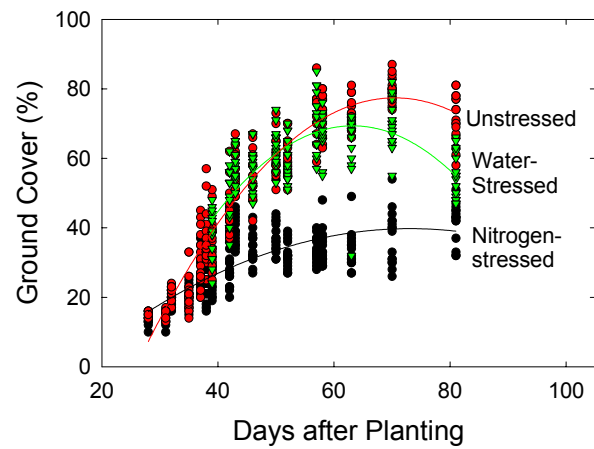
Figure 21. Comparison of ground cover for unstressed, water-stressed, and nitrogen-stressed plots.
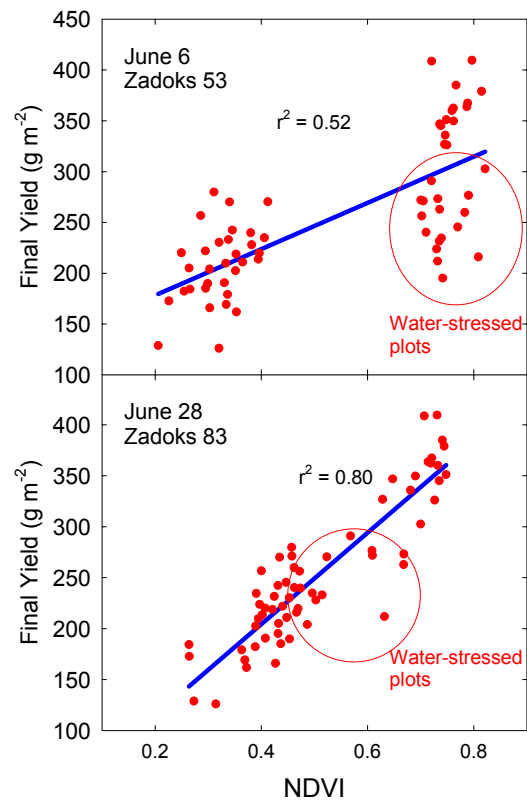
Figure 22. Comparison of NDVI during the 2002 growing season with final yield.

Table 8 Relationship between NDVI and final yield during 2002 by date.

Date (2002)	Days after planting	Stage (Zadoks)	Linear r^2 (Yield vs. NDVI)	Slope	Intercept
6 May	28	13	0.03		
9 May	31	21	0.05		
13 May	35	23	0.10		
15 May	37	30	0.21		
16 May	38	30	0.26		
17 May	39	31	0.15		
18 May	40	31	0.25		
20 May	42	32	0.13		
21 May	43	32	0.31		
23 May	45	32	0.22		
24 May	46	33	0.36		
25 May	47	33	0.30		
28 May	50	41	0.39	267	116
29 May	51	41	0.42	284	91
30 May	52	45	0.42	261	115
3 June	54	49	0.56	274	86
4 June	56	50	0.53	246	107
5 June	57	52	0.53	241	119
6 June	58	53	0.52	227	133
13 June	66	60	0.62	289	86
14 June	67	61	0.62	284	98
17 June	70	69	0.72	336	65
19 June	72	71	0.77	379	26
20 June	73	73	0.74	362	40
23 June	76	75	0.80	390	41
25 June	78	80	0.80	406	39
28 June	81	83	0.80	448	25
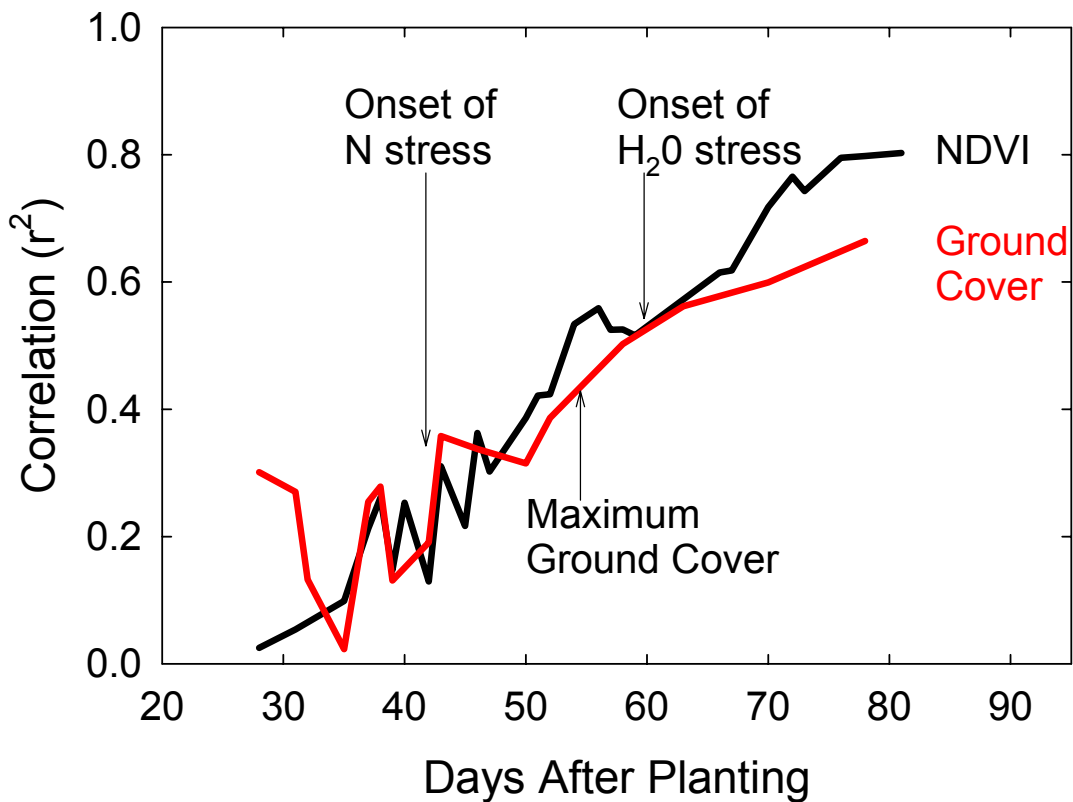
Figure 23. Correlation of NDVI and ground cover with final seed yield (summer 2002).

INTEGRATED ABSORPTION AND CROP YIELD

The integrated absorption of radiation by each plot was estimated by NDVI values. Previous studies of wheat with satellite imagery have compared NDVI with final yield, but no relationship between NDVI and absorption has been discussed. Discussion: note that estimated yield was higher than predicted yield for most water stressed plots. Compare this with Boissard's paper on ear hydric status after anthesis. Because ear hydric status after anthesis can affect yield

dramatically, this probably explains why the estimated yield was higher than the

actual yield for these plots. The following references relate to NDVI and yield

estimates… (Benedetti and Rossini, 1993; Boissard et al., 1993; Labus et al.,

2002; Manjunath et al., 2002)

Figure 24. Correlation of NDVI with radiation absorption estimates based on light
bar measurements. At high NDVI values (above 0.8), radiation absorption slope
increased.

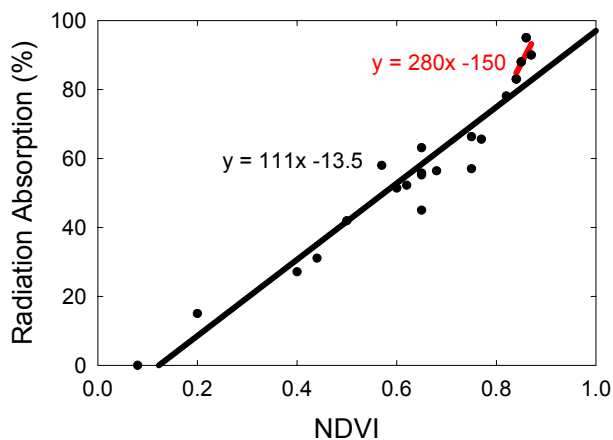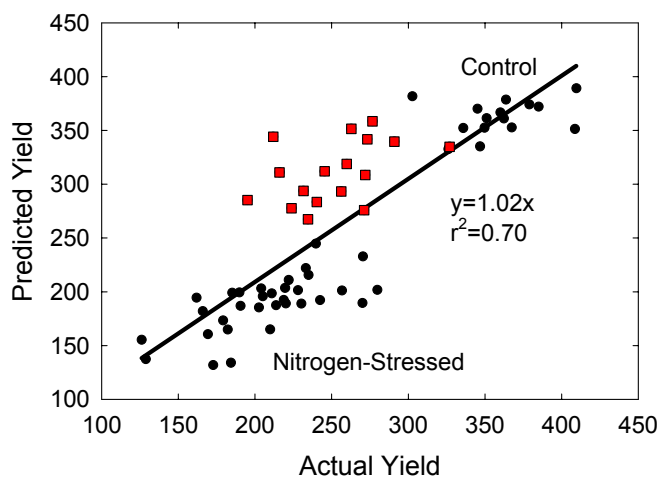Figure 25. Comparison of actual and predicted yield based on radiation
absorption model.

The high correlation values for unstressed plots at anthesis suggest that predictions of plant yield can be highly accurate at anthesis if plant stress does not occur afterwards. Although NDVI became a less accurate predictor of yield after anthesis in 2001 (Table 7), much of this may have been due to early leaf senescence due to the long period of plant stress observed in this experiment. Correlation did not show a decrease during the 2002 test with time, suggesting that the onset of stress can be identified well into dough development.

The peak correlation of ground cover with final yield at anthesis agrees with Dusek et al. (1985), who state that ground cover has a peak correlation with plant biomass near anthesis. The superior correlation of NDVI with final yield after anthesis may be due to the ability of this vegetation index to estimate only green, actively photosynthetic tissue. The digital images do not discriminate yellow leaves from green leaves, resulting in higher variability due to early senescence of leaves on stressed plants.

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APPENDIX 2: LEAF REFLECTANCE, TRANSMITTANCE

AND CHLOROPHYLL CONCENTRATION

ABSTRACT

Chlorophyll concentration is a useful indicator of plant health and nitrogen concentration. Transmittance and reflectance of visible and NIR radiation are commonly used to estimate chlorophyll concentration of plant leaves. Chlorophyll concentration is commonly estimated using differential transmittance of multiple visible and NIR wavelengths. However, more research during the past few years has focused on reflectance as an indicator of chlorophyll concentration. This study compared the accuracy of transmittance and reflectance measurements in determining leaf chlorophyll concentration of spring wheat (Triticum aestivum cv. Westbred 936). Normalized reflectance and transmittance data showed a similar linear correlation with leaf chlorophyll concentration (highest r^2=0.90 and 0.91, respectively). However, reflectance data required normalization to a wavelength to attain this high correlation, while transmittance data did not.

INTRODUCTION

Chlorophyll properties dominate leaf reflectance and transmittance of visible radiation. Nitrogen is a principle component of chlorophyll (Taiz and Zeiger, 1998), and chlorophyll concentration often correlates closely with nitrogen concentration in plant leaves (Costa et al., 2001; Fernández et al., 1994; Filella et

al., 1995; Serrano et al., 2000). Chlorophyll absorbs the majority of incident red and blue radiation, resulting in little red or blue reflectance by green vegetation. The blue absorbance peak of chlorophyll overlaps with the absorbance of carotenoids, so blue reflectance is not generally used to estimate chlorophyll concentration (Sims and Gamon, 2002). Maximum red absorbance occurs between 660 and 680 nm (Curran, 1989), but relatively low chlorophyll concentrations can saturate this absorption region (Sims and Gamon, 2002). Therefore, chlorophyll concentration is usually predicted from reflectance in the 550 nm or 700 nm ranges, because these regions require higher chlorophyll concentrations to saturate. Leaf chlorophyll concentration has also been well-correlated with reflectance in the green and red spectral regions. Leaf reflectance factors in the 550 nm and 660 nm range show high correlation with leaf nitrogen concentration (Fernández et al., 1994) and chlorophyll concentration (Adams et al., 1999). The shape of the visible reflectance spectra of leaves changes between the maximum reflectance near 550 nm and the minimum near 660 nm as they become chlorophyll-deficient, and changes in this shape can also be used to identify chlorosis in some instances (Adams et al., 1999; Carter and Spiering, 2002).

Leaf mesophyll reflects a large proportion of NIR radiation (Huete et al., 1984; Taiz and Zeiger, 1998). The region of rapid increase in reflectance between the red and infrared regions of the spectrum, called the red edge, is frequently used to indicate plant health (e.g. Dawson and Curran, 1998; Horler et al., 1983a; Horler et al., 1983b; Jago et al., 1999). Horler et al. (1983b) observed

that chlorophyll concentration in leaves correlated with the maximum slope of reflectance at the boundary between the red and NIR spectral domains. Spectral indices have been derived for both single-leaf and plant canopy reflectance measurements. Single-leaf measurements offer the advantage of higher signal-to-noise ratio and more control over the operating environment, while canopy measurements allow measurements over a broader scale.

MATERIALS AND METHODS

Wheat plants were grown at seven nitrogen treatments to allow a wide range of leaf chlorophyll concentration. At heading, the reflectance of the top two or three leaves for the main stem of each plant was measured using an ASD FieldSpec Pro spectrometer with high-intensity contact probe (ASD, Boulder, CO). Transmittance of the leaves was also measured using a StellarNet EPP2000 VIS/NIR spectrometer (StellarNet, Inc. Tampa, FL) with a custom-built transmittance probe, and a SPAD-502 chlorophyll meter (Minolta, Ramsey, NJ). After measurements, 15-mm wide circles were cut from each measured leaf. Chlorophyll was extracted from each leaf disk in 7 mL DMSO and an incubation period of 30 minutes (Hiscox and Israelstam, 1979). The extractions were stored at 4° C for 2 days, then analyzed for chlorophyll concentration using the method described by Porra et al. (1989).

RESULTS

Leaf transmittance and reflectance of green and far-red radiation showed a high linear correlation (highest r^2=0.91) with tissue chlorophyll concentrations. Normalized and raw transmittance data showed similar correlation to chlorophyll concentration by wavelength, with the maximum correlation in the green and red-edge regions of the transmittance spectrum (Figure 26).

Raw reflectance data showed significantly lower correlation and different peak correlation wavelengths than normalized data. One likely reason for this deviation is that leaves did not fill the entire viewing area of the ASD reflectance probe. Normalization should be performed prior to wavelength analysis to increase the accuracy of reflectance in estimating leaf chlorophyll concentration when using this setup. Chlorophyll concentration correlated well with normalized leaf transmittance and reflectance (highest r^2 = 0.91). Both reflectance and transmittance showed a decreased correlation value in the red, with correlation minima occurring near 675 nm. The correlation (r^2) minimum for leaf reflectance was 0.08, and the correlation minimum for leaf transmittance was 0.67 (Figure 26).
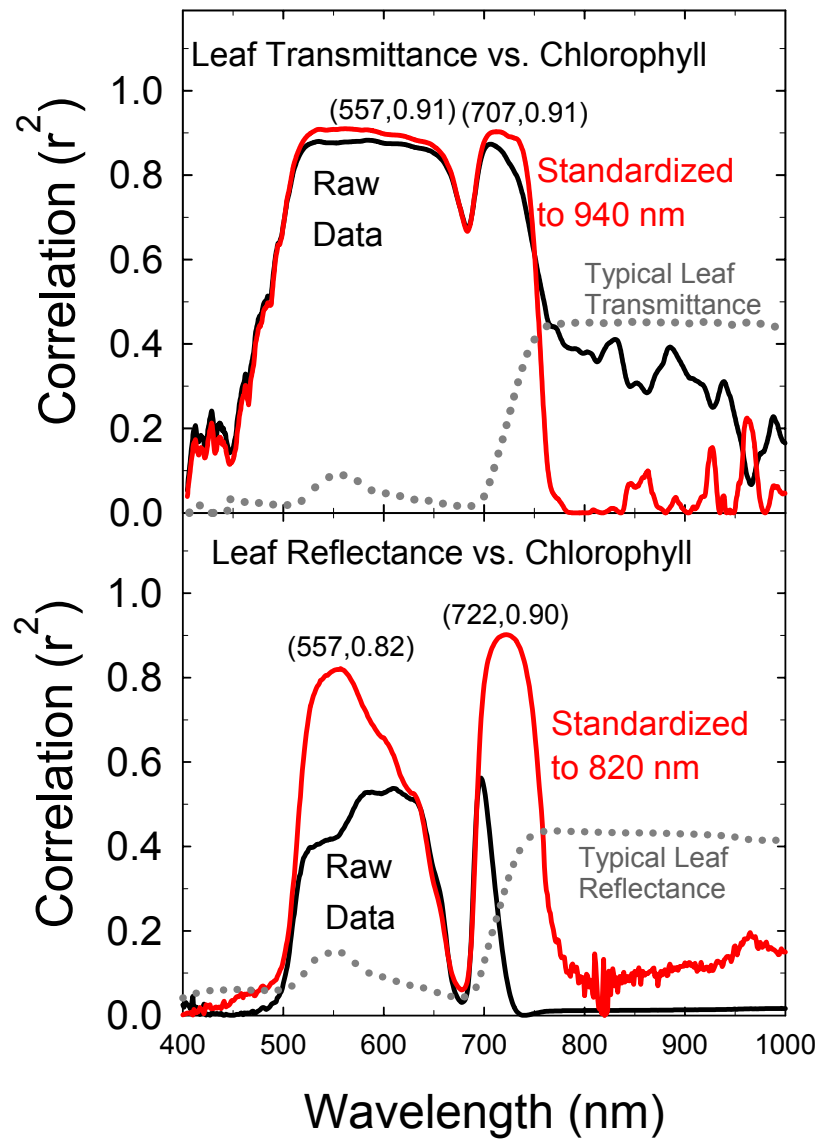
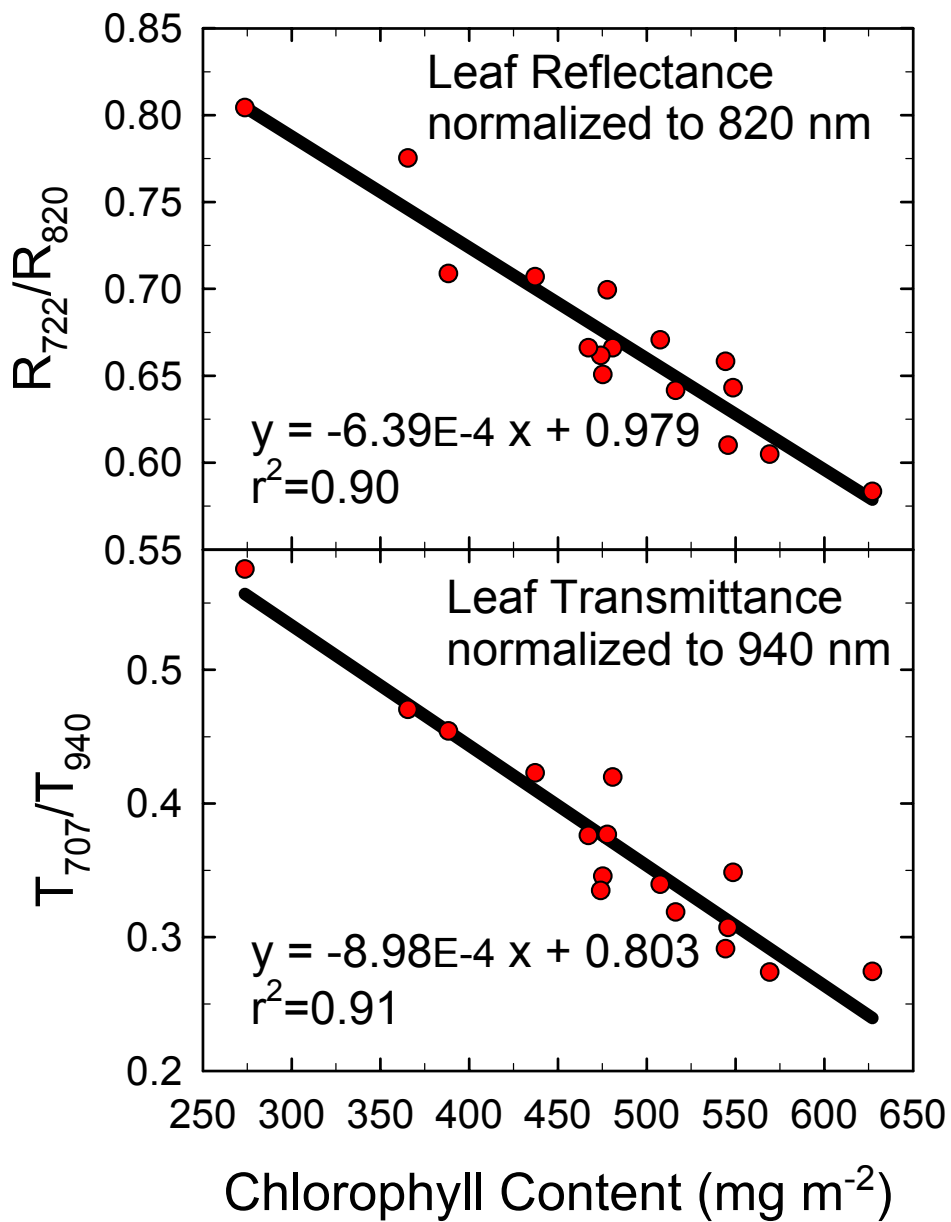Figure 26. Comparison of leaf reflectance and transmittance with chlorophyll concentration.

Figure 27. Correlation of normalized leaf reflectance and transmittance with leaf chlorophyll concentration.

DISCUSSION

Chlorophyll concentration correlated well with normalized leaf transmittance and reflectance (highest r^2 = 0.91). Both reflectance and transmittance showed a decreased correlation value in the red, with correlation minima occurring near 675 nm. This agrees with the work of Carter and Spiering (2002), who noted the trend but did not account for their findings. Sims and Gamon (2002) offer an explanation for this phenomenon, stating that relatively low chlorophyll concentrations are sufficient to saturate absorption in the 660-680 nm range, which reduces the sensitivity of these wavelengths for determining high chlorophyll concentrations. The data from this experiment supported this idea, because deviations occurred at the higher chlorophyll concentrations.

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APPENDIX 3: BACKGROUND EFFECTS

ON SPECTRAL INDICES

ABSTRACT

Vegetation indices that measure plant growth are based on deviations from the reflectance of a bare soil to a plant-covered soil. Spectral indices such as the normalized difference vegetation index (NDVI) correlate well with ground cover and leaf area index (LAI) for a particular soil, but can be widely different for the same crop with a different soil color. Previous research has attempted to minimize this effect. Indices used to correct for the soil background include perpendicular vegetation indices (PVI), the soil-adjusted vegetation index (SAVI), and derivative vegetation indices. These indices treat the soil as a reflective medium that has a nearly linear slope in the visible and NIR spectral regions. However, many plants are grown on backgrounds that do not match the typical soil line. Examples include greenhouse plants and row crops that have plastic placed under them. The objective of this study was to evaluate vegetation indices with standard and nonstandard reflective backgrounds to determine which index best determines leaf area index over nonstandard backgrounds. Backgrounds included white, red, gray, soil-covered, and black. It was determined that the best indicators of LAI over a wide variety of backgrounds were the difference vegetation index and SAVI with custom coefficients. An alternative method of mathematically subtracting the background from the

plant/background spectrum was examined, and this method increased the correlation between LAI and spectral indices.

INTRODUCTION

A significant issue in whole-canopy reflectance experiments is the variation between green plant cover and the soil background. High reflecting, light-colored soils influence indices more than do dark, low-reflecting soils (Jackson et al., 1983). Spectral differences between soils may be attributed to variations in surface moisture, particle size distribution, soil mineralogy, soil structure, surface roughness, crusting and presence of shadow (Huete et al., 1984; Huete et al., 1985). Many reflectance indices are sensitive to ground cover because of the changes in red and infrared reflectance with increased green ground cover. Jackson et al. (1983) stated that the change in soil reflectance ratios changes little due to wetting, following the fact that a change in soil reflectance due to water concentration is about the same in the visible and near-IR regions of the spectrum. They also stated that since the opposite is true for vegetation, the ratio of red to NIR reflectance is theoretically a good discriminator of vegetation. Jackson et al. (1983) concluded that the ratio is not a good discriminator for green vegetation covers less than 50%, but becomes a very sensitive indicator as the ground cover increases.

During early stages of growth, the soil constitutes a large portion of canopy reflectance. An early attempt to correct for the soil line was introduced by Kauth and Thomas (1976), and is referred to as the perpendicular vegetation

index (PVI). The PVI estimates soil brightness by supplying a soil slope (a) and an offset (b) derived from the NIR vs. red soil baseline. The soil-adjusted vegetation index (SAVI) simplifies the soil relationship to canopy reflectance by adding a simple brightness factor (L), which is typically set to 0.5, but can range from 0 to 1 (Elvidge and Chen, 1995; Huete, 1988).

Another technique used to minimize soil background effects on canopy spectral signatures is the use of high-resolution derivative spectra (Demetriades-Shah et al., 1990; Elvidge and Chen, 1995; Hall et al., 1990; Peñuelas et al., 1994). Spectral derivatives can be used for both single-leaf and whole-plant spectral analysis. Peñuelas et al. (1994) identified derivative spectral differences in reflectance between healthy, water-stressed, and nitrogen-stressed sunflower leaves. Demetriades-Shah et al. (1990) demonstrated the use of derivative spectra to suppress low-frequency background noise, resulting in derivative spectral indices that were superior to conventional broad-band spectral indices for their studies.

MATERIALS AND METHODS

Background effects were analyzed for their influence on plant reflectance. Seven single Westbred 936 spring wheat seeds were germinated on blotter paper. After emergence, the seedling were placed in open-cell foam plugs and transplanted into 2-liter hydroponic bottles at a rate of one seedling per bottle. A plant nitrogen level of 3.5% was used as the control and nitrogen need was based on Equation 4.

$$gH_2O \times \frac{1g\ dry\ matter}{400gH_2O} \times \frac{0.035\ g\ N_{100\%}}{g\ dry\ matter} \times \frac{1\ mol\ N}{14\ g\ N} \times \frac{treatment}{100\%} = mol\ N\ needed$$

[5]

$$\frac{6 * 10^{-4}\ mol\ N}{L} \times L\ nutrient\ solution\ added = mol\ N\ already\ added$$

[6]

The quantity of nitric acid to add each day was then calculated as follows:

$$\frac{(mol\ N\ needed - mol\ N\ added)}{1.0 * 10^{-3}\ mol\ N\ mL\ acid^{-1}} = mL\ nitric\ acid\ needed\ in\ sol'n$$

[7]

The nitrogen treatments for this experiment were 140% of control, 100 % control, 80%, 60%, 40%, 20%, and scant nitrogen based on formulas. Treatments commenced at Zadoks stage 13. A hydroponic solution mix with scant nitrogen (0.1 mM $Ca(NO_3)_2$ and 0.4 mM KNO_3) and ample other nutrients was used to water the tubs daily. The amount of water added to each tub was measured. In addition, nitrogen was added in the form of 1M nitric acid to each treatment tub separately based on desired plant nitrogen level. A water use requirement (g water required per g of plant dry mass increase) of 400 g g^{-1} was assumed. A plant nitrogen level of 3.5% was used as the control and nitrogen need was based upon equations 5, 6, and 7.

These equations were entered into a spreadsheet, and nitrogen addition each day was based upon water use and treatment level. Treatment levels included 100% N control, 140%, 80%, 60%, 40%, 20%, and scant nitrogen.

Each plant was held in place using a stand to prevent positional variability while pictures and spectral readings were taken. Five colored cardboard disks (diameter = 56 cm) were designed as backgrounds to slide under each wheat

plant for spectral measurements. One disk was covered with soil, and the other four disks was spray-painted white, red, black, or gray. The plants were mounted one at a time in a stationary holder, upon which each disk was placed under the plant in turn to simulate changes in soil background. The spectrometer fiber optic cable was mounted 70 cm above the center of the disk for an estimated 24 cm wide circular field-of-view. At heading, plant ground cover was measured using a digital image of each plant. Leaf chlorophyll concentration was measured on the upper two to three leaves for each plant using a SPAD-502 chlorophyll meter, an ASD reflectance probe, and a custom-built leaf transmittance probe that is compatible with the StellarNet spectrometer system. Each plant was then harvested one tiller at a time, and spectral readings were performed at each stage of harvest with the five background disks. Leaf area was measured for each harvested portion of each plant using a LICOR LI-3100 leaf area meter (LICOR, Inc. Lincoln, NE), and LAI was calculated as the leaf area divided by 452 cm^2, the area of a circle with a 24 cm diameter.

Leaf area was estimated using broadband and narrowband versions of five indices: red NDVI, green NDVI, SAVI, green DVI, and red DVI. The SAVI tests included both a standard L of 0.5 and a best-fit L for each background.

Mathematical Subtraction Method

The spectral properties of each background were systematically subtracted from the reflectance spectrum of each plant/background combination. The property of this correction was the idea that the reflectance of a

plant/background combination is the sum of the plant component and the visible

background component, and that the background component can be estimated

separately from the plant component. This idea is visible in a comparison of

background reflectance with plant/background reflectance (Figure 28). This

required an initial reflectance measurement of each background, which was

performed with an ASD FieldSpec Pro JR spectroradiometer and a high intensity

contact probe. A reference wavelength of 450 nm was chosen as a

standardization wavelength. This wavelength was chosen because leaves reflect

only a small fraction of radiation in this region, and this region of the spectrum is

not generally used to estimate chlorophyll concentration (Sims and Gamon,

2002). Wavelengths below 450 nm have less available signal, and it was

observed that the reference wavelength is more effective if it is close to the

wavelengths used for spectral indices. The subtraction formula is described in

Equation 8.

$$R_{corrected} = R_{initial} - \frac{R_{initial\,@\,450nm} - 0.02}{R_{bckgrnd\,@\,450nm}} \times R_{bckgrnd} \qquad [8]$$

In this equation, $R_{corrected}$ is the corrected reflectance at a given

wavelength, $R_{initial}$ is the uncorrected reflectance, and $R_{bckgrnd}$ is the background

reflectance. The subtraction of 0.02 is meant to account for the reflectance of

plant tissue at 450 nm and is based on the observed reflectance of a single leaf

in this region. This value can be adjusted up or down, but by its nature will tend

to undercorrect for the background at high values and to overcorrect near zero.

RESULTS

Spectral Indices and Leaf Area Index

Soil background had a pronounced effect on spectral data (Figure 28). The most useful indicators of leaf area index with the colored backgrounds was the difference in reflectance between the NIR and red spectral domains, and in soil-adjusted vegetation index (SAVI). The first derivative indices corrected for background color less than the SAVI.

Mathematical elimination of the soil background worked best with backgrounds that did not show a heavy edge effect (Figure 29). This correction also tended to under-correct for the soil effects, although a combination of mathematical background elimination and SAVI showed the highest correlation with leaf area index.

Table 9 Comparison of narrowband and broadband indices with leaf area index (LAI)

| Index compared with LAI	Correlation (r^2) Uncorrected	Correlation (r^2) Corrected	Correlation (r^2) Black Background
NDVI broadband	0.237	0.539	0.802
NDVI narrowband	0.234	0.531	0.801
GNDVI broadband	0.040	0.212	0.770
GNDVI narrowband	0.027	0.100	0.758
SAVI broadband L=0.50	0.266	0.762	0.805
SAVI narrowband L=0.50	0.289	0.767	0.802
Green Derivative (DVI)$_{500:550nm}$	0.210	0.493	0.755
Red Derivative (DVI)$_{670:770 nm}$	0.635	0.732	0.800
SAVI broadband custom L	0.576	0.799	0.817
SAVI narrowband custom L	0.549	0.767	0.822
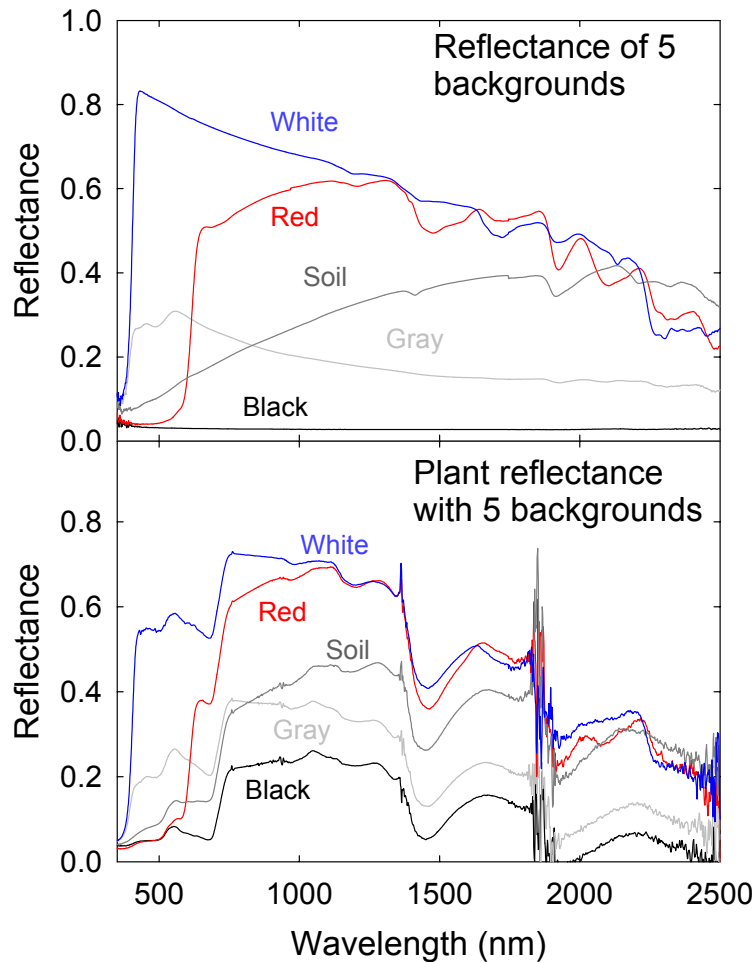
Figure 28. Effects of five backgrounds on plant reflectance.

Mathematical Background Subtraction

Mathematical background subtraction had a pronounced effect on plant/background spectra. Most corrected spectra were similar, regardless of background. The exception was the corrected reflectance from the red background (Figure 29). The red background was difficult to correct for, because of its low reflectance at 450 nm and its pronounced reflectance edge (Figure 28).

The mathematical subtraction method also tended to undercorrect for the brightness component in some cases, resulting in higher overall corrected reflectance values for plants with brightly colored backgrounds than for dark backgrounds.

Therefore, use of SAVI after correction resulted in further improvement of the correlation between spectral data and LAI (

Table 9). Correlation of all indices was improved with use of the mathematical subtraction, although improvement of SAVI was most notable. Use of a constant L value showed almost the same correlation after mathematical correction as use of a custom L value.
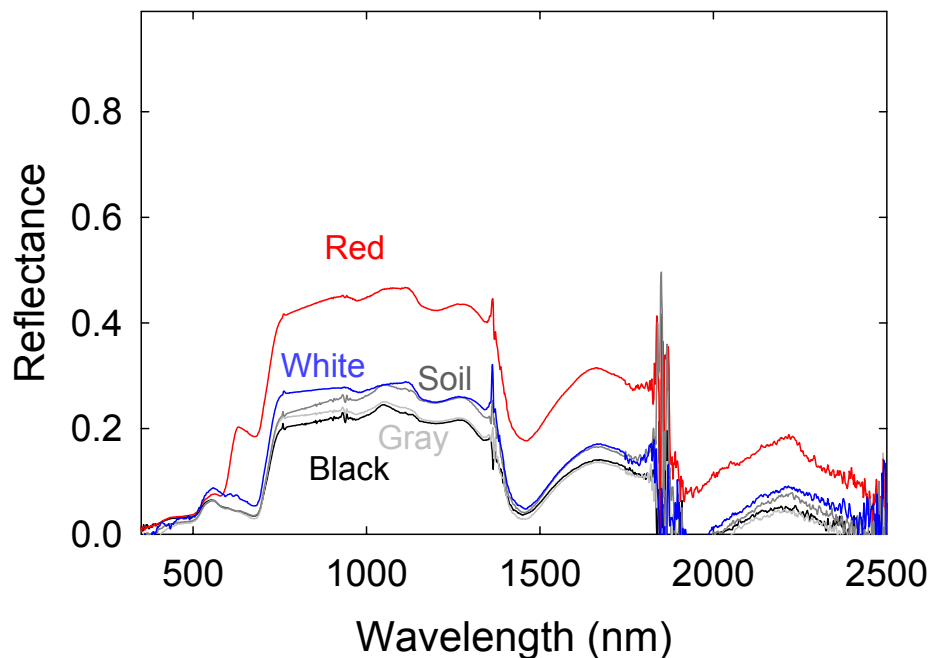

Figure 29 Corrected spectra resulting from mathematical background subtraction.

DISCUSSION

**Vegetation Indices and Background
Effects**

In the absence of a soil-adjustment correction, the difference vegetation index provides the highest correlation with LAI (r^2 = 0.635) of any of the tested indices. The primary reason for this high correlation is that difference vegetation measurements are unaffected by the background offset. Therefore, the index is affected only by the plant reflectance and the slope of reflectance of the background between the wavelengths of interest. Backgrounds with gradual slopes show only minor DVI variations based on background. However, DVI can be sensitive to changes in solar angle and slight experimental error, so it should be used with caution. DVI tends to be less accurate as an estimator of ground cover during a growing season than are ratio indices in a normal field setting (Table 4).

The other index with relatively high correlation was SAVI before mathematic correction, although SAVI indices with best-fit L values showed significantly higher correlation with LAI than did SAVI indices with the common L value of 0.5 (0.576 and 0.549 vs. 0.266 and 0.289 for broadband and narrowband SAVI, respectively (

Table 9).

Mathematic Correction

Mathematical correction of background can decrease background effects in spectral measurements. However, mathematical correction alone is often insufficient to completely correct for the background. The most common cases where this occurs involve a background with a reflectance shoulder. Of particular concern are backgrounds with a region of high reflectance slope at a wavelength higher than the reference wavelength. Correction of these backgrounds is difficult and often erratic.

Correcting for a unique soil background is also more accurate if similar wavelengths are used. The green NDVI performed well below the red NDVI and other red indices for both uncorrected and corrected data.

The challenge of correcting for a soil background by subtracting the background is that this requires a predetermined background reflectance. The post processing also makes this method less user-friendly than most vegetation indices. However, in cases where the background does not match standard soil reflectance parameters, correction allows a more valid estimate of plant parameters than uncorrected data.

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APPENDIX 4: SOLAR ANGLE AND VEGETATION INDICES

ABSTRACT

Plants and soil exhibit non-lambertian spectral characteristics and specular reflectance properties. Spectral measurements are often performed near solar noon to minimize the influence of solar angle on spectral indices. However, plant stress indicators are not necessarily the most identifiable near the middle of the day. For instance, excessive light changes short-term plant photosynthetic activity, and water stress is most readily identifiable in the afternoon as soil water deficit increases. Therefore, identification of plant reflectance changes based on solar angle may be useful to determine stress at angles other than near solar noon. This research was performed to quantify the effects of solar angle on reflectance of a wheat canopy. Solar angle was found to have a pronounced effect on plant canopy reflectance, and common vegetation indices that correct for soil brightness were unable to correct for this effect.

INTRODUCTION

Reflectance measurements are affected by both the angle of the sun and the angle of the sensor from zenith (Figure 30), where zenith is a point normal to the earth. Vegetated terrain exhibits strong forward and backscattering, which varies by plant cover and type (Otterman et al., 1995). Satellite measurements are predominantly conducted from the zenith or near the zenith, so many

measurements of reflectance at the ground level are made near the zenith to

allow estimates of satellite data and decrease the sensor and solar angle effects.

The effects of sensor angle on reflectance have been recognized for many

years. For instance, Woolley reported changes in leaf reflectance between 400

and 2500 nm based on sensor angle in 1971. Woolley demonstrated that

absolute reflectance increases as sensor angle changes from nadir. Pinter et al.

(1987) and Rahman et al. (1999) observed that spectral band ratios are

significantly affected by off-nadir viewing, with maximum index values coming

when the sensor angle and the solar angle corresponded closely with each other.

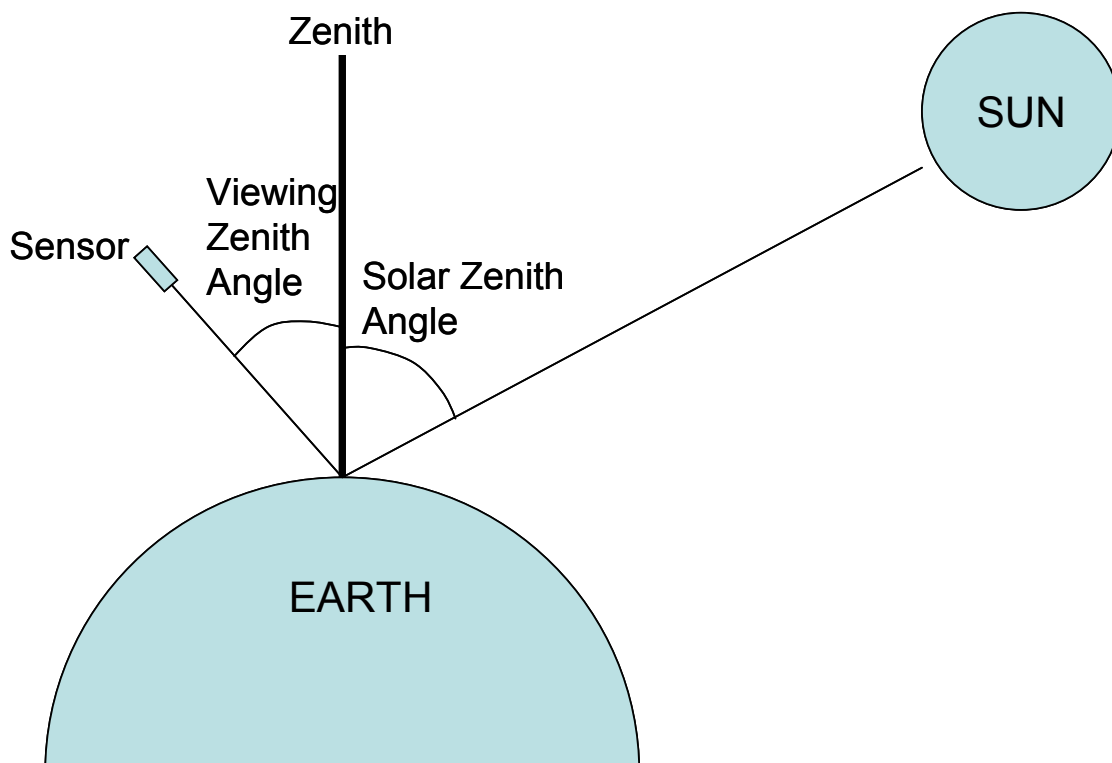This angular phenomenon has been termed the hot spot.

Figure 30. Solar zenith angle and viewing zenith angle in comparison to zenith.

Spectral studies are often performed near solar noon (the time of day that the sun is closest to the zenith) to decrease the effects of solar angle on canopy reflectance (Osborne et al., 2002; Otterman et al., 1995; Serrano et al., 2000). However, the angle of the sun at solar noon is dependent on the day of the year. Diurnal reflectance measurements of wheat canopies over the visible and NIR regions of the spectrum suggest that visible reflectance remains roughly constant throughout the day and infrared reflectance increases as angle from solar azimuth increases. Asrar et al. (1985) observed that increased solar zenith angle generally increased LAI estimates that used red and infrared spectral indices and attributed these changes to increased infrared reflectance. Pinter et al. (1987) reported that maxima in the NIR/red ratio were attained mid-morning and mid-afternoon, and minima coincided with the high solar position near midday. Rahman et al. (1999) also observed that reflectance amplitude varied with sensor angle in relation to solar angle.

Solar position may also influence plant reflectance by influencing the quantity and quality of light that are incident to the plant. For example, Gamon et al. (1992) suggested that the xanthophyll chemical changes due to changes in light intensity are partly responsible for changes in absorption efficiency and changes in leaf reflectance between morning and afternoon. Antheraxanthin undergoes deepoxidation to zeaxanthin under excessive photosynthetically active radiation (PAR) and undergoes epoxidation under limiting PAR. The concentrations of these component pigments are identifiable by reflectance between 500 nm and 550 nm.

Another physiological indicator of plant health is water concentration. Water stress is usually identified best late in the afternoon, because high rates of evapotranspiration increase leaf water deficit (Kramer, 1969). Therefore, measurements at a non-optimal solar angle may provide useful estimates of plant health.

MATERIALS AND METHODS

Spectral indices were tested for sensitivity to solar angle. Reflectance measurements were performed on sixty-four wheat (Triticum aestivum cv. Westbred 936) plots at 6:30 p.m. on May 29, 2002, and at 8:30 a.m., 10:15 a.m., 12:15 p.m., and 3:30 p.m. on May 30, 2002 using a StellarNet EPP2000 spectrometer mounted in a wheelbarrow. All plots consisted of wheat at boot stage. Atmospheric conditions were clear and cloudless for all measurements. Solar angle was calculated from the following equation:

$$\sin(\beta) = \sin(\phi) \times \sin(D) - \cos(\phi) \times \cos(D) \times \cos(15 \times t_{UTC} - \psi) \, [9]$$

In this equation, β is the solar angle, ϕ is the latitude, D is the solar declination, t_{UTC} is the universal time correction, and ψ is the longitude. Latitude was estimated as 41.78° N, longitude was estimated as 111.85°, t_{UTC} was calculated as local daylight savings time plus 6 hours, and D was calculated as 21.83. Spectral indices were compared with 12:00 noon values.
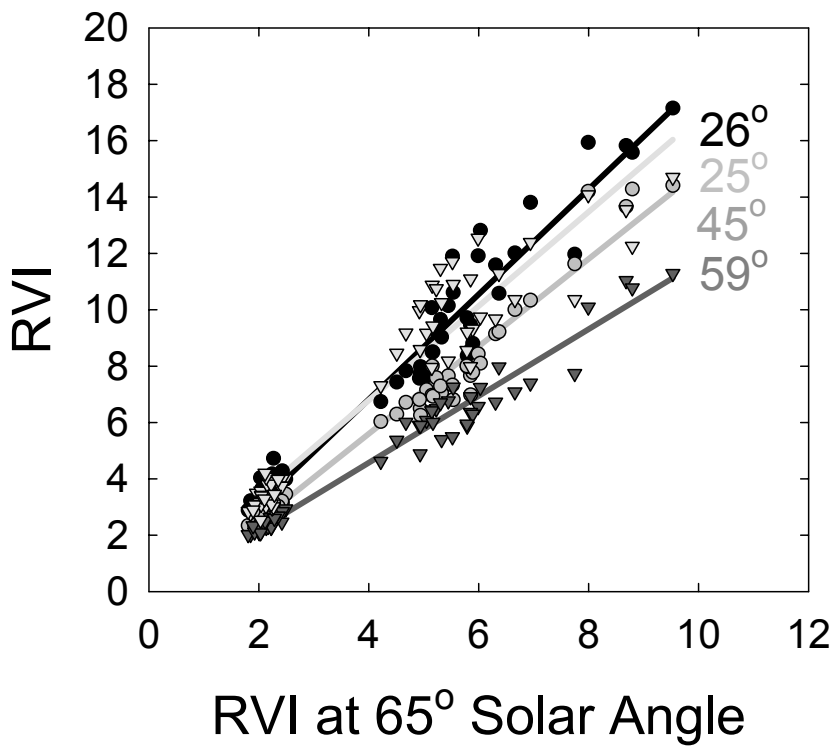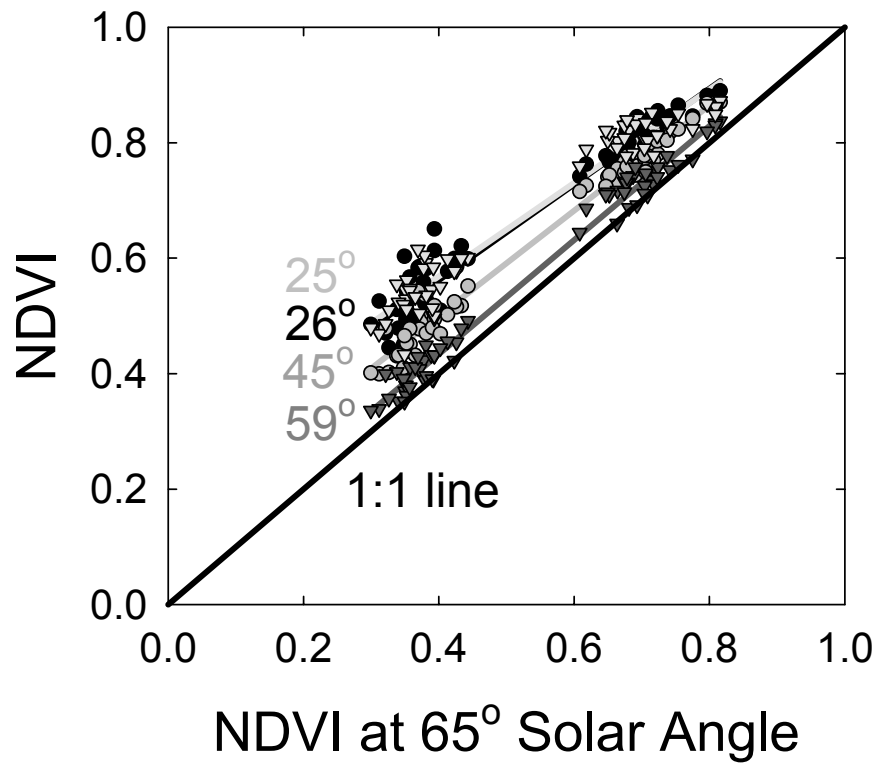
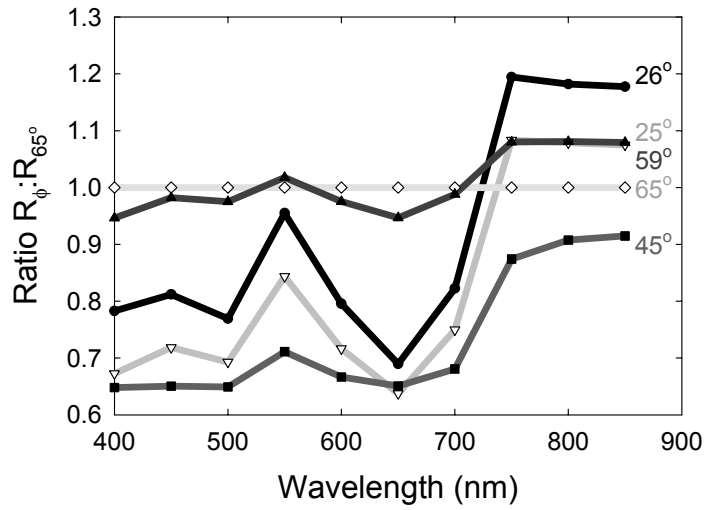Figure 31. NDVI and RVI by solar angle.

Figure 32. Ratio of reflectance at lower solar angle to reflectance at 65° solar angle by wavelength. Points represent means of 16 plot readings.
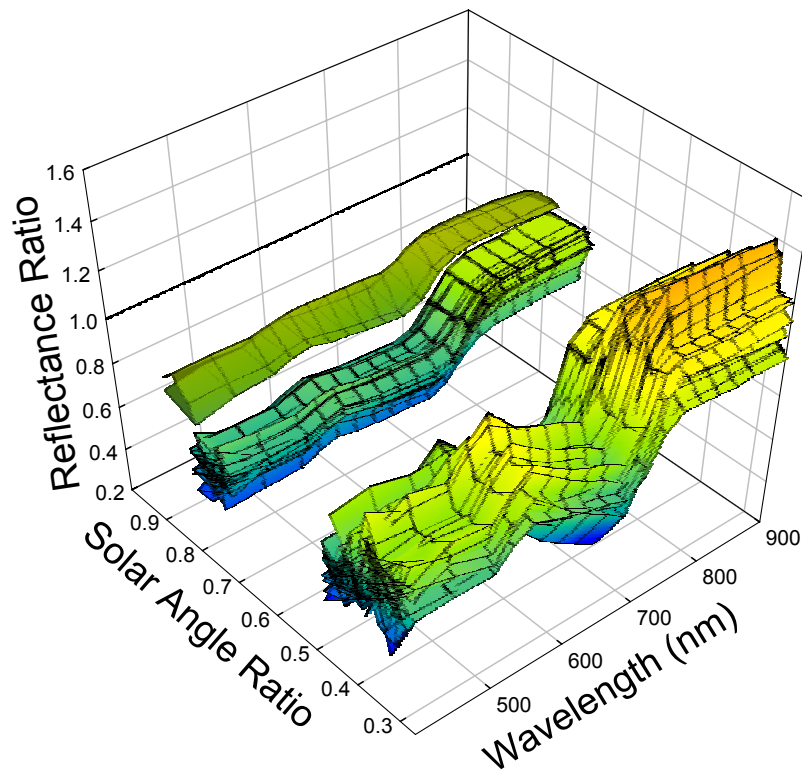

Figure 33. Comparison of reflectance ratio by wavelength with solar angle ratio to maximum solar angle. Lower solar angle resulted in increased ratio scatter, a

general decrease of baseline reflectance, and an increase of reflectance peaks in the green and NIR regions.

RESULTS

Decreasing solar angle decreased baseline reflectance and increased reflectance peaks at 550 nm and in the NIR. The 45° solar angle had a similar reflectance baseline as reflectance measurements at lower solar angles (Figure 33). Spectral indices that attempt to remove soil background do not correct for this feature, since it is wavelength dependent and follows vegetation patterns. Therefore, spectral estimates of ground cover and greenness should be taken at similar solar angle where possible.

Measurements at different solar angles showed differences in plant reflectance at all wavelengths. Asrar et al. (1985) noted that both red and NIR reflectance are affected by solar angle, but that NIR reflectance is less sensitive to solar angle. This experiment confirms these results. However, sensitivity to solar angle appears to be tied more to reflectance quantity at a wavelength than to the wavelength itself.

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APPENDIX 5: GRAPHS AND STATISTICS FOR NITROGEN AND WATER-

STRESS PAPER

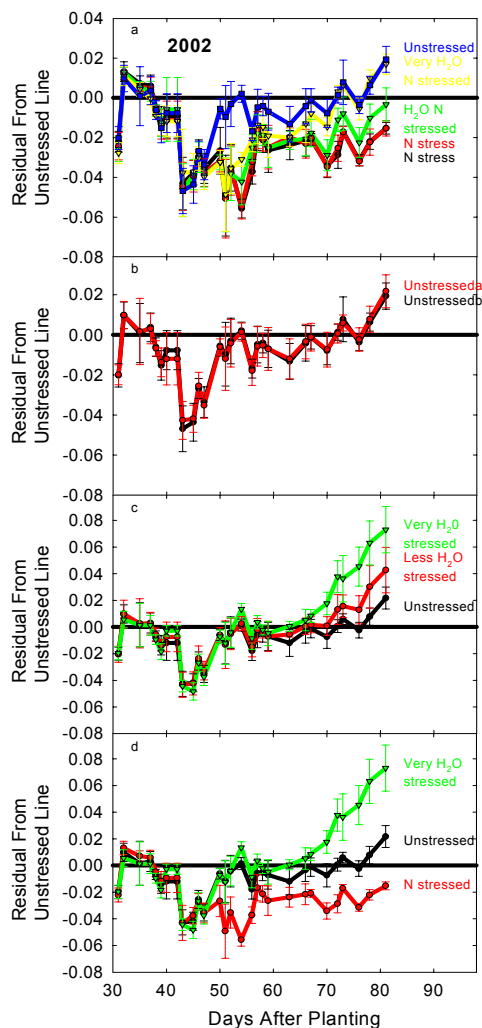**COMPARISON OF THE UNSTRESSED LINE AMONG TREATMENTS**

Figure 34. Comparison of the residuals of all treatments from the unstressed line during 2002. Graph a compares the unstressed treatment to nitrogen-stressed treatments with varying levels of water stress. Graph b compares the unstressed plots nearest the line source with those that were further from the line source. Graph c compares the unstressed treatment with two levels of water stress. Graph d compares the unstressed treatment with nitrogen-stressed plots and very water-stressed plots. All error bars represent $\pm$ 1 standard deviation from the mean by date.

Figure 34 demonstrates the predictive power of the unstressed line during the growing season for all treatments in the 2002 study. Graph a in this figure compares the effect of water-stress on nitrogen-stressed plots during the growing season. A heavy water stress increases the GNDVI:NDVI ratio significantly, which in turn makes the residual from the unstressed line more positive. This attribute is shown to a lesser extent in cases of less water stress.

Graph b compares the effects of proximity to the line source on changes in spectral attributes of plots. It was suggested that plots closest to the line source will be over-watered, and that the resultant leaching of nutrients will result in mild nitrogen-deficiency. These results suggest that if any such stress did occur, it was too mild to be detected by this method. This suggestion is supported by the SPAD data for these treatments during the growing season (Figure 35). The plots showed similar, and usually overlapping, SPAD values throughout the growing season, suggesting that the placement of the unstressed plots did not significantly affect plant nitrogen status for this study.

Graph c of Figure 34 compares the effects of two levels of water stress on the average residual from the unstressed line. The very water-stressed plots had a higher residual at the end of the season than the less water-stressed plots, which in turn had a higher residual than the unstressed plots. This pattern of increasing residual with increasing water stress follows the same pattern as with the nitrogen-stressed plots (graph a). Graph d shows the broad separation between nitrogen-stress, water-stress, and control plots by the end of the season based on the unstressed line.
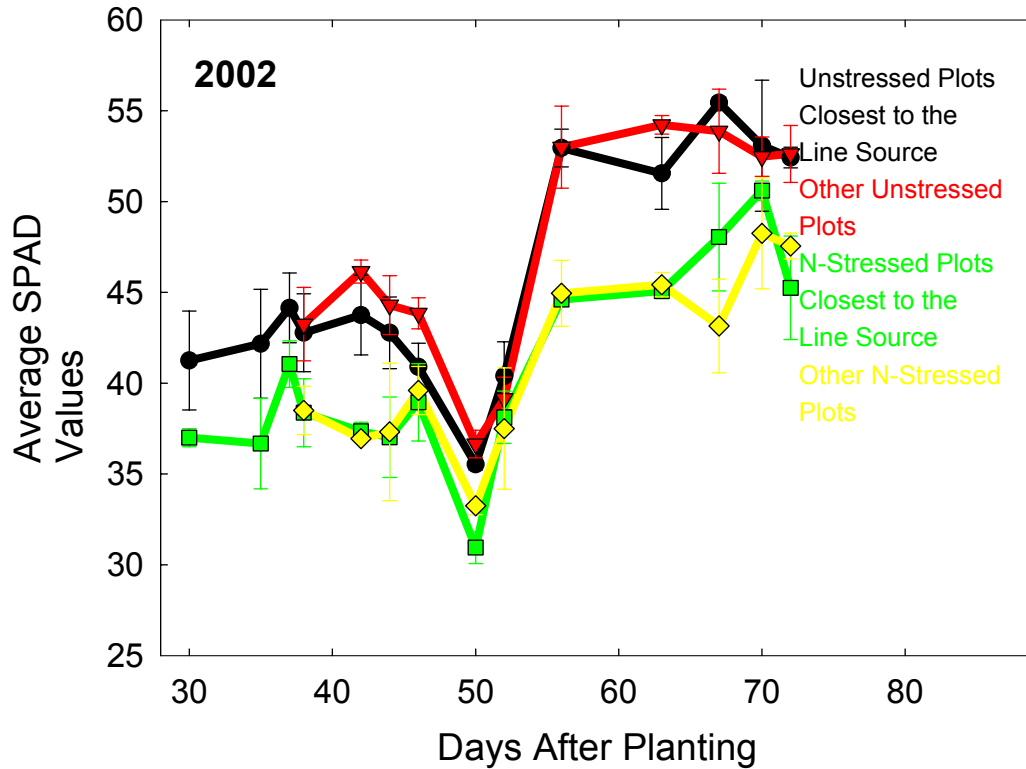
Figure 35. Comparison of the SPAD values of unstressed and N-stressed plots closest to the line source compared to other unstressed and N-stressed plots plots. Plots showed similar, and usually overlapping, SPAD values throughout the growing season. This suggests that the placement of the unstressed plots did not significantly affect plant nitrogen status for either treatment due to leaching. Error bars represent ± 1 standard deviation from the mean by date.

The following is the derivation of the conversion of the normalized green:red relationship (NGR) from an NDVI relationship to a DVI relationship.

$$\frac{(NIR - R)\big/(NIR + R)}{(NIR - G)\big/(NIR + G)} = \frac{(NIR - R) \times (NIR + G)}{(NIR - G) \times (NIR + R)} \sim \frac{DVI_{red}}{DVI_{green}}$$

STATISTICAL TEST OF 2001 AND 2002 UNSTRESSED LINES

The test statistic ((slope a - slope b) - 0)/(variance of slope a) = t (degrees of freedom of slope a), as described by Neter et al. (1996), was performed to determine if the 2001 and 2002 regression lines were statistically different. In this case, the degrees of freedom of the slope is determined as n-2, with n being the number of sample points that the regression is fitted to. The null hypothesis in this case is that the slope of line 1 (a) is not different than the slope of line 2 (b). The following table includes the parameters for both the 2001 and 2002 regression lines and the results. Tests of both lines reveal P-values greater than 0.05, and we cannot reject the null hypothesis that the lines are not statistically different.

Table 10. Statistical parameters of the 2001 and 2002 regression equations used to estimate whether the slopes are different.

Year	Regression Line	Slope	Standard Error of Slope	Degrees of Freedom	t value	Test of Significance
2001	GNDVI = 0.67 x NDVI + 0.17	0.67	0.613	57	0.075	P>0.4
2002	GNDVI = 0.70 x NDVI + 0.14	0.70	0.095	513	0.486	P>0.3

REFERENCE

Neter, J., M.H. Kutner, C.J. Nachtsheim, and W. Wasserman. 1996. Applied linear statistical models. 4th ed. Irwin, Chicago.

APPENDIX 6: GRAPHS OF DVI, RVI, NDVI, AND GNDVI COMPARED WITH

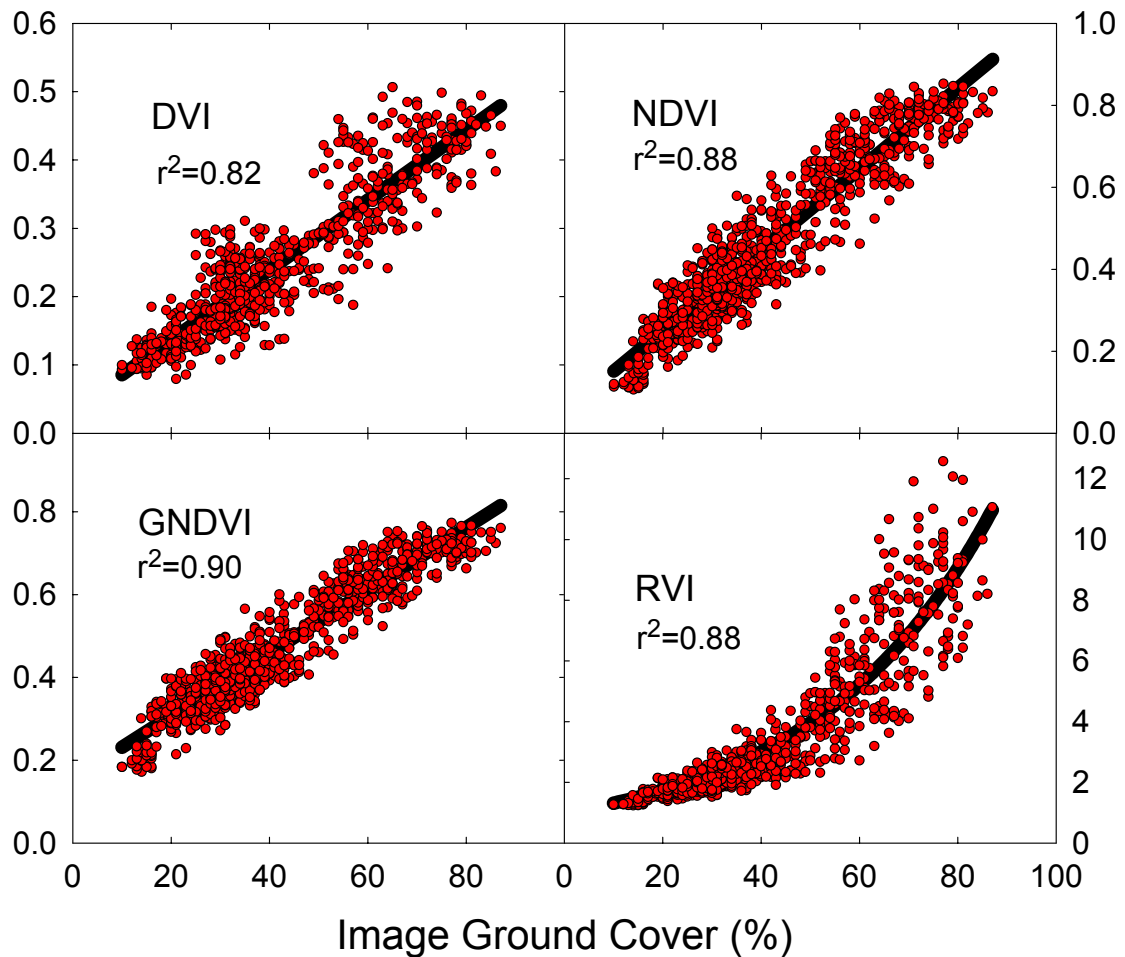**DIGITAL IMAGES OF GROUND COVER**

Figure 36. Comparison of DVI, NDVI, GNDVI, and RVI with digital images of ground cover. DVI had a lower correlation with ground cover than the ratio indices. RVI had a similar correlation with ground cover as NDVI, but the relationship was nonlinear. NDVI and GNDVI both had a high linear correlation with ground cover.