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Monitoring Nitrogen Levels in the Cotton Canopy using Real-Time Active-Illumination Spectral Sensing

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To the Graduate Council:

I am submitting herewith a thesis written by Marisol Benitez Ramirez entitled "Monitoring Nitrogen Levels in the Cotton Canopy using Real-Time Active-Illumination Spectral Sensing." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Biosystems Engineering.

John B. Wilkerson, Major Professor

We have read this thesis and recommend its acceptance:

Paul D. Ayers, Arnold M. Saxton

Accepted for the Council:
Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Monitoring Nitrogen Levels in the Cotton Canopy using Real-Time Active-Illumination Spectral Sensing

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Marisol Benitez Ramirez
May 2010

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Abstract

Managing nitrogen (N) fertilizer is fundamental to efficient cotton production. Traditional N management strategies often utilize N inefficiently through sub-optimal rate prescriptions and inappropriately timed applications. This leads to reduced production efficiency and increased environmental risk. Both deficiency and excess of N in cotton crop negatively affects lint yield and fiber quality. Thus, the aim is to monitor in-season cotton N levels in real-time at a growth stage where supplemental N can be applied. Research has shown high correlation of cotton leaf N concentrations with spectral reflectance of plants. The GreenSeeker® sensor is a ground-based active-light sensor developed to nondestructively evaluate N status in crops. However, the Normalized Difference Vegetation Index (NDVI) reported by the sensor is subject to influence by the soil background. The objective of this research was to develop an algorithm that improves a ground-based sensing system's ability to discriminate between plant biomass and soil, allowing it to better estimate N status in cotton. Three cotton varieties, three seeding rates, and four N rates were established in a field experiment in Milan, TN. GreenSeeker readings and ultrasonic plant height data were collected and analyzed to investigate the influence of these crop management factors on NDVI. Strong positive correlation ($r>0.72$) between NDVI and plant height was confirmed. Seeding rate affected NDVI throughout the season, confirming an effect of soil background noise on NDVI values. To aid in algorithm creation, NDVI data were collected from a subset of plots, the plant population was thinned, and re-sensed. Difference in NDVI of these populations was minimized when data below a threshold was removed prior to index calculation. Two algorithms were identified that reduced vegetation indices difference to within the published error of the sensor. The reduction of plant population effect on NDVI was validated by post-processing a larger data set using both algorithms.

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Chapter 1 - Introduction

Managing nitrogen (N) fertilizer is fundamental to efficient cotton production. Traditional N management strategies often utilize N inefficiently through sub-optimal rate prescriptions and inappropriately timed applications. This has both economic and environmental implications. Deficiency and excess of N in a cotton crop can negatively affect lint yield and fiber quality. An excess of N supply often results in excessive growth of biomass and lower yield (Faircloth, 2005; Fritschi et al., 2003) and a deficiency of N translates to lower biomass production resulting in lower lint yield and fiber quality (Gerik et al., 1994; Girma et al., 2007; Setatou and Simonis, 1996).

One of the main goals of cotton farm managers is to detect N status of the plant and respond with supplemental N in a timely manner. This would increase N utilization efficiency, improve yield, increase profit, and minimize N losses to the environment. Variable rate N application methods have been shown to reduce the amount of fertilizer spread on the field and improve N utilization efficiency (Raun et al., 2002; Teal et al., 2004). However, cotton N management has historically been based on pre-plant soil tests or in-season petiole tests. These tests are expensive, time consuming, and poorly synchronize fertilizer application with crop demand, leading to less than optimum yield (Shanahan et al., 2008).

Real-time knowledge of cotton plant N status is the key to timely application of supplemental N at a rate matched to the spatial variability of plant N need. Several studies (Feibo et al., 1998; Gerik et al., 1994; Neves et al., 2005) have shown that cotton leaf N concentration is an important indicator of N status. Other studies (Bronson et al., 2003; Bronson et al., 2005; Buscaglia and Varco, 2002; Fridgen and Varco, 2004; Li et al., 2001; Read et al., 2002; Tarpley et al., 2000; Zhao et al., 2005) have found high correlation of cotton leaf N concentration with spectral reflectance of plants. Many of these studies have been performed under greenhouse conditions (Buscaglia and Varco, 2002; Read et al., 2002; Tarpley et al., 2000) while others have been conducted under field conditions (Bronson et al., 2003; Bronson et al., 2005; Fridgen and Varco, 2004; Li et al., 2001; Zhao et al., 2005). The effectiveness of leaf N concentration as an indicator of N status combined with the correlation between leaf N concentration and spectral reflectance suggest spectral reflectance could be effective for real-time monitoring of cotton plant N status and N fertilizer management in the field.

The effectiveness of satellite and aerial remote sensing technologies for estimating cotton leaf N concentration is often limited by atmospheric conditions (Slater and Jackson, 1982a), soil brightness (Huete, 1987; Huete et al., 1985), and vegetation density (Huete, 1987). Ground based active-light remote sensing devices have been developed to reduce the impact of atmospheric conditions. However, the Normalized Difference Vegetation Index (NDVI) reported by these sensors is still subject to background noise from the soil surface. The following research addresses non-canopy influences on measurements taken with a commercially-available active-light spectral reflectance sensor.

Objectives

The overall objective of this research is to develop a method to improve *in situ* prediction accuracy of cotton plant N by a ground-based sensing system to a level sufficient to control real-time application of supplemental N. Specific objectives include:

Objective 1

Investigate the effect of cotton variety, plant population (seeding rate), soil applied N rate, and plant height (growth stage), on NDVI measurements and evaluate the inclusion of plant height as a covariate to address biomass influence on NDVI measurements.

Objective 2

Investigate the environmental factors (including soil moisture and diurnal cycle) that impact NDVI measurements.

Objective 3

Investigate the effect of soil background on noise content in NDVI measurements and develop an algorithm to minimize the impact.

Chapter 2 - Literature review

Cotton management

Accuracy and timely prediction of nitrogen (N) deficiency in cotton production is vital for farm managers making N management decisions. Nitrogen is an essential nutrient required by cotton plants. Both N deficiency and N excess negatively affect cotton production. Under-fertilization of cotton reduces vegetative and reproductive growth leading to potential yield losses (Bell et al., 2003; Fritschi et al., 2003; Gerik et al., 1994). Over-fertilization of cotton does not only increases production costs and potential adverse environmental impacts, but also delays maturity and reduces lint quality and yield (Faircloth, 2005; Fritschi et al., 2003; Girma et al., 2007; Setatou and Simonis, 1996). Therefore, cotton producers must accurately determine plant N status to maximize N use efficiency, increase lint quality and yield, and minimize N losses to the environment.

Historically, plant N status has been estimated through destructive sampling techniques (samples taken physically from the plant and soil) and laboratory chemical analysis. Although conventional laboratory analysis techniques like soil N content and plant tissue N concentration are commonly available to help predict N status in plants, these analyses are generally time consuming, costly and labor intensive (Sui et al., 2005; Tracy et al., 1992; Zhao et al., 2005). As a result management decisions are often based on a minimal amount of random samples collected within the field when using laboratory analysis techniques. Since small number of samples typically do not encompass the in-field variability, management decisions are usually based on an average of a relatively small number of samples, or on samples taken only from the more demanding areas of the field (Shanahan et al., 2008). Furthermore, these N status estimation techniques generally suggest the use of conventional uniform rate applications instead of variable rate applications due to a lack if information on spatial variability (Shanahan et al., 2008).

The terms variable rate application and site-specific management were introduced to agriculture in the 1980’s when new technologies were initially combined to create what is currently know as precision agriculture. Precision agriculture offers an alternative to traditional uniform application of agricultural inputs, such as tillage, seeds, herbicide, pesticide, and disease control. One of the most important aspects of precision agriculture is the ability to vary the rate of the application of agricultural inputs. According to Clark and McGuckin (1996), a geographic information system (GIS) is the brain precision farming system and enables profit optimization through knowledge-based farming decisions. Variable rate application systems utilize a computer or controller to adapt the application rate to various sites throughout each field. The computer or controller receives information from peripheral sources, such as global positioning systems (GPS), sensors, and other equipment as a function of field position and uses this information to make decisions about application rates.

Remote sensing in agriculture

Emerging technologies, such as remote sensing, are used in conjunction with global positioning systems (GPS) and site-specific management to provide farmers with tremendous quantities of real-time geo-referenced data. The ability to locate coordinates in the same field over time allows for making repeated measurements that can be used to calculated change over time and build data layers for fields (Leon et al., 2003). Geo-referenced nondestructive measurement of soil and canopy spectral reflectance provides the data necessary to predict *in situ* crop and field conditions in real-time (Fridgen and Varco, 2004; Leon et al., 2003; Sui et al., 2005; Zhao et al., 2005). Remote sensing involves the use of ground-, aircraft-, or satellite-based sensors to monitor the reflection of electromagnetic radiation from the target. Spectral reflectance has the capability of delineating stress anomalies within crop production fields, as well as mapping spatial and temporal variation in crop growth (Fridgen and Varco, 2004). These new technologies provide an alternative resource for in-season plant N evaluation and spatially variable N application improving crop management decisions.

Remote sensing in agriculture is based on the principle of changes in light interception and reflectance of vegetation, indicating leaf color. Chlorophyll (Chl) is the primary contributor of green color in leaves. The major component of the Chl molecule is nitrogen. When N availability to plants is limited, Chl formation is restricted and green color in the leaves is affected. Thus, a quick measurement of leaf Chl content could offer a technique for predicting cotton N requirements (Tracy et al., 1992). Researchers (Bronson et al., 2003; Feibo et al., 1998; Neves et al., 2005; Zhao et al., 2005) have found a high correlation of leaf N content with leaf Chl content in cotton using a SPAD meter (Minolta SPAD-502 Chl meter, Minolta Corp., Osaka, Japan). The SPAD meter is a hand-held tool that measures differences in light attenuation through the leaf at the 430 nm and 750 nm wavelengths. The 430 nm wavelength (blue region) is a spectral transmittance peak for both chlorophyll a and b, while the 750 nm is in the near infrared (NIR) region, where limited transmittance occurs (Tracy et al., 1992). However, using the SPAD meter for field-scale management decisions is problematic due to the amount of time it takes to collect sufficient data to accurately represent a whole field. Also, it does not lend itself to real-time variable rate nitrogen management.

The chlorophyll in leaves absorbs the blue (~ 450 nm) and red (~ 670 nm) light, and reflects green (~ 550 nm) and NIR (~750 nm) wavelengths (Shanahan et al., 2008; Tracy et al., 1992). In remote sensing, leaf reflectance properties in the visible region (400 to 700 nm) depend on plant Chl concentration for healthy crops (Buscaglia and Varco, 2002).. Longstreth and Nobel (1980) published that N deficiency in cotton decreased Chl content in plants leading to thinner and yellowish-green leaves. Moreover, Buscaglia and Varco (2002) reported that leaf reflectance correlated more strongly with leaf tissue N concentration than chlorophyll SPAD meter readings at squaring and flowering stages. This suggests that spectral reflectance could be a more sensitive means of estimating cotton N status during early growth stages. Thus, using remote sensing for Chl is possible as an estimate of in-season N-status (Read et al., 2002).

Remote sensing has been widely studied in cotton crops to correlate vegetation indices with plant growth (Bronson et al., 2003; Leon et al., 2003; Li et al., 2001; Plant et al., 2000; Thenkabail et al., 2000; Zhao et al., 2007), nutrients status (Bronson et al., 2003; Bronson et al., 2005; Buscaglia and Varco, 2002; Fridgen and Varco, 2004; Kostrzewski et al., 2002; Leon et al., 2003; Li et al., 2001; Lough and Varco, 2000; Read et al., 2002; Saranga et al., 1998; Tarpley et al., 2000; Zhao et al., 2005) and lint yield (Leon et al., 2003; Li et al., 2001; Plant et al., 2000; Thenkabail et al., 2000; Zhao et al., 2007).

Vegetation indices

Vegetation indices are values generated using reflectance measurements from two or more spectral wavelengths. Reflectance is the ratio of the total amount of radiation (energy) reflected by a surface to the total amount of radiation incident on the surface. The correlation of vegetation indices with biomass, leaf area index (LAI), N status, or yield depend on the index used (Thorp and Tian, 2004). There are many vegetation indices such as ratio vegetation index (RVI), difference vegetation index (DVI), green vegetative index (GVI), land perpendicular vegetation index (PVI), among others. However, one of the most common vegetation index used in crop management is the normalized difference vegetation index (NDVI) (Tucker, 1979). The NDVI is expressed as:

$$NDVI = \frac{R_{NIR} - R_{red}}{R_{NIR} + R_{red}}$$

where R_{NIR} is the reflectance in the near infrared (NIR) region (770 ± 15 nm) and R_{red} is the reflectance in the red region (650 ± 10 nm) of the electromagnetic spectrum. Reflectance in NIR region is known to positively correlate with leaf area, whereas reflectance in the red region is known to negatively correlate with green leaf area (Knipling, 1970). Also, vegetation reflectance is affected by the contribution of stems and leaf orientation to canopy reflectance (Carter and Miller, 1994).

Several authors have associated cotton canopy and leaf reflectance at different wavelengths depending on crop characteristics (leaf thickness, internal leaf structure, external characteristics, and chlorophyll content) and the type of remote sensing system. In 1993, Carter published that the most reliable leaf reflectance response of cotton plant stress was the reflectance at visible wavelengths (400-700 nm). Among six vascular plant species, visible reflectance ranges of 535-640 nm and 685-700 nm were the most sensitive wavelengths to eight stress agents. Carter (1994) later found that the ratios R_{695}/R_{420} (red/violet) and R_{695}/R_{760} (red/NIR) were stronger indicators of N stress in those six plant species. Wilkerson et al. (1998) found that cotton features, such as plant N content and expected yield, could be predicted using a blue band (460-490 nm), green band (545-565 nm), amber band (red-yellow) (570-680 nm) and NIR band (740-770 nm) ratio (four wavebands ratio) from the cotton canopy, as early as pin-head square using a SD-1000 fiber optic spectrometer (Ocean

Optics, Inc., Dunedin, FL). Lough and Varco (2000) evaluated the effect of N and K nutrition on cotton leaf reflectance using a LI-COR 1800 spectroradiometer (LI-COR Co., Lincoln, NE) and reported that given an adequate supply of K the greatest separation of NDVI between N treatments occurred at the 550 nm waveband and red-edge shift. Tarpley et al. (2000) calculated vegetation indices from spectral reflectance of individual cotton leaves to estimate leaf N. They found that the leaf reflectance ratios between wavebands in the red-edge (700-716 nm) and the very NIR region (755-920 nm and 1000 nm) measured with a GER 1500 spectroradiometer (Spectra Vista Corporation, Poughkeepsie, NY) provided excellent precision and accuracy on prediction of cotton leaf N concentration. Read et al. (2002) reported that the use of remote sensing in cotton canopy reflectance to assess N status is achievable for narrow wavelength reflectance ratios that involve the violet or blue region of the wavelength (400 nm to 450 nm) and red-edge region. For leaf reflectance the wavebands ratio was 695nm/755 nm. Measurements were taken using a GER 1500 spectroradiometer. Buscaglia and Varco (2002) found a better cotton leaf N concentration estimation at the green (550 nm) wavelength using leaf reflectance at squaring and flowering stages measured with a LI-COR 1800 spectroradiometer. They suggested that better detection of N status occurs at earlier cotton growth stages. Zhao et al. (2005) using a portable ASD Field Spec FR spectroradiometer (ASD, Inc., Boulder, CO) found high correlation of cotton leaf reflectance and leaf N concentration at 517 nm and 701 nm wavelengths, and the best linear relationship among leaf N concentration and spectral reflectance ratio at 517 and 413 nm (R_{517}/R_{413}). Leaf Chl concentration was related with spectral ratios of either R_{708}/R_{915} or R_{551}/R_{915}.

A number of studies (Leon et al., 2003; Li et al., 2001; Plant et al., 2000; Thomasson et al., 2004; Zhao et al., 2007) found a high correlation between the canopy/leaf spectral reflectance and cotton yield using the green-red ratio and NDVI. Leon et al. (2003) and Thenkabail et al. (2000) found high correlation among cotton plant height and green, red, NIR bands, and vegetation indices such as NDVI, NIR/red ratio, NIR-red difference, and others. Other studies like that published by Bronson et al. (2003) reported that green vegetation index (GVI) and green normalized difference vegetation index (GNDVI) could be used to estimate cotton leaf N and red vegetation index (RVI) and red normalized difference vegetation index (RNDVI) for estimating cotton biomass. Fridgen and Varco (2004) studied the dependency of cotton leaf N, leaf chlorophyll, and leaf reflectance on N and K availability. They concluded that a distinct N and K deficiency, given that no other nutrients are deficient, might be detected by leaf reflectance in the visible to NIR range. Additionally, red-edge shift was related to cotton leaf N status with a possible dependence on the availability of other nutrients, such as K. Li et al. (2001) related cotton plant biomass and N uptake to NIR reflectance. Saranga et al. (1998) punched a 2.5 cm disk out of cotton leaves to analyze N status in plant using spectral reflectance. They reported that cotton yield was linearly correlated with leaf N concentration, and leaf N concentration was reliably monitored by near-infrared analysis (NIRA).

The existing body of research indicates that remote sensing throughout different wavelength ratios and vegetation indices offers the opportunity to detect biophysical parameters such as leaf N content, plant biomass, and plant height. Differences in these parameters result in change in leaf and canopy spectral reflectance, allowing for detection of nutrient deficiency in crops and predicting yield. Most of these ratios and vegetation indices are composed of reflectance response at the visible and near infrared wavelength and are dominated by the red and NIR wavelength combinations.

Limitations of remote sensing in agriculture

One of the limitations that remote sensing technology has in production agriculture is the change in canopy density throughout the season. The resulting impact on spectral response, makes it difficult to relate spectral variations to crop properties. This limitation is especially prevalent early in the season when the plant biomass covers a small fraction of the soil background. It is difficult to determine whether differences in spectral reflectance are due to a crop condition that alters canopy reflectance (e.g. nitrogen, water stress) or if they are an artifact of changing canopy density (Barnes et al., 2000). Carlson and Ripley (1997) published a dependence of the NDVI of vegetation canopy on vegetation cover. NDVI readings were sensitive to different partial vegetation covers when it was less than 100%. They also concluded that LAI and partial vegetation cover are not completely independent quantities, and that after full cover, a further increase in LAI results in a slightly increase in

NDVI readings. On the other hand, Behrens et al. (2004) reported that the sensor's measuring angle has a significant influence on the calculation of vegetation indices. Significant differences in vegetation indices due to varieties were also found, which could affect the estimation of crop stress.

Other restrictions in the use of satellite or aerial remote sensors for measuring canopy/leaf reflectance have been published by several researchers. Slater and Jackson (1982) documented that atmospheric parameters may affect remote sensing measurement of vegetation by obstructing and delaying the discrimination of stressed and unstressed canopies by 3 to 7 days. Kostrzewski et al. (2002) stated that remote sensing data obtained through satellite and aerial images requires three or more weeks to be post-processed and made available for management decisions. This delays make the information less useful in crop management.

The limitations of ground-based remote sensors have been published as well. Huete et al. (1985) found that both soil brightness and soil spectra influence greenness measures for both low vegetation densities and canopy coverage approaching 75 percent. Darker soils were found to increase vegetation indices values. Later, Huete (1987) confirmed that vegetation indices depend on the quantity of vegetation available for irradiance and soil brightness. Soil-dependent reflectance can dominate canopy reflectance for low levels of canopy coverage, but the canopy reflectance dominates the soil reflectance for high levels of canopy coverage. Brighter soils caused the greatest deviation of measured canopy reflectance from vegetation spectra. Principal component analysis (PCA) results showed that reflectance from soil mixes with the various vegetation indices inhibiting reliable vegetation discrimination. Soil brightness affected the NIR/red ratio differently than the PVI and the GVI. Brighter soils generated greater greenness signals in PVI and GVI, while darker soils generated greater greenness signals when NIR/red ratio was used. Huete suggested that an enhancement in the vegetation indices analysis could be developed by filtering soil background response from canopy reflectance.

Differences in variety may also be of concern when comparing spectral reflectance from a ground-based platform. Sims and Gamon (2002) studied previous published vegetation indices used for estimation of leaf pigment content and found that those indices provide relatively poor correlation with leaf chlorophyll content when applied across a wide range of species and plant functional types. Leaf surface reflectance seems to be the most influential factor in this variation. These results could be attributed to the fact that these vegetation indices have been tested for only one or few related species. They developed a new spectral index that reduces the effect of difference in leaf surface reflectance, improving the correlation with leaf chlorophyll. They also found that the red-edge region is less sensitive to leaf structure variation. Behrens et al. (2004) revealed significant spectral differences between twelve different varieties of winter oil seed rape, which could reduce the estimation accuracy of crop N status. They suggested variety-specific calibrations are necessary. Despite these limitations, researchers at Oklahoma State University (Arnall et al., 2006; Freeman et al., 2007; Girma et al., 2006; Martin et al., 2007; Raun et al., 2005; Raun et al., 2002; Teal et al., 2004) have developed and extensively tested the ground-based GreenSeeker hand-held optical sensor, achieving good correlations of N uptake, Chl content, plant height, plant biomass and yield with NDVI spectral reflectance on crops such as wheat and corn.

Adjusted soil background vegetation indices

According to Demetriades-Shah et al. (1990) “the classical methods in analytical chemistry for reducing errors due to turbid matrix are to take the differences or the ratio at two wavelengths. The analytical signal at a wavelength in an absorption band of the species of interest is normalized by the signal at another wavelength where there is not specific absorption, close to the analytical wavelength. So, any level background signal or absorption will be similar at both wavelengths and will be eliminated when the ratio or a difference is taken”. However, ratios and differences of wavelengths only completely correct background signals if they have a constant slope from sample to sample (spectrally level). Soil background signals do not have a constant slope (is not spectrally level) but increases gradually from the visible to the NIR region. Additionally, the rate of increase differs for different soils. For this reason, the wavelengths typically used in difference calculations, such as NDVI, do not always reduce soil background noise to acceptable levels. Many attempts have been made to minimize the soil impact of soil on spectral reflectance measurements (Arnall et al., 2006; Baret and Guyot, 1991; Demetriades-Shah et al., 1990; Hall et al., 1990; Huete, 1988; Major et al., 1990; Qi et al., 1994; Richardson and Weigand, 1977; Rondeaux et al., 1996). A number of vegetation indices have been published that address soil background. Some of the most discussed in the literature are:

Perpendicular vegetation index (PVI): proposed by Richardson and Weigand (1977), this index is the perpendicular distance between bare soil red and NIR reflectance.

$$PVI = \alpha * R_{NIR} - \beta * R_{red}$$

where α and β are soil line parameters, and R_{NIR} and R_{red} are reflectance in the NIR and red wavelengths, respectively. The soil line is a straight line formed by the bare soil NIR and red spectral reflectance. Reflectance in the red wavelength decreases and in the NIR increases as vegetation growth which generates a vegetation point away from the soil line. Therefore, the measure of vegetation present in soil is the perpendicular distance from the vegetation point to the soil line (Jackson, 1983). Although PVI is better than NDVI at low vegetation densities (when soil and plant interaction are minimal), it is still significantly affected by soil background (Thorp and Tian, 2004).

Soil adjusted vegetation index (SAVI): proposed by Huete (1988), SAVI introduces the *L* factor into the NDVI equation to minimize first order soil interaction on canopy reflectance (especially for *L* from 0.5 to 1). This *L* factor varies inversely with the amount of vegetation coverage. The optimal adjustment was found at *L*=0.5 where soil noise is reduced considerably throughout different vegetation densities.

$$SAVI = \left[\frac{R_{NIR} - R_{red}}{R_{NIR} + R_{red} + L}\right] * (1 + L)$$

However, the ideal adjustment *L* factor does not remain constant because the nature of the soil-vegetation interaction varies with canopy closure. “Graphically, the transformation involves a shifting of the origin of reflectance spectral plotted in NIR-red wavelengths to account for first order soil-vegetation interactions and differential red and NIR flux extinction through vegetated canopies” (Huete, 1988).

Transformed soil adjusted vegetation index (TSAVI): SAVI is a solution for bare soil only when soil line parameters are a=1 and b=0. As this is not generally the case, Baret and Guyot (1991) developed TSAVI which is a measure for the other cases ($a \neq 1$ and $b \neq 0$).

$$TSAVI = \frac{a(R_{NIR} - aR_{red} - b)}{aR_{NIR} + R_{red} - ab + X(1 + a^2)}$$

where a and b are slope and intercept of the soil line ($R_{NIR_{soil}} = a * R_{red_{soil}} + b$), respectively. X corresponds to the coefficient value adjusted to minimize soil effects (X=0.08 optimal). TSAVI seems to be a more reliable than SAVI and PVI when leaf inclination angle is known.

Soil adjusted vegetation index ($SAVI_2$): proposed by Major et al. (1990), this index add an adjusted parameter (θ) to the red reflectance to reduce solar angles, leaf angles and leaf area index effects.

$$SAVI_2 = \left[\frac{R_{NIR}}{R_{red} + \theta} \right]$$

where $\theta = b/a$ is the soil adjustment factor, with b as the intercept and a as the slope of the vegetation isolines.

Modified soil adjusted vegetation index (MSAVI): developed by Qi et al. (1994), MSAVI increases the dynamic range of the vegetation signal while minimizing the soil background effect using a variable L instead of the constant L.

$$MSAVI = \left[\frac{R_{NIR} - R_{red}}{R_{NIR} + R_{red} + L} \right] * (1 + L)$$

$$\text{with } L = 1 - 2a * NDVI * WDVI$$

where $WDVI = R_{NIR} - aR_{red}$ is the weighted difference vegetation index and a is the slope of the soil line. At higher vegetation densities, L approaches 0, and the MSAVI behaves like a NDVI; while a lower vegetation densities, L approaches 1, and the MSAVI behaves like PVI. The great difference between SAVI and MSAVI is that SAVI uses a manual adjustment L, while MSAVI uses a self-adjustment L. MSAVI seems to have a greater dynamic range response and be less sensitive to soil background effects than the above vegetation indices.

Optimal soil adjusted vegetation index (OSAVI): proposed by Rondeaux et al. (1996), the SAVI *L* factor is called *X* value and it was found to be optimal at 0.16 for low and high vegetation cover.

$$OSAVI = \frac{R_{NIR} - R_{red}}{R_{NIR} + R_{red} + 0.16}$$

The multiplication factor in *SAVI* (1+*L*) has been left out because it only significantly affects the index at relative large *L* values (*L*>0.4). Higher values of *X*, such as for the SAVI (*X*=0.5) result in higher standard deviation. This vegetation index becomes attractive because of its simplicity and previous knowledge of the soil line parameters is not required.

High spectra resolution derivative: proposed by Hall et al. (1990) and Demetriades-Shah et al. (1990), this method relates the second derivative of the spectral reflectance versus the wavelength function for vegetation. The derivative of a spectrum is its rate of change with respect to wavelength. A curve that varies linearly with the wavelength represents the soil background signal (y_1) and a third-order polynomial represents the vegetation signal (y_2) represented with a:

$$\begin{aligned} y_1 &= a_1 + b_1 x \\ y_2 &= a_2 + b_2 x + c_2 x^2 + d_2 x^3 \\ Y &= F y_1 + (1 - F) y_2 \end{aligned}$$

where *Y* is a composite spectrum containing the useful signals and F (<1) is the relative weighting factor for the background. Differentiating twice gives:

$$d^2Y / dx^2 = (1 - F)(2c_2 + 6d_2 x)$$

This function seems to be less sensitive to the reflectance of non-photosynthetically active material such as soil background and canopy structures. Demetriades-Shan et al. showed that derivative spectral indices are superior to conventional broadband spectral indices such as NIR/red reflectance ratio. Nonetheless, the second derivative is very sensitive to noise; hence precise calibration of the sensor for each band is required. Furthermore, the generality of these results is unclear since this vegetation index has only been applied to one species (sugar beet) (Sims and Gamon, 2002).

A different approach to investigate soil background noise was published by Arnall et al. (2006). They studied the relationship between the coefficient of variation (CV) in spectral reflectance measurements and plant density at early growth stage in winter wheat. The prediction of $RI_{Harvest}$ (response index yield) improved when CV was integrated into the RI_{NDVI} (response index vegetation index) calculation. The CV increased when plant population was poor. They concluded that CV could be used as an estimate of variation in plant density to identify the areas where the plant stand is so poor that N fertilization would be ineffective.

Although these indices appear to be more reliable and less noisy than the NDVI, they are not widely used in practice, perhaps because of the complexity of the vegetation indices formulation or due to the fact that they have not convincingly demonstrated improved estimation of the vegetation parameters as compared to NDVI. The interaction between reflectance signals is complex and difficult to correct. For this reason researchers still emphasize the need to refine vegetation indices to avoid or correct soil background effect (Rondeaux et al., 1996). Meanwhile, NDVI remains the most used index for remote sensing applications in agriculture (Thorp and Tian, 2004).

Ground-based remote sensing

As stated before, atmospheric turbidity affects aircraft and satellite remote sensing of vegetation. An approach presented in the literature to correct this problem is the use of ground-based sensors positioned a meter or so above the crop canopy. Images obtained by near-remote sensing offer several advantages over the far-remote sensing images. These advantages include improvement of spatial resolution and less distance between sensor and target. As a result, less atmospheric interference or turbidity affects the sensors (Shanahan et al., 2008).

The advantages that ground-based sensors have over aircraft- or satellite-based sensors help justify the development of on-the-go sensing systems for site-specific management applications. Heege and Thiessen (2002) published the Kiel-system, an on-the-go sensor for site-specific N top dressing based on the red and NIR crop reflectance signals. The design was based on the fact that every farmer knows that the N supply affects the color of the leaves and the growth of the crops. The reflected radiation from the canopy depends on the incident solar radiation (irradiance) in addition to plant condition. This dependence make it necessary to measure the incident solar radiation so that the crop reflectance can be normalized to eliminate the impact of fluctuations in the incident solar radiation on crop reflectance. Kostrzewski et al. (2002) used the remote sensing system called Agricultural Irrigation Imaging System (AgIIS) constructed at the University of Arizona Maricopa Agricultural Center. They collected 1-meter resolution data to separate water and N stress using the coefficient of variation of the water and N stress indices measured. They found that the CV of water and N stress indices were more reliable measurements of water and N status than the mean value of the indices.

However, the spectral reflectance measured using these Kiel and AgIIS systems have the disadvantage of being influenced by solar radiation on crop canopy. Pinter (1993) and Pinter et al. (1985) shown that solar zenith angles and atmospheric conditions change the incident light on the crop canopy, which affected the spectral reflectance data accuracy. Sui et al. (2005) designed, implemented and tested a multi-spectral optical sensor for detection of N status in cotton to address this disadvantage of the Kiel and AgIIS systems. This sensor used modulated halogen light as the illumination source and measured spectral reflectance from cotton canopy in the blue, green, red, and NIR wavebands. Since the sensor was only sensitive to modulated light, changes in ambient light did not affect the spectral reflectance measurements.

Commercial on-the-go optical sensing devices have been recently developed for real-time variable rate N application. These sensors utilize internal light emitting diodes (LED) for illumination rather than ambient light. This modulated light source allows day and night operation under varying environmental conditions (ignoring fog, clouds and shading from the tractor). GreenSeeker® (NTech Industries Inc., Ukiah , CA) and CropCircle™ (Holland Scientific Inc., Lincoln, NE), and N-Sensor ALS (Yara UK Ltd., Lincolnshire, UK) are the three commercially-available sensor-based systems for site-specific application of N. All of them can measure crop spectral reflectance and calculate a number of vegetation indices that are related to N status in plants, depending on plant biomass and chlorophyll content. These vegetation indices are used to calculate optimal N rates in real-time using empirically determined algorithms.

Nevertheless, it seems these commercially available systems are susceptible to the same soil background noise problem as the other systems discussed in the literature (Arnall et al., 2006; Barnes et al., 2000; Qi et al., 1994). The effect of this noise is a concern when using ground-based sensor for fertilizer application. The soil background noise decreases the resulting canopy/leaf reflectance signal, leading to an increase in fertilizer applications. Frequent patches of bare soils present within the crop can also lead to overuse of fertilizer.

GreenSeeker sensor

The commercially available GreenSeeker® RT100 sensor (NTech Industries Inc, Ukiah, CA) developed by Oklahoma State University is a ground-based active-light sensor. Active-light signifies that the sensor uses its own generated light, making it independent of solar illumination. The light generated is reflected off the crop and measured by a photodiodes located in front of the sensor head (Figure 1). NTech Industries Inc. (2007) define the GreenSeeker® sensor as a mapping system which uses red (656 nm ± 25 nm) and NIR (774 nm ± 25 nm) bands to measure two specific wavelengths of light reflected off the plant. It was designed to operate 28 to 44 inches over the plant canopy to be sensed. NDVI readings remain similar through the 28-44 inches range because it is a normalized ratio. The field of view of the sensor is an area of 24 in. ± 4 in. x 0.6 in. ± 0.2 in. The default system program takes 1000 samples per second and reports an average value every second. However, the user is allowed to define the update rate, increasing or decreasing the interval between average values. The GreenSeeker® sensor is able to generate NDVI and other vegetation indices such as soil adjusted NDVI (SA-NDVI), wide dynamic range NDVI (WDR-NDVI), ratio vegetation index (RVI) and inverse ratio vegetation index (IRVI)) of soil and plant matter. Each of these vegetative indices can be used as indicators of N status in plants.

Several studies have been published using GreenSeeker® sensors for detecting N status in crops (Arnall et al., 2006; Freeman et al., 2007; Girma et al., 2006; Martin et al., 2007; Raun et al., 2005; Raun et al., 2002; Shanahan et al., 2008; Teal et al., 2004). However, most of these studies were conducted in corn and winter wheat crops. Only a few studies have been reported for cotton crops. Bronson et al. (2005) reported that NDVI measured with a GreenSeeker® was poorly correlated with cotton leaf N, biomass, and lint

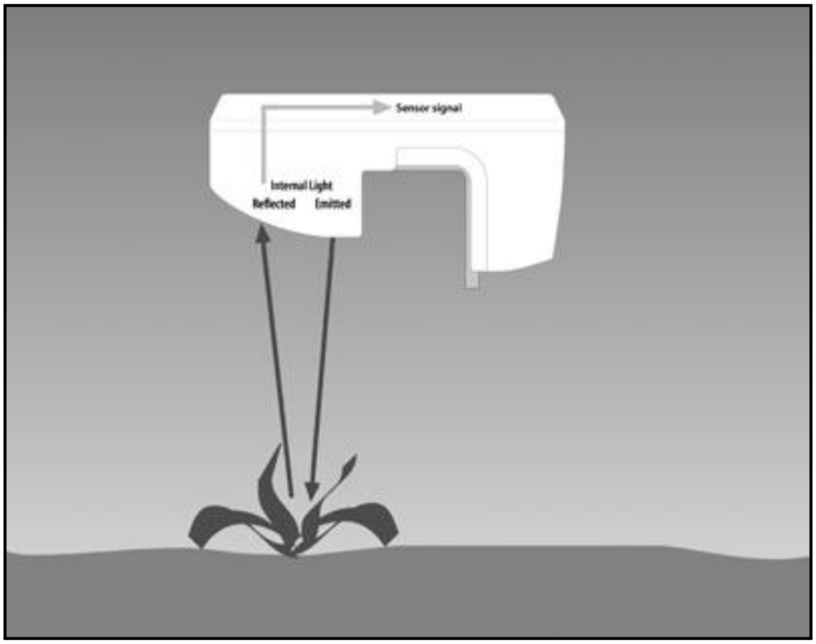

Figure 1. Illustration of GreenSeeker's light emission and reflectance measurement system. (Image was taken from http://www.lesspub.com).

yield. Khalilian et al. (2007) used a GreenSeeker® to measure NDVI and found a strong correlation between NDVI and plant parameters such as plant height and cotton yield.

Few studies have been published investigating the impact that cotton variety, seeding rate, and environmental conditions (such as solar radiation, cloudiness, etc.) could have on NDVI readings using GreenSeeker® sensors. On the other hand, research publications have suggested an ample number of adjusted vegetation indices to correct soil background influence on vegetation indices without a complete success. The impact of these factors must be understood to effectively utilize NDVI readings generated by GreenSeeker® sensors as a feasible N measuring tool and to control variable rate N applicators based solely on spectral reflectance.

Summarizing, ground-based active-light sensor is a non-destructive technology able to map crop biophysical characteristics of plants using spectral reflectance. Although active-light sensors were designed to minimize solar illumination effect on spectral reflectance measurement, it is still affected by other external factors such as soil background noise. Soil background noise is a significant concern when farm management decisions are based on plant N status estimated from spectral reflectance. This noise decreases the canopy/leaf reflectance measurements leading to an increase in fertilizer applications. Given the environmental and economic impacts of over-fertilization, reducing the impact of soil background noise was chosen as a primary goal for this research.

Chapter 3 - Characterizing cotton canopy reflectance by crop management factors

Introduction

Nitrogen fertilization management is fundamental in cotton production. Recently, new technologies such as on-the-go sensing devices have been developed to address variable rate N application. These sensing devices measure crop spectral reflectance and calculate vegetation indices that relate to N status in plants based on plant biomass density and chlorophyll content. These indices are used to calculate optimal N rates in real-time using empirically determined algorithms to decrease cost of production and increase yield. However, Behrens et al. (2004) found significant differences in the vegetation indices of twelve varieties of winter oilseed rape. They suggest considering the influence of variety differences on vegetation index in order to avoid misunderstandings when interpreting vegetation indices. Physiological differences in leaves from different cotton varieties could have a similar impact on spectral reflectance. Detecting spectral reflectance difference among cotton varieties will help to characterize and quantify the impact of variety on NDVI readings and to predict N status in cotton crops more accurately.

Several researches(Arnall et al., 2006; Barnes et al., 2000; Qi et al., 1994) have reported that the soil background effect on vegetation indices is a problem in ground-based sensors. Characterizing spectral reflectance differences due to plant density will provide the data necessary to better understand and minimize the impact of soil background on vegetation indices.

The objective of this chapter is to present the results from a large-scale experiment conducted in Milan, TN during the 2007 and 2008 growing seasons. The primary goal was to investigate the effects of variety, plant population (seeding rate), and soil-applied N rate on NDVI readings throughout the growing season. In addition, the correlations between leaf N concentration and soil-applied N rate, manually-counted plant population and seeding rate, and plant height and NDVI readings, were investigated through small scale experiments conducted during the 2007 and 2008 growing seasons.

Experiments were conducted in a forty acre field located at the University of Tennessee Research and Education Center in Milan, TN. The field, labeled as A202, was a non-irrigated cotton production field, managed such that phosphorus (P), potassium (K), and pH were not yield limiting factors over the course of the experiment. Historically the field was planted in a corn and cotton rotation. The experiment was conducted using the commercially-available GreenSeeker® sensor. Because the 2007 growing season turned out to be such a dry year, data collected in 2007was used as preliminary data for designing the 2008 study.

Two different varieties were selected in 2007 and three different varieties were selected in 2008 to represent distinctly different leaf textures and to characterize potential leaf spectral reflectance differences due to cotton variety. Three different seeding rates were planted to generate different in-row plant spacing to investigate the impact of early season plant spacing on NDVI readings. In order to study the capability of the sensor to detect N status differences in plants, four supplemental N rates were used in the field experiment for both 2007 and 2008 growing seasons.

Large-scale experimental design 2007

Materials and Methods

The A202 field experiment was configured as a Randomized Incomplete Block Design (RIBD) split-plot. The field was divided into four quadrants defined as blocks. Two cotton varieties (DP143 (smooth leaf) and DP555 (semi-smooth leaf)) were assigned to the blocks as shown in Figure 2. Each block was divided into plots 240 ft wide by 300 ft long. On May 9th, cotton was planted in these plots at one of three seeding rates (16,400, 28,700 and 50,225 seeds/ac) using a John Deere vacuum planter (Deere & Co., Moline, IL) equipped with a Rawson variable seeding rate controller (Rawson Control Systems, Oelwein, IA) (Figure 3). Each plot was split into subplots 40 ft wide (12 rows) by 100 ft long. These subplots will be referred to as the experimental units. Four supplemental N rates (0, 30, 60, 90 lb/ac of liquid UAN) were applied randomly in each experimental unit at 55 days after planting (July 3rd). Pre-plant N was broadcast over the whole field at 30 lbs/ac; thus, a total of 30, 60, 90, and 120 lb/ac were applied in the experimental units (Figure 4).

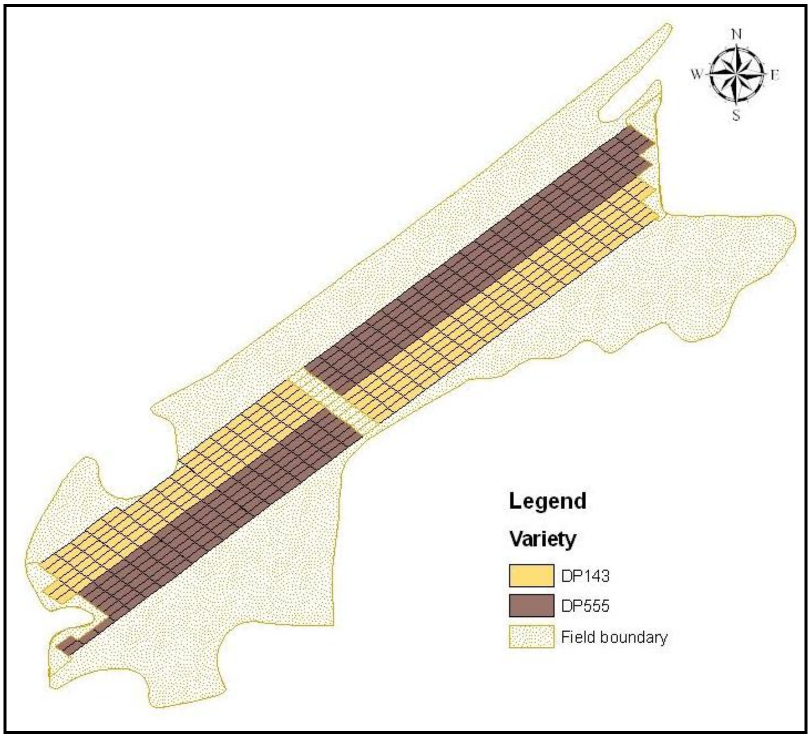

Figure 2. The field was divided into four quadrants. Each quadrant was planted with one of two varieties (DP143 and DP555)

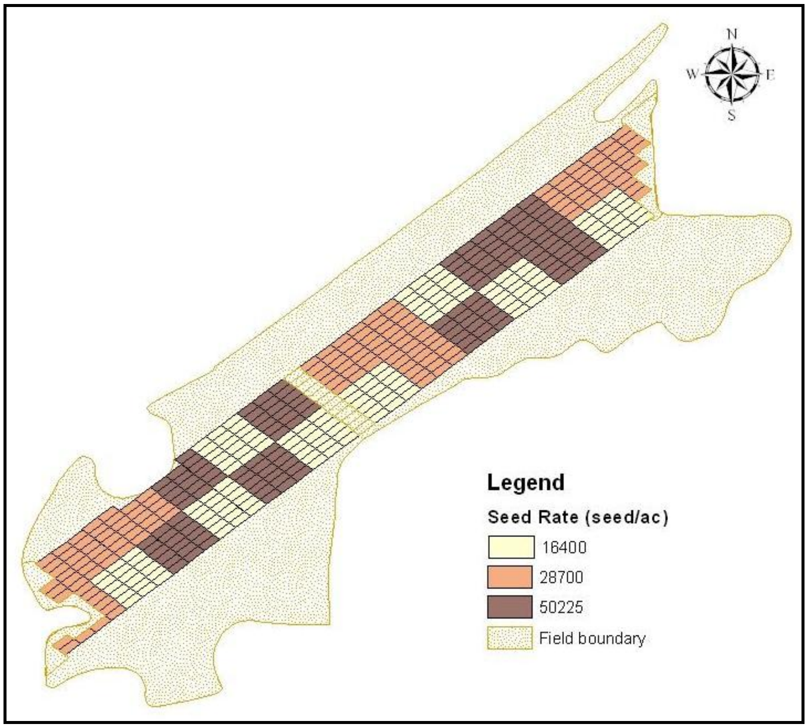

Figure 3. Three seeding rates were planted during 2007 (16,400, 28,700, and 50,225 seeds/ac)

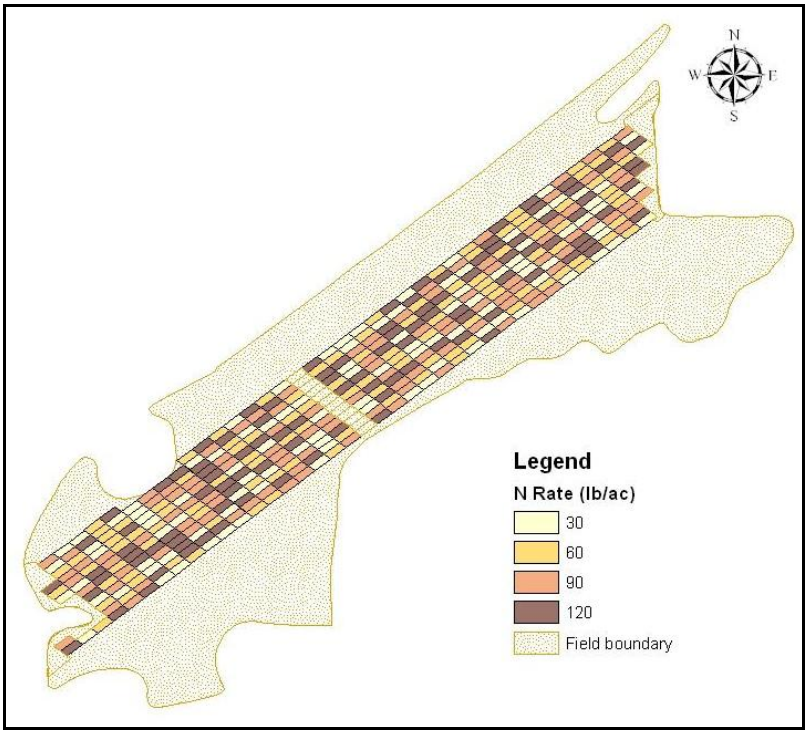

Figure 4. Four nitrogen rates were used during 2007 (30, 60, 90, and 120 lb/ac)

NDVI and plant height data were collected at 10 Hz (one point every 1/10 sec) using two GreenSeeker® RT100 sensors (NTech Industries Inc., Ukiah , CA) and an custom build ultrasonic distance sensors (Sensor and Controls Lab, Biosystems Engineering and Soil Science, University of Tennessee, Knoxville, TN), respectively. Each point was geo-referenced using a Trimble Ag332 GPS (Trimble Navigation Limited, Sunnyvale, CA). All sensing equipment was mounted on a modified Spirit™ plot sprayer (Figure 5). The GreenSeeker® sensors were maintained at a height of 36 inches above the crop canopy on a boom controlled manually or using an ultrasonic distance sensor in the hydraulic feedback loop. The ultrasonic distance sensors for plant height measurement were mounted on a fixed boom at the rear of the modified platform. Height of the boom was set to a fixed distance from ground and remained constant during each of the data collection events. NDVI and plant height were measured in four rows out of every experimental unit, driving the modified platform at 3-6 mph. At this speed, a spatial resolution of 2.27 to 1.14 observations per foot of row was obtained, respectively. Data were recorded starting at the pin-head square growth stage until full bloom. Specifically, for 2007 data were collected during early (June 27th), middle (July 12th) and late (July 23rd) periods of the cotton growing season.

ArcMap (ESRI, Redlands, CA) was used prior to statistical analysis to remove data points that fell within ten feet of experimental unit/treatment boundaries to remove areas where the variable rate planter/nitrogen applicator would be in transition between rates. Averages and standard deviations for NDVI and ultrasonic plant height were then calculated for each experimental unit. Descriptive univariate statistics and exploratory analysis were performed for each data set with PROC UNIVARIATE in SAS (SAS Institute Inc., 2003). Histograms, box plots, and normality probability plots were used to examine the distribution of NDVI residual values. Normality was tested with Shapiro-Wilk's test. Analysis of variance (ANOVA) and statistical test of least significant difference (LSD) were conducted using PROC MIXED in SAS. Ultrasonic plant height was incorporated as a covariate variable during statistical analysis to address biomass influence on NDVI readings.

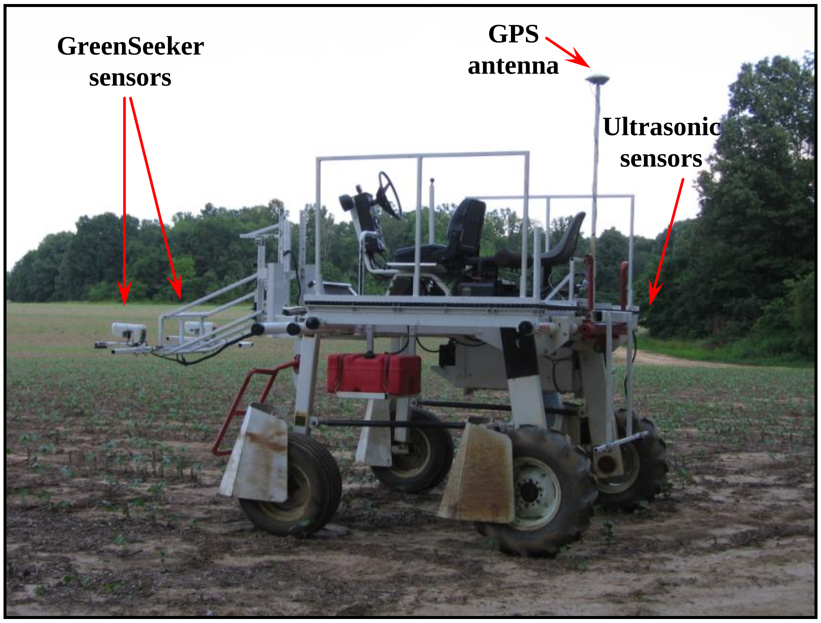

Figure 5. Mobile platform modified from a Spirit™ plot sprayer with GreenSeeker® sensors, ultrasonic distance sensors, and GPS.

Results and Discussion

The 2007 season was one of the driest years on record with low precipitation and high temperature records (20 days during the season exceeding 100 ˚F). Only 9 inches of total rain fall was recorded during the first 120 days of the growing season (Figure 6). Consequently, data collected throughout 2007 growing season was used as preliminary data for the experimental design implemented in 2008, since the growing conditions were representative of extreme rather than average growing conditions.

Table 1 shows a summary of the seeding and N rate effects on spectral reflectance for one of the two varieties (DP555) and one of the two GreenSeeker® sensors (GreenSeeker#1) for 2007 growing season. No significant differences in NDVI means were observed among the four N rates across the growing season. However, a significant effect of seeding rate on NDVI mean and CV were observed for all three sampling dates. It was interesting to observe the behavior of NDVI mean differences. Early in the season (June 27th), the lower the seeding rate, the lower the NDVI mean. However, for the later two dates of the growing season, the trend shifted such that differences were inverted; the lower the seeding rate, the higher the NDVI mean. These results could be attributed to a couple of factors. First, early in the season the between-plant spacing or soil background noise could be lowering the NDVI means for the lower seeding rate. Second, the between-plant spacing associated with the lower seeding rate could benefit the growth of the canopy resulting in wider canopy coverage and a correspondingly higher NDVI reading.

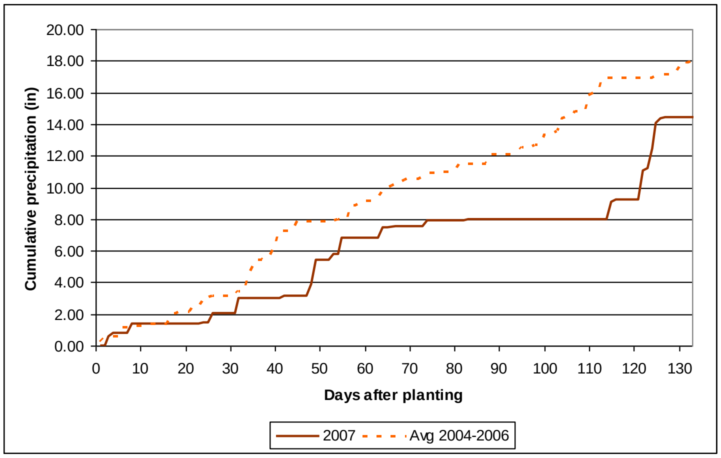

Figure 6. Cumulative precipitation throughout the growing season (Dashed line represents average precipitation for 2004 to 2006. Solid line represents precipitation for 2007).

Table 1. Mean NDVI and CV for N rate and seeding rate treatments. This table contains data for one of the two GreenSeeker® sensors (GreenSeeker#1) and one of the two cotton varieties (DP555). Means with the same letter are not statistically different within a given date (protected LSD (P<0.05)).

Dates	6/27/07		7/12/07		7/23/07	
N Rate (lb/ac)	**NDVI mean**	**CV (%)**	**NDVI mean**	**CV (%)**	**NDVI mean**	**CV (%)**
30	0.6897 a	9.3434 a	0.8429 a	3.5018 a	0.7365 a	5.4876 b
60	0.6882 a	9.2788 a	0.8435 a	3.3836 a	0.7427 a	5.5462 b
90	0.6900 a	8.7168 a	0.8427 a	3.5556 a	0.7411 a	5.7675 ab
120	0.6831 a	8.6943 a	0.8451 a	3.3421 a	0.7388 a	6.4081 a
Seed Rate (seeds/ac)						
16,400	0.6192 b	13.414 a	0.8322 b	5.4206 a	0.7520 a	6.0298 ab
28,700	0.7185 a	7.6926 b	0.8446 ab	2.8424 b	0.7288 b	6.4269 a
50,225	0.7256 a	5.9182 b	0.8538 a	2.0743 b	0.7385 b	4.9503 b

The coefficient of variation helps us to understand the variability in the NDVI readings due to seeding rate. Figure 7 shows graphically the seeding rate effect on CV of NDVI means measured using GreenSeeker#1 and DP143 cotton variety. These results suggest that when the experimental field was planted at a lower seeding rate (lower plant density), more space in between plants was produced, increasing the percentage of the reflectance readings from the soil surface, and therefore increasing the variability in the NDVI readings. Hence, coefficient of variation decreased as seeding rate (plant biomass) increased. However, on July 23rd CV results showed a different behavior. The middle seeding rate (28,700 seeds/ac) showed the higher CV, but it was not significantly different from the lower seeding rate and only a weak significant difference from the higher seeding rate was observed. As the crop matured, a diminishing impact from plant density was observed in NDVI readings. The minimal impact on CV late in the season is likely due to canopy closure within a cotton row, regardless the seeding rate. Similar results were obtained for the other GreenSeeker sensor and for the other cotton variety (DP143) (Appendix 1).

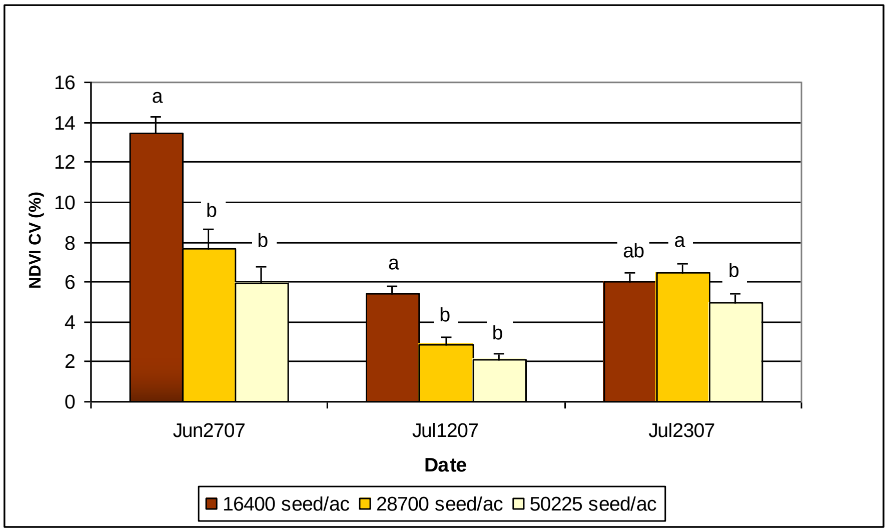

Figure 7. Coefficient of variation (%) of NDVI by seeding rate (seeds/ac) and dates for DP143 cotton variety (Different letter indicates significant difference in NDVI's CV by date (protected LSD (P<0.05))).

Large-scale experimental design 2008

Materials and Methods

To accomplish the objectives proposed and based on the results obtained during the 2007growing season, the experimental design for 2008 was simplified to a Randomized Complete Block Design (RCBD), split–plot, with factorial in the subplots. The entire field was divided into six blocks (Figure 8). Each block was divided into three plots that spanned the length of the field. On May 9th, each plot was planted with one of the three cotton varieties selected to represent three different leaf textures (hairy (DP432), semi-smooth (DP444) and smooth leaves (DP434)) (Figure 9). Cotton varieties were selected according to cotton seed producer specifications. Plots were split into subplots 100 feet in length and eight rows of cotton wide (40 inch row spacing), for an average of 36 subplots per plot. These subplots will be referred to as the experimental units. Experimental units received a randomized factorial combination of seeding rate and N rate. Three seeding rates (16,400, 28,700 and 50,225 seeds/ac) (Figure 10) and four supplemental N rates (0, 30, 60, 90 lb/ac of liquid UAN) (Figure 11) were used. This provided 12 factorial combinations and three replicates per plot.

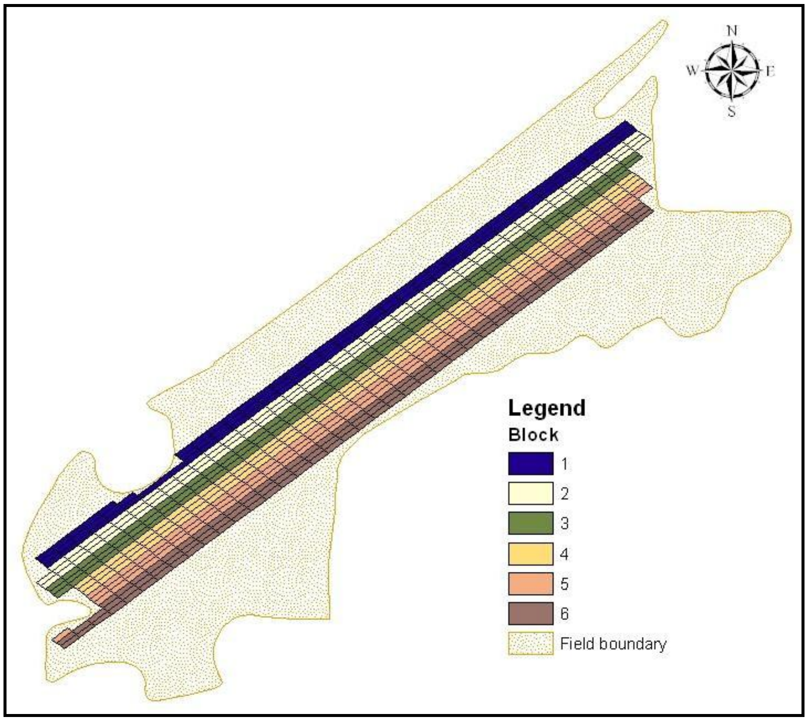

Figure 8. A202 field layout for block. Numbers 1 through 6 represent the six long blocks.

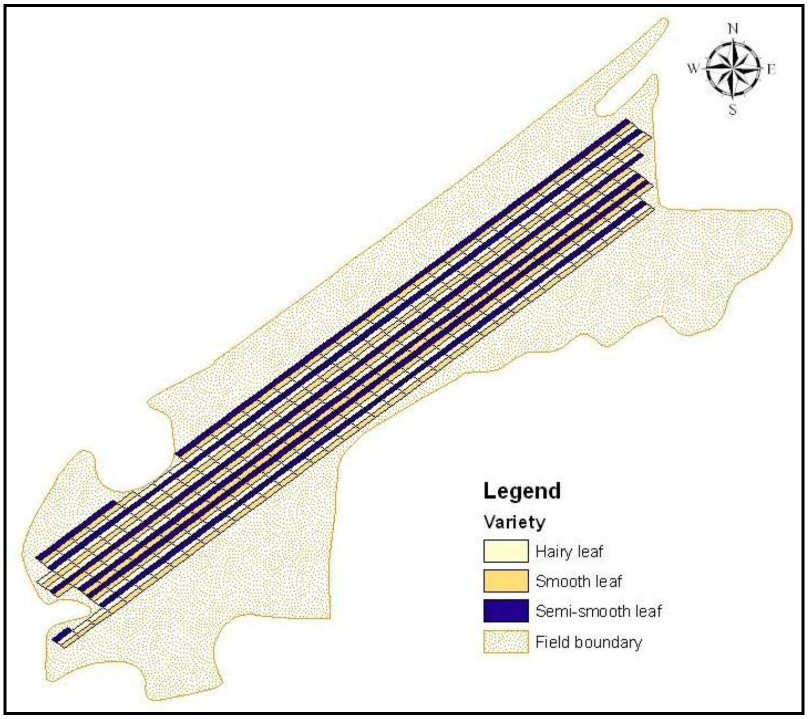

Figure 9. A202 field layout for plant variety (leaf texture - hairy, semi-smooth and smooth leaf).

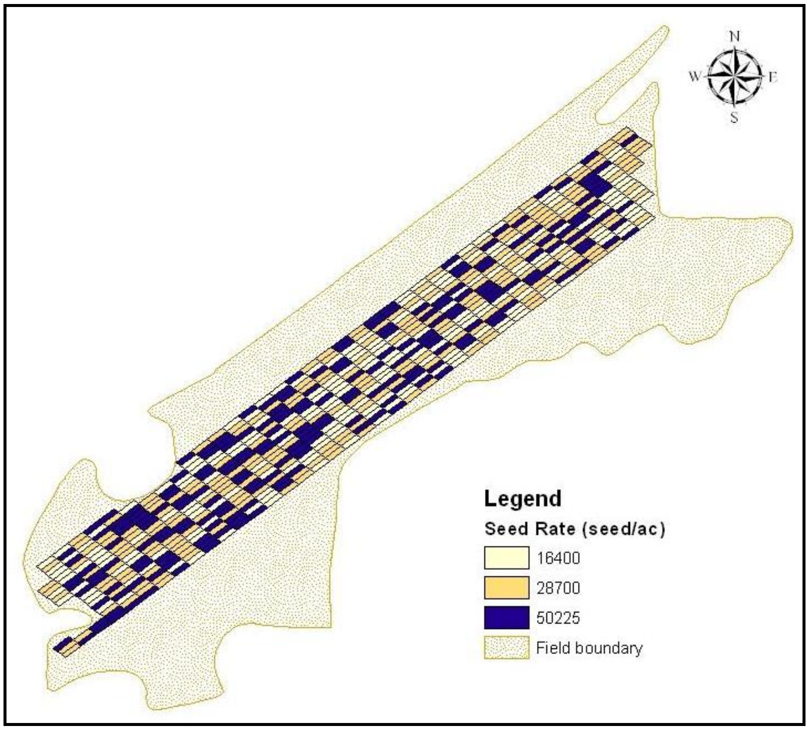

Figure 10. A202 field layout for seeding rate (16,400, 28,700, and 50,225 seeds/ac).

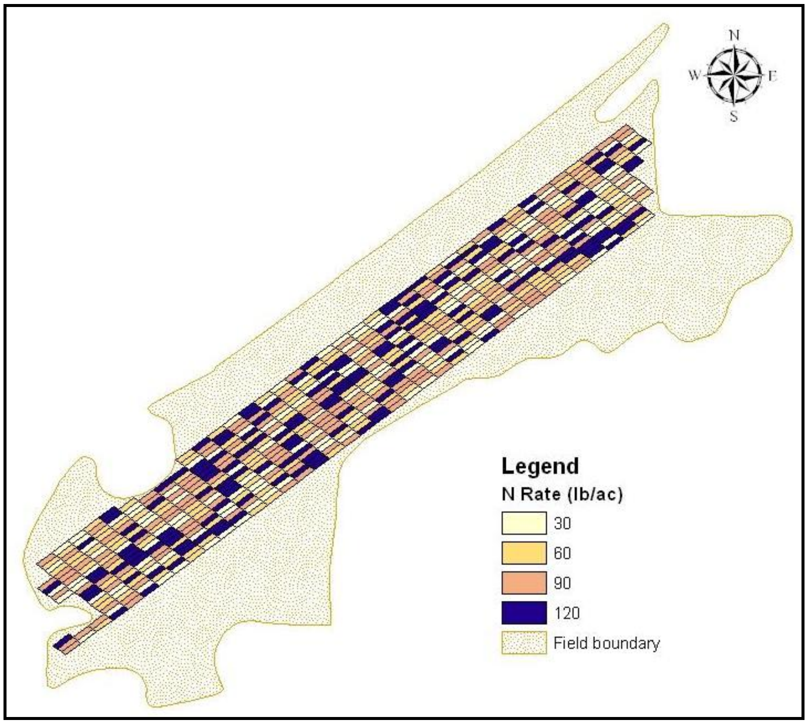

Figure 11. A202 field layout for N rate (30, 60, 90, and 120 lb/ac).

Thirty pounds per acre of N were broadcast over the whole field as pre-plant N. Supplemental N fertilization was applied on July 2nd to each experimental unit such that four levels of N could be obtained (30, 60, 90 and 120 lb/ac). Nitrogen rates were assigned to experimental units in 2008 and the possibility of residual N from 2007 was considered. For instance, if a plot received 120 lbs/ac in 2007, it received 120 lbs/ac for the 2008 growing season. Due to the reduction in the size of the plots from 12 rows in 2007 to 8 rows in 2008, some plots were divided by two of the previous year's N rate. Nitrogen treatments for 2008 were assigned randomly to subplots within these plots.

Field experiment data collection for 2008 was performed using the modified Spirit™ plot sprayer described above. All sensing and GPS equipment was operated following the same protocol used in 2007. An additional ultrasonic distance sensor was added to the rear boom of the modified platform for the 2008 experiments. NDVI was measured using the two GreenSeeker® sensors in the two middle rows of each subplot (Figure 12). Plant height was measured in the same rows using the two ultrasonic height sensors. Data were collected every week of the cotton growing season, from just prior to pin-head square (June 18th) until full bloom (August 5th).

A custom ArcMap script was used to post-process all nine data sets from 2008 and spatially adjust the data points to account for the offset between the sensors and the GPS antenna. As in 2007, ArcMap (ESRI, Redlands, CA) was used prior to statistical analysis to remove data points that fell within ten feet of subplot/treatment boundaries to remove areas where the variable rate planter/nitrogen applicator would be in transition between rates. An average of the NDVI measurements per GreenSeeker® sensor and per subplot was calculated for each of the nine dates. Both GreenSeeker® sensor means were then averaged per subplot and the NDVI mean values were used for statistical analysis. Descriptive univariate statistics and exploratory analysis were performed for every analysis as in 2007. ANOVA and LSD statistical test were conducted using PROC MIXED. Statistical analysis was performed to investigate cotton variety, seeding rate, and N rate effects on NDVI data. Ultrasonic plant height was incorporated as a covariate variable during statistical analysis to address biomass influence on NDVI readings.

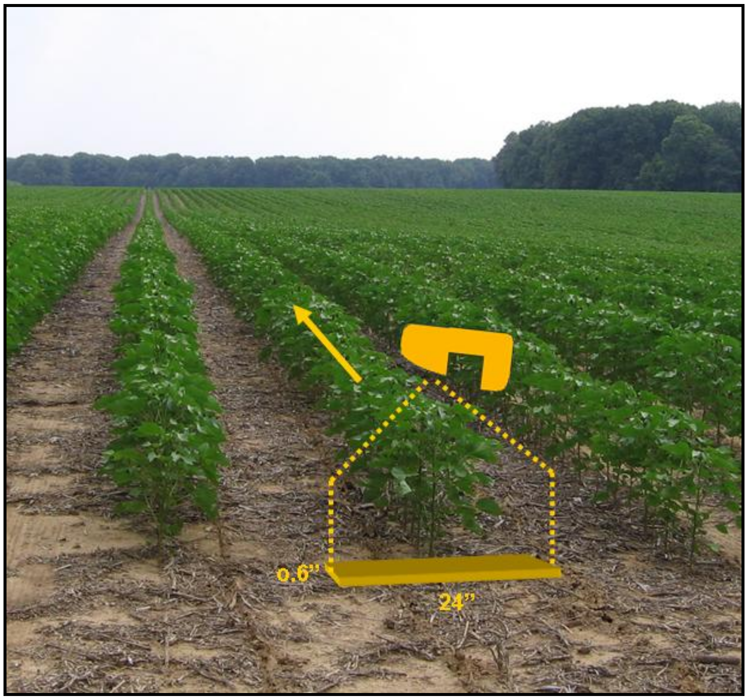

Figure 12. GreenSeeker® sensor followed rows direction when collecting data. Field of view for each measurement from the GreenSeeker® sensor is approximately 24x 0.6 inches.

Results and Discussion

NDVI readings were significantly different by cotton variety throughout the 2008 growing season. Five of the nine sampling dates confirmed that cotton variety had a significant effect on NDVI readings ($P<0.05$) (Figure 13). In general, DP432 (hairy leaf) had the higher NDVI values across the field, while the other two varieties (DP434 and DP444) did not differ from each other. DP444 had the lower value on June 26th and was significantly different from the other two cotton varieties (Table 2).

This result would normally suggest that cotton leaf texture affects NDVI readings, with the hairier leaves having higher NDVI values. However, these differences are more likely due to architectural differences by variety (Pinter et al., 1985). The DP432 cotton variety may have a canopy that spreads more that the other two varieties, resulting in increased canopy coverage within the GreenSeeker®'s field-of-view. In addition, significant plant height differences were found among cotton varieties (results can be found below under

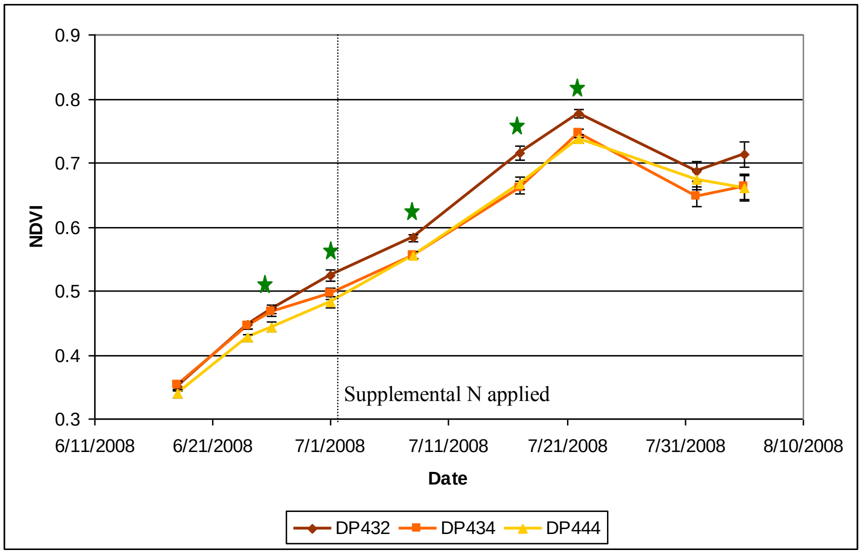

Figure 13. NDVI by variety (DP432, DP434, and DP444) for nine sampling dates (Green stars indicate significant differences in NDVI means (protected LSD (P<0.05))).

plant height experiment analysis). DP432 was significantly shorter than the other two varieties. Although, plant height influence on NDVI readings was controlled using ultrasonic plant height as a covariate variable during the statistical analysis, it could be an indication of certain architectural differences by variety. Moreover, early in the season plants were not large enough to show differences due to variety. Likewise, when full coverage was reached by the canopy of the plants, no variety difference was detected.

Statistical analyses showed that NDVI was significantly different due to seeding rate (16,400, 28,700, and 50,225 seeds/ac) for all nine dates (P<0.05) (Figure 14). The lower seeding rate (16,400 seeds/ac) led to lower NDVI readings, while the higher seeding rate (50,225 seeds/ac) led to higher NDVI readings (Table 2). These results were expected and could be attributed to the influence of soil background noise on NDVI readings, as concluded in the 2007 growing season. The more space in-between plants (lower plant density), the more influence the soil background spectral reflectance would decrease NDVI values. Consequently, a reduction of the effect of soil background spectral reflectance on NDVI readings will attenuate these differences.

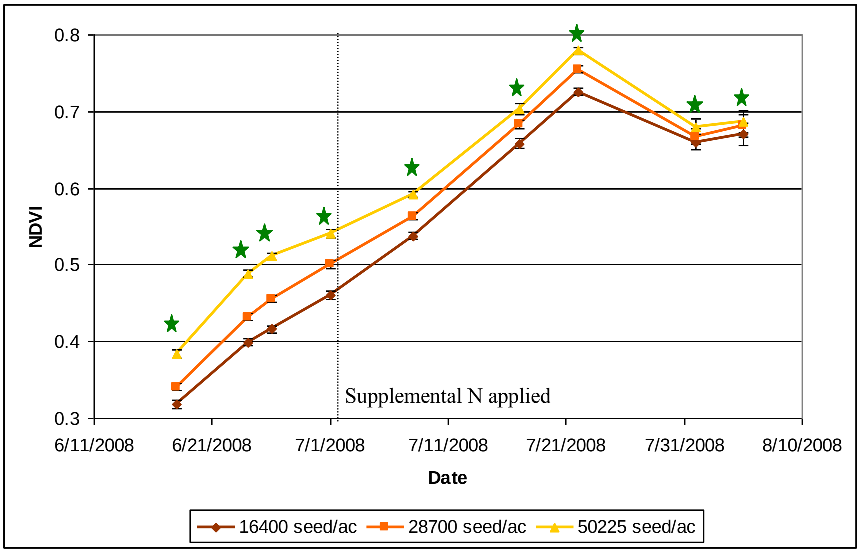

Figure 14. NDVI by seeding rate (16,400, 28,700, and 50,225 seeds/ac) for nine sampling dates (Green stars indicate significant differences in NDVI means (protected LSD (P<0.05)))

Statistical analysis from the nine sampling dates also showed a significant difference in NDVI across the four N rates (30, 60, 90, and 120 lb/ac) applied in the field experiment. Three out of the nine sampling dates showed significant differences (P<0.05) and all three of these days occurred after supplemental N application (Figure 15). The lower N rate (30 lb/ac) led to lower NDVI readings, while the higher N rate (120 lb/ac) led to higher NDVI readings (Table 2). From these results it is reasonable to conclude that no significant residual N from previous year treatments was affecting the cotton crop at early stage. Supplemental N was applied on July 2nd (54 days after planting). The natural delay of plant N uptake plus the lack of precipitation after supplemental N application (the following 10 days no precipitation was registered) could explain why no difference was observed in NDVI readings due to N rate for July 8th sampling date. A slight significant difference was observed later, for July 17th, August 1st, and August 8th sampling dates with F value=3.10, 4.11, and 2.88, respectively.

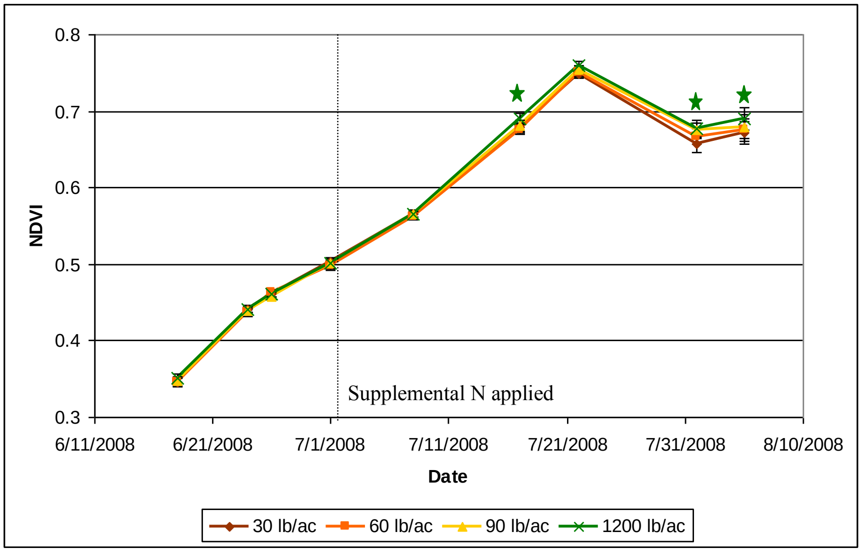

Figure 15. NDVI by N rate (30, 60, 90, and 120 lb/ac) for nine sampling dates (Green stars indicate significant differences in NDVI means (protected LSD (P<0.05))).

Table 2. NDVI means for three cotton varieties, three seeding rates, and four N rates for nine sampling dates. Means with the same letters are not statistically different within a given date and treatment (protected LSD (P<0.05).

Variety		Date								
		06/18/08	06/24/08	06/26/08	07/01/08	07/08/08	07/17/08	07/22/08	08/01/08	08/05/08
DP432	NDVI	0.3506 a	0.4464 a	0.4719 a	0.5240 a	0.5825 a	0.7154 a	0.7760 a	0.6866 a	0.7135 a
	Std Error	0.0069	0.0059	0.0068	0.0088	0.0057	0.0102	0.0066	0.0155	0.0196
DP434	NDVI	0.3522 a	0.4461 a	0.4672 a	0.4953 b	0.5558 b	0.6608 b	0.7470 b	0.6468 a	0.6631 ab
	Std Error	0.0069	0.0059	0.0068	0.0088	0.0057	0.0102	0.0065	0.0156	0.0195
DP444	NDVI	0.3398 a	0.4267 b	0.4438 b	0.4832 b	0.5555 b	0.6681 b	0.7371 b	0.6740 a	0.6612 b
	Std Error	0.0069	0.0060	0.0068	0.0088	0.0058	0.0102	0.0066	0.0155	0.0196
Seeding rate (seeds/ac)										
16400	NDVI	0.3179 c	0.3989 c	0.4160 c	0.4609 c	0.5380 c	0.6578 c	0.7258 c	0.6602 b	0.6697 b
	Std Error	0.0055	0.0043	0.0046	0.0059	0.0041	0.0065	0.0046	0.0103	0.0145
28700	NDVI	0.3409 b	0.4321 b	0.4558 b	0.5006 b	0.5636 b	0.6832 b	0.7552 b	0.6674 b	0.6809 a
	Std Error	0.0053	0.0039	0.0044	0.0058	0.0039	0.0064	0.0044	0.0102	0.0145
50225	NDVI	0.3837 a	0.4883 a	0.5111 a	0.5410 a	0.5922 a	0.7033 a	0.7791 a	0.6798 a	0.6872 a
	Std Error	0.0055	0.0043	0.0046	0.0059	0.0040	0.0064	0.0045	0.0102	0.0145
Nitrogen rate (lb/ac)										
30	NDVI	0.3464 a	0.4419 a	0.4616 a	0.5028 a	0.5664 a	0.6783 b	0.7490 b	0.6571 b	0.6724 b
	Std Error	0.0055	0.0043	0.0046	0.0060	0.0042	0.0067	0.0049	0.0105	0.0147
60	NDVI	0.3456 a	0.4366 a	0.4627 a	0.4987 a	0.5617 a	0.6761 b	0.7503 ab	0.6671 ab	0.6751 b
	Std Error	0.0054	0.0043	0.0046	0.0060	0.0042	0.0066	0.0048	0.0105	0.0147
90	NDVI	0.3471 a	0.4394 a	0.4580 a	0.5012 a	0.5653 a	0.6808 ab	0.7544 ab	0.6747 a	0.6800 ab
	Std Error	0.0054	0.0043	0.0046	0.0060	0.0042	0.0067	0.0049	0.0105	0.0147
120	NDVI	0.3511 a	0.4410 a	0.4616 a	0.5007 a	0.5650 a	0.6906 a	0.7599 a	0.6776 a	0.6896 a
	Std Error	0.0054	0.0043	0.0046	0.0060	0.0042	0.0067	0.00483	0.0105	0.0147

Soil moisture

Materials and Methods

Soil moisture data were collected to investigate the potential impact of water stress on NDVI readings. Thirty six Watermark™ soil moisture sensors (Spectrum Technologies, Inc., Plainfield, IL) (Figure 16) were inserted 12 inches below the soil surface to measure water tension at 36 random locations (Figure17). To obtain quasi-static soil moisture conditions, the Watermark™ sensors were not read within 24 hours after a rainfall event. Soil moisture sensors were measured and recorded four times throughout the growing season in 2007.

Water tension data were gathered from the soil moisture sensors coinciding with the GreenSeekers® NDVI data collection scheduled on June 27th, to be able to investigate possible relationships between water tension and NDVI measurements. Water tension data were compared across the seeding and N rates to study potential differences in water tension due to the treatments. Descriptive univariate statistics and exploratory analysis were performed for each data set using PROC UNIVARIATE. Histograms, box plots, and normality probability plots were used to examine the distribution of NDVI and water tension (kPa) residual values. Normality was tested with Shapiro-Wilk's test. Analysis of variance (ANOVA) and statistical test of least significant difference (LSD) were conducted for each of the four soil moisture sampling dates using PROC MIXED. Statistical analysis was performed using a RIBD to investigate potential seeding and N rates effects on soil-water tension. Ultrasonic plant height was incorporated as a covariate variable during statistical analysis in order to address biomass influence on NDVI readings. Additionally, soil-water tension and NDVI means were correlated using Pearson correlation (r) in PROC CORR, to consider soil-water stress influence on NDVI readings.

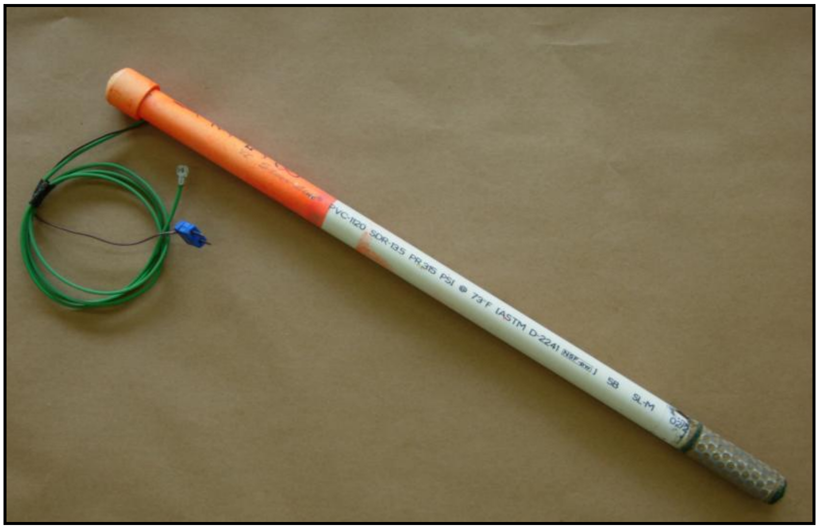

Figure 16. Watermark™ soil moisture sensor. Sensors are positioned ~ 12 inches below the soil surface.

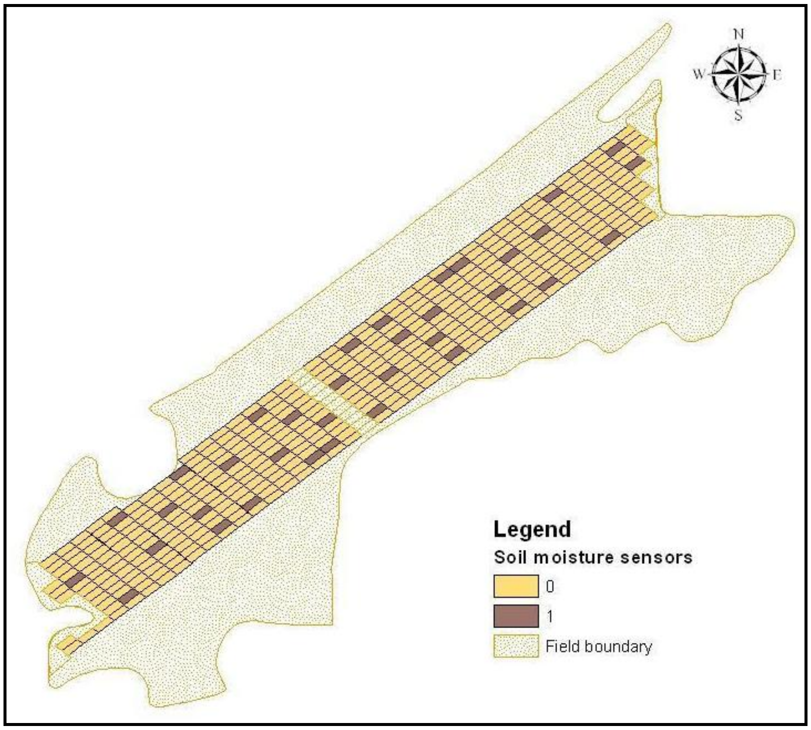

Figure 17. Soil moisture sensors plots for 2007 growing season. Sensors are located in experimental units represented by the number 1.

Results and Discussion

Due to lack of precipitation, soil moisture readings were concluded on August 10th, 2007 (93 days after planting). Soil moisture sensors showed an exponential trend in the water tension during the season (Figure 18). Additionally, soil-water tension was positively correlated (r= 0.66098 P<0.0001) with NDVI mean on June 27th (49 days after planting) (Figure 19). This result suggested that when water is more available to cotton plants, the leaf characteristics are affected in such manner that a lower NDVI is measured. A possible explanation of this unexpected result could be that NDVI response to water tension could be confounded by plant population. As observed in Table 1 (above) on June 27th, lower NDVI means were measured at the lower seeding rate. This could imply a lower demand for water because of the lower plant density; hence lower water tension. However, no significant differences on water tension were observed due to seeding rate on the respective date.

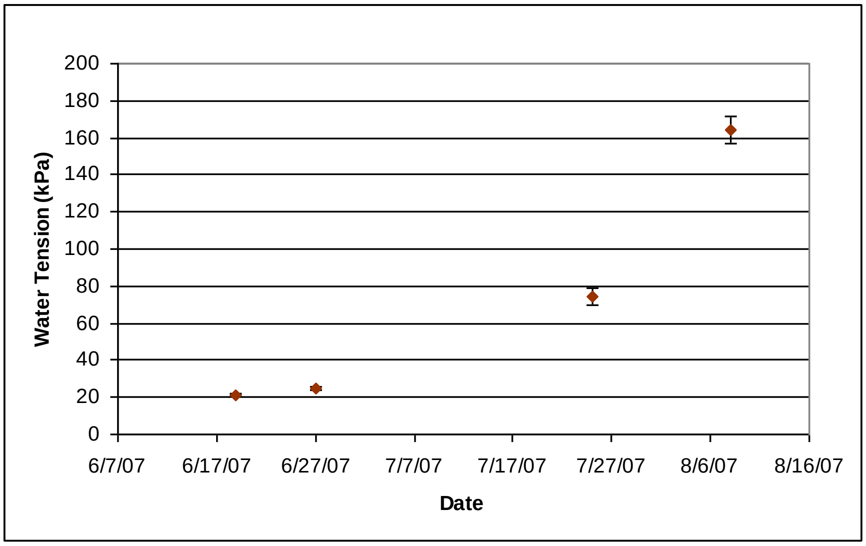

Figure 18. Water tension readings (kPa). Exponential increase of water tension throughout the growing season due to lack of precipitation.

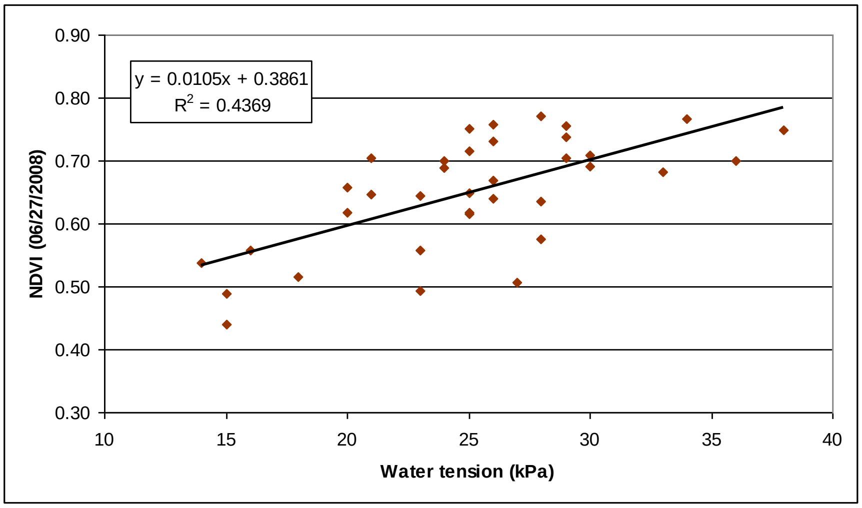

Figure 19. Linear correlation of NDVI and soil-water tension (kPa) from June 27th, 2007.

Leaf N concentration

Materials and Methods

A small scale experiment was designed to compare leaf N concentration and NDVI as predictors of N status in cotton crops. In 2007, leaf N concentration was measured at bloom (August 10th). Twelve plants were randomly selected for sampling from each of 50 experimental units. Recently matured leaves were sampled from the main stem at the fourth leaf below the first quarter-sized leaf of each plant. The 12 leaves were removed, combined, and stored in paper bags identified by experimental unit. Samples were dried in a convection oven at 60˚C for 3 days. Dry leaves were finely ground and analyzed for N concentration using a CE Elantech dry combustion carbon-nitrogen analyzer (CE Elantech, Lakewood, NJ).

From the experimental field in 2008, six subplots were chosen randomly from 2 plots selected from each of the 6 blocks (12 plots in total). Thus, leaf samples were collected from twenty four randomly chosen subplots per cotton variety (DP432, DP434, and DP444), for a total of 72 subplots (Figure 20). Subplots that included more than one of the 2007 treatments were excluded from this selection. Twelve plants were randomly selected from the fourth and fifth rows within each subplot. The fourth leaf below the first quarter-sized leaf from the main stem was sampled. The same protocol used in 2007 for leaf sample collection and

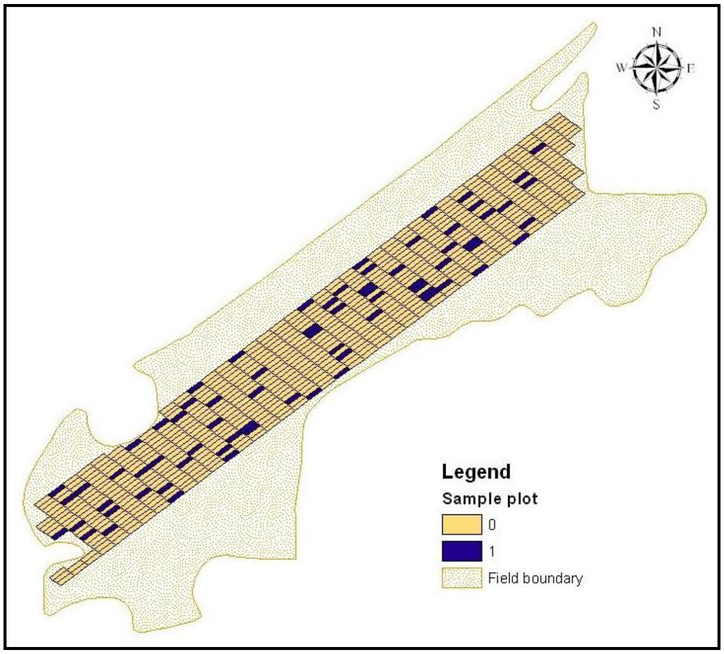

Figure 20. A202 field layout for 72 randomly assigned sampling subplots. Number 1 represents a sampling plot.

analysis was used in 2008. This sampling was repeated twice during the growing season. The first sampling was conducted one day before supplemental N application (pin-head square stage). The second sampling was approximately 30 days after supplemental N application (first bloom stage).

Descriptive univariate statistics and exploratory analysis were performed for each data set with PROC UNIVARIATE. Histograms, box plots, and normality probability plots were used to examine the distribution of NDVI and leaf N concentration (%N) residual values. Normality was tested with Shapiro-Wilk's test. ANOVA and LSD statistical test were conducted for each of the leaf N concentration sampling dates using PROC MIXED. For 2007, statistical analysis was performed by cotton variety (DP143 and DP555) using a RIBD to investigate potential differences on leaf N concentration due to applied N rates. For 2008, a RIBD split-plot with factorial treatment in the subplots was used to study differences on leaf N concentration due to cotton variety, seeding rate, and N rate treatment effects. Not all of the three varieties were present in every block which is what resulted in the incomplete block analysis. Pearson correlation coefficient (r) was calculated among leaf N concentration and NDVI means for every sampling date using PROC CORR.

Results and Discussion

Statistically significant differences in leaf N concentration were found between seeding rate treatment, but no significant differences were found between N rate treatments during the 2007 growing season. At bloom stage, the lower seeding rate (16,400 seeds/ac) showed the higher leaf N concentration, as was expected. However, this trend was not follow by the other two seeding rates (Figure 21). The highest seeding rate (50,225 seeds/ac) showed higher leaf N concentration than the middle seeding rate (28,700 seeds/ac). These results were unexpected and no logical explanation could be found.

Although it is not statistically significant, figure 22 shows that leaf N concentration for the lowest applied N rate (30 lb/ac) followed the expected trend and was lower than the other three rates. However, analysis also showed a slight decrease in leaf N concentration for N rates greater than 60 lb/ac. This suggest that N application higher than 60 lb/ac could be considered as over-fertilization since no additional N-uptake seems to occur in plants at rates higher than 60 lb/ac. This result also tends to agree with the findings of Bell et al. (2003). The critical leaf N value published for that time of the season was 4.1%. The minimum for any treatment in A202 during the 2007 growing season was 5.6%. According to this, there was no N deficient cotton. This helps to explain the lack of difference in NDVI readings across N treatments. The lack of NDVI difference across the field and in the leaf N concentrations could be due to the lack of precipitation and its effect on nitrogen uptake.

Results obtained from the 2008 leaf N concentration experiment showed no significant difference in leaf N concentration due to cotton variety at either the pin-head square or first bloom stages (Figure 23). However, significant differences in leaf N concentration due to seeding rate ($P<0.05$) were observed at pin-head square (53 days after planting). Later in the season significant differences due to seeding rate and N rate treatments ($P<0.05$) were observed at first bloom stage (84 days after planting), as seen in Figure 24 and 25, respectively.

Lower seeding rates showed higher leaf N concentrations at pin-head square (July 1st) as expected. This could be attributed to the plant's nutrient demand from the soil in relationship with plant population. The higher the plant population, the higher the nutrient demand, and the less available N in the soil. No significant differences in leaf N

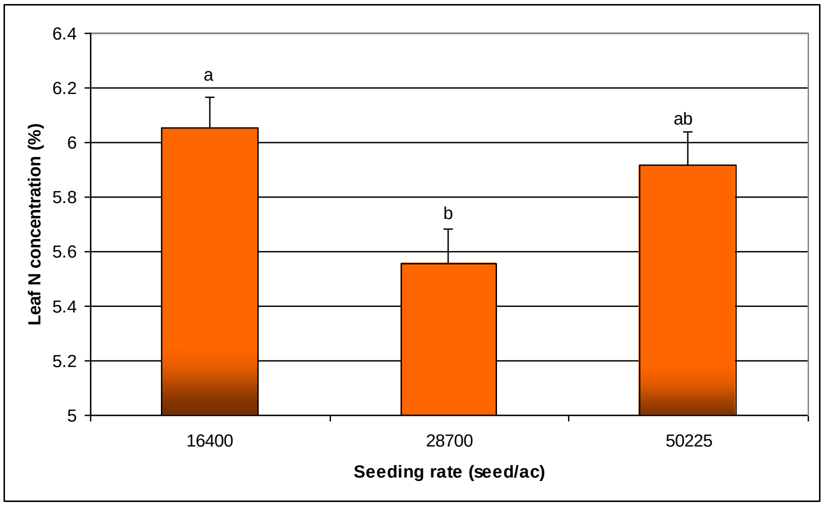

Figure 21. Leaf N concentration (%) by seeding rates (seeds/ac) for 2007 (Different letter indicates significant difference in leaf N concentration by seeding rate (protected LSD (P<0.05))).

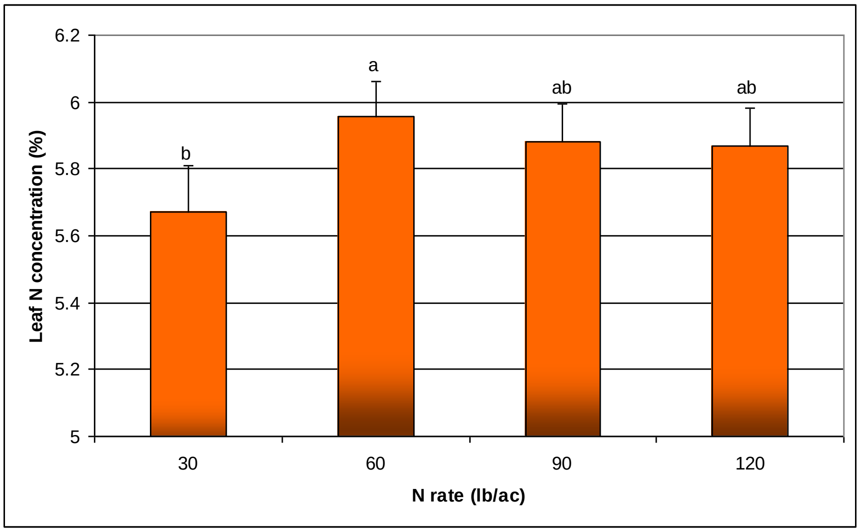

Figure 22. Leaf N concentration (%) by applied N rates (lb/ac) for 2007 (Different letter indicates significant difference in leaf N concentration by N rate (protected LSD (P<0.05))).

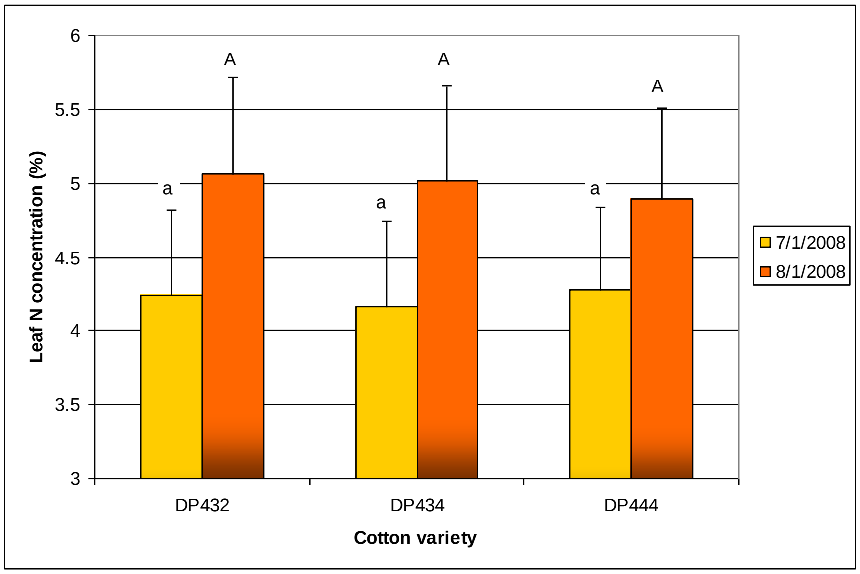

Figure 23. Leaf N concentration (%) by cotton variety (DP432, DP434, and DP444) for both sampling dates (July 1st and August 1st, 2008) (Different letter indicates significant difference in plant height by date (protected LSD (P<0.05))).

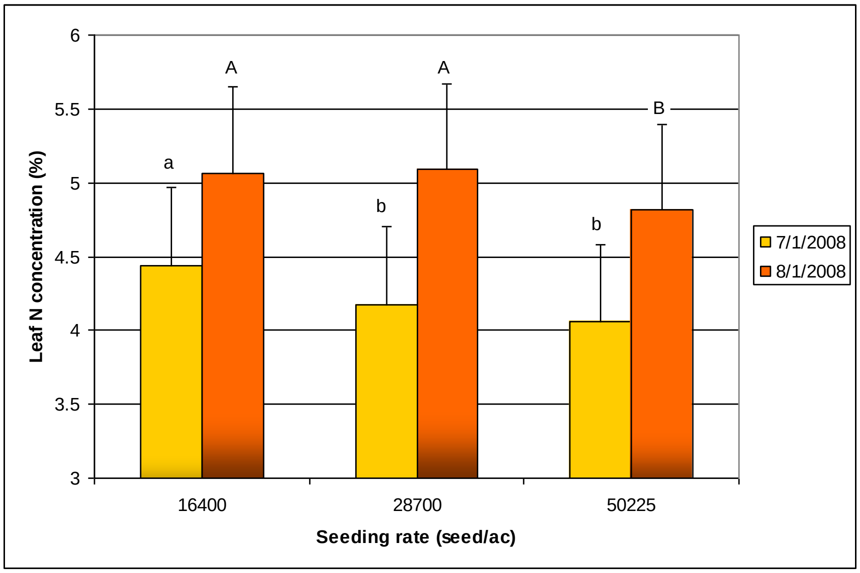

Figure 24. Leaf N concentration (%) by seeding rate (16,400, 28,700, and 50,225 seeds/ac) for both sampling dates (July 1st and August 1st, 2008) (Different letter indicates significant difference in plant height by date (protected LSD (P<0.05))).

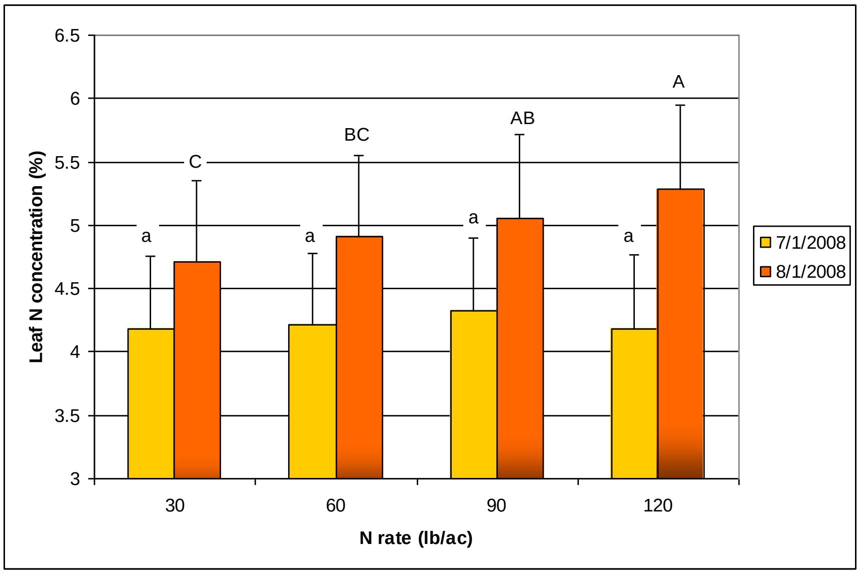

Figure 25. Leaf N concentration (%) by N rate (30, 60, 90, and 120 lb/ac) for both sampling dates (July 1st and August 1st, 2008) (Different letter indicates significant difference in plant height by date (protected LSD ($P<0.05$))).

concentration were observed due to N rate early in the season, which suggests there was no significant variability in residual N availability across the field before supplemental fertilization. Supplemental N was applied one day after first plant sampling across the entire field.

Leaf N concentration showed significant differences due to seeding rate at first bloom stage (August 1st), following the same trend as early season cotton. Higher seeding rates led to lower leaf N concentration. The two lower seeding rates (16,400 and 28,700 seeds/ac) showed the same leaf N concentration values (~5.1%) while the higher seeding rate (50,225 seeds/ac) showed a significantly lower value (4.81%). On the other hand, results showed that higher N rates led to higher leaf N concentration. The study published by Bell et al. (2003) concluded that the critical leaf N concentration value is 4.3% at early bloom. Lower values from this critical leaf N concentration value led to yield losses. All measured leaf N concentrations were greater than the critical concentration for all four supplemental N rates, which suggests that all four supplemental N fertilization rates were adequate to prevent yield loss. Moreover, these results are in concordance with the results obtained from the NDVI measurements from the 2008 growing season. No significant differences due to N rate were observed on NDVI readings from July 1st (before supplemental N application), but significant differences were observed on August 1st. Therefore, from the results we can conclude that GreenSeeker® sensor's have the potential to predict N status in a cotton crop, similar to destructive leaf N sampling techniques. However, no significant correlation was found among NDVI readings and leaf N concentration for July 1st (r= -0.04830, P=0.6870) (Figure 26) and August 1st (r=0.18821, P=0.1134) (Figure 27). On July 1st, the seeding rate effect on NDVI readings was the opposite of the effect on leaf N concentration: higher seeding rate led to higher NDVI readings. This could be attributed to plant density or reduce soil background effects. On August 1st, the seeding rate effect on NDVI readings was similar to the effect on leaf N concentration. However, better correlation between NDVI readings and leaf N concentration would be expected after applying an algorithm to minimize in-between plant spacing noise, which would improve the prediction of plant N status.

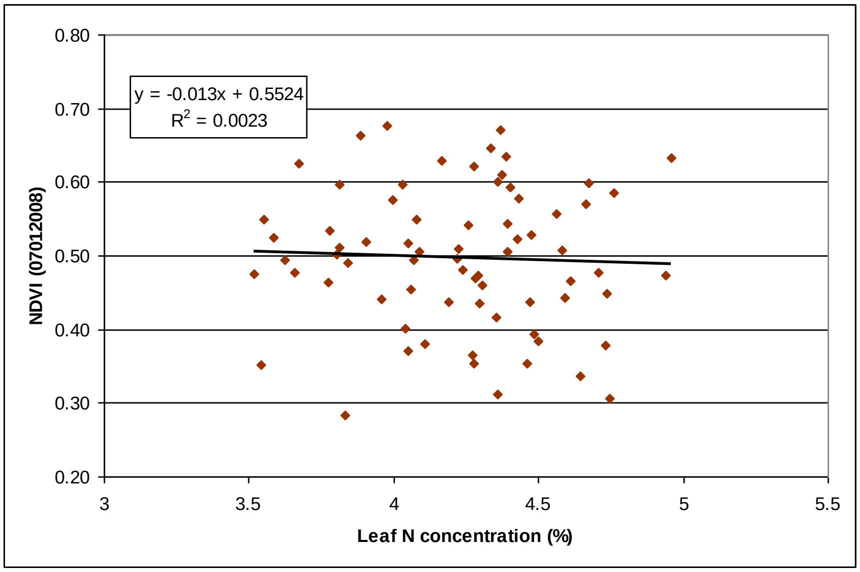

Figure 26. Linear correlation of NDVI and leaf N concentration (%) from July 1st, 2008.

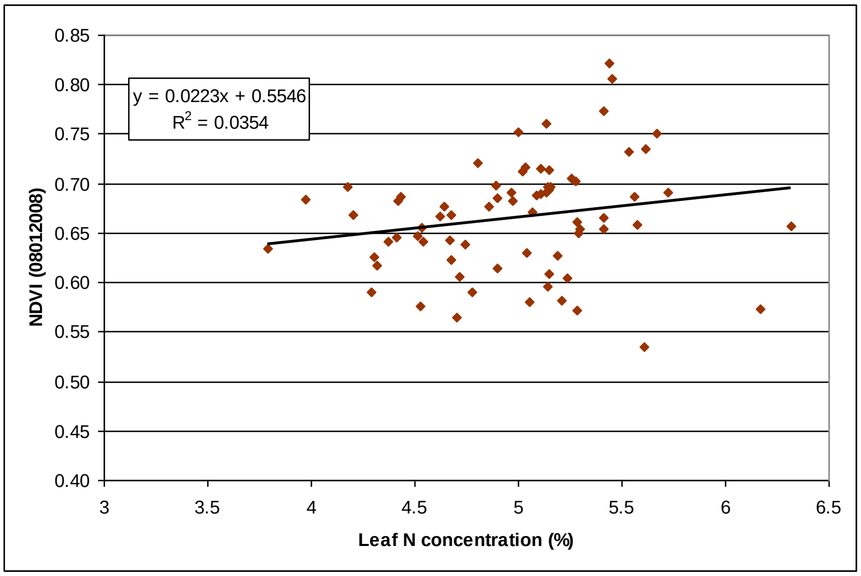

Figure 27. Linear correlation of NDVI and leaf N concentration (%) from August 1st, 2008.

Plant population

Materials and Methods

Plant population was manually counted for 48 experimental units for the 2007 growing season. Experimental units were chosen to cover the three seeding rates (16,400, 28,700, and 50,225 seeds/ac). For 2008, plant population was manually counted on June 9th in the same 72 subplots used for leaf N concentration experiment. These subplots represent the three cotton varieties (DP432, DP434, and DP44), three seeding rates (16400, 28700, and 50225 seeds/ac), and four N rates (30, 60, 90, and 120 lb/ac) used in the large-scale experiment. For both the 2007 and 2008 growing seasons, the number of plants over a 20 foot length of the two center cotton rows within experimental units was manually counted and recorded.

Descriptive univariate statistics and exploratory analysis were performed for each data set with PROC UNIVARIATE. Histograms, box plots, and normality probability plots were used to examine the distribution of NDVI and manually counted plant population residual values. Normality was tested with Shapiro-Wilk's test. ANOVA and LSD statistical test were conducted for both of the manually counted plant population sampling dates using PROC MIXED. For 2007, statistical analysis was performed using a RIBD to investigate potential differences on plant population due to seeding rate. For 2008, a RIBD split-plot with factorial treatment in the subplots was used to study potential differences between seeding rates. The estimated population was scaled and reported on a plants per acre basis.

Results and Discussion

Statistical analysis verified in both seasons three significantly different plant populations. The estimated population was then scaled and reported on a plants per acre basis for every season. In 2007, a plant populations of 16,302, 29,704, and 48,868 plants/ac were obtained from target seeding rates of 16,400, 28,700, and 50,225 seeds/ac, respectively. Similarly in 2008, a plant population of 11,099, 17,096, and 32,534 plants/ac were measured based on the same target seeding rates, respectively. Table 3 shows the significant difference between the three plant populations for both 2007 and 2008 season, as well as, the small difference between the plant count and target seeding rates for the 2007 growing season. The discrepancy between the target seeding rate and the measured plant population in 2008 growing season was due to a planter calibration error.

Table 3. Manually counted plant population (plants/ac) by seeding rate (seeds/ac) for the 2007 and 2008 growing seasons. Different letter indicates significant difference in plant population by seeding rate (protected LSD (P<0.05)).

Target seeding rate (seeds/ac)	Growing season			
	2007		2008	
	Manually counted plant population (seeds/ac)	Std Error	Manually counted plant population (seeds/ac)	Std Error
16,400	16302 c	757.94	11099 c	487.46
28,700	29704 b	836.35	17096 b	593.95
50,225	48868 a	973.57	32534 a	748.09

Plant height

Materials and Methods

Plant height was measured using an ultrasonic distance sensor. Plant height data were collected simultaneously with NDVI readings during the entire 2007 and 2008 growing seasons. The ultrasonic sensor measures the distance from the canopy of the plant to the sensor. Therefore, to obtain plant canopy height values, every data point from the ultrasonic sensor was subtracted from a fixed sensor height (measured from the rear Spirit's boom) to the ground surface. An average of the plant height measurements per ultrasonic sensor was calculated for each experimental unit. During the analysis it was determined that one of the ultrasonic sensors malfunctioned during the 2008 season, so the analyses for 2008 were based on one ultrasonic sensor.

Additionally, plant height was manually measured in both 2007 and 2008 growing seasons, in order to validate ultrasonic distance sensor performance. In 2007, plant height was manually measured at 50 experimental units within the field. Five random points within each selected experimental unit were measured, averaged, and recorded. Manual plant height measurements were made on July 12th (64 days after planting). In 2008, plant height was manually measured from the same 72 sampling subplots used for leaf N concentration experiment. Three random plants per row were measured in each of the two middle rows within each subplot for a total of six samples per subplot. Manual plant height measurements were repeated twice during 2008, once at pin-head square on July 1st (53 days after planting) and again at first bloom on August 1st (84 days after planting). For both 2007 and 2008 datasets, the manual plant height measurements were averaged per experimental unit and the averages were used for statistical analysis.

Descriptive univariate statistics and exploratory analysis were performed for each data set with PROC UNIVARIATE. Histograms, box plots, and normality probability plots were used to examine the distribution of NDVI, ultrasonic and manual plant height residual values. Normality was tested with Shapiro-Wilk's test. A linear correlation was performed between manual and ultrasonic plant height measurements using PROC CORR. Ultrasonic plant height and NDVI means correlation for every sampling date was conducted using Pearson correlation coefficient (r). In 2008, ANOVA and LSD statistical test were conducted for each of the manual plant height sampling dates and each of the ultrasonic plant height sampling dates using PROC MIXED to analyze for differences in manual and ultrasonic plant height measurements due to cotton variety, seeding rate, and N rate treatment effects. Statistical analysis was performed using a RIBD split–plot with factorial treatments in the subplots for manual plant height, and a RCBD split–plot with factorial in the subplots for ultrasonic plant height.

Results and Discussion

During the 2007 growing season, the two plant height measurement techniques (manual and ultrasonic) were highly correlated (r= 0.80821, P<0.0001) (Figure 28). Similarly in 2008, ultrasonic and manual plant height were highly correlated (r= 0.96311, P<0.001) after combining both plant height datasets (Figure 29). The manually measured plant height represents an average of a small number of samples within an experimental unit, measuring the highest tip in the plant. The ultrasonic sensor measurements were averaged for all plant height measurements within an experimental unit. Hence, the two values cannot be directly compared. The intention of this analysis was to illustrate the correlation between both measurements techniques and not to validate the calibration of the ultrasonic distance sensor.

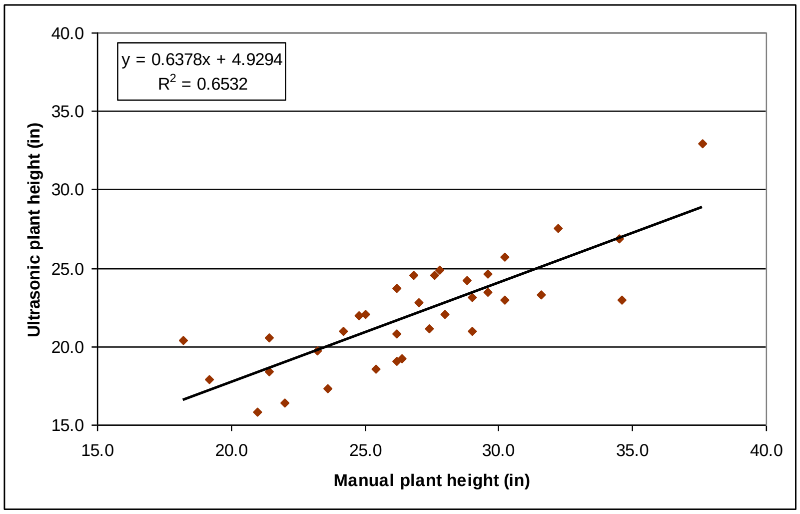

Figure 28. Correlation of ultrasonic and manual plant height (in) measurements sampled on July 12th 2007.

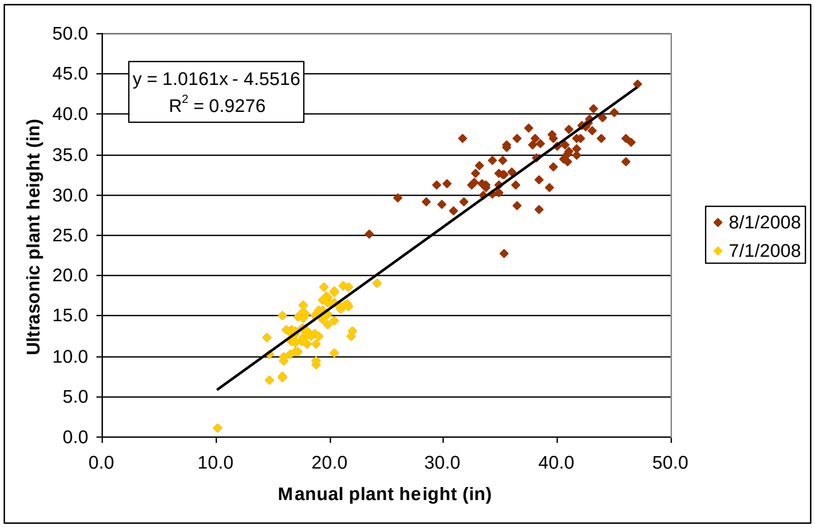

Figure 29. Correlation of ultrasonic and manual plant height (in), including both sampling dates (July 1st and August 1st, 2008).

Plant height measured with the ultrasonic distance sensor was highly correlated with NDVI measurements throughout the 2007 growing season. A strong correlation was observed for all sampling dates for GreenSeeker#1 (r= 0.68884, 0.69007, and 0.90183 (P<0.0001) for June 27th, July 12th, and July 23rd, respectively). Figure 30 represents the correlation between NDVI readings and ultrasonic plant height for July 23rd. Similar results were obtained for GreenSeeker#2 (Appendix 2). In 2008, ultrasonic plant height was strongly positive correlated with NDVI means on eight of the nine sampling dates (r >0.72 and P<0.0001). The other date was moderately correlated (r= 0.52 and P<0.0001). Thus, larger plants led to higher NDVI readings. This result was the motivation to use ultrasonic plant height as a covariate in all NDVI statistical models in order to address plant biomass influence on NDVI readings since plant height is correlated with plant biomass (Freeman et al., 2007). Figure 31 represents the correlation between NDVI readings and ultrasonic plant height for June 18th, 2008 (40 days after planting). Similar results were found for the other data collection dates (Appendix 3).

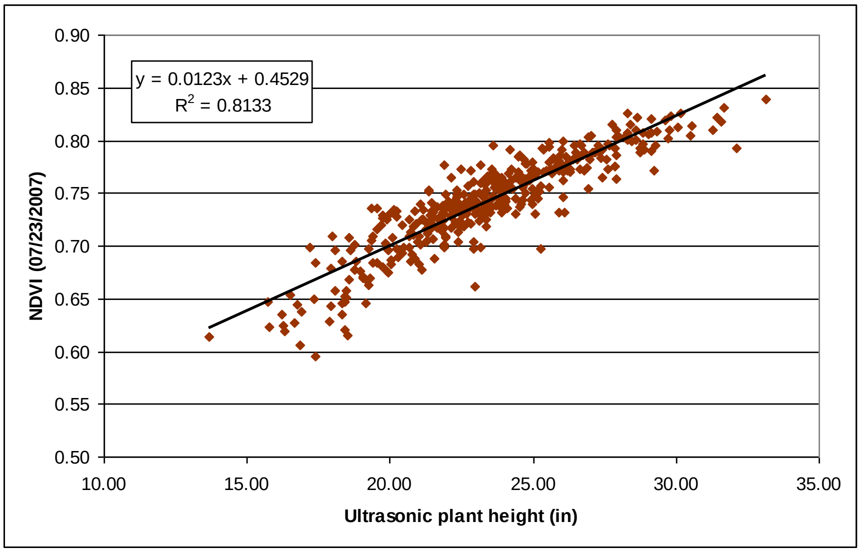

Figure 30. Correlation of NDVI and ultrasonic plant height (in) for July 23rd, 2007.

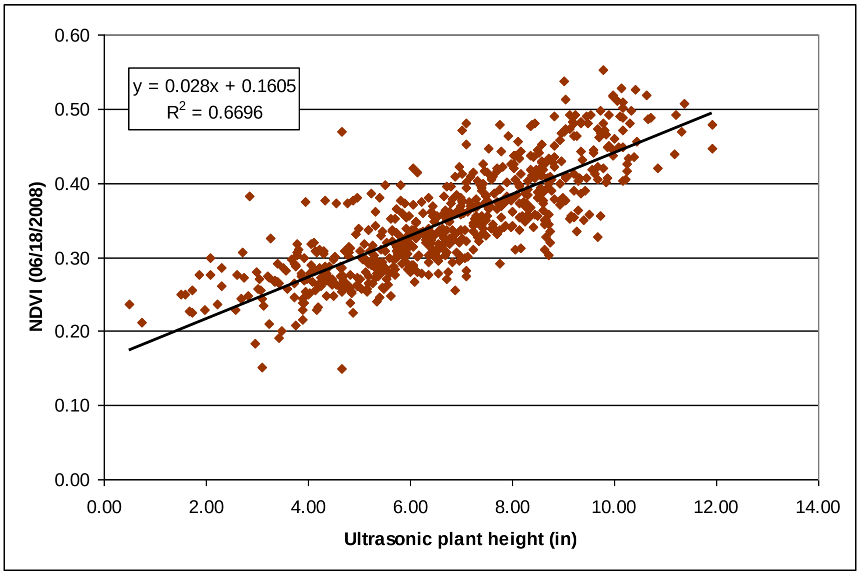

Figure 31. Correlation of NDVI and ultrasonic plant height (in) for June 18th, 2008.

The effects of cotton variety, seeding rate, and N rate on ultrasonic plant height were studied during the 2008 growing season. Plant height (ultrasonic sensor) was significantly affected by cotton variety and seeding rate treatments throughout the growing season, and only affected by N rate treatment late in the season ($P<0.05$). No significant interactions between cotton variety, seeding rate, and N rate treatments were observed.

Nine plant height and NDVI data sets were collected over the course of the 2008 growing season. Plant height for all of these dates significantly differed ($P<0.05$) by cotton variety (Figure 32). The hairy leaf variety (DP432) was shorter than the other two cotton varieties (DP434 and DP444). Variety DP434 (smooth leaf) was the shortest cotton most of the season except at the end when varieties DP434 and DP444 seem to grow approximately to similar heights. These results support the explanation given for differences found on NDVI readings due to cotton variety, crediting these differences to architectural variations.

Eight of the nine sampling dates showed that plant height differed significantly ($P<0.05$) by seeding rate (Figure 33). Results indicated that in the early and middle parts of the season, plants were tallest at the highest plant population (50,225 seeds/ac) and decreased in sizes as the plant population decreased. Later in the season, this relationship was reversed (Appendix 3). Therefore, given that enough nutrient resources were available in the field early in the season, plants were likely competing for sunlight. Plant population would likely affect available light to individual plants. This could cause the plants to grow taller at greater plant densities. Meanwhile, later in the season, the available N becomes the limiting factor and reduces the rate of plant growth.

Finally, in the ultrasonic plant height analysis, the last two dates of the nine sampling dates showed that plant height was significantly affected ($P<0.05$) by N rate (Figure 34). Plant height was shorter at the lower N rate (30 lb/ac) for both dates. The other three N rates did not show any differences. These results support the previous discussion about effect of limited nutrient resources on plant height.

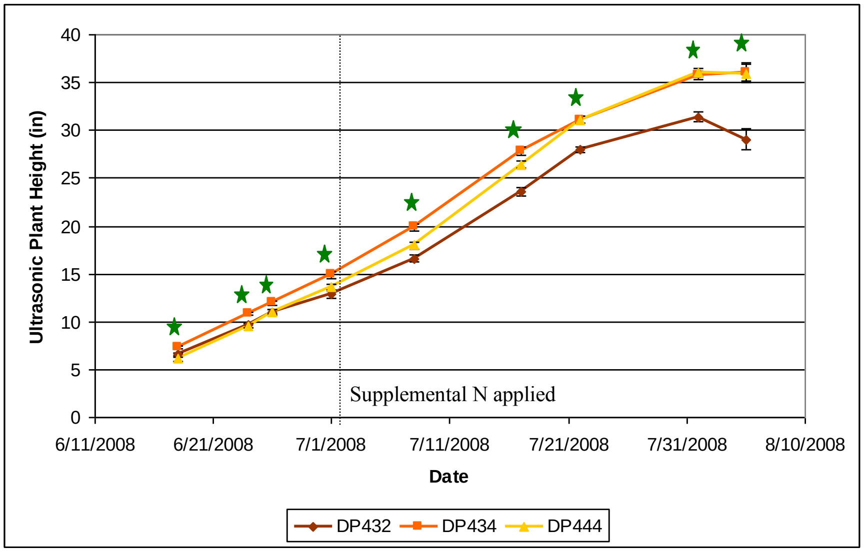

Figure 32. Plant height (in) by variety (DP432, DP434, and DP444) for nine sampling dates (Green stars indicate significant differences in plant height (protected LSD (P<0.05))).

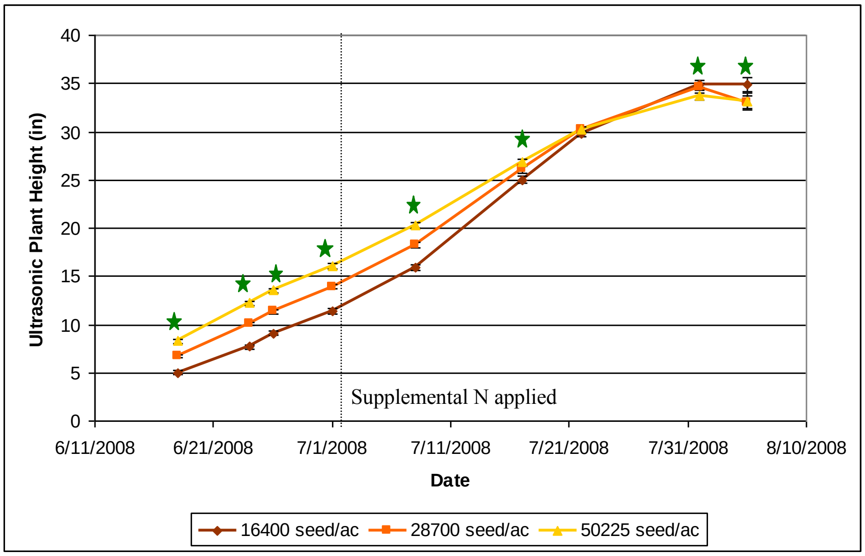

Figure 33. Plant height (in) by seeding rate (16,400, 28,700, and 50,225 seeds/ac) for nine sampling dates (Green stars indicate significant differences in plant height (protected LSD (P<0.05))).

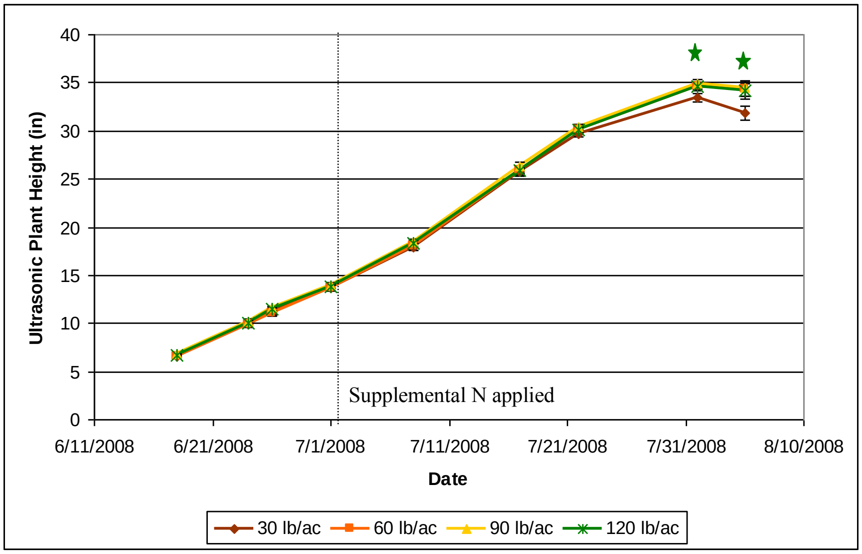

Figure 34. Plant height (in) by nitrogen rate (30, 60, 90, and 120 lb/ac) for nine sampling dates (Green stars indicate significant differences in plant height (protected LSD (P<0.05))).

Manual plant height measurements early in the growing season (53 days after planting) did not significantly differ by cotton variety and seeding rate treatments. However, it was observed that manual plant height differed significantly due to cotton variety (Figure 35) and seeding rate (Figure 36) later in the season (84 days after planting) ($P<0.05$). These results suggest that variety DP432 is comparatively shorter than the other two varieties (DP434 and DP444) at the same maturity stage. Lower seeding rates (16,400 seeds/ac) led to taller plant growth at plant maturity. Figure 37 shows that no significant differences using manual plant height measurements were detected due to N rate either at 53 days after planting or at 84 days after planting (after supplemental N application).

Summary

Throughout the 2007 growing season there was a significant impact of seeding rate on NDVI measurements. During the early season, lower seeding rates led to lower NDVI measurements. The opposite response was observed at the end of the season once the plant canopy closed. Conversely, as the seeding rate increased, the CV of the NDVI means decreased

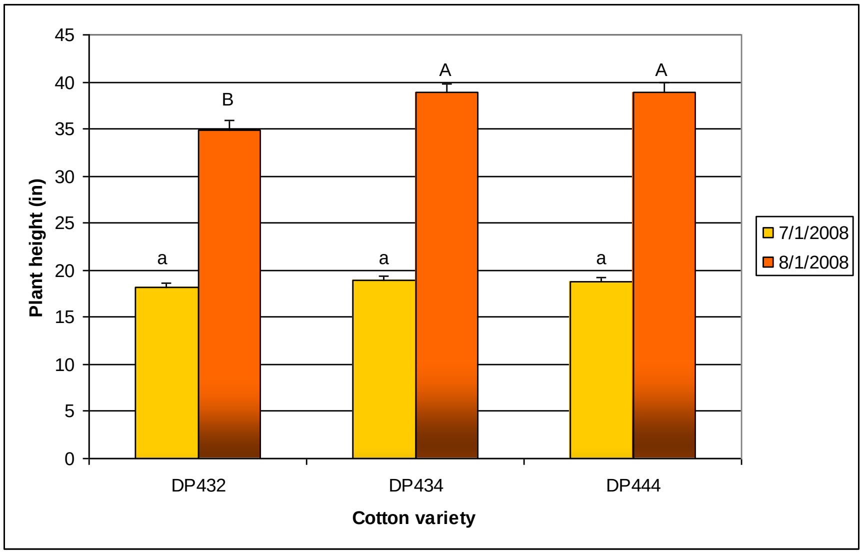

Figure 35. Manual plant height (in) by cotton variety for both sampling dates (July 1st and August 1st, 2008) (Different letter indicates significant difference ($P<0.05$) in plant height by date).

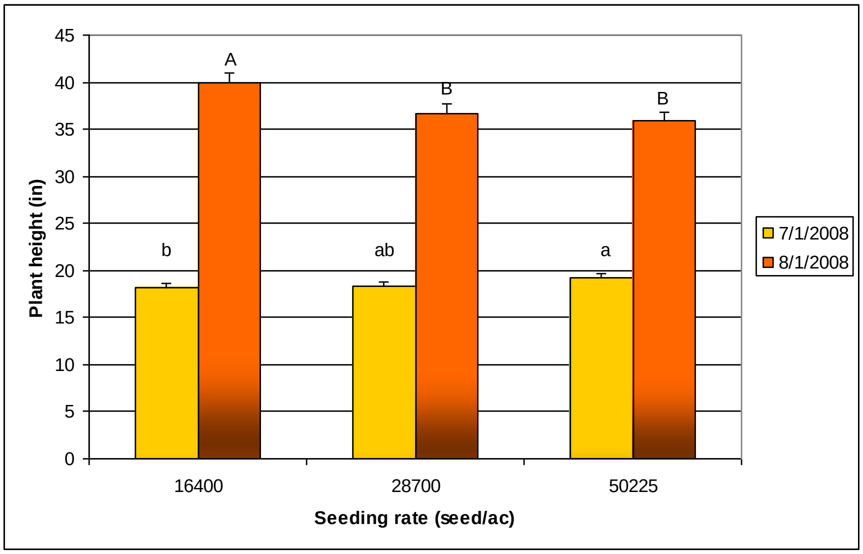

Figure 36. Manual plant height (in) by seeding rate (seeds/ac) for both sampling dates (July 1st and August 1st, 2008) (Different letter indicates significant difference in plant height by date (protected LSD ($P<0.05$))).

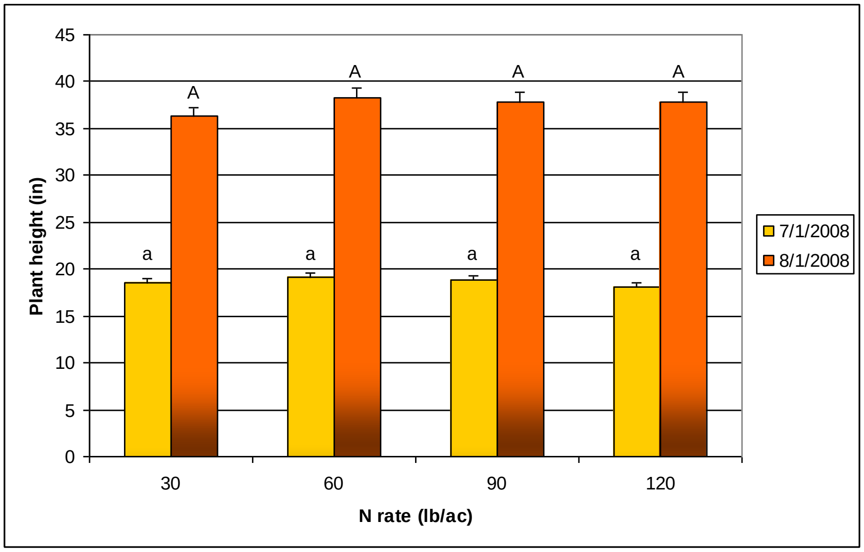

Figure 37. Manual plant height (in) by N rate (lb/ac) for both sampling dates (July 1st and August 1st, 2008) (Different letter indicates significant difference in plant height by date (protected LSD (P<0.05))).

for all sampling dates. Similarly in 2008, data analyses showed significant differences due to seeding rate throughout the growing season. The lower the seeding rates, the lower the NDVI readings. These results suggest that plant population or soil background strongly influences the NDVI measurement mean and CV. Leaf N concentration analysis showed significant differences due to seeding rates before and after supplemental N application in the 2008 season. These differences showed that lower seeding rates led to higher leaf N concentration. These results suggest that plant population influences available N.

No significant difference due to supplemental N rates was observed in NDVI measurements in 2007. Likewise, results showed that different supplemental N rates did not result in significant differences on leaf N concentration. These results were expected for the early-season sampling dates, since fertilizer was applied on July 3rd. But differences were expected later in season (July 23rd) since several authors (Bell et al., 2003; Buscaglia and Varco, 2002; Fridgen and Varco, 2004; Gerik et al., 1994; Zhao et al., 2005) have demonstrated relationship between applied N and leaf N concentration. Only leaf N concentration at 30 lb/ac N rate was significantly lower than the 60lb/ac rate. The lack of differences could be due to the un-seasonably dry conditions of the 2007 growing season as compared to the 2008 season. The soil-water tension increased exponentially over the course of the season until the soil-water tension exceeded 160 kPa, so cotton plants were most likely under moisture stress during the leaf sampling periods. This could have affected N-uptake late in season.

For the 2008 growing season, significant differences were measured in NDVI by N rate later in the season after supplemental N was applied on July 2nd. Lower soil-applied N rates led to lower NDVI readings as expected. These results are in agreement with the results obtained from the leaf N concentration analysis. Results showed significant differences on leaf N concentration caused by N rates only after supplemental N was applied. In this case, lower N rates led into lower leaf N concentrations. Therefore, these results would suggest that GreenSeeker® sensor' NDVI measurements are capable of predicting the mid-season N status in a cotton crop, with results similar to a destructive leaf N analysis.

No leaf N concentration significant differences were observed due to supplemental N application early in the season for both 2007 and 2008. This would suggest that no significant variability in residual N was available across the field before supplemental fertilization was applied.

No significant correlation was found among NDVI measurements and leaf N concentration before and after supplemental N application. This could be attributed to the soil background effect caused by seeding rate on NDVI measurements. NDVI readings were significantly different by cotton variety throughout 2008 growing season. DP432 (hairy leaf) had the higher NDVI values across the field, while the other two varieties (DP434 and DP444) did not differ from each other. These differences are more likely due to architectural differences by variety (Pinter et al., 1985) and not the surface texture of the leaf. The DP432 cotton variety may have a canopy that spreads more than the other varieties, resulting in increased canopy coverage within the GreenSeeker®'s field-of-view. The DP432 variety was significantly shorter than the other two varieties indicating certain architectural differences by variety. Cotton variety differences could be detected in middle season before full canopy coverage was reached. However, leaf N concentration analysis showed no significant differences due to cotton variety.

Plant population (manual count and ultrasonic sensed) was strongly correlated with seeding rates for both 2007 and 2008 growing seasons. Similarly, plant height had a strong positive correlation with NDVI for both seasons. This result agrees with data published by Leon et al. (2003) and Thenkabail et al. (2000) where high correlations between cotton plant height and vegetation indices such as NDVI, NIR/red ratio, and NIR-red difference were found. This result supports the use of plant height as a covariate when analyzing NDVI measurements in order to address the influence of plant biomass on NDVI readings.

Ultrasonic plant height was significantly affected by cotton variety and seeding rate treatment throughout 2008 growing season, and only affected by N rate treatment later in the season. Plants height increased at higher seeding rate relative to the lower seeding rates early in season but decreased late in the growing season. Results suggest that under high plant population densities, plants were likely competing for sunlight resources early in the season when nutrients were not in high demand. Later in season, plants were likely competing for N resources when the demand was greater at higher plant densities. Also, the ultrasonic plant height analysis showed significant differences due to N rate treatment late in season (after August 1st, 2008). These results support the previous discussion about the effect of limited nutrient resources on plant height late in the season. Similarly, no significant differences were found in manual plant height due to cotton variety, seeding rate, or N rate treatments on July 1st, 2008. However, cotton variety and seeding rate did affect manual plant heights on August 1st, 2008.

Finally, correlation analysis showed a high relationship among ultrasonic and manual plant heights. A linear regression where the slope was not one and the intercept was not zero was anticipated since manual plant height was measured at the highest leaf from selected plants within a measurement unit, while the ultrasonic sensor reported the average plant height for the canopy within a measurement unit. Therefore, this analysis was used as a relative comparison between ultrasonic and manual measurements.

Chapter 4 - Investigating the effect of environmental factors on cotton canopy NDVI measurements

Introduction

It is well known that environmental factors can cause inconsistency in NDVI readings. A study published by Pinter (1993) revealed that variations in solar zenith angle, cloudiness, haziness, and environmental conditions affect spectral reflectance. In order to overcome this limitation, sensors that use modulated active light sources have become dominant for real-time crop sensing applications.

The GreenSeeker® is an active-light sensor that uses light emitting diodes (LED) to generate its own red and NIR modulated light sources. Frequency modulation allows the sensor to reject ambient light, reducing the variability from natural illumination. The GreenSeeker® sensor is capable of calculating a number of vegetation indices combining the red and NIR spectral reflectance responses (NTech Industries, Inc. 2007). Ambient conditions could produce changes in cotton leaves affecting NDVI in a period of time as short as a single diurnal cycle. Additionally, water stress by itself could cause changes in cotton leaves, affecting NDVI readings. This chapter will present the results from two small scale experiments conducted during the 2008 growing season with the purpose of addressing effects of diurnal cycle and soil-water availability on GreenSeeker® sensor measurements.

Diurnal cycle

Materials and Methods

A small experiment was performed on July 18th (70 days after planting) to investigate the potential impact of diurnal cycle on leaf spectral reflectance obtained from GreenSeeker® sensors. NDVI and plant height data were collected from three long plots (~ 3600 feet) nine times throughout the day using the sensing equipment described in Chapter 3.

Plots were randomly chosen to represent the three cotton varieties (DP432, DP434, and DP444) used in the experiment (Figure 38). The sensors were positioned over the middle two rows of each sampling plot. The first NDVI measurements began at 8:21 (CST), and continued consecutively for every plot approximately on the hour until the last measurement at 15:58 (CST). NDVI data were always collected traveling in the same direction for each plot and repeated over the course of the day to incorporate different solar radiation angles and plant conditions. NDVI readings were compared across the diurnal cycle to detect and quantify potential differences in spectral reflectance due to environmental effects.

An average of the NDVI measurements per GreenSeeker® sensor, per subplot was calculated for each of the nine sampling periods. Both GreenSeeker® sensor means were then averaged per subplot and the NDVI mean values were used for statistical analysis. An average plant height measurement was made using the ultrasonic sensor over one of the two rows.

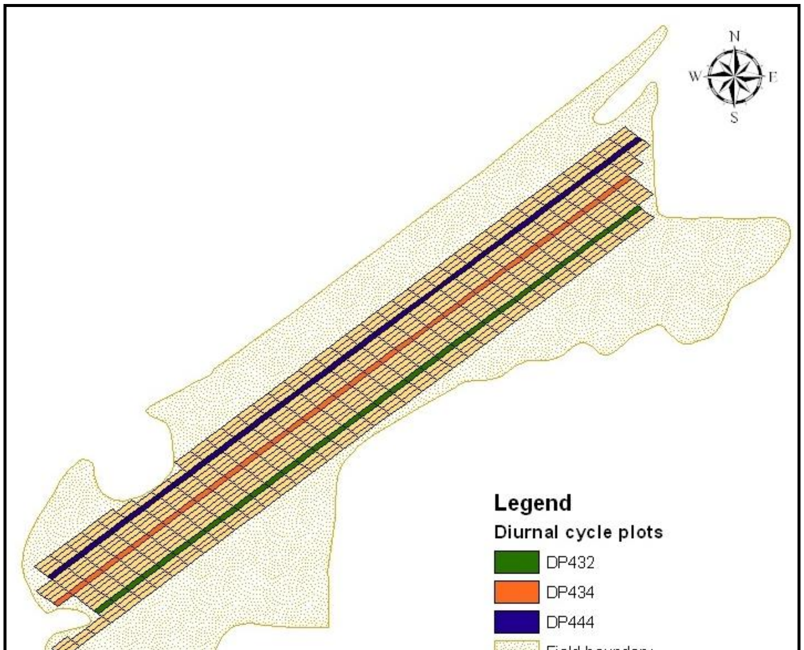

Figure 38. A202 field layout for diurnal cycle sampling plots. One plot for each of the three cotton varieties (DP432, DP434, and DP444).

Descriptive univariate statistics and exploratory analysis were performed using PROC UNIVARIATE. Histograms, box plots, and normality probability plots were used to examine the distribution of NDVI residual values. Normality was tested with Shapiro-Wilk's test. Analysis of variance (ANOVA) and statistical test of least significant difference (LSD) were conducted using PROC MIXED. A randomized block design (RBD) with replication, covariate and repeated measures was used to study diurnal cycle effects on NDVI measurements. Cotton varieties were considered a block effect. Every subplot contained a combination of seeding rate and N rate treatment was considered an experimental unit for this analysis. Three replications of each treatment combination were used in the analysis. Plant height measurements were incorporated as a covariate variable during statistical analysis to address the biomass influence on NDVI readings.

Results and Discussion

Figure 39 shows the time effect on NDVI readings. In the morning, the NDVI was higher and progressively decreased over the course of the day until the lowest value was obtained around 14:00 (CST). NDVI increased again during the later part of the day. Around 13:00 (CST) the NDVI measurements appear to have been affected by an external factor as evidenced by a sudden increase in NDVI. This could be attributed to a possible rapid change in solar radiation (presence of clouds) but no change in the sky condition was noted. The mean NDVI was statistically different between any two consecutive time periods for all periods except the 11:10 – 12:07 periods. Trends in this data suggest that ambient conditions are affecting NDVI measurements using the GreenSeeker®. It could be due to different factors such as water stress, cloudiness, wind, or heat that could be affecting cotton leaf orientation and reflective properties consequently affecting NDVI readings. Therefore, ambient light intensity, solar radiation angle, or weather conditions may still be influencing the GreenSeeker® sensors even though it was designed as an active-light sensor to avoid these effects. These results suggest that attention should be paid to external conditions and lapse of time during data collection with the GreenSeeker® sensors.

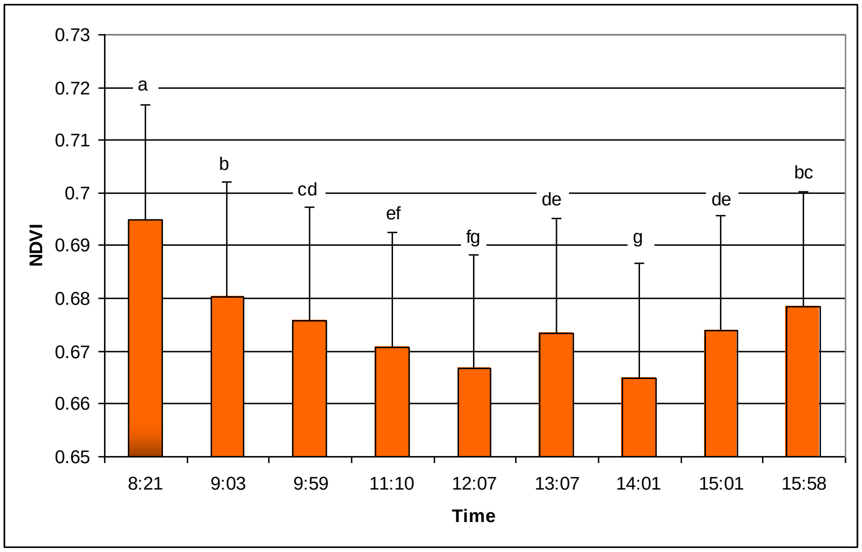

Figure 39. NDVI by time during a diurnal cycle (July 18th, 2008) (Different letter indicates significant difference in NDVI (protected LSD ($P<0.05$))).

Soil moisture

Materials and Methods

Soil moisture data were collected to investigate the potential impact of water stress on NDVI readings. Thirty-six Watermark™ soil moisture sensors (Spectrum Technologies, Inc., Plainfield, IL) were inserted 12 inches below the soil surface (Figure 11 in Chapter 3). Soil moisture sensors were placed in 24 of the 72 intensively monitored subplots. All 24 of these subplots were of a single cotton variety (DP444), but 12 combinations of seeding rate (16,400, 28,700, and 50,225 seeds/ac) and N rate (30, 60, 90, and 120 lb/ac) were represented. The other 12 soil moisture sensors were stratified subjectively in the same cotton variety across the field to account for variability in the field due to field topography and soil type (Figure 40).

Soil-water tension was measured and recorded five times throughout the growing season. Data were not collected within 24 hours of a rainfall event allowing time for the soil-water tension to equilibrate within the Watermark™ sensors. Water tension data were gathered from the soil moisture sensors coinciding with scheduled GreenSeekers® NDVI data collection events.

Descriptive univariate statistics and exploratory analysis were performed using PROC UNIVARIATE. Histograms, box plots, and normality probability plots were used to examine the distribution of NDVI and water tension (kPa) residual values. Normality was tested with Shapiro-Wilk’s test. ANOVA and LSD statistical tests were conducted for each of the five soil moisture sampling dates using PROC MIXED. An incomplete block design (IBD) with factorial treatments was used to study the effect of seeding rate and N rate on soil-water tension. Plots running the length of the field were used as the blocking factor to control variability within the field such that every subplot contained a combination of seeding rate and N rate treatment. Each field-length plot was considered an experimental unit for this analysis. Not all treatment combinations were present in every plot/block resulting in the incomplete block analysis.

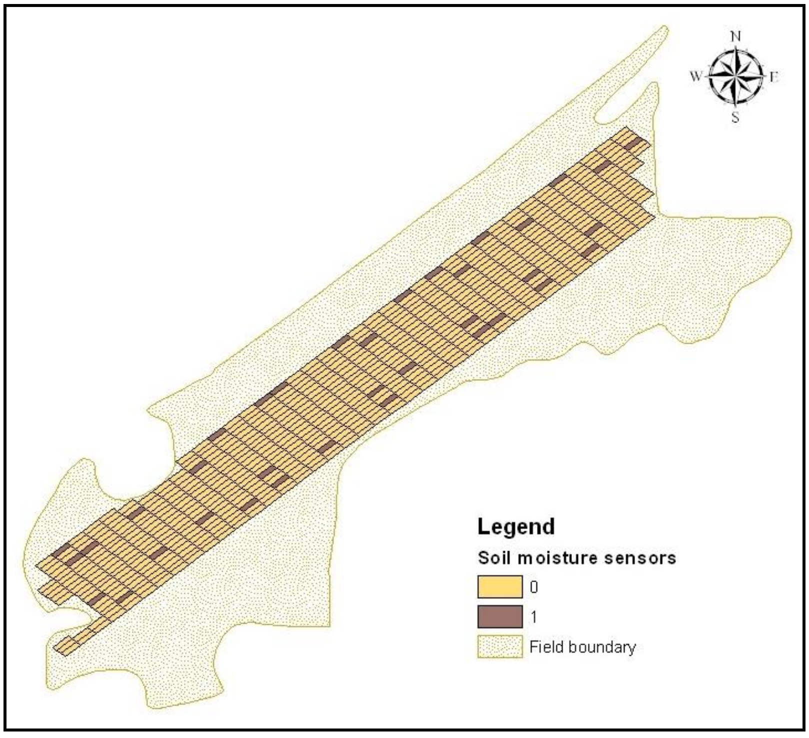

Figure 40. A202 field layout for soil moisture sensors plots. Plots with a number 1 indicate where the sensors were installed.

An average of the NDVI measurements per GreenSeeker® sensor, per subplot was calculated for each of the five sampling times when soil-water tension was measured. Both GreenSeeker® sensor means were then averaged per subplot and the NDVI mean values were used for statistical analysis. Pearson correlation coefficient (r) was calculated for soil-water tension and NDVI means for each of the five time periods using PROC CORR.

Results and Discussion

Water tension was 33 percent lower in 2008 (109kPa, Aug 5th) compared to the same 2007 time period (164kPa, Aug 8th). Figure 41 illustrates the increase in water tension over the course of the growing season. However, total amount of precipitation in 2008 was distributed more uniformly during the growing season than in 2007 (Figure 42). Cumulative precipitation through the growing season in 2008 was always higher than in 2007, although in 2008 cumulative precipitation was 12 inches while it was 14 inches in 2007. Hence, 2008 was expected to be a more representative year for cotton production compared with the previous year.

For most of the 2008 sampling periods, soil-water tension did not differ by seeding rate (Figure 43) or N rate treatments. No significant interaction between seeding rate and N rate treatments was observed with the soil-water tension data. Only on July 17th (69 days after planting) did soil-water tension significantly differ due to seeding rate ($P<0.05$). On this date, soil-water tension was higher where the seeding rate was higher suggesting that the larger plant population negatively impacted available water for plants.

The NDVI measurements were not linearly related to soil-water tension for the two early dates (June 18th and 26th) when plants were small (40 and 50 days after planting, respectively). However, the relationship of soil-water tension and NDVI measurements follow a significant linear trend for the consecutive two dates (July 8th and July 17th with $r=$ 0.60349, and 0.5957, respectively ($P\leq0.001$)), corresponding to 62 and 71 days after planting (Figure 44). Although it is not a strong relationship, NDVI data were positively correlated with soil-water tension on those two dates. For the last date (August 5th or 90 days after planting), no significant linear relationship was found among NDVI readings and soil-water tension (Appendix 4).

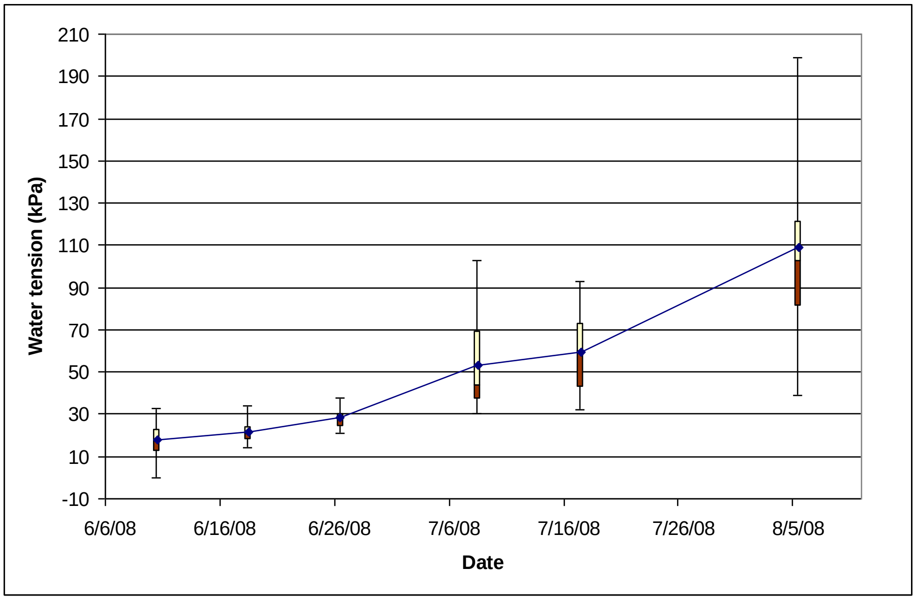

Figure 41. Soil-water tension (kPa) data from 2008 growing season using Watermark™ soil moisture sensors.

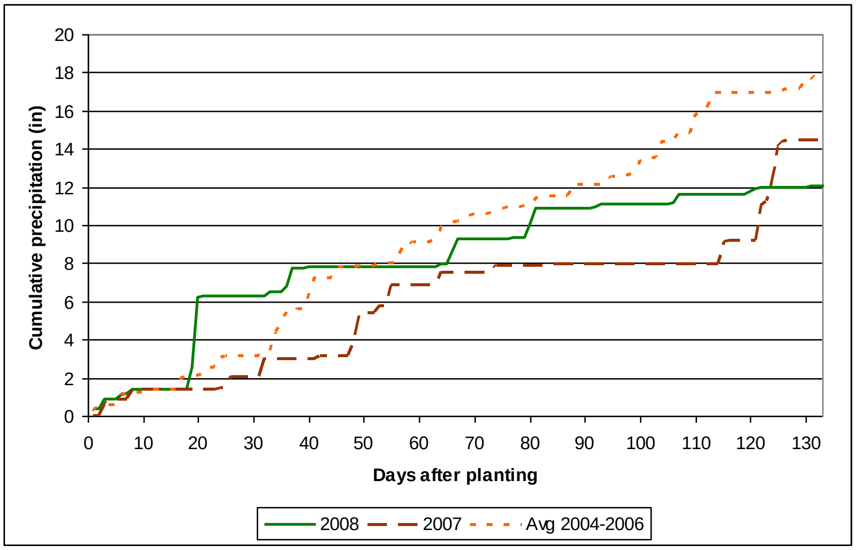

Figure 42. Cumulative precipitation (in) through growing season (Pointed line shows average cumulative precipitation for 2004 to 2006. Dashed line shows cum. prec. for 2007. Solid line shows cum. prec. for 2008).

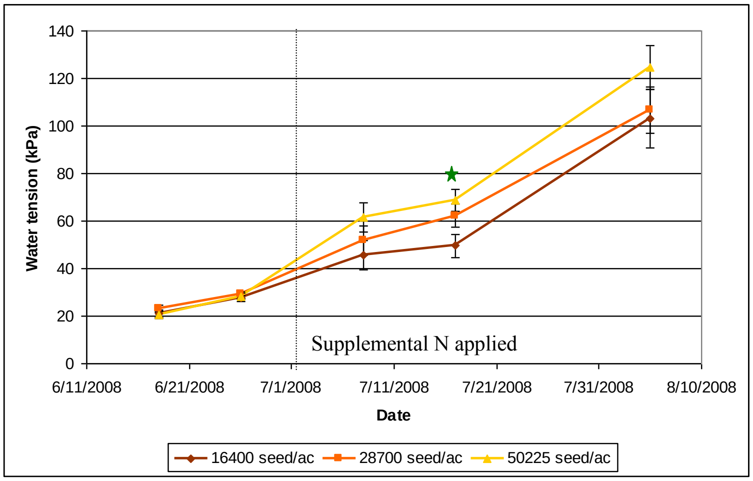

Figure 43. Soil-water tension (kPa) by seeding rate (seeds/ac) for five sampling dates (Green star indicates significant difference in soil-water tension (protected LSD (P<0.05))).

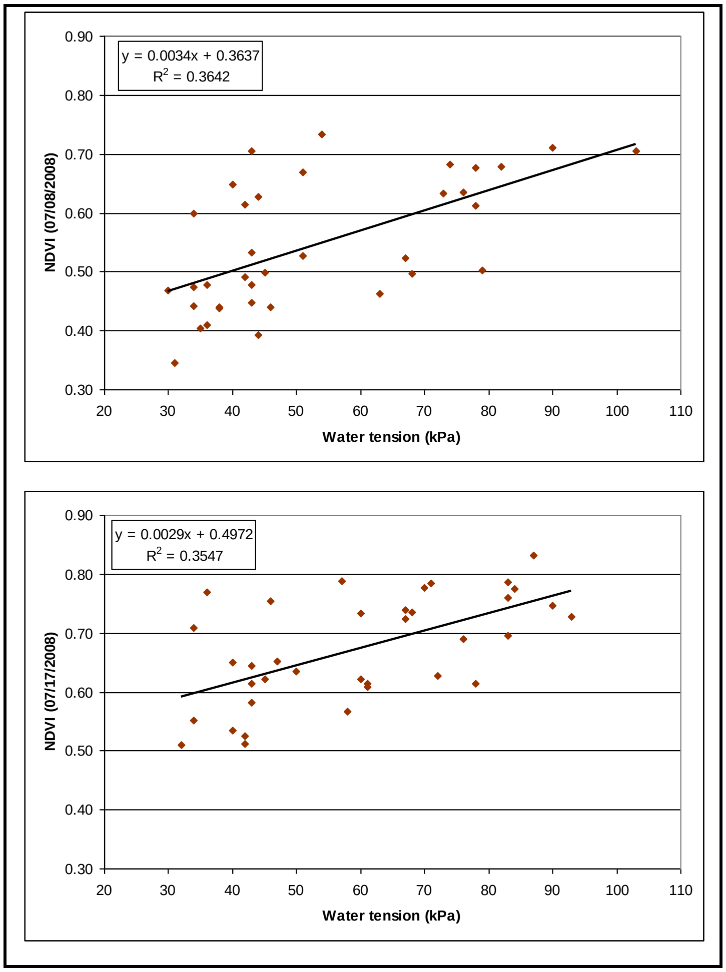

Figure 44. Correlation of NDVI and soil-water tension (kPa) for July 8th and July 17th, 2008 (P<0.001and P=0.001, respectively).

Figure 41 and 42 clearly illustrate that during the period between June 18th and June 26th water tension was low because of the occurrence of precipitation around that period. Little water tension variability across the field was observed on both dates. Therefore, the cotton crop did not show water stress signals early in season that could be detected and correlated with NDVI data. However, water tension and its variability across the field increased for the following two dates (July 8th and July 17th) due to low precipitation accumulation. Approximately 1.5 inches of precipitation was accumulated during that period. The increase in correlation of soilwater tension and NDVI agree with what several researchers (Barnes et al., 2000; Ko et al., 2006; Kostrzewski et al., 2002; Plant et al., 2000) have concluded: NDVI is susceptible to changes in leaves of plants when the crop is under a water stressed condition. Higher soil-water tension led to higher NDVI readings. A possible explanation of this unexpected result could be that plant population could confound NDVI response to soil-water tension. Moreover, lower plant density implies a lower demand of water and hence less water tension. Significant differences on soil-water tension due to seeding rate effect were observed on July 17th where the lower seeding rate led to lower soil-water tension. Conversely, mature cotton plants seemed to be less affected by water stress than younger plants. On August 5th water tension was higher than in the mid-season (July 8th and July 17th), but no correlation of soil-water tension and NDVI was shown in the statistical results. Therefore, users should be aware of crop water stress when NDVI data is used as a predictor of N status in crop, since results could be confounded by external factors other than the actual status of the nutrient in plant.

Summary

Two small scale experiments were conducted in the A202 field during 2008 growing season to address the influence of water stress and diurnal cycle on NDVI measurements. From the results of these experiments it is possible to conclude that during a diurnal cycle the time of sampling affected NDVI measurements. Ambient factors affecting cotton leaves (such as water availability, wind, heat, ambient light intensity, solar radiation angle, or weather conditions) are still affecting GreenSeeker® sensors' NDVI data, even though the active-light design was developed to avoid these effects.

Results suggested that NDVI measurements are susceptible to changes in the leaves of the plants when crop is under water stress conditions. No impact on NDVI measurements was observed early in the growing season when soil-water tension was low. NDVI measurements were only affected by soil-water tension during the middle of the 2008 season, a period of higher soil-water tension. However, the impact of soil-water tension on NDVI readings also seems to be related to crop stage since late in season no significant effect was found even though high soil-water tension was observed. Therefore, GreenSeeker® sensors users should pay attention to external factors, such as ambient conditions and crop water stress, when NDVI data is used as a predictor of plant N status.

Chapter 5 - Developing an algorithm to minimize soil background noise influence on canopy NDVI readings

Introduction

It is well known that variability in soil background spectral reflectance produces noise in canopy NDVI measurements (Huete, 1987; Huete et al., 1985). This noise will vary depending on the design and performance of the remote sensor itself, as well as the architecture of the plants (Pinter et al., 1985). The GreenSeeker® sensor is a commercially available ground-based active light remote sensing device with a field of view of 24 in. ± 4 in. wide by 0.6 in. ± 0.2 in. long in the direction of travel (NTech Industries Inc, (2007)). This sensor generates 1000 measurements/sec of spectral reflectance response. These measurements are averaged and reported at a user-specified rate (>10 Hz). Due to this averaging, canopy gaps are integrated with canopy reflectance and reported as a composite NDVI measurement. These reported measurements appear as lower NDVI values as compared to readings from 100 percent canopy coverage. Thus, these points have been influenced by the soil background noise. Thorp and Tian (2004) concluded that NDVI is the most used vegetation index as a predictor of plant N status. The aim of this chapter is to investigate the impact of between-plant soil background noise on NDVI readings and develop an algorithm to minimize such noise to be able to predict N status in cotton crops.

Manipulating plant spacing

Plant spacing was manipulated in twelve random subplots within the 28,700 seeds/ac seeding rate. Manipulation of plant spacing was made to accentuate between-plant spacing to be able to discriminate between canopy and soil background spectral reflectance.

Four NDVI data collection and plant removal passes were performed in the two middle rows of every subplot. The GreenSeeker® sensors were set to report a data point every 1/10 sec (average of 100 readings). The ground speed of the Spirit™ platform was maintained at approximately 0.3 mile per hour over 20 foot length to increase the spatial density of the data points (~2 samples per inch). The first NDVI collection pass over these subplots was made prior to any in-row plant manipulation. The plant population was then incrementally reduced and NDVI measurements repeated until only 25 percent of the plants remained. Plant removal consisted of pulling one plant every "fourth" plant, and then every "third" plant and so on (Figure 45). NDVI data collection was repeated after each plant population reduction. Plant populations were thinned three times for a total of four NDVI measurements per subplot. So, pass one corresponded to NDVI measurements with the entire plant population intact for each subplot. Pass two corresponded to NDVI measurements after removing every fourth plant in each subplot and so on for pass three and pass four. The aim was to manage the NDVI means from passes two, three, and four to be similar to or at least reduce differences between the NDVI mean of pass one. The assumption was that measurements collected with the whole plant population would represent the "real" NDVI value at which we wanted to approach. Coming near to this "real" NDVI value implies that plant spacing noise generated by plant removal was minimized by a post-processing algorithm. Descriptive univariate statistics and exploratory analysis were performed with PROC UNIVARIATE.

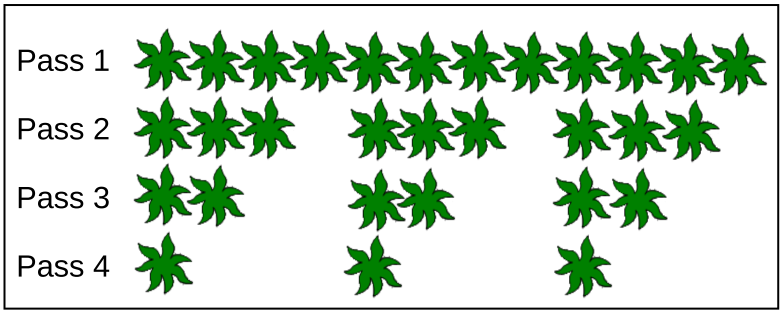

Figure 45. Plant removal by pass. Empty spaces indicate plant removal.

Developing algorithms

Materials and Methods

Several approaches were pursed in order to minimize the between-plant background effect using the spectral reflectance data collected over the manipulated plant spacing rows. To analyze the impact of soil background from in-row plant removal, NDVI data was collected before and after plant removal and analyzed using ANOVA and LSD using PROC MIXED. A randomized complete block design (RBD) with variety and N rate treatment combinations as blocks were used to study vegetation indices or wavelength ratios differences among the four passes.

The first approach attempted to minimize differences among the four passes was the analysis of wavelength ratios. These ratios were considered because of their simplicity. They were defined as:

- The ratio of red and NIR wavelengths: $RR = \frac{R_{red}}{R_{NIR}}$
- The normalized red and NIR ratio: $NRR = \frac{R_{red}}{(R_{NIR} + R_{red})}$
- The normalized NIR and red ratio: $NNR = \frac{R_{NIR}}{(R_{NIR} + R_{red})}$

where R_{red} and R_{NIR} are the red and NIR wavelength readings taken with the GreenSeeker® sensors. After been analyzed, NRR and NNR ratios offered the greater minimization of difference among the four passes; however, results were not satisfactory.

The second approach was the analysis of the optimal soil adjusted vegetation index (OSAVI) introduced by Rondeaux et al. (1996):

$$OSAVI = \frac{R_{NIR} - R_{red}}{R_{NIR} + R_{red} + 0.16}$$

OSAVI was chosen from the previously defined indices because it is recommended for low and high vegetation cover and it is calculated by the GreenSeeker® sensor’s internal algorithm. The resulting data showed a reduction in the differences among passes, although better results were expected.

The third approach shifted NDVI values using the standard deviation of manipulated plant spacing data. NDVI values within a window of 1.2 inches were grouped (2 or 3 points) and their mean and standard deviation (SD) were calculated for each subplot. The window of 1.2 inches was chosen in order to cover at least 2 fields-of-view of the GreenSeeker® sensor. Then, a revised NDVI value (*NDVI')* was calculated using the mean and SD as follow: $NDVI' = \overline{NDVI} + a * SD_{NDVI}$, where a is the number of SD's used in the analysis ($a = 1$ or 2). *NDVI'* was analyzed and compared using the models and statistical tests stated above. Results were not satisfactory and the approach was discarded.

The fourth approach trimmed NDVI values lower than a*SD from the mean of the manipulated plant spacing data. The standard deviation was calculated by subplot and then all NDVI values lower than one SD were trimmed from the dataset. The same procedure was repeated for NDVI values lower than two SDs. The resulting datasets were analyzed using the model and statistical tests stated above. NDVI measurements trimmed by 2*SD showed better results than NDVI trimmed by 1*SD or any of the previous approaches.

To this point OSAVI and trimming NDVI data values lower than 2*SD approaches provided the best results at reducing or minimizing differences among manipulated plant spacing passes due to soil background noise. For this reason a combination of both algorithms was pursued as a fifth approach. OSAVI standard deviation was calculated by subplot. Then, OSAVI values lower than 2*SD were deleted from the dataset. Statistical analysis of this modified data set indicated satisfactory minimization of the differences among the four manipulated plant spacing passes.

The sixth approach applied to the data trimmed all NDVI values below a threshold of 0.4 (*Note*: this 0.4 threshold set subjectively will vary greatly depending on plant's growth stage). NDVI values lower than 0.4 were trimmed because it was assumed that values lower than 0.4 were highly influenced by soil background spectral reflectance. The maximum NDVI value was then chosen from a moving window of 10 consecutive data points. These NDVI values represented the new dataset called "maximum canopy values". "Maximum canopy values" dataset was analyzed using the model and statistical test described above. Table 4 shows the six different approaches pursed in order to minimize the between-plant background effect using the manipulated plant spacing spectral reflectance.

Table 4. Approaches pursed to minimize between-plant background noise on plant canopy reflectance from the 12 subplots with manipulated plant spacing.

Approach	Ratio or Vegetation index	Description
1st	$RR = \frac{R_{red}}{R_{NIR}}$ $NRR = \frac{R_{red}}{(R_{NIR} + R_{red})}$ $NNR = \frac{R_{NIR}}{(R_{NIR} + R_{red})}$	Red and NIR ratio. Normalized Red and NIR ratio. Normalized NIR and Red ratio.
2nd	$OSAVI = \frac{R_{NIR} - R_{red}}{R_{NIR} + R_{red} + 0.16}$	Optimal soil adjusted vegetation index.
3rd	$NDVI' = \overline{NDVI} + a * SD_{NDVI}$	NDVI mean plus one or two standard deviations.
4th	*NDVI trimmed a*SD*	Trim NDVI values lower than one or two standard deviations.
5th	*OSAVI trimmed 2*SD*	Trim OSAVI values lower than two standard deviations.
6th	*NDVI "maximum canopy values"*	Trim NDVI values lower than 0.4, then choose maximum NDVI values from a window of 10 consecutive points.

Results and Discussion

Figure 46 shows the four NDVI data collection passes from one of the twelve subplots sampled. Statistical analysis was conducted on manipulated plant spacing datasets to identify the differences in NDVI measurements among the four passes. Analysis was first conducted before applying any adjustment algorithm to the data to provide a baseline for comparison. Significant differences in NDVI values were found between each of the four passes (F value=252.73, $P<0.05$) and NDVI values decreased by approximately 0.1 each time plants were removed (Figure 47). NDVI differences between passes are attributed to between-plant soil background noise since only plant population was modified at this point and plant spectral characteristics were not altered.

No significant improvement in reducing difference was observed using the RR ratio as compared to NDVI values. A statistically significant reduction in the difference between the four passes was observed when the NRR and NNR ratios were used instead of NDVI values. NRR and NNR differences were approximately 0.05 between passes (F value=253.01, $P<0.05$) (Figure 48). Statistical analysis of the OSAVI data revealed a reduction in the difference among the passes of approximately 0.07 (F value=207.19, P<0.05), which is similar to those observed with the NRR and NNR ratios (Figure 48). The advantage of OSAVI over the NRR and NNR ratios is that the GreenSeeker® sensor' internal algorithm already calculates this vegetation index.

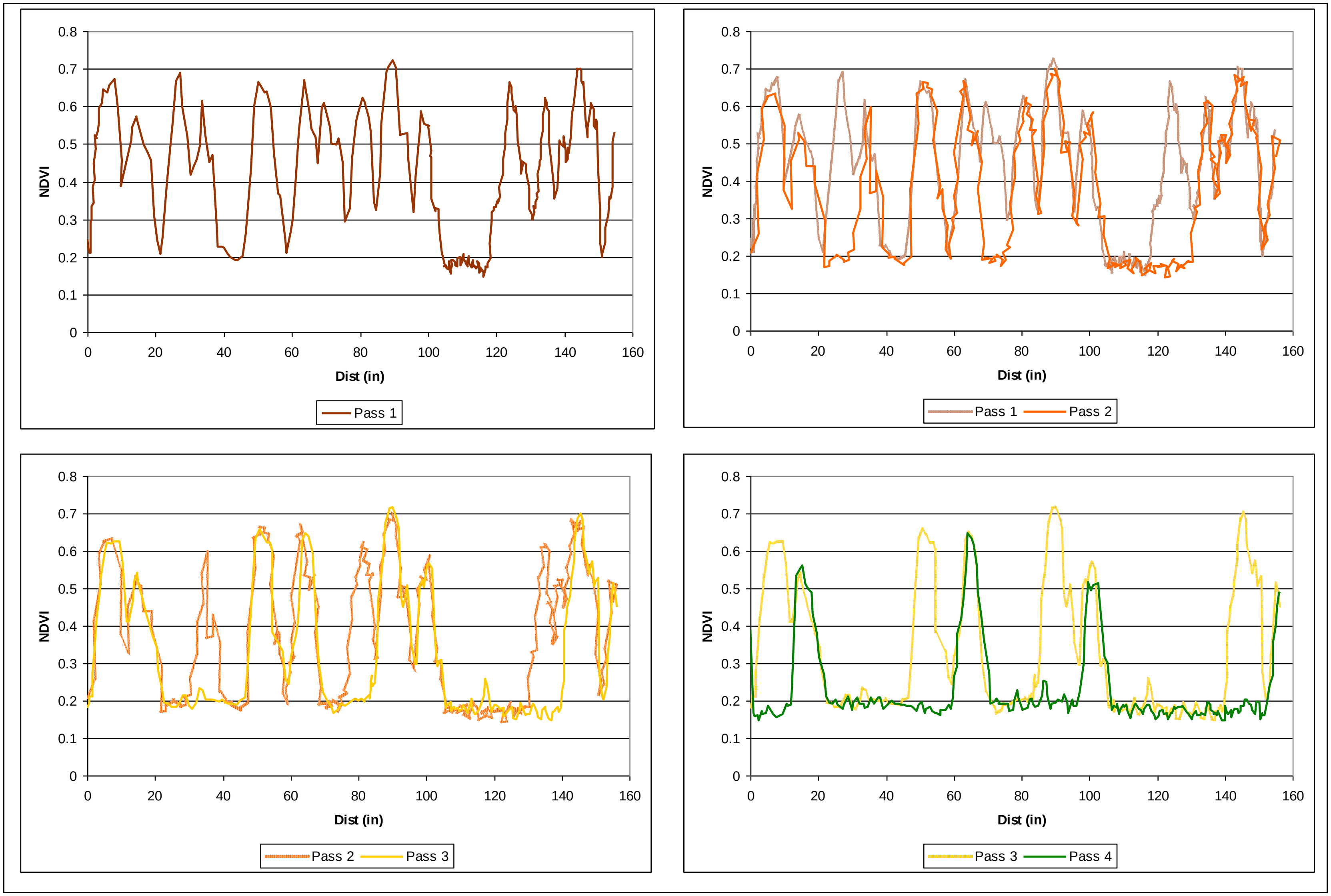

Figure 46. NDVI measurements for the entire plant population (Pass 1) and for every Pass (Pass 2, Pass 3, and Pass 4) after removing plant from the field (Data corresponding to plot 75).

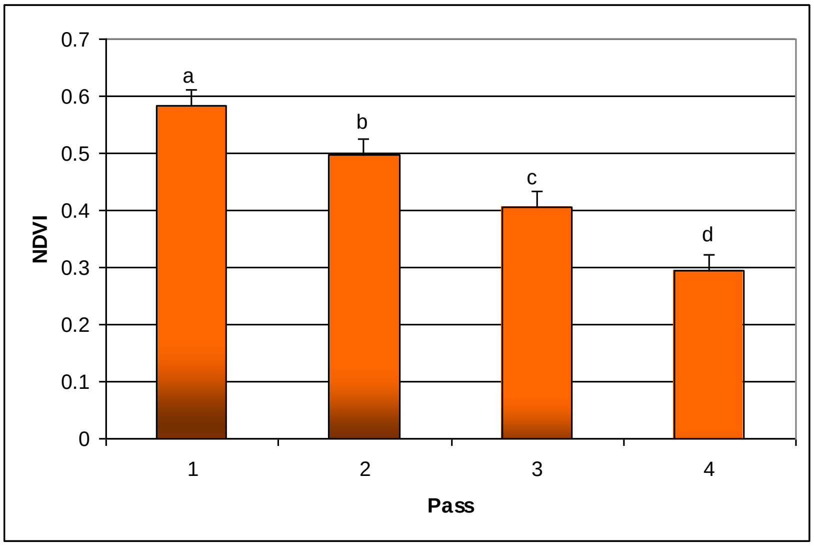

Figure 47. NDVI by pass for the 12 manipulated plant population subplots (Different letter indicates significant difference in NDVI (protected LSD (P<0.05))).

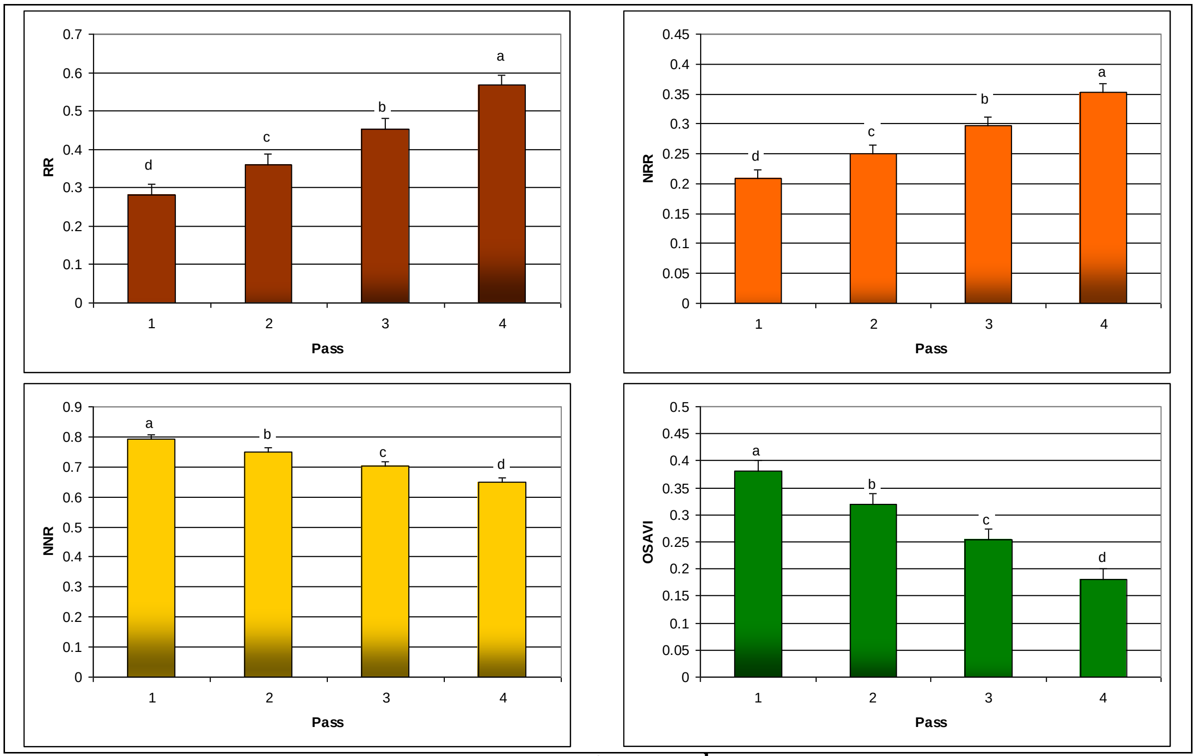

Figure 48. RR, NRR, NNR, and OSAVI (1st and 2nd approach, respectively) by pass for the 12 manipulated plant population subplots (Different letter indicates significant difference in the ratio or vegetation index (protected LSD (P<0.05))).

The *NDVI'* algorithm did impact the differences in NDVI values among passes, but not in a consistent or desirable manner (Table 5). For example, the application of the algorithm with *a*=1 did eliminate the significant difference between passes 1 and 2, but the significant difference between passes 1 and two returned after applying the algorithm with *a*=2. The change in the NDVI values is desirable for *a*=1 because the NDVI values for passes 1 and 2 are closer, but not for *a*=2 because the additional increase in the NDVI value for pass 2 causes it to overshoot the value for pass 1. The fact that the standard error is steadily increasing is even more concerning, since the goal is to maintain or minimize the variability in the data while reducing the differences between the mean NDVI values. Elimination of the differences between the mean NDVI values by increasing the standard error in the data is not desirable. This algorithm was omitted from further analysis due to unsatisfactory results.

Statistical analyses of NDVI trimmed 1*SD and NDVI trimmed 2*SD datasets showed a reduction on NDVI difference between passes as compared to unmodified NDVI differences. Although the differences between passes obtained in both post-processed datasets were statistically significant, a greater reduction of the differences between passes was obtained when data were trimmed for values lower than 2*SD (Figure 49). NDVI trimmed 2*SD differences were around 0.03 between passes (F value=48.48, P<0.05).

Table 5. *NDVI'* means by pass for the 12 manipulated plant population subplots. Different letter means statistically different between passes (protected LSD (P<0.05)) (3rd approach).

Vegetation index	Pass	NDVI	Std Error
NDVI_1.2	1	0.6026 a	0.03025
	2	0.4859 b	0.03025
	3	0.4124 c	0.03025
	4	0.3249 d	0.03025
***NDVI'₁*=NDVI_1.2+SD**	1	0.6017 a	0.03696
	2	0.5946 a	0.03696
	3	0.5246 b	0.03696
	4	0.4280 c	0.03696
***NDVI'₂*=NDVI_1.2+2*SD**	1	0.6009 b	0.04495
	2	0.7033 a	0.04495
	3	0.6368 ab	0.04495
	4	0.5310 c	0.04495

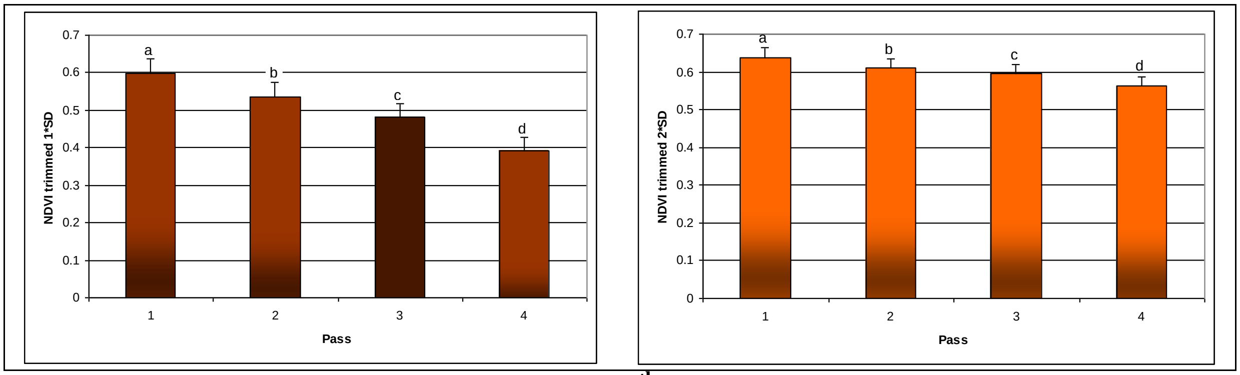

Figure 49. NDVI trimmed one and two SD (4th approach) by pass for the 12 manipulated plant population subplots (Different letter indicates significant difference in NDVI (protected LSD ($P<0.05$))).

The 2*SD trimming approach was applied to OSAVI because these two techniques had yielded the best reductions in differences by "pass". The resulting differences were even smaller than any of the previous analyses (Figure 50). Statistically significant OSAVI trimmed 2*SD differences were around 0.02 between passes (F value=32.7, $P<0.05$).

The final approach generated the "maximum canopy values" dataset for each of the 12 manipulated plant spacing subplots. This approach was pursued with the purpose of obtaining the best algorithm that allows the minimization of between-plant noise. Results showed no significant NDVI difference between passes two and three, and significant but small differences among passes one-two and three-four (F value=22.75, $P<0.05$) (Figure 51). Thus, a further minimization of the NDVI differences was achieved using "maximum canopy values" datasets compared with the results obtained using OSAVI trimmed 2*SD values algorithm.

The objective was to generate an algorithm to minimize differences due to between-plant noise in the manipulated plant spacing subplot datasets. This aim was reached in that the NDVI values for passes two, three, and four were closer, if not equal, to the NDVI values for pass one, which represented the unmodified plant population. This aim was better achieved when using the filtered "maximum canopy values" and OSAVI trimmed 2*SD algorithm. Due to the similarly of the results obtained with both algorithms, they were applied in the entire experimental field datasets to test the minimization of between-plant noise.

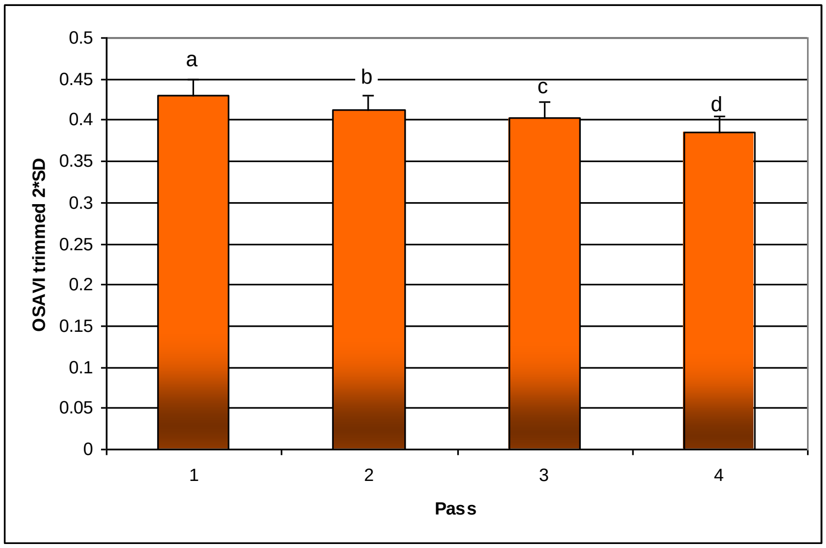

Figure 50. OSAVI trimmed two SD (5th approach) by pass for the 12 manipulated plant population subplots (Different letter indicates significant difference in OSAVI (protected LSD (P<0.05)).

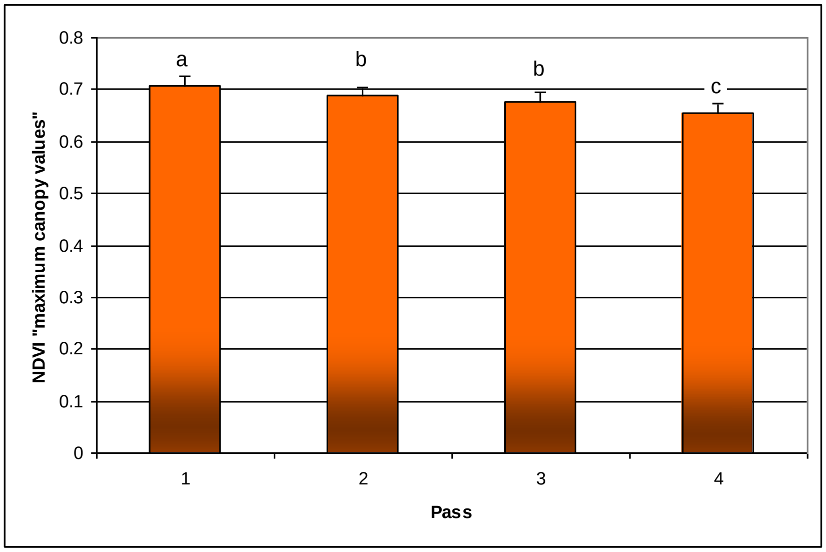

Figure 51. NDVI "maximum canopy values" (6th approach) by pass for the 12 manipulated plant population subplots (Different letter indicates significant difference in NDVI (protected LSD (P<0.05)).

Testing algorithms

Materials and Methods

The two best performing algorithms (OSAVI trimmed 2*SD and NDVI "maximum canopy values") were applied to all experimental field data sets collected over nine sampling dates throughout the 2008 growing season. These data sets follow the experimental design described in Chapter 3. Performing these tests allowed comparison with previous results to determine if there was a reduction in the NDVI measurements differences, especially across the three seeding rates. This was used as an indicator of the minimization of soil background noise effect on NDVI data.

The OSAVI and NDVI readings (for the post-processed data) were both averaged per GreenSeeker® sensor and per subplot for each of the nine sampling dates. Both GreenSeeker® sensor means were then averaged per subplot and the OSAVI and NDVI mean values were used for statistical analysis. ANOVA and LSD statistical tests were conducted for each of the nine sampling dates using PROC MIXED in SAS to study differences in OSAVI and NDVI mean values due to variety, plant spacing (seeding rate) and N rate treatment effects. Ultrasonic plant height was incorporated as a covariate variable to address biomass influence on NDVI readings.

Results and Discussion

Analyses of the unmodified data collected from the whole field showed significant differences in NDVI by cotton variety in five out of the nine sampling dates (Figure 13 Chapter 3). These NDVI differences were attributed to possible architectural differences by cotton variety. Significant differences in NDVI were observed for all seeding rate on all nine measurement dates (Figure 14 in Chapter 3). Lower NDVI values were observed at lower seeding rates. These significant differences were attributed to the influence of soil background noise on NDVI readings. The more space between plants, the more influence soil background had on spectral reflectance. Additionally, data from three out of the nine sampling dates showed significant NDVI differences due to supplemental N rate after supplemental N application (Figure 15 in Chapter 3).

The major aim was to evaluate the algorithms developed to attenuate the NDVI differences caused by soil background spectral reflectance noise. As stated before, two algorithms were chosen to be applied to the data set collected on the nine sampling date for the entire experimental field. Analyses of the results from both algorithms were divided into two sections for more comprehensibility to the reader.

<u>OSAVI trimmed 2*SD analysis.</u> After filtering the data using OSAVI trimmed 2*SD algorithm, statistical analysis confirmed that cotton variety had a significant effect on OSAVI readings ($P<0.05$) in three out of the nine sampling dates. Variety DP432 had significantly higher OSAVI values than the other two varieties (DP434 and DP444) later in season. OSAVI values for DP444 were significant lower than the other two varieties early in season. For all significant dates, the F values were low, particularly on June 18th and August 5th, which indicated that OSAVI differences were not strong enough to use as a basis for management decisions. Therefore, the differences between cotton varieties were minimized after using the OSAVI trimmed 2*SD algorithm as compared to the differences between cotton varieties obtained before filtering the datasets (Figure 52).

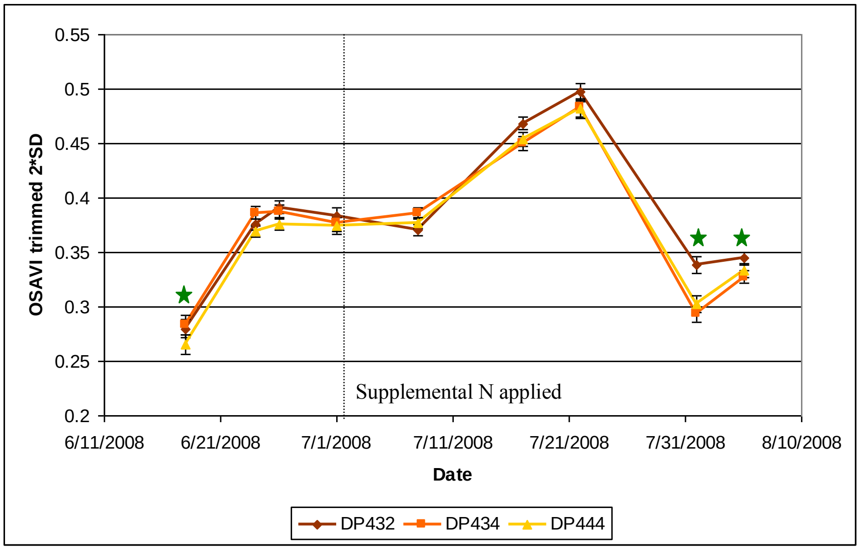

Figure 52. OSAVI trimmed 2*SD by cotton variety (DP432, DP434, and DP444) for nine sampling dates (Green stars indicate significant differences in OSAVI (protected LSD ($P<0.05$)).

Statistical analysis showed that seeding rate had a significant effect on OSAVI readings ($P<0.05$) after filtering the data using OSAVI trimmed 2*SD algorithm. For six of the nine sampling dates, OSAVI readings were greater for the higher seeding rate (50,225 seeds/ac) as compared to the two lower seeding rates (16,400 and 28,700 seeds/ac) (Figure 53). However, OSAVI differences among seeding rates were reduced by applying the trimmed 2*SD algorithm (Appendix 7). For all significant dates, F values were lower once the data were filtered using the OSAVI trimmed 2*SD algorithm. The statistical test was particularly significant on the July 22nd data set ($P<0.05$) but with an F value=6.72, indicating that OSAVI differences were not strong enough to support management decisions. Hence, spectral reflectance differences due to soil background noise were minimized. Although there is a considerable reduction in OSAVI differences early in the season, results suggest that the influence of soil background noise on the spectral reflectance could be reduced by eliminating the impact of between plant spacing noise, but the lack of control in the field of view for the sensor made it difficult to completely minimize the influence of soil background. Statistical analysis of the filtered data using OSAVI trimmed 2*SD algorithm showed basically the same results as obtained previously with non filtered OSAVI readings. Three out of the nine sampling dates confirmed differences in filtered OSAVI readings due to the effect of N rate treatment ($P<0.05$) (Figure 54). Although the differences were statistically significant, F values were low, suggesting that those differences would not be relevant enough for field practices. However, filtered OSAVI difference were statistically stronger than for non filtered OSAVI readings with higher F values (F value = 3.09, 6.7 and 7.69, for July 22nd, August 1st and August 5th, respectively). For those three dates, the lower N rate (30 lb/ac) differed from the rest of the rates (60, 90, and 120 lb/ac). These differences in level of significance could be attributed to the removal of the confounding effect of soil background noise in filtered OSAVI readings, which provide a clearer measure of the "greenness" of plants.

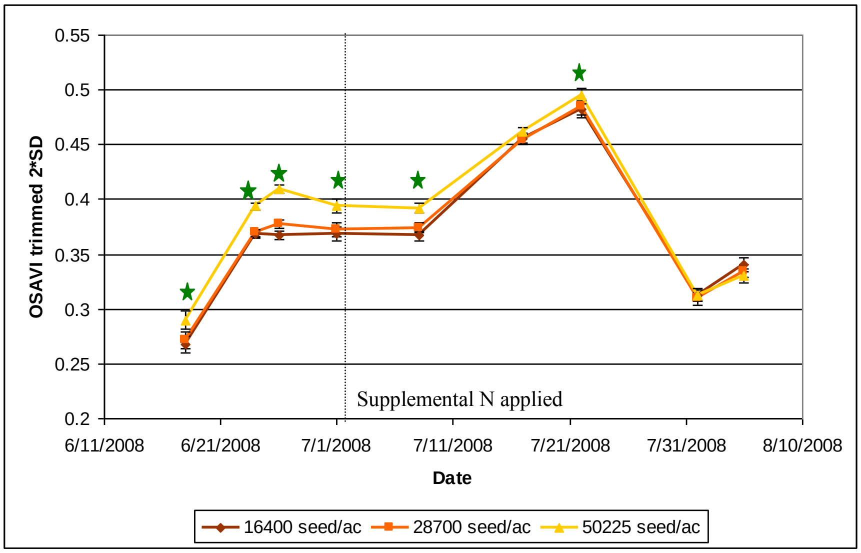

Figure 53. OSAVI trimmed 2*SD by seeding rate (16400, 28700, and 50225 seeds/ac) for nine sampling dates (Green stars indicate significant differences in OSAVI (protected LSD (P<0.05)).

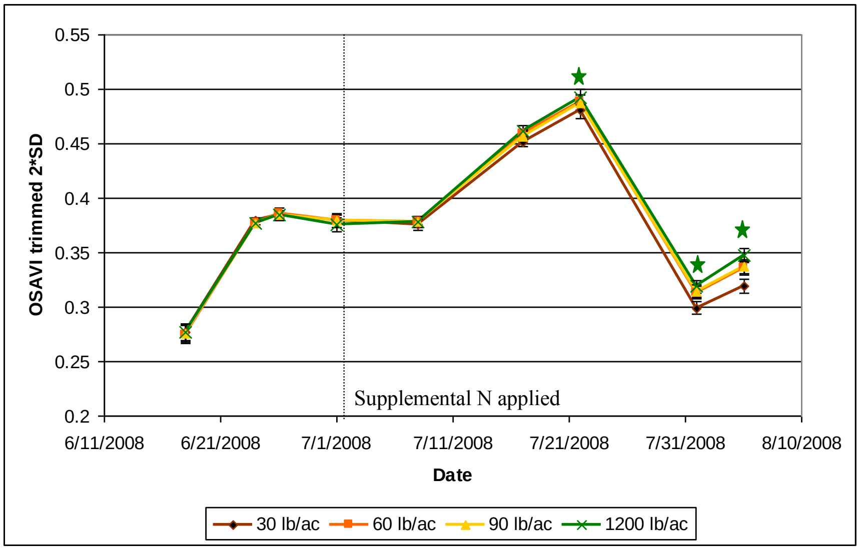

Figure 54. OSAVI trimmed 2*SD by N rate (30, 60, 90, and 120 lb/ac) for nine sampling dates (Green stars indicate significant differences in OSAVI (protected LSD (P<0.05)).

"Maximum canopy values" analysis. Statistical analysis of filtered data using the "maximum canopy values" algorithm showed no significant differences due to cotton variety on NDVI readings throughout the growing season except on June 18th when variety DP444 had lower NDVI compared with the other two cotton varieties (DP432 and DP434) (Figure 55). Although statistically significant, the F value=5.61 (P=0.0225) suggests that the difference found was not strong enough to be of practical consideration. These results are satisfactory since a minimization of the NDVI difference due to cotton variety was achieved.

A statistically significant effect on filtered NDVI readings due to seeding rate was observed in eight of the nine sampling dates ($P<0.05$) (Figure 56). Lower NDVI values were observed at lower seeding rates. Significant differences in NDVI readings due to seeding rate were greater early in the season, which was similar to the non filtered NDVI data analysis. Differences decreased through the seasons while the canopy gaps between plants were closed. Although results for filtered data showed similar number of dates with significant differences, these differences were weaker (with smaller F values) as compared to the results obtained with the non filtered NDVI data. Furthermore, although significant differences in filtered NDVI readings were observed throughout the season, the trend in the early season was opposite to that found late in the season. One possible explanation of these results is that lower plant density allows the development of wider canopies, besides it could be lowering the N demand, resulting in larger, greener, and healthier plants.

Statistical significant differences NDVI readings due to N rate treatment were observed in the "maximum canopy values" datasets. Only the last two sampling dates (August 1st and August 5th) showed significant differences, the lower N rate (30 lb/ac) was statistically different from the rest of the rates (60, 90, and 120 lb/ac) (Figure 57). NDVI difference on both dates were statistically stronger than for non filtered NDVI readings with F value= 6.54 and F value=10.25, respectively. These differences in significance, like those observed in the OSAVI trimmed 2*SD algorithm, could be attributed to the attenuation of soil background noise in NDVI readings and the resulting improved characterization of the "greenness" of the plants. Additionally, these results could suggest that the lower N rate did not reach the minimum requirements for cotton plants, since it was the only rate with statistically significant differences as compared to the other three rates.

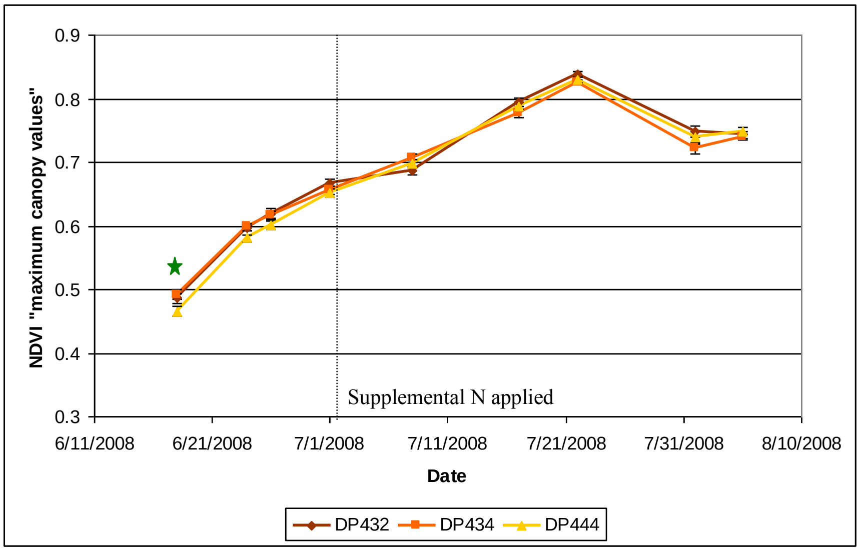

Figure 55. NDVI maximum canopy values by cotton variety (DP432, DP434, and DP444) for nine sampling dates (Green star indicates significant difference in NDVI (protected LSD (P<0.05))).

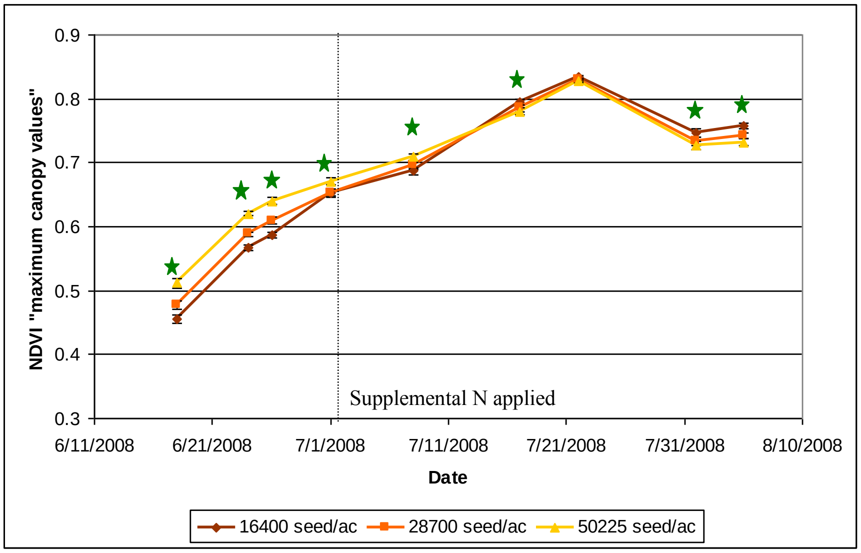

Figure 56. NDVI maximum canopy values by seeding rate (16400, 28700, and 50225 seeds/ac) for nine sampling dates (Green stars indicate significant differences in NDVI (protected LSD (P<0.05))).

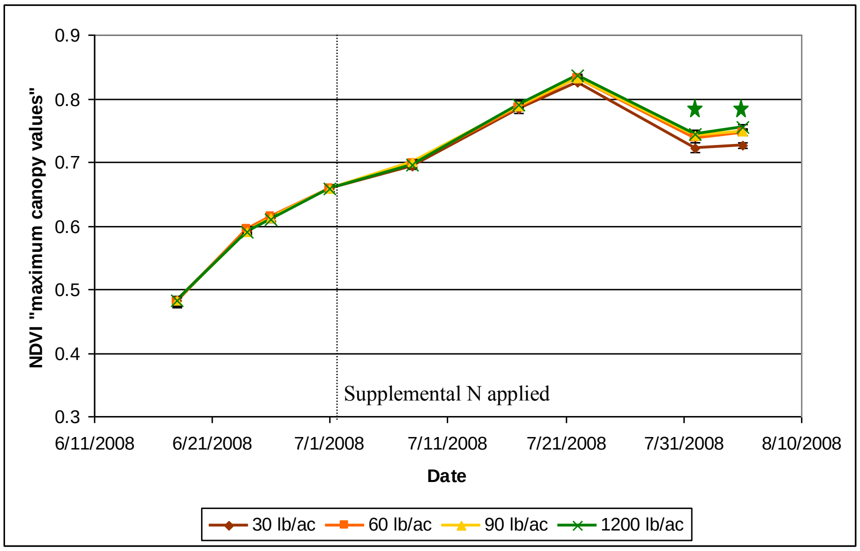

Figure 57. NDVI maximum canopy values by N rate (30, 60, 90, and 120 lb/ac) for nine sampling dates (Green stars indicate significant differences in NDVI (protected LSD (P<0.05))).

Filtered data and Leaf N concentration correlation. The correlation of OSAVI trimmed 2*SD values and NDVI "maximum canopy values" with leaf N concentration was investigated to identify any improvement from using the minimizing soil background noise algorithms. For July 1st, no significant correlation was found between OSAVI and NDVI filtered values with leaf N concentration (r= 0.09072, P=0.4485 and r= 0.11338, P=0.3430, respectively) (Figures 58 a,b). However, an interesting result was obtained after using both algorithms to minimize between-plant spacing noise: the change in sign of the correlation equation lines of leaf N concentration with the spectral reflectance (OSAVI and NDVI). Contrary to the results obtained using the non filtered values, lower leaf N concentration led to lower spectral reflectance after filtering the data (graphs of non filtered NDVI values can be found in Figure 26 and 27 in Chapter 3 and graph of non filtered OSAVI values can be found in Appendix 8). On August 1st, significant correlation was observed between OSAVI and NDVI filtered values with leaf N concentration (r= 0.40275, P=0.0005 and r= 0.28010, P=0.0172, respectively) (Figures 59 a,b). Once OSAVI trimmed 2*SD and NDVI "maximum canopy values" algorithms were applied, correlations became statistically significant, compared with the non filtered data. These results suggest an improvement in NDVI readings as predictor of N status in cotton crop.

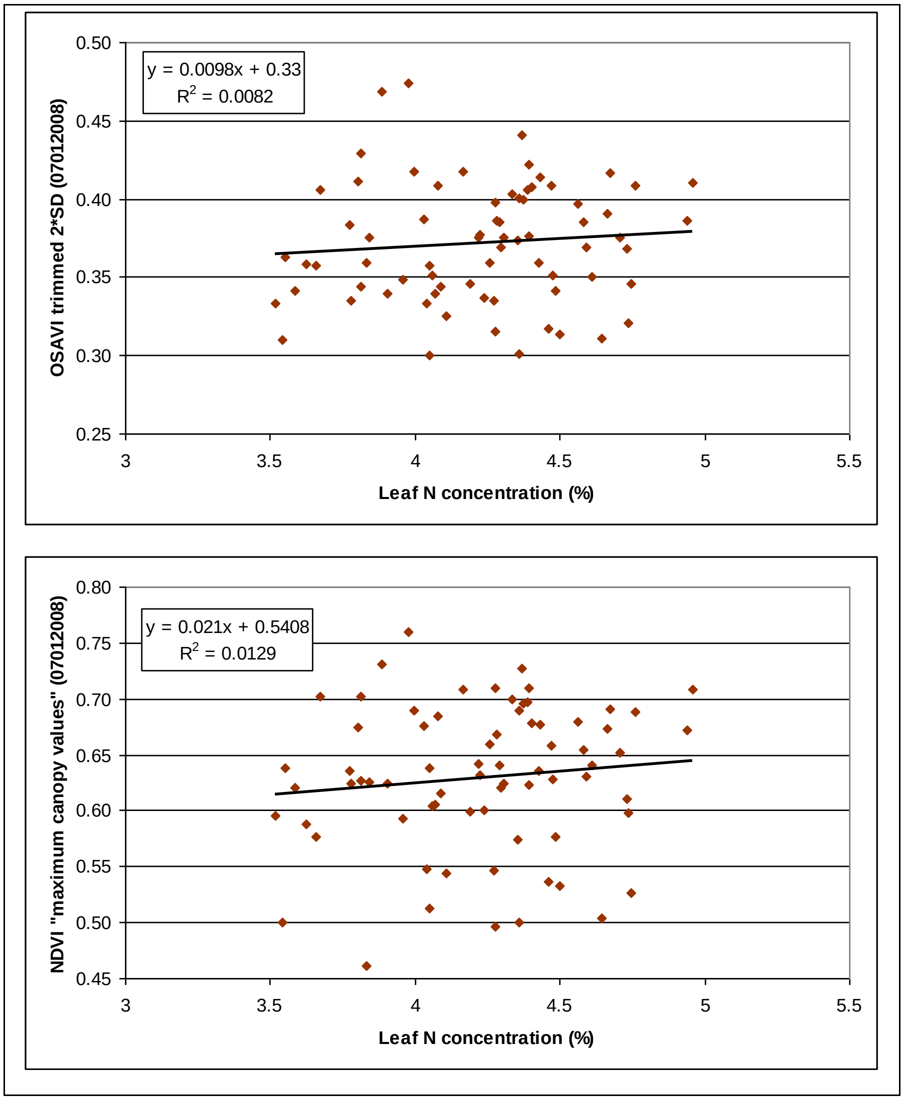

Figure 58. a. Linear correlation of OSAVI trimmed 2*SD and leaf N concentration (%) from July 1st, 2008. b. Linear correlation of NDVI "maximum canopy values" and leaf N concentration (%) from July 1st, 2008.

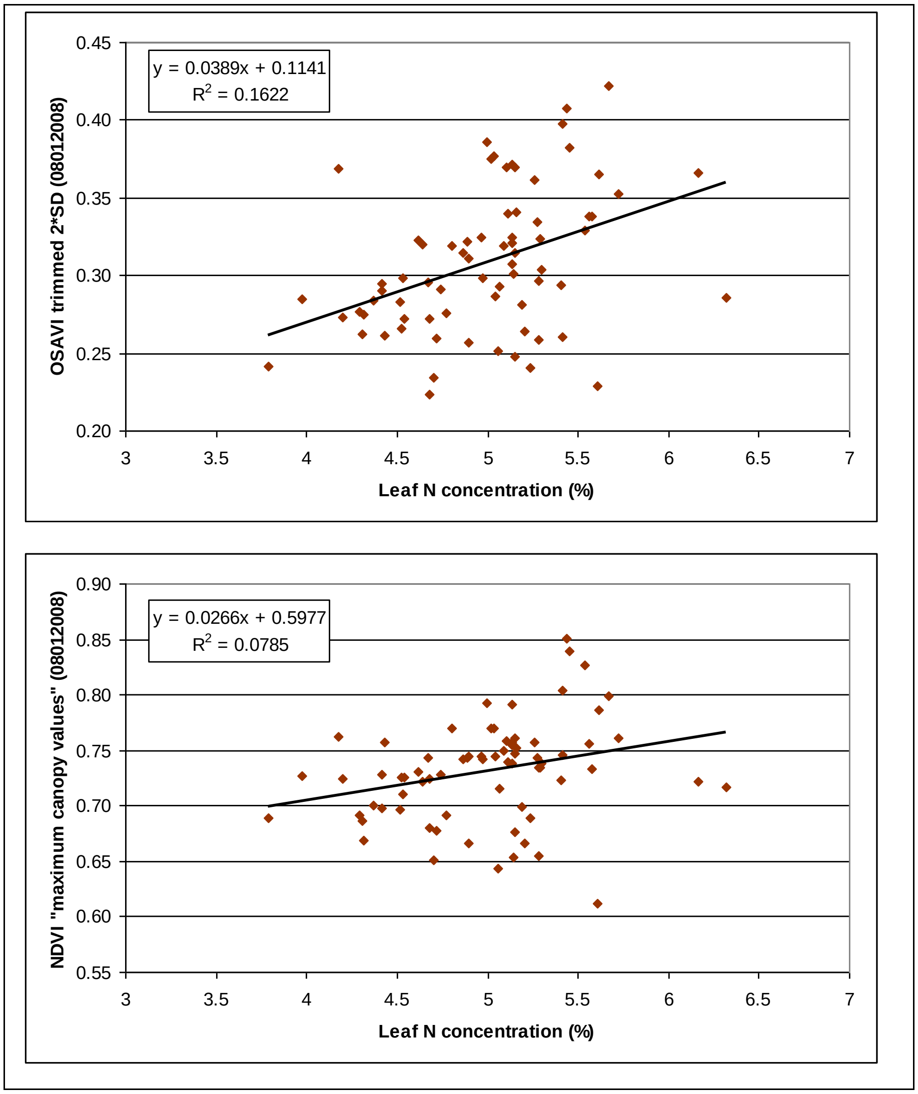

Figure 59. Linear correlation of NDVI canopy values and leaf N concentration (%) from July 1st and August 1st, 2008.

Summary

After studying several approaches, two algorithms gave the best results in minimizing differences among passes in the manipulated plant spacing experiment. The aim was that after applying the best algorithm to the manipulated plant spacing subplot datasets, the spectral reflectance for passes two, three, and four would be closer, if not equal, to the spectral reflectance corresponding to pass one which was conducted before the plant population was modified. The OSAVI trimmed 2*SD and NDVI "maximum canopy values" algorithms achieved a reduction in the spectral reflectance differences among the four measurements passes. After the application of these algorithms, differences were within the published error of the GreenSeeker® sensor. These two algorithms were then applied to the entire field experimental datasets with the aim of minimizing differences due to between-plant spacing noise. OSAVI trimmed 2*SD and NDVI "maximum canopy values" datasets were generated, analyzed, and compared to each other and to the non filtered OSAVI and NDVI data analyses.

After applying the two algorithms and comparing results with the non filtered data analyses, we can conclude that both algorithms minimized the differences in spectral reflectance due to variety, seeding rate, and N rate treatments. However, the vegetation index differences were lower when NDVI "maximum canopy values" were used than with OSAVI trimmed 2*SD data. It was particularly interesting that differences were minimized at the point where N management decisions will be made (around 50 days after planting). Another interesting result is the positive correlation of leaf N concentration with the vegetation indices after both algorithms were applied to minimize the impact of between plant spacing. Although moderately significant, it suggests an improvement in accuracy of the vegetation indices as a predictor of N status in cotton crops.

Chapter 6 - Conclusions

- NDVI GreenSeeker® sensor's measurements were able to characterize cotton variety differences in middle-season. Differences were more likely due to plant architectural dissimilarity by variety. Differences were attenuated later in season, when full plant canopy coverage was reached.
- NDVI GreenSeeker® sensor's measurements were affected by plant density throughout the growing season. Greater plant spacing along the row produced lower NDVI measurements early in the season. Differences were primarily attributed to soil background within the field-of-view. Differences were attenuated later in season, when full plant canopy coverage is reached.
- NDVI GreenSeeker® sensor's measurements were able to discriminate between supplemental N rate applications. Lower N rates yielded lower NDVI readings at the end of the growing season, after supplemental fertilization was applied.
- Available soil moisture affected not only the growth of cotton plants but the N uptake and affected NDVI measurements and leaf N concentration analysis.
- Leaf N concentration analyses differed by planted seeding rate before and after supplemental N application. Lower seeding rates led to higher leaf N concentrations. Differences were attributed to plant demand of N from the soil in relationship with plant population.
- Leaf N concentration analyses confirmed differences between N rates after supplemental N applications. Lower N rates led to lower leaf N concentrations later in season.
- Strongly positive correlation between NDVI GreenSeeker® sensor measurements and ultrasonic plant height sensor measurements was confirmed. Taller plants led to higher NDVI measurements.
- Ultrasonic plant height sensor measurements differed by cotton variety, confirming plant architectural differences between varieties.

- Ultrasonic plant height sensor measurements differed by seeding rate throughout the growing season. Higher seeding rates led to taller plants early in season. This relationship changed later in the season: lower seeding rates led to taller plants. Differences were more likely due to competition for sunlight resources early in season and for soil nutrients resources later in season.
- Ultrasonic plant height sensor measurements only differed by N rate later in the season. Only the lower N rate (30 lb/ac) resulted in smaller cotton plants, confirming the effect of soil nutrient limitation on plant height.
- NDVI GreenSeeker® sensor measurements were statistically affected by the time of sampling in a diurnal cycle. Differences were attributed to ambient factors affecting cotton leaf, such as water availability, wind, heat, ambient light intensity, solar radiation angle, or weather conditions.
- OSAVI trimmed 2*SD and NDVI "maximum canopy values" algorithms provided the best results by minimizing differences caused by soil background noise on NDVI GreenSeeker® measurements.
- No significant correlation between vegetation indices and leaf N concentration was achieved before using both algorithms. Moderately significant positive correlation between vegetation indices and leaf N concentration was achieved after using both algorithms to minimize soil background noise effect.
- "Maximum canopy values" algorithm gave better results than the to OSAVI trimmed 2*SD algorithm. NDVI GreenSeeker® measurements differences were greatly minimized by the "maximum canopy values" algorithm. This has strong implications when N management decisions are being made.

Recommendations

- GreenSeeker® sensor users should pay attention to crop water stress when NDVI data is used as a predictor of N status in cotton crops.
- A more detailed experiment should be design in order to confirm the diurnal cycle experiment results. Meanwhile, GreenSeeker® sensor users should pay attention to external factors such as ambient conditions when NDVI data is used as a predictor of N status in cotton crops.
- OSAVI GreenSeeker® measurements are available in the internal algorithm of the sensor. Comparison of this data with the results obtained for this experiment should be studied in future works; in order to identify potential differences between applied methods of manipulating data.

Bibliography

Arnall, D.B., W.R. Raun, J.B. Solie, M.L. Stone, G.V. Johnson, K. Girma, K.W. Freeman, R.K. Teal, and K.L. Martin. 2006. Relationship Between Coefficient of Variation Measured by Spectral Reflectance and Plant Density at Early Growth Stages in Winter Wheat. Journal of Plant Nutrition 29:1983–1997.

Baret, F., and G. Guyot. 1991. Potentials and limits of vegetation indices for LAI and APAR assessment. Remote Sensing of Environment 35:161-173.

Barnes, E.M., T.R. Clarke, S.E. Richards, P.D. Colaizzi, J. Haberland, M. Kostrzewski, P. Waller, C. Choi, E. Riley, T. Thompson, R.J. Lascano, H. Li, and M.S. Moran. 2000. Coincident detection of crop water stress, nitrogen status and canopy density using ground-based multispectral data. Fifth International Conference of Precision Agriculture.

Behrens, T., M. Kraft, and F. Wiesler. 2004. Influence of measuring angle, nitrogen fertilization, and variety on spectral reflectance of winter oilseed rape canopies. J. Plant Nutr. Soil Sci. 167:99-105.

Bell, P.F., D.J. Boquet, E. Millhollon, S. Moore, W. Ebelhar, C.C. Mitchell, J. Varco, E.R. Funderburg, C. Kennedy, G.A. Breitenbeck, C. Craig, M. Holman, W. Baker, and J.S. McConnell. 2003. Relationships between Leaf-Blade Nitrogen and Relative Seedcotton Yields. Crop Science 43:1367-1374.

Bronson, K.F., T.T. Chua, J.D. Booker, J.W. Keeling, and R.J. Lascano. 2003. In-Season Nitrogen Status Sensing in Irrigated Cotton: II. Leaf Nitrogen and Biomass. Soil Sci. Soc. Am. J. 67:1439-1448.

Bronson, K.F., J.D. Booker, J.W. Keeling, R.K. Boman, T.A. Wheeler, R.J. Lascano, and R.L. Nichols. 2005. Cotton Canopy Reflectance at Landscape Scale as Affected by Nitrogen Fertilization. Agronomy Journal 97:654-660.

Buscaglia, H.J., and J.J. Varco. 2002. Early Detection of Cotton Leaf Nitrogen Status Using Leaf Reflectance. Journal of Plant Nutrition 25:2067-2080.

Carlson, T.N., and D.A. Ripley. 1997. On the Relation between NDVI, Fractional Vegetation Cover, and Leaf Area Index. Remote Sensing of Environment 62:241-252.

Carter, G.A. 1993. Responses of Leaf Spectral Reflectance to Plant Stress. American Journal of Botany 80:239-243.

Carter, G.A. 1994. Ratios of leaf reflectances in narrow wavebands as indicators of plant stress. International Journal of Remote Sensing 15:697-703.

Carter, G.A., and R.L. Miller. 1994. Early detection of plant stress by digital imaging within narrow stress-sensitive wavebands. Remote Sensing of Environment 50:295-302.

Clark, R.L., and R.L. McGuckin. 1996. Variable rate application equipment for precision farming. 1996 Beltwide Cotton Conference.

Demetriades-Shah, T.H., M.D. Steven, and J.A. Clark. 1990. High Resolution Derivative Spectra in Remote Sensing. Remote Sensing of Environment 33:55-64.

Faircloth, J. 2005. Cotton fertility. 2005 Virginia Cotton Production Guide.

Feibo, W., W. Lianghuan, and X. Fuhua. 1998. Chlorophyll meter to predict nitrogen sidedress requirements for short-season cotton (Gossypium hirsutum L.). Field Crops Research 56:309–314.

Freeman, K., K. Girma, D.B. Arnall, R.W. Mullen, K.L. Martin, R.K. Teal, and W.R. Raun. 2007. By-Plant Prediction of Corn Forage Biomass and Nitrogen Uptake at Various Growth Stages Using Remote Sensing and Plant Height. Agronomy Journal 99:530–536.

Fridgen, J.L., and J.J. Varco. 2004. Dependency of Cotton Leaf Nitrogen, Chlorophyll, and Reflectance on Nitrogen and Potassium Availability. Agronomy Journal 96:63-69.

Fritschi, F.B., B.A. Roberts, R.L. Travis, D.W. Rains, and R.B. Hutmacher. 2003. Response of Irrigated Acala and Pima Cotton to Nitrogen Fertilization. Agronomy Journal 95:133-146.

Gerik, T.J., B.S. Jackson, C.O. Stockle, and W.D. Rosenthal. 1994. Plant Nitrogen Status and Boll Load of Cotton. Agronomy Journal 86:514-518.

Girma, K., R.K. Teal, K.W. Freeman, R.K. Boman, and W.R. Raun. 2007. Cotton Lint Yield and Quality As Affected by Applications of N, P, and K Fertilizers. The Journal of Cotton Science 11:12-19.

Girma, K., K.L. Martin, R.H. Anderson, D.B. Arnall, K.D. Brixey, M.A. Casillas, B. Chung, B.C. Dobey, S.K. Kamenidou, S.K. Kariuki, E.E. Katsalirou, J.C. Morris, J.Q. Moss, C.T. Rohla, B.J. Sudbury, B.S. Tubana, and W.R. Raun. 2006. Mid-Season Prediction of Wheat-Grain Yield Potential Using Plant, Soil, and Sensor Measurements. Journal of Plant Nutrition 29:873–897.

Hall, F.G., K.F. Huemmrich, and S.N. Goward. 1990. Use of Narrow-Band Spectra to Estimate the Fraction of Absorbed Photosynthetically Active Radiation. Remote Sensing of Environment 32:47-54.

Heege, H.J., and E. Thiessen. 2002. On-the-Go Sensing for Site-Specific Nitrogen Top Dressing. Proceedings 2002 ASAE Annual International Meeting / CIGR XVth World Congress ASAE Paper Number: 021113.

Huete, A.R. 1987. Soil-Dependent Spectral Response in a Developing Plant Canopy. Agronomy Journal 79:61-68.

Huete, A.R. 1988. A Soil-Adjusted Vegetation Index (SAVI). Remote Sensing of Environment 25:295-309.

Huete, A.R., R.D. Jackson, and D.F. Post. 1985. Spectral Response of a Plant Canopy with Different Soil Backgrounds. Remote Sensing of Environment 17:37-53.

Jackson, R.D. 1983. Spectral indices in N-Space. Remote Sensing of Environment 13:409-421.

Khalilian, A., W. Henderson, and R. Hallman. 2007. Site-specific application of plant growth regulators in cotton. 2007 Beltwide Cotton Conferences.

Knipling, E.B. 1970. Physical and physiological basis for the reflectance of visible and near-infrared radiation from vegetation. Remote Sensing of Environment 1:155-159.

Ko, J., S.J. Maas, S. Mauget, G. Piccinni, and D. Wanjura. 2006. Modeling Water-Stressed Cotton Growth Using Within-Season Remote Sensing Data. Agronomy Journal 98:1600-1609.

Kostrzewski, M., P. Waller, P. Guertin, J. Haberland, P. Colaizzi, E. Barnes, T. Thompson, T. Clarke, E. Riley, and C. Choi. 2002. Ground-Based Remote Sensing of Water and Nitrogen Stress. Transactions of the ASAE 46:29-38.

Leon, C.T., D.R. Shaw, M.S. Cox, M.J. Abshire, B. Ward, and M.C. Wardlaw III. 2003. Utility of Remote Sensing in Predicting Crop and Soil Characteristics. Precision Agriculture 4:359-384.

Li, H., R.J. Lascano, E.M. Barnes, J. Booker, L.T. Wilson, K.F. Bronson, and E. Segarra. 2001. Multispectral Reflectance of Cotton Related to Plant Growth, Soil Water and Texture, and Site Elevation. Agronomy Journal 93:1327-1337.

Longstreth, D.J., and P.S. Nobel. 1980. Nutrient Influences on Leaf Photosynthesis. Effects of Nitrogen, Phosphorus, and Potassium for *Gossypium hirsutum L.* Plant Phisiology 65:541-543.

Lough, L.J., and J.J. Varco. 2000. Effect of Varying N and K Nutrition on the Spectral Reflectance Properties of Cotton. Fifth International Conference on Precision Agriculture.

Major, D.J., F. Baret, and G. Guyot. 1990. A ratio vegetation index adjusted for soil brightness. International Journal of Remote Sensing 11:727-740.

Martin, K.L., K. Girma, K.W. Freeman, R.K. Teal, B. Tubana, D.B. Arnall, B. Chung, O. Walsh, J.B. Solie, M.L. Stone, and W.R. Raun. 2007. Expression of Variability in Corn as Influenced by Growth Stage Using Optical Sensor Measurements. Agronomy Journal 99:384–389.

Neves, O.S.C., J.G.d. Carvalho, F.A.D. Martins, T.R.P.d. Pádua, and P.J.d. Pinho. 2005. Use of SPAD-502 in the evaluation of chlorophyll contents and nutritional status of herbaceous cotton to nitrogen, sulphur, iron and manganese. Pesq. agropec. bras. 40:517-521.

NTech Industries Inc. 2007. GreenSeeker RT100 data collection and mapping unit. Operating manual.

Pinter, P.J. 1993. Solar angle independence in the relationship between absorbed PAR and remotely sensed data for alfalfa. Remote Sensing of Environment 46:19-25.

Pinter, P.J., R.D. Jackson, C.E. Ezra, and H.W. Gausman. 1985. Sun-angle and canopy-architecture effects on the spectral reflectance of six wheat cultivars. International Journal of Remote Sensing 6:1813-1825.

Plant, R.E., D.S. Munk, B.R. Roberts, R.L. Vargas, D.W. Rains, R.L. Travis, and R.B. Hutmacher. 2000. Relationship Between Remotely Sensed Reflectance Data and Cotton Growth and Yield. Transactions of the ASAE 43:535-546.

Qi, J., A. Chehbouni, A.R. Huete, Y.H. Kerr, and S. Sorooshian. 1994. A Modified Soil Adjusted Vegetation Index. Remote Sensing of Environment 48:119-126.

Raun, W.R., J.B. Solie, K.L. Martin, K.W. Freeman, M.L. Stone, G.V. Johnson, and R.W. Mullen. 2005. Growth Stage, Development, and Spatial Variability in Corn Evaluated Using Optical Sensor Readings. Journal of Plant Nutrition 28:173–182.

Raun, W.R., J.B. Solie, G.V. Johnson, M.L. Stone, R.W. Mullen, K.W. Freeman, W.E. Thomason, and E.V. Lukina. 2002. Improving Nitrogen Use Efficiency in Cereal Grain Production with Optical Sensing and Variable Rate Application. Agronomy Journal 94:815-820.

Read, J.J., L. Tarpley, J.M. McKinion, and K.R. Reddy. 2002. Narrow-Waveband Reflectance Ratios for Remote Estimation of Nitrogen Status in Cotton. J. Environ. Qual. 31:1442–1452.

Richardson, A.J., and C.L. Weigand. 1977. Distinguishing Vegetation from Soil-Background Information. Photogrammetric Engineering and Remote Sensing 43:1541-1542.

Rondeaux, G., M. Steven, and F. Baret. 1996. Optimization of Soil-Adjusted Vegetation Indices. Remote Sensing of Environment 55:95-107.

Saranga, Y., A. Landa, Y. Shekel, A. Bosak, and U. Kafkafi. 1998. Near-Infrared Analysis of Cotton Leaves as a Guide for Nitrogen Fertilization. Agronomy Journal 90:16-21.

SAS Institute Inc. 2003. SAS for Windows. Version 9.1.3. Cary, NC: SAS Institute Inc.

Setatou, H.B., and A.D. Simonis. 1996. Effect of time and rate of nitrogen application on cotton. Fertilizer Research 43:49-53.

Shanahan, J.F., N.R. Kitchen, W.R. Raun, and J.S. Schepers. 2008. Responsive in-season nitrogen management for cereals. Computers and Electronics in Agriculture 61:51-62.

Sims, D.A., and J.A. Gamon. 2002. Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. Remote Sensing of Environment 81:337-354.

Slater, P.N., and R.D. Jackson. 1982a. Atmospheric effects on radiation reflected from soil and vegetation as measured by orbital sensors using various scanning directions. Applied Optics 21:3923-3931.

Slater, P.N., and R.D. Jackson. 1982b. Atmospheric effects on radiation reflected from soil and vegetation as measured by orbital sensors using various scanning directions. Applied Optics 21.

Sui, R., J.B. Wilkerson, W.E. Hart, L.R. Wilhelm, and D.D. Howard. 2005. Multi-Spectral Sensor for Detection of Nitrogen Status in Cotton. Applied Engineering in Agriculture 21:167-172.

Tarpley, L., K.R. Reddy, and G.F. Sassenrath-Cole. 2000. Reflectance Indices with Precision and Accuracy in Predicting Cotton Leaf Nitrogen Concentration. Crop Science 40:1814-1819.

Teal, R.K., W.R. Raun, J.B. Solie, M.L. Stone, K.W. Freeman, G.V. Johnson, and K.L. Martin. 2004. By-Plant Precision Snesing for Variable Nitrogen Rate Application in Corn. Proceedings Precision Agriculture Conference.

Thenkabail, P.S., R.B. Smith, and E. De Pauw. 2000. Hyperspectral Vegetation Indices and Their Relationships with Agricultural Crop Characteristics. Remote Sensing of Environment 71:158-182.

Thomasson, J.A., J.R. Wooten, S. Gogineni, R. Sui, and B.M. Kolla. 2004. Remote-sensing and weather information in cotton yield prediction, p. 127-135, *In* W. S. Gao, David R, ed. Ecosystems' Dynamics, Agricultural Remote Sensing and Modeling, and Site-Specific Agriculture, Vol. 5153. Proceedings of the SPIE.

Thorp, K.R., and L.F. Tian. 2004. A Review on Remote Sensing of Weed in Agriculture. Precision Agriculture 5:477-508.

Tracy, P.W., S.G. Hefner, C.W. Wood, and K.L. Edmisten. 1992. Theory Behind the Use of Instantaneous Leaf Chlorophyll Measurements for Determining Mid-Season Cotton Nitrogen Recommendations. Proceedings 1992 Beltwide Cotton Conferences:1099-1100.

Tucker, C.J. 1979. Red and photographic infrared linear combinations for monitoring vegetation. Remote Sensing of Environment 8:127-150.

Wilkerson, J.B., R. Sui, W.E. Hart, and D.D. Howard. 1998. Development of a Variable-Rate Nitrogen Applicator Based on Spectral Reflectance Characteristics of Cotton Plants. Proceedings 1998 Beltwide Cotton Conferences:439-443.

Zhao, D., K.R. Reddy, V.G. Kakani, J.J. Read, and S. Koti. 2005. Selection of Optimum Reflectance Ratios for Estimating Leaf Nitrogen and Chlorophyll Concentrations of Field-Grown Cotton. Agronomy Journal 97:89-98.

Zhao, D., K.R. Reddy, V.G. Kakani, J.J. Read, and S. Koti. 2007. Canopy reflectance in cotton for growth assessment and lint yield prediction. European Journal of Agronomy 26:335-344.

Appendices

Appendix 1. Cotton Crop Management Effects 2007

Table 6. NDVI and CV mean for N rate and seeding rate treatments. This table contains data for one of the two GreenSeeker® sensors (GreenSeeker#1) and one of the two cotton varieties (DP143). Means with the same letter are not statistically different within a given date (protected LSD (P<0.05).

Dates	6/27/07		7/12/07		7/23/07	
N Rate (lb/ac)	**NDVI mean**	**CV (%)**	**NDVI mean**	**CV (%)**	**NDVI mean**	**CV (%)**
30	0.6567 a	10.583 a	0.8430 a	3.1009 b	0.7361 a	5.4876 b
60	0.6528 a	10.657 a	0.8393 a	4.2981 a	0.7384 a	5.5462 b
90	0.6601 a	10.217 a	0.8436 a	3.3387 ab	0.7412 a	5.7675 ab
120	0.6532 a	11.269 a	0.8401 a	4.4238 a	0.7357 a	6.4081 a
Seed Rate (seeds/ac)						
16400	0.6041 a	14.912 a	0.8231 a	6.3882 a	0.7435 a	6.0298 ab
28700	0.6684 a	9.9580 b	0.8462 a	2.7370 a	0.7283 a	6.4269 a
50225	0.6945 a	7.1747 b	0.8552 a	2.2459 a	0.7418 a	4.9503 b

Table 7. NDVI and CV mean for N rate and seeding rate treatments. This table contains data for one of the two GreenSeeker® sensors (GreenSeeker#2) and one of the two cotton varieties (DP555). Means with the same letter are not statistically different within a given date (protected LSD (P<0.05).

Dates	6/27/07		7/12/07		7/23/07	
N Rate (lb/ac)	**NDVI mean**	**CV (%)**	**NDVI mean**	**CV (%)**	**NDVI mean**	**CV (%)**
30	0.6607 a	9.9369 a	0.8289 a	4.6299 a	0.04019 b	10.506 a
60	0.6624 a	10.009 a	0.8298 a	4.5722 a	0.04042 b	10.954 a
90	0.6578 a	10.166 a	0.8305 a	4.4347 a	0.04216 ab	11.301 a
120	0.6620 a	9.5349 a	0.8291 a	4.7183 a	0.04633a	11.148 a
Seed Rate (seeds/ac)						
16400	0.5928 b	13.860 a	0.8224 b	5.7946 a	0.04493 ab	11.889 a
28700	0.6926 a	8.7595 b	0.8272 ab	4.4144 ab	0.04598 a	11.087 ab
50225	0.6968 a	7.1156 b	0.8391 a	3.5573 b	0.03591 b	9.9556 a

Table 8. NDVI and CV mean for N rate and seeding rate treatments. This table contains data for one of the two GreenSeeker® sensors (GreenSeeker#2) and one of the two cotton varieties (DP143). Means with the same letter are not statistically different within a given date (protected LSD (P<0.05).

Dates	**6/27/07**		**7/12/07**		**7/23/07**	
N Rate (lb/ac)	**NDVI mean**	**CV (%)**	**NDVI mean**	**CV (%)**	**NDVI mean**	**CV (%)**
30	0.6450 a	10.017 a	0.8329 a	3.5508 a	0.03935 b	10.777 a
60	0.6396 a	10.018 a	0.8300 a	4.3554 a	0.04312 b	10.517 a
90	0.6386 a	10.512 a	0.8320 a	4.0308 a	0.04288 b	11.289 a
120	0.6309 a	11.193 a	0.8262 a	4.7760 a	0.05015 a	11.499 a
Seed Rate (seeds/ac)						
16400	0.5849 b	14.321 a	0.8126 b	6.2649 a	0.05495 a	12.367 a
28700	0.6500 ab	9.5363 b	0.8334 ab	3.4660 b	0.04152 ab	11.147 ab
50225	0.6807 a	7.4469 b	0.8448 a	2.8039 b	0.03515 b	9.5477 b

Appendix 2. NDVI and Ultrasonic Plant Height Correlation 2007

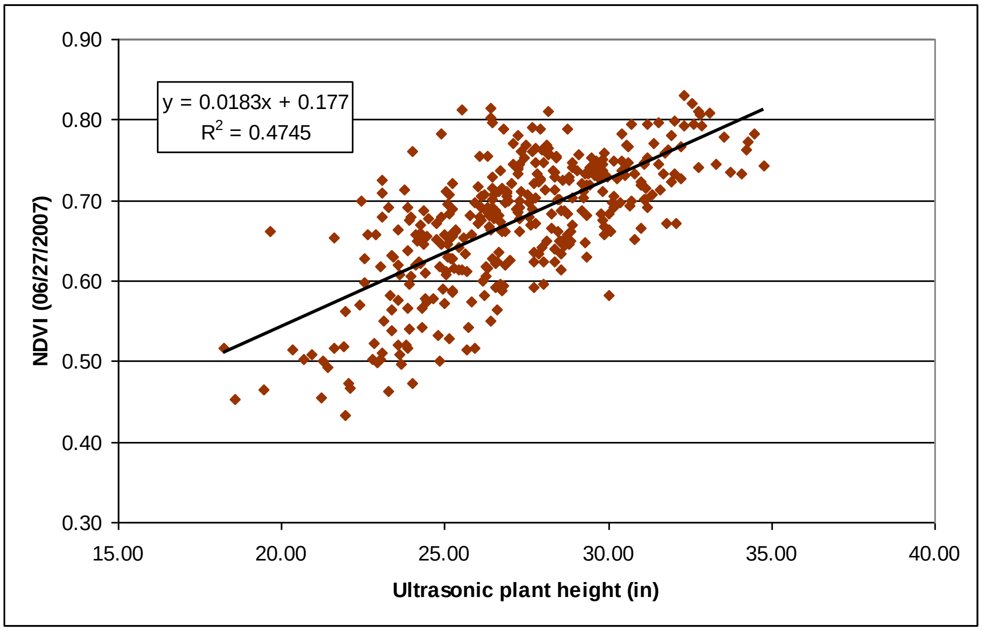

Figure 60. Linear correlation of NDVI and ultrasonic plant height (in) measurements per plot using GreenSeeker#1. Data collected in early season (06/27/2007).

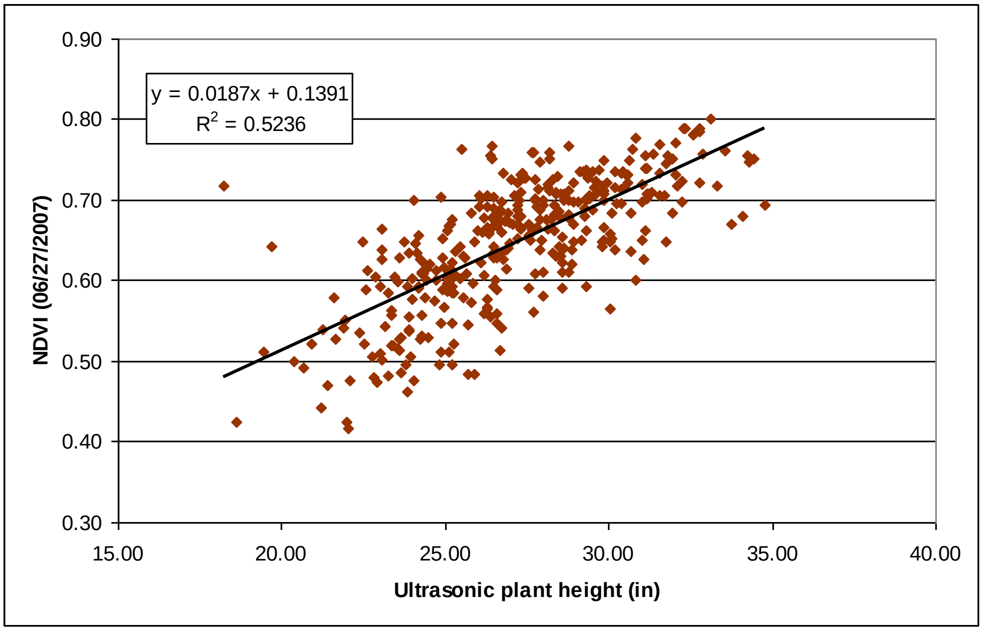

Figure 61. Linear correlation of NDVI and ultrasonic plant height (in) measurements per plot using GreenSeeker#2. Data collected in early season (06/27/2007).

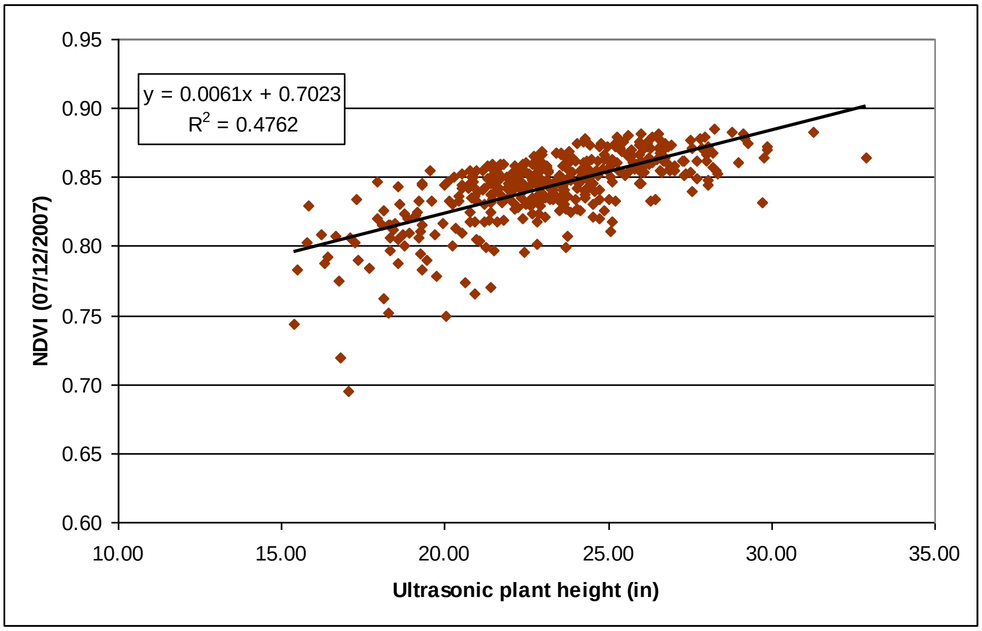

Figure 62. Linear correlation of NDVI and ultrasonic plant height (in) measurements per plot using GreenSeeker#1. Data collected in middle season (07/12/2007).

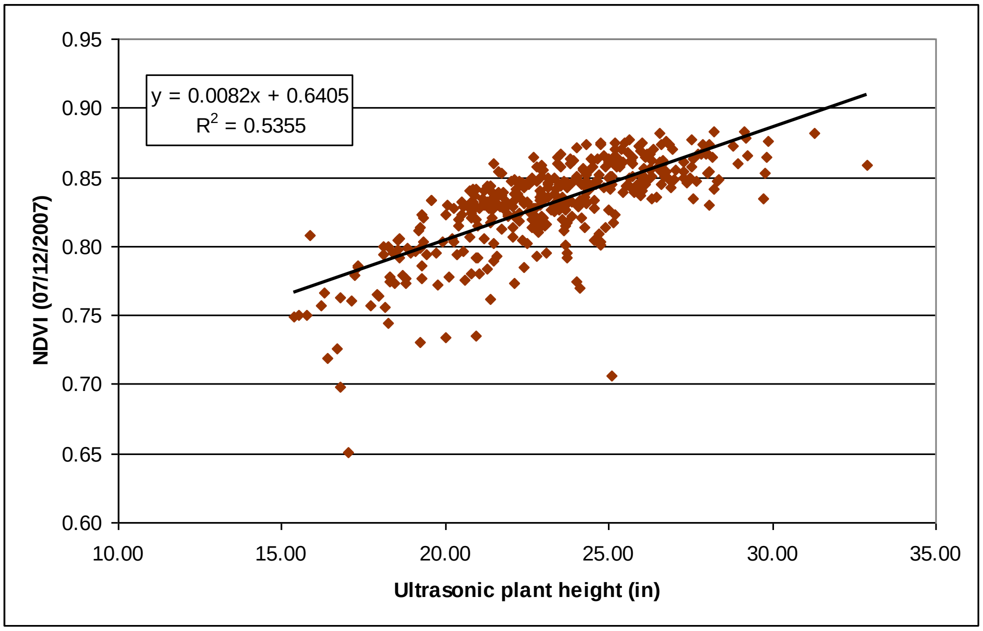

Figure 63. Linear correlation of NDVI and ultrasonic plant height (in) measurements per plot using GreenSeeker#2. Data collected in middle season (07/12/2007).

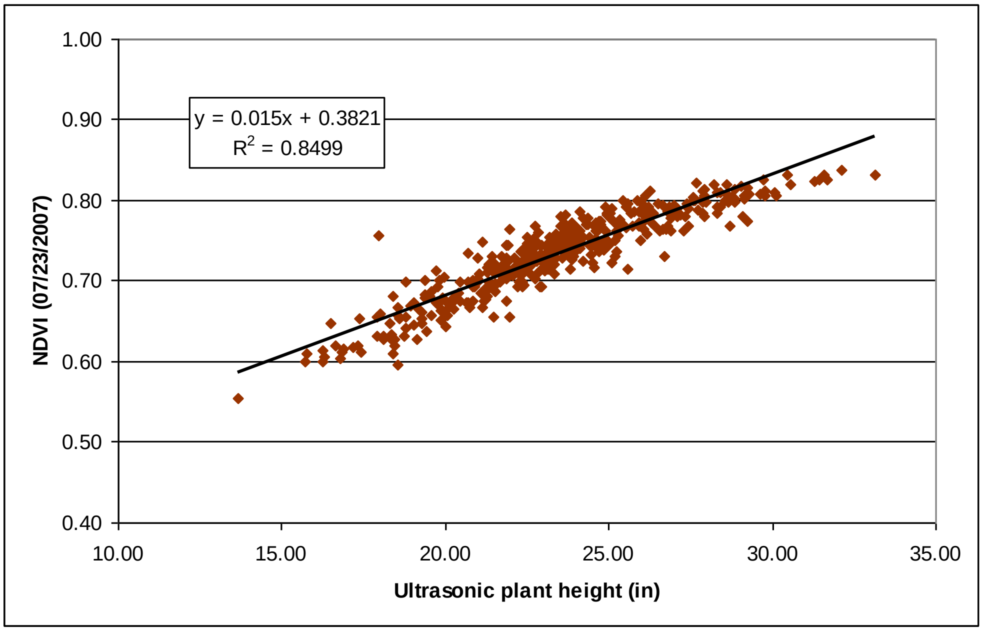

Figure 64. Linear correlation of NDVI and ultrasonic plant height (in) measurements per plot using GreenSeeker#2. Data collected late in season (07/23/2007).

Appendix 3. Plant Height Experiment 2008

Table 9. Manual plant height (in) mean three cotton varieties, three seeding rates, and four N rates for two sampling date. Means with the same letter are not statistically different within a given date (protected LSD (P<0.05).

Variety		Date	
		07/01/2008	08/01/2008
DP432	NDVI	18.1414 a	34.9022 b
	Std Error	0.4567	1.0090
DP434	NDVI	18.8801 a	38.8387 a
	Std Error	0.4631	1.0193
DP444	NDVI	18.8392 a	38.9088 a
	Std Error	0.4391	0.9758
Seeding rate (seeds/ac)			
16400	NDVI	18.2048 b	40.0010 a
	Std Error	0.4105	0.9229
28700	NDVI	18.3712 ab	36.7068 b
	Std Error	0.4143	0.9289
50225	NDVI	19.2846 a	35.9419 b
	Std Error	0.4048	0.9129
Nitrogen rate (lb/ac)			
30	NDVI	18.5096 a	36.3013 a
	Std Error	0.4542	0.9984
60	NDVI	19.1642 a	38.3063 a
	Std Error	0.4647	1.0168
90	NDVI	18.7857 a	37.7970 a
	Std Error	0.4606	1.0098
120	NDVI	18.0214 a	37.7950 a
	Std Error	0.4635	1.0152

Table 10. Ultrasonic plant height (in) means for three cotton varieties, three seeding rates, and four N rates for nine sampling dates. Means with the same letter are not statistically different within a given date and treatment (protected LSD (P<0.05).

Variety		Date								
		06/18/08	06/24/08	06/26/08	07/01/08	07/08/08	07/17/08	07/22/08	08/01/08	08/05/08
DP432	NDVI	6.5842 b	9.6052 b	10.9857 b	12.8403 b	16.6153 c	23.5686 c	27.9410 b	31.4275 b	29.0725 b
	Std Error	0.1903	0.911	0.2277	0.3230	0.3415	0.3988	0.3171	0.4562	1.1581
DP434	NDVI	7.3459 a	10.8485 a	11.9606 a	14.9013 a	19.8951 a	27.8352 a	31.1281 a	35.7716 a	36.0916 a
	Std Error	0.1910	0.1931	0.2297	0.3252	0.3446	0.4004	0.3191	0.4213	0.9274
DP444	NDVI	6.0558 c	9.5479 b	11.0151 b	13.6558 b	17.9549 b	26.4390 b	31.1202 a	36.0949 a	35.9273 a
	Std Error	0.1929	0.1967	0.2335	0.3283	0.3504	0.4063	0.3247	0.4472	0.9288
Seeding rate (seeds/ac)										
16400	NDVI	5.0116	7.6855 c	9.1049 c	11.4138 c	15.9259 c	24.9720 c	29.7458 a	34.9447 a	34.9528 a
	Std Error	0.1724	0.1808	0.2028	0.2497	0.3504	0.3681	0.2841	0.3832	0.7268
28700	NDVI	6.7236 b	10.0993 b	11.3328 b	13.9264 b	18.2469 b	26.0601 b	30.2273 a	34.6566 a	32.9586 b
	Std Error	0.1691	0.1736	0.1952	0.2419	0.3390	0.3608	0.2747	0.3748	0.7338
50225	NDVI	8.2507 a	12.2168 a	13.5236 a	16.0573 a	20.2925 a	26.8106 a	30.2162 a	33.6927 b	33.1801 b
	Std Error	0.1711	0.1786	0.2005	0.2464	0.3470	0.3650	0.2801	0.3821	0.7763
Nitrogen rate (lb/ac)										
30	NDVI	6.6085 a	9.8622 a	11.1848 ab	13.6322 a	17.9204 a	25.6996 a	29.6839 a	33.4909 b	31.8296 b
	Std Error	0.1838	0.2049	0.2261	0.2722	0.3922	0.3962	0.3140	0.4117	0.7600
60	NDVI	6.5650 a	9.8595 a	11.0510 b	13.6926 a	18.0040 a	25.9206 a	30.0911 a	34.6756 a	34.3790 a
	Std Error	0.1820	0.2005	0.2215	0.2692	0.3844	0.3930	0.3102	0.4084	0.8018
90	NDVI	6.7964 a	10.2152 a	11.6223 a	13.9900 a	18.4389 a	26.3354 a	30.3659 a	34.9229 a	34.4123 a
	Std Error	0.1836	0.2052	0.2245	0.2706	0.3899	0.3933	0.3134	0.4094	0.8148
120	NDVI	6.6779 a	10.0653 a	11.4236 ab	13.8818 a	18.2571 a	25.8347 a	30.1116 a	34.6360 a	34.1677 a
	Std Error	0.1828	0.2024	0.2236	0.2706	0.3873	0.3917	0.3116	0.4129	0.8085

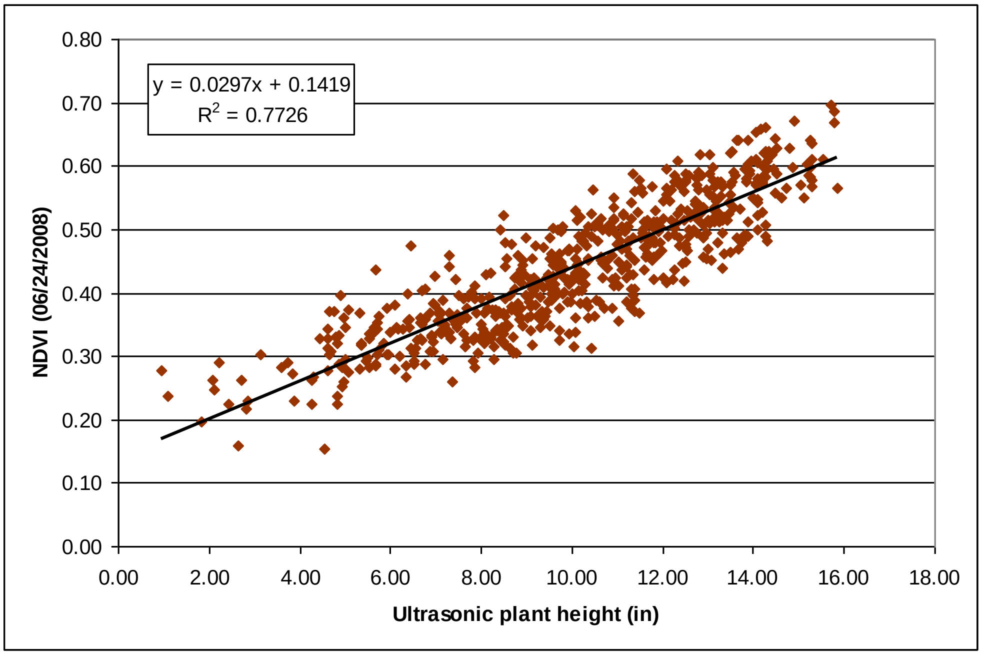

Figure 65. Linear correlation of NDVI and ultrasonic plant height (in) from June 24th, 2008.

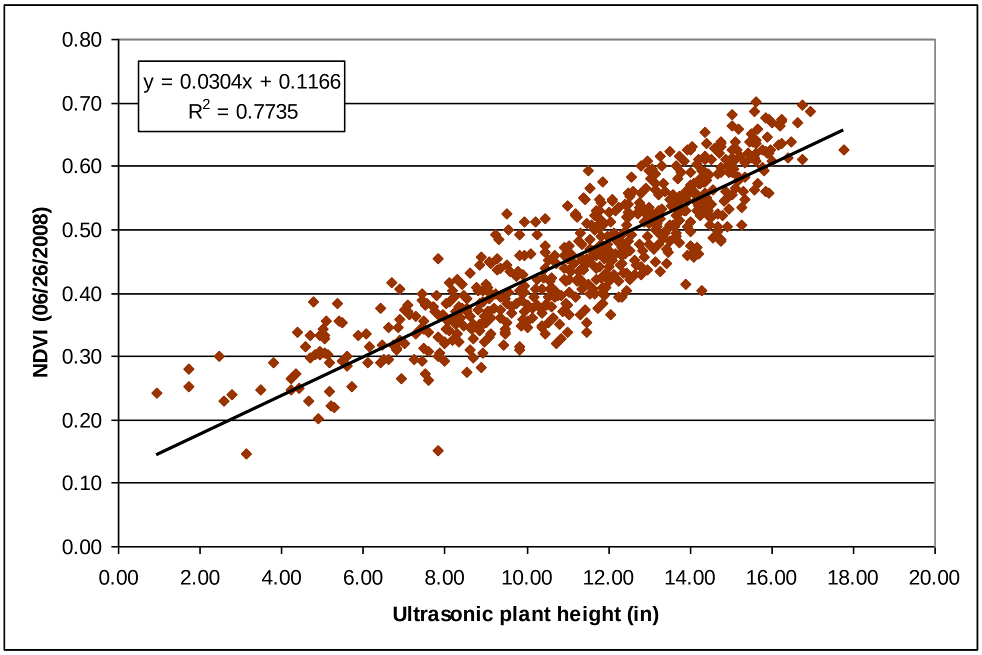

Figure 66. Linear correlation of NDVI and ultrasonic plant height (in) from June 26th, 2008.

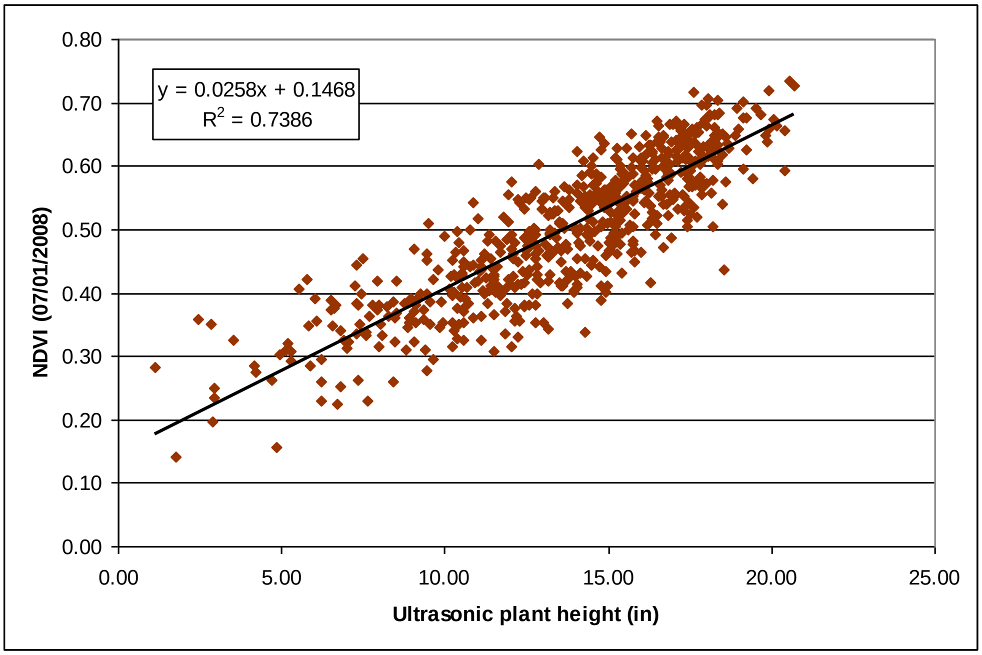

Figure 67. Linear correlation of NDVI and ultrasonic plant height (in) from July 1st, 2008.

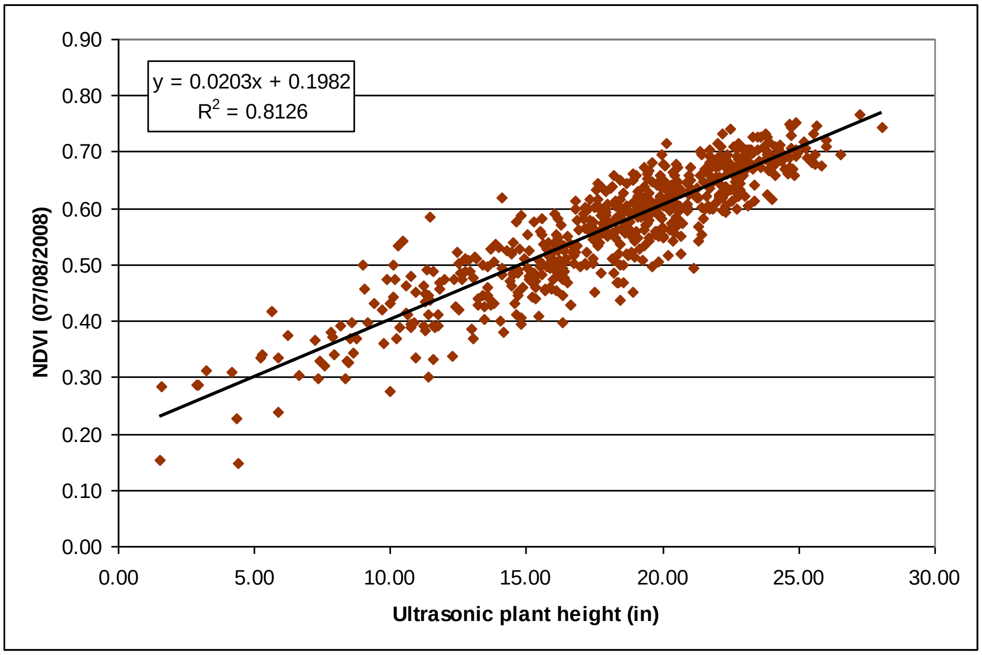

Figure 68. Linear correlation of NDVI and ultrasonic plant height (in) from July 8th, 2008.

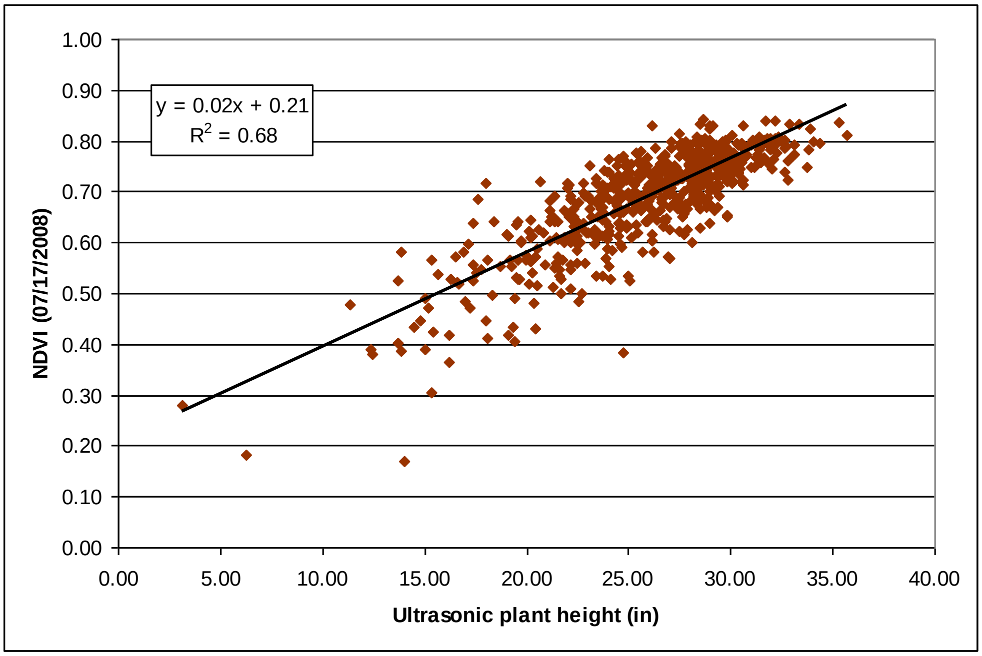

Figure 69. Linear correlation of NDVI and ultrasonic plant height (in) from July 17th, 2008.

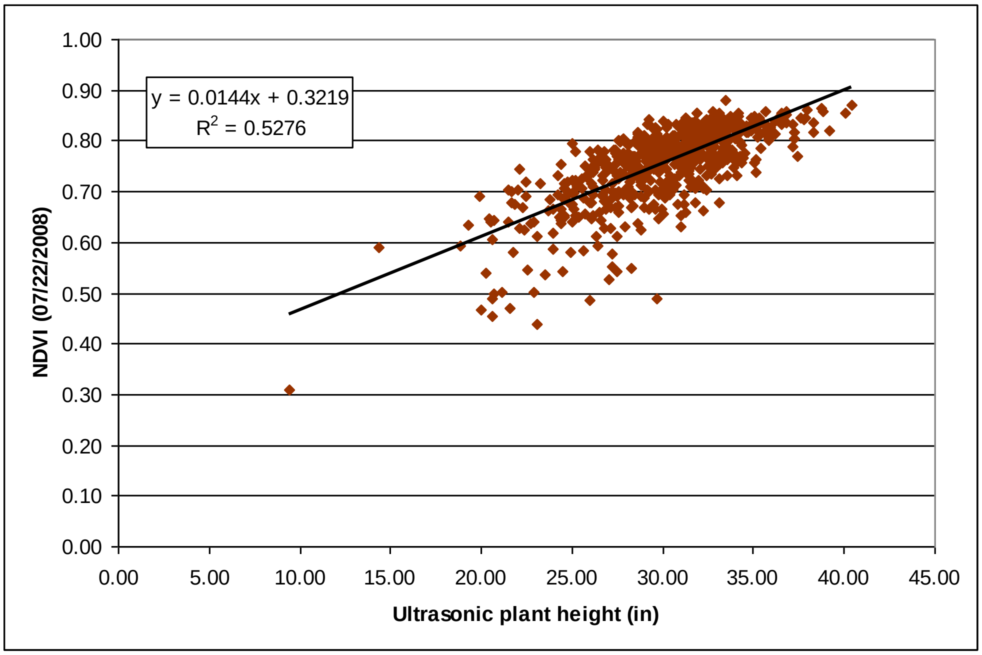

Figure 70. Linear correlation of NDVI and ultrasonic plant height (in) from July 22nd, 2008.

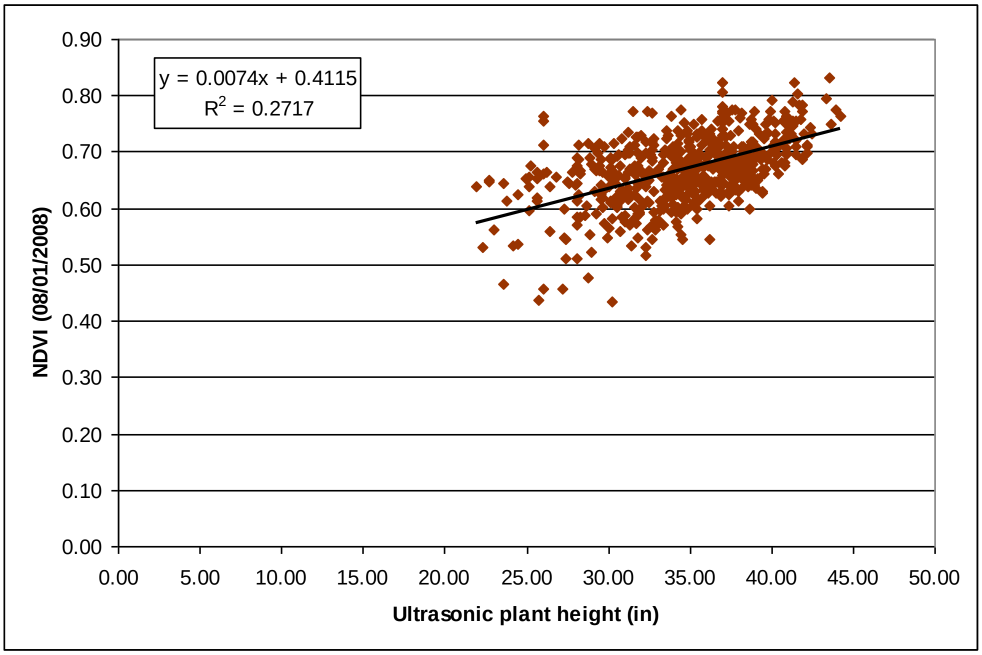

Figure 71. Linear correlation of NDVI and ultrasonic plant height (in) from August 1st, 2008.

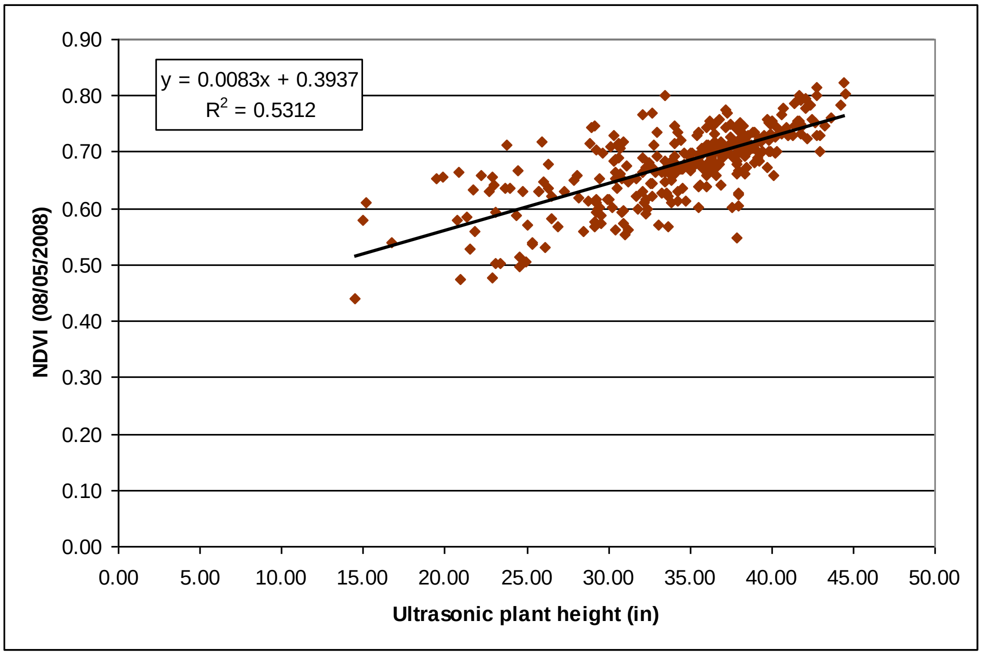

Figure 72. Linear correlation of NDVI and ultrasonic plant height (in) from August 5th, 2008.

Appendix 4. Soil Moisture Experiment 2008

Table 11. Water tension (kPa) mean differences for three seeding rates and four N rates for five sampling dates. Means with the same letter are not statistically different within a given date (protected LSD (P<0.05).

Seeding rate (seeds/ac)		Date				
		06/18/2008	06/26/2008	07/08/2008	07/17/2008	08/05/2008
16400	NDVI	21.1319 a	27.4583 a	45.5674 a	49.5258 b	102.96 a
	Std Error	1.6563	1.1818	6.2962	4.9078	12.427
28700	NDVI	23.1048 a	29.0000 a	51.6675 a	62.2857 ab	106.71 a
	Std Error	1.6232	1.1689	6.2100	4.8346	9.7289
50225	NDVI	20.3164 a	28.4375 a	61.5074 a	68.8538 a	124.63 a
	Std Error	1.5969	1.1359	6.0551	4.7213	9.2026
Nitrogen rate (lb/ac)						
30	NDVI	22.8169 a	28.6667 a	49.9029 a	55.6137 a	105.83 a
	Std Error	1.4994	1.0646	5.6746	4.4251	8.9279
60	NDVI	21.1626 a	27.4722 a	55.8762 a	64.1266 a	108.11 a
	Std Error	1.6826	1.2183	6.4649	5.0297	10.309
90	NDVI	21.0403 a	28.6667 a	51.4701 a	60.3136 a	124.67 a
	Std Error	2.1038	1.5585	8.2298	6.3863	14.5792
120	NDVI	21.0509 a	28.3889 a	54.4073 a	60.8331 a	107.11 a
	Std Error	1.9894	1.4693	7.7631	6.0260	13.959

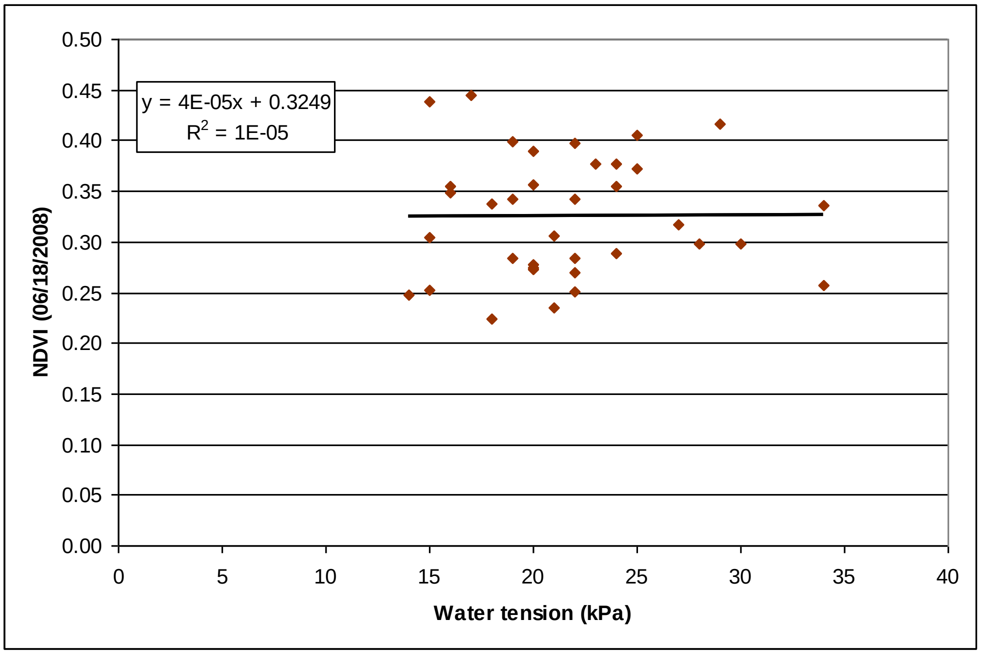

Figure 73. Linear correlation of NDVI and soil-water tension (kPa) from June 18th, 2008.

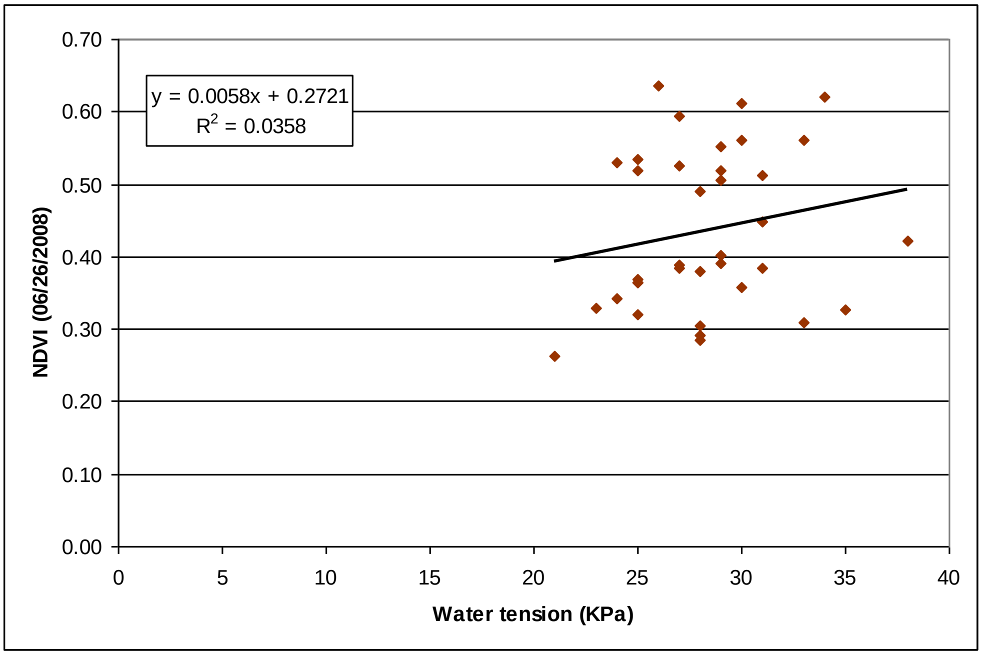

Figure 74. Linear correlation of NDVI and soil-water tension (kPa) from June 26th, 2008.

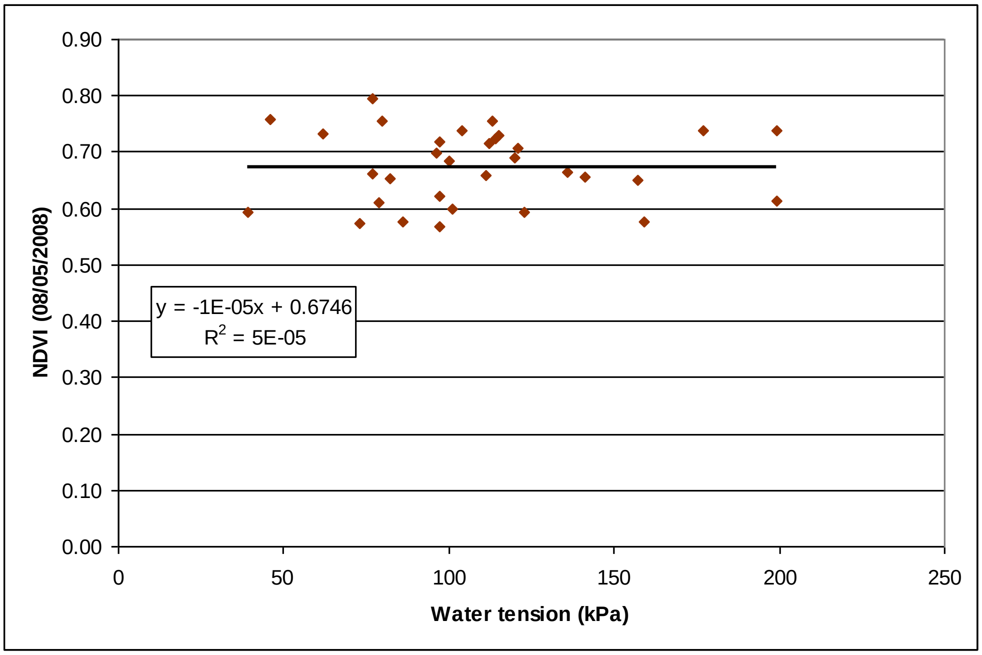

Figure 75. Linear correlation of NDVI and soil-water tension (kPa) from August 05th, 2008.

Appendix 5. Diurnal Cycle Experiment 2008

Table 12. NDVI mean for nine sampling times during a diurnal cycle. Means with the same letter are not statistically different between Times (protected LSD (P<0.05).

	Time								
	8:21	9:03	9:59	11:10	12:07	13:07	14:01	15:01	15:58
NDVI	0.695	0.6803	0.6757	0.6707	0.6667	0.6734	0.665	0.674	0.6785
Std Error	0.0217	0.0217	0.0217	0.0217	0.0217	0.0217	0.0217	0.0217	0.02171
Letter group	a	b	cd	ef	fg	de	g	de	bc

Appendix 6. Manipulated Plant Spacing Experiment 2008

Table 13. NDVI means by Pass for the 12 manipulated plant spacing subplots. Different letter means statistically different between Passes (protected LSD (P<0.05).

Pass	NDVI	Std Error
1	0.5833 a	0.02819
2	0.4980 b	0.02817
3	0.4056 c	0.02818
4	0.2953 d	0.02820

Table 14. RR, NRR, NNR, and OSAVI means by Pass for the 12 manipulated plant spacing subplots. Different letter means statistically different between Passes (protected LSD (P<0.05).

Ratio	Pass	NDVI	Std Error
RR	1	0.2812 d	0.02668
	2	0.3609 c	0.02666
	3	0.4539 b	0.02667
	4	0.5674 a	0.02669
NRR	1	0.2085 d	0.01408
	2	0.2510 c	0.01407
	3	0.2972 b	0.01407
	4	0.3524 a	0.01408
NNR	1	0.7915 a	0.01408
	2	0.7490 b	0.01407
	3	0.7028 c	0.01407
	4	0.6476 d	0.01408
OSAVI	1	0.3816 a	0.01966
	2	0.3202 b	0.01965
	3	0.2544 c	0.01965
	4	0.1811 d	0.01966

Table 15. NDVI trimmed one or two standard deviations means by Pass for the 12 manipulated plant spacing subplots. Different letter means statistically different between Passes (protected LSD (P<0.05).

Vegetation index	Pass	NDVI	Std Error
NDVI trimmed SD	1	0.5994 a	0.03693
	2	0.5364 b	0.03693
	3	0.4815 c	0.03695
	4	0.3913 d	0.03701
NDVI trimmed 2*SD	1	0.6389 a	0.02401
	2	0.6100 b	0.02401
	3	0.5944 c	0.02404
	4	0.5633 d	0.02416

Table 16. OSAVI trimmed one or two standard deviation means by Pass for the 12 manipulated plant spacing subplots. Different letter means statistically different between Passes (protected LSD (P<0.05).

Vegetation index	Pass	NDVI	Std Error
OSAVI trimmed SD	1	0.4002 a	0.02433
	2	0.3643 b	0.02433
	3	0.3351 c	0.02435
	4	0.2913 d	0.02442
OSAVI trimmed 2*SD	1	0.4297 a	0.01900
	2	0.4116 b	0.01901
	3	0.4024 c	0.01903
	4	0.3851 d	0.01913

Table 17. NDVI “maximum canopy values” means by Pass for the 12 manipulated plant spacing subplots. Different letter means statistically different between Passes (protected LSD (P<0.05).

Pass	NDVI	Std Error
1	0.7088 a	0.01734
2	0.6879 b	0.01742
3	0.6769 b	0.01753
4	0.6538 c	0.01816

Appendix 7. Testing Algorithms with Entire Field Experiment Data 2008

Table 18. OSAVI means for three cotton varieties, three seeding rates, and four N rates for nine sampling dates. Means with the same letter are not statistically different within a given date and treatment (protected LSD (P<0.05).

Variety		Date								
		06/18/08	06/24/08	06/26/08	07/01/08	07/08/08	07/17/08	07/22/08	08/01/08	08/05/08
DP432	NDVI	0.2401 a	0.3102 ab	0.3258 a	0.3301 a	0.3270 b	0.4458 a	0.4821 a	0.3295 a	0.3353 a
	Std Error	0.009794	0.006780	0.007232	0.008924	0.006184	0.006715	0.009595	0.008661	0.007015
DP434	NDVI	0.2440 a	0.3223 a	0.3242 a	0.3159 a	0.3440 a	0.4185 b	0.4629 ab	0.2846 b	0.3193 b
	Std Error	0.009803	0.006790	0.007243	0.008932	0.006244	0.006702	0.009577	0.008716	0.006968
DP444	NDVI	0.2257 a	0.2995 b	0.3044 a	0.3091 a	0.3355 ab	0.4197 b	0.4556 b	0.2951 b	0.3230 ab
	Std Error	0.009810	0.006807	0.007273	0.008946	0.006306	0.006715	0.009596	0.008705	0.007053
Seeding rate (seeds/ac)										
16400	NDVI	0.2149 c	0.2788 c	0.2725 c	0.2868 c	0.3023 c	0.4086 c	0.4455 c	0.2980 b	0.3256 a
	Std Error	0.008671	0.004761	0.004784	0.007065	0.005487	0.005000	0.007906	0.006310	0.006619
28700	NDVI	0.2317 b	0.3032 b	0.3113 b	0.3159 b	0.3342 b	0.4280 b	0.4674 b	0.3007 b	0.3261 a
	Std Error	0.008607	0.004584	0.004593	0.006953	0.005282	0.004923	0.007855	0.006235	0.006482
50225	NDVI	0.2633 a	0.3501 a	0.3706 a	0.3524 a	0.3699 a	0.4474 a	0.4877 a	0.3105 a	0.3258 a
	Std Error	0.008659	0.004720	0.004742	0.007041	0.005425	0.004969	0.007889	0.006279	0.006566
Nitrogen rate (lb/ac)										
30	NDVI	0.2369 a	0.3125 a	0.3168 a	0.3205 a	0.3344 a	0.4234 b	0.4608 b	0.2916 b	0.3099 c
	Std Error	0.008663	0.004767	0.004853	0.007107	0.005771	0.005223	0.008041	0.006523	0.006994
60	NDVI	0.2349 a	0.3088 a	0.3179 a	0.3168 a	0.3326 a	0.4265 ab	0.4672 ab	0.3027 a	0.3252 b
	Std Error	0.008655	0.004739	0.004814	0.007083	0.005699	0.005195	0.008020	0.006489	0.006925
90	NDVI	0.2360 a	0.3102 a	0.3174 a	0.3189 a	0.3372 a	0.4268 ab	0.4668 ab	0.3070 a	0.3286 ab
	Std Error	0.008662	0.004762	0.004845	0.007106	0.005749	0.005210	0.008027	0.006511	0.006958
120	NDVI	0.2386 a	0.3111 a	0.3205 a	0.3174 a	0.3377 a	0.4352 a	0.4726 a	0.3110 a	0.3397 a
	Std Error	0.008659	0.004752	0.004836	0.007097	0.005736	0.005190	0.008022	0.006503	0.006940

Table 19. OSAVI trimmed 2*SD means for three cotton varieties, three seeding rates, and four N rates for nine sampling dates. Means with the same letter are not statistically different within a given date and treatment (protected LSD (P<0.05).

Variety		Date								
		06/18/08	06/24/08	06/26/08	07/01/08	07/08/08	07/17/08	07/22/08	08/01/08	08/05/08
DP432	NDVI	0.2801 a	0.3752 ab	0.3914 a	0.3837 a	0.3700 b	0.4682 a	0.4971 a	0.3386 a	0.3443 a
	Std Error	0.008520	0.005290	0.006022	0.007442	0.005120	0.005919	0.008234	0.007390	0.006173
DP434	NDVI	0.2833 a	0.3864 a	0.3872 a	0.3765 a	0.3864 a	0.4501 b	0.4828 a	0.2931 b	0.3275 b
	Std Error	0.008527	0.005301	0.006040	0.007455	0.005166	0.005913	0.008217	0.007450	0.006145
DP444	NDVI	0.2654 b	0.3696 b	0.3760 a	0.3747 a	0.3767 ab	0.4538 ab	0.4817 a	0.3028 b	0.3330 ab
	Std Error	0.008533	0.005313	0.006053	0.007467	0.005195	0.005912	0.008233	0.007435	0.006212
Seeding rate (seeds/ac)										
16400	NDVI	0.2676 b	0.3682 b	0.3675 c	0.3683 b	0.3670 b	0.4555 ab	0.4823 b	0.3123 a	0.3403 a
	Std Error	0.007848	0.003516	0.004222	0.006088	0.004592	0.004457	0.007116	0.005388	0.006095
28700	NDVI	0.2713 b	0.3693 b	0.3775 b	0.3726 b	0.3741 b	0.4546 b	0.4847 b	0.3093 a	0.3344 ab
	Std Error	0.007795	0.003356	0.004099	0.006007	0.004460	0.004381	0.007069	0.005306	0.005972
50225	NDVI	0.29000 a	0.3936 a	0.4096 a	0.3939 a	0.3920 a	0.4619 a	0.4946 a	0.3129 a	0.3301 b
	Std Error	0.007837	0.003478	0.004197	0.006068	0.004555	0.004425	0.007098	0.005354	0.006046
Nitrogen rate (lb/ac)										
30	NDVI	0.2772 a	0.3791 a	0.3846 a	0.3797 a	0.3758 a	0.4519 b	0.4804 b	0.2992 b	0.3194 b
	Std Error	0.007846	0.003534	0.004288	0.006157	0.004806	0.004634	0.007251	0.005609	0.006421
60	NDVI	0.2749 a	0.3764 a	0.3862 a	0.3790 a	0.3779 a	0.4585 ab	0.4882 ab	0.3133 a	0.3361 a
	Std Error	0.007839	0.003512	0.004272	0.006138	0.004762	0.004616	0.007231	0.005573	0.006359
90	NDVI	0.2760 a	0.3763 a	0.3843 a	0.3790 a	0.3784 a	0.4570 ab	0.4876 ab	0.3146 a	0.3373 a
	Std Error	0.007846	0.003532	0.004286	0.006158	0.004792	0.004621	0.007242	0.005602	0.006396
120	NDVI	0.2770 a	0.3764 a	0.3845 a	0.3756 a	0.3787 a	0.4620 a	0.4926 a	0.3188 a	0.3470 a
	Std Error	0.007842	0.003519	0.004275	0.006147	0.004775	0.004605	0.007232	0.005588	0.006372

Table 20. NDVI “maximum canopy values” means for three cotton varieties, three seeding rates, and four N rates for nine sampling dates. Means with the same letter are not statistically different within a given date and treatment (protected LSD (P<0.05).

Variety		Date								
		06/18/08	06/24/08	06/26/08	07/01/08	07/08/08	07/17/08	07/22/08	08/01/08	08/05/08
DP432	NDVI	0.4860 a	0.5966 ab	0.6187 a	0.6669 a	0.6869 b	0.7950 a	0.8385 a	0.7483 a	0.7431 a
	Std Error	0.007608	0.005255	0.007692	0.006835	0.006356	0.007150	0.004315	0.008128	0.005266
DP434	NDVI	0.4917 a	0.5984 a	0.6154 a	0.6566 a	0.7069 a	0.7773 a	0.8263 b	0.712 b	0.7397 a
	Std Error	0.007646	0.005313	0.007728	0.006862	0.006410	0.007153	0.004298	0.008154	0.005285
DP444	NDVI	0.4658 b	0.5811 b	0.6020 a	0.6524 a	0.6977 ab	0.7883 a	0.8287 ab	0.7395 ab	0.7494 a
	Std Error	0.007672	0.005316	0.007747	0.006870	0.006464	0.007145	0.004322	0.008176	0.005353
Seeding rate (seeds/ac)										
16400	NDVI	0.4550 c	0.5676 c	0.5870 c	0.6523 b	0.6874 b	0.7947 a	0.8340 a	0.7466 a	0.7581 a
	Std Error	0.006895	0.004011	0.005282	0.005475	0.005450	0.005020	0.003520	0.006543	0.004740
28700	NDVI	0.4770 b	0.5881 b	0.6094 b	0.6532 b	0.6952 b	0.7853 b	0.8307 a	0.7344 b	0.7419 b
	Std Error	0.006733	0.003687	0.005049	0.005302	0.005284	0.004960	0.003458	0.006461	0.004601
50225	NDVI	0.5114 a	0.6205 a	0.6397 a	0.6704 a	0.7089 a	0.7806 b	0.8288 a	0.7280 b	0.7322 c
	Std Error	0.006825	0.003938	0.005231	0.005426	0.005377	0.004995	0.003494	0.006511	0.004684
Nitrogen rate (lb/ac)										
30	NDVI	0.4814 a	0.5918 a	0.6130 a	0.6592 a	0.6941 a	0.7828 b	0.8259 b	0.7230 b	0.7266 b
	Std Error	0.006921	0.004072	0.005406	0.005550	0.005869	0.005166	0.003682	0.06759	0.005072
60	NDVI	0.4792 a	0.5948 a	0.6136 a	0.6591 a	0.6989 a	0.7866 ab	0.8316 ab	0.7375 a	0.7464 a
	Std Error	0.006903	0.004048	0.005366	0.005518	0.005801	0.005147	0.003660	0.006727	0.005027
90	NDVI	0.4817 a	0.5904 a	0.6112 a	0.6576 a	0.6991 a	0.7871 ab	0.8319 ab	0.7414 a	0.7479 a
	Std Error	0.006908	0.004060	0.005391	0.005540	0.005841	0.005153	0.003677	0.006752	0.005061
120	NDVI	0.4822 a	0.5911 a	0.6103 a	0.6586 a	0.6966 a	0.7909 a	0.8353 a	0.7433 a	0.7553 a
	Std Error	0.006915	0.004039	0.005371	0.005536	0.005808	0.005142	0.003667	0.006741	0.005046

Appendix 8. OSAVI and Leaf N Concentration Correlation 2008

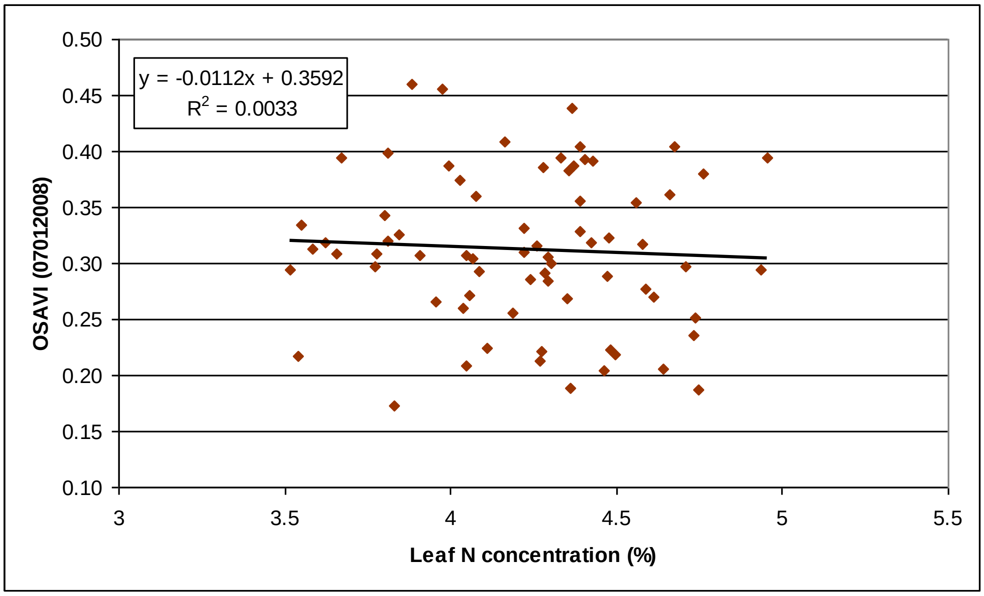

Figure 76. Linear correlation of OSAVI and leaf N concentration (%) from July 1st, 2008.

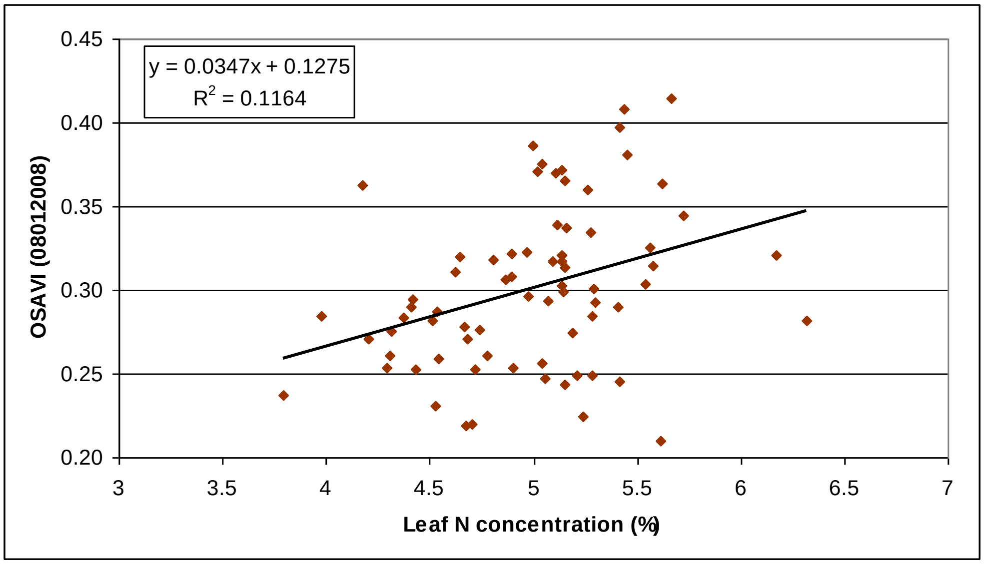

Figure 77. Linear correlation of OSAVI and leaf N concentration (%) from August 1st, 2008.

Vita

Marisol Benitez Ramirez was born on October 2nd, 1971 in San Cristobal, Venezuela. She grew up in Merida, Venezuela. She studied in the "Colegio Arzobispo Silva" her secondary school and graduated in 1988. Marisol began her academic career in September of 1989 studying Systems Engineering at the University of Los Andes. Through October of 1995 to May of 1996, she was awarded with a fellowship from the Foundation for the Development of Science and Technology (FUNDACITE – Mérida, Venezuela). The last year of her undergraduate studies she served as a Teaching Assistant in Servomecanisms. Marisol finished her Bachelor of Science degree in Systems Engineering with a major concentration in Control Systems and minors in Computer Engineering and Operation Research in July 1998. After graduation she worked for a year as Research Associate in the *Project "Integration of Technologies of Heterogeneous Software Systems" CONICIT* at the Systems Engineering School of the University of Los Andes in Merida, Venezuela.

In January 2000, Marisol moved to the US to study English. From April of 2001 through August 2003, she worked as a laboratory and field technician in the Crop Sciences Department at the University of Illinois at Urbana-Champaign. Marisol began a Master of Science program in the Crop Science Department at UIUC in August 2003. Throughout her program she worked as a Research Assistant. She graduated in May of 2005. Marisol worked from January through December of 2005 in Clough's Lab of the Crop Science Department at UIUC developing algorithms for microarray data statistical analysis.

Marisol moved back to her home country Venezuela during 2006. In January 2007, she began a Master of Science program in the Biosystems Engineering and Soil Sciences Department at the University of Tennessee. Throughout her program she worked as a Graduate Teaching Assistant as a Computer Lab Coordinator. Member of the Honor Society of Agriculture Gamma Sigma Delta, Marisol finished her Master of Science degree in July 2009 with a major in Biosystems Engineering and a minor in Statistics.