REFLECTANCE SENSORS TO PREDICT MID-SEASON NITROGEN NEED OF COTTON

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by

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The undersigned, appointed by the dean of the Graduate School, have examined the thesis entitled:

REFLECTANCE SENSORS TO PREDICT MID-SEASON NITROGEN NEED OF COTTON

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a candidate for the degree of **Master of Science**,

and hereby certify that, in their opinion, it is worthy of acceptance.

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To my father and mother, Jose Claudio de Oliveira and Rosa Maria Farias de Oliveira,
My sisters, Camila and Luana Farias de Oliveira,
And to my husband and beautiful daughter, Jason Miller and Leilah Oliveira Miller

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LITERATURE REVIEW

Nitrogen (N) is an essential nutrient for cotton (*Gossypium hirsutum* L.) production. A five year irrigated study in California concluded that cotton plants need a total of 200 to 220 kg ha^{-1} of plant available N (soil NO_3-N plus applied N) to achieve 1500 kg lint ha^{-1} yield (Hutmacher et al., 2004). A long-term study found that in furrow flow and center pivot irrigation (high and medium frequency), the maximum cotton yield was obtained by N fertilization of 67 to 134 kg N ha^{-1} (McConnell et al., 1993). Little difference in soil nitrate accumulation was found between the N rates that ranged from 0 to 134 kg N ha^{-1}. This large range in N fertilization needed for maximum yield is due to the amount of nitrogen already present in the soil. A two year study in Louisiana indicated that plants that had a N rate of 168 kg N ha^{-1} did not use nitrogen as efficiently as plants receiving 84 kg N ha^{-1} and did not have high yields (Boquet and Breitenbeck, 2000).

However, if the N supply is insufficient or exceeds the N requirement of cotton, some production problems may occur. Nitrogen deficiency causes reduced vegetative growth, reduced number of bolls and seed, low fiber strength and length, and low fiber yields (Malavolta et al., 2004; Read et al., 2006). On the other hand, excessive N application leads to excessive vegetative growth (McConnell et al., 1993). Excessive vegetative growth increases production cost since it increases the need for growth regulator (McConnell et al., 1992), defoliant (McConnell et al., 1993), and insecticide due to more insect pressure (Cisneros and Godfrey, 2001). Over application of N can also delay crop maturity (McConnell et al., 1993). Delayed crop maturity can affect profit due to rainy weather at the end of the season that will cause boll rot and not allow the harvest equipment to operate.

Determining the amount of nitrogen that is needed by cotton is a difficult task because of the spatial variability of N in the soil between and within fields. Within a field there can be spatial variability of N in the soil (Elms et al., 2001). In this three year study, the analyzed soil samples collected from the center of each of the 57 grid cells for a 5.3 ha field showed a high variability of soil nitrate (C.V. = 54.4). Similarly, a corn (*Zea mays* L.) study found that the economically optimal N rate (EONR) within a field varied from 0 to 280 kg N ha^{-1} in five of the eight areas (Scharf et al., 2005). The remaining three areas had smaller within-field variability. If a uniform rate of nitrogen is applied throughout the field, ignoring N variability in the soil, some areas will probably have excess nitrogen, while others will have too little N to meet cotton's needs.

Diagnosing N status will help to avoid nitrogen deficiency and excess N application, and therefore help to minimize production cost and maximize yield and quality. The current diagnostic tools available to the cotton producers are soil testing for nitrate and ammonium, petiole nitrate concentration, and leaf total N concentration. These tools are not well-suited for variable N diagnosis and application since they are labor-intensive and time-consuming. Furthermore, most of these tools are used mainly to diagnose N deficiency that already exists, but not to select N fertilizer rate. There is a need both to calibrate diagnostic tools to predict how much N fertilizer is needed, and to develop tools that can be used to diagnose N need in a spatially variable manner. Reflectance sensors have the potential to meet both of these needs.

SPECTRAL SENSORS AS INDICATORS OF N STATUS

Recently, with the advance of precision agriculture and remote sensing, newer methods of N status detection have been developed. These tools are based on the spectral properties (absorption or reflectance) of a single leaf or a canopy, and have the potential to sense reflectance change cause by N deficiency and guide N application. Numerous studies have investigated the capability of these sensors to detect N status.

Few studies have shown yield response to N and reflectance relationships. A nitrogen-rate experiment conducted in Tennessee seemed to indicate that spectral reflectance at mid-early square was highly related to yield response to N (Wilkerson et al., 1998). In this experiment, yield was very responsive to N application. The size of the relative spectral index [(Near infrared + blue)/(green + amber)] response to N was related to size of yield response to N. The spectral index pointed out the nitrogen rate that had the best yield and economical advantage. The index was also capable of determining the N needed in nitrogen deficient areas. The fact that these results were obtained from spectral data at mid-early square is encouraging for timely N application. However, this was a one location experiment and more data are needed to confirm these results.

On the other hand, in a group of five N response experiments with low and moderate yield response to N, the size of the yield response was at best weakly related to relative 'NDVI-days' (the integral of NDVI over time) (Plant et al., 2000). The location with the largest yield response to N was only intermediate in terms of spectral differences between N rate treatments. Even if there was a consistent relationship between the size of the spectral response to N and the size of the yield response to N, the 'NDVI-days' reported in this research were accumulated until far too late in the season to be used in making N application decisions.

Most studies do not mention the relationship between yield response to total nitrogen and spectral data. For instance, an experiment with different N rates might not have reflectance differences between the treatments if the area has enough N in the soil. Nonetheless, these studies can help us understand how reflectance behaves at different N statuses and how it relates to other N diagnostic tools.

Several studies have focused on determining the effects of varying N rates on leaf reflectance (Buscaglia and Varco, 2002; Zhao et al., 2005; Fridgen and Varco, 2004). A greenhouse experiment found that reflectance on the visible part of the spectrum (380-750 nm) increased as nitrogen deficiency increased (Buscaglia and Varco, 2002). Nitrogen deficiency lowers chlorophyll content, and consequently decreases the amount of light absorbed by leaf, increasing reflectance. In this study conducted in Mississippi, leaf reflectance was recorded on the most recently expanded leaf using a spectroradiometer. Readings were taken at second week of squaring (SWS) and second week of flowering (SWF) from four N treatments. The lowest N rate had consistently higher leaf reflectance than the higher N treatments in the visible range for both SWS and SWF.

Accordingly, comparable results were obtained by two different field studies that recorded reflectance from detached fully expanded leaves (Zhao et al., 2005; Fridgen and Varco, 2004). In the first study, the results indicated that reflectance in the visible range and red edge was affected by the N rate (Zhao et al., 2005). For instance, a decrease in N application resulted in an increase in reflectance at 556 and 710nm. Their field study consisted of four N rates (0 to 168 kg N ha^{-1}). The reflectance measurements were taken weekly or biweekly, using a spectroradiometer. In agreement with the previously mentioned results, a field study in Mississippi reported that their cotton treatment with 0 N/ha had higher reflectance than the other four N treatments near 550nm at early flower and peak bloom (Fridgen and Varco, 2004). Leaf reflectance was measured from each plot using a spectroradiometer.

Other studies tried to relate the sensor's data with N rate and more commonly used N diagnostic tools, such as leaf N and soil nitrate test, to prove the concept that N status can be predicted from color (Feibo et al., 1998; Boggs et al., 2003; Bronson et al., 2005). A two-location experiment in China was conducted to determine the relationship between chlorophyll meter with N application and leaf N (Feibo et. al., 1998). The results indicated that cotton N rate can be predicted from color. All five growth stages had highly significant relationships (R^2 = 0.67 to 0.91; significant at 0.01 P level) between N rate and SPAD (chlorophyll meter) values. Additionally, linear regression between area-based leaf N and SPAD were significant at each of the five growth stages ($R^2 > 0.78$; $P < 0.01$). Chlorophyll meter readings were taken on the most recently expanded leaves.

Other research seemed to indicate that chlorophyll meter was a good indicator of N status, but that hyperspectral data collected with the sensors mounted above the cotton canopy could not detect N status. A three nitrogen rate field experiment in Alabama found that SPAD readings had significant correlations with hyperspectral data between 776 to 817 nm (Boggs et al., 2003). However, chlorophyll meter readings had a better correlation with soil nitrate than did the spectral reflectance. The low correlations were attributed to the fact that hyperspectral data included readings from the soil and from a number of leaves, while the chlorophyll readings were collected from five leaves, which increased the accuracy by avoiding the effect of bare soil in the readings. Another factor that could have caused the low correlation between the spectral data and the soil-nitrate is the fact that there was no N response difference between the two highest N rates out of only three N treatments in this experiment. Spectral readings and soil samples were obtained 15 weeks after planting.

Furthermore, a study found that the reflectance measurements had poor correlation with leaf N, indicating that spectral data from a hand-held sensor might not be able to determine N status (Bronson et al., 2005). Three N treatments were tested: 0 N, blanket-rate N, and variable N rate application based on soil nitrate test and a yield goal of 1110 kg lint ha^{-1}. Reflectance was measured at the early square growth stage during a three year period (2002-2004). Samples for leaf N were also collected at early square. Passive sensors, which measure the sunlight reflected from the plants, and active sensors, which measures the reflected light emitted by their own light source, were used in this study. Three NDVIs (Normalized Difference Vegetation Index) were computed from the reflectance data: NDVI red = (780 - 670nm)/(780 + 670nm) from both passive and active sensors and NDVI green = (780 - 550nm)/(780 + 550nm) from a passive sensor. Generally, most of the NDVIs and leaf N had greater values from the fertilized plots than the 0 N plots. Partial least square regression on spectral reflectance successfully estimated leaf N in 2002 and 2004 ($R^2 < 0.63$). However, the NDVIs had low coefficients of determination of leaf N ($R^2 < 0.33$). Moreover, when the individual wavelengths were related to leaf N, the correlations were generally low. The low R^2s could have been a result of no statistical difference on the leaf N concentrations between the blanket N rate and variable N rate in any of the three years. Also, in 2003 there was a lack of response to N due to weather.

So far, few studies have compared the spectral readings to the yield response to nitrogen. Most studies have researched how spectral reflectance is affected by different N rates or relationships between spectral data and N diagnostic tools. More research is needed to study how N recommendations based on reflectance measurements can be developed.

COMPARISONS BETWEEN WAVELENGTHS AND INDICES AS AN INDICATOR OF COTTON N STATUS

Several studies have focused on the wavelengths that can better diagnose N status (Zhao et al., 2005; Buscaglia and Varco, 2002; Fridgen and Varco, 2004; Sui and Thomasson, 2004). Some wavelengths are more related to leaf chlorophyll and consequently are more sensitive to a change in N status. In recent field research it was reported that the reflectance for 556 (green) and 710 (red) nm had the best correlations with N rate (Zhao et al., 2005). The results also showed that the reflectance for 517 (green) and 701 (red) nm had the best correlations with tissue leaf N. The reflectance measurements were taken weekly or biweekly, from detached fully expanded leaves using a spectroradiometer. Their study consisted of four N rates from 0 to 168 kg N ha^{-1}. Leaf N was measured on both a dry weight and leaf area basis.

Similar results were obtained by a greenhouse experiment Buscaglia and Varco (2002) and a field study Fridgen and Varco (2004). Buscaglia and Varco (2002) reported that the best wavelengths to predict leaf N were 550 and 728nm. Leaf reflectance was recorded at second week of squaring (SWS) and second week of flowering (SWF) on the most recently expanded leaf of four N treatments. Five leaves for each treatment were sampled to determine total leaf N. Similarly, Fridgen and Varco (2004) results showed that the cotton with no N applied had greater reflectance at 550 nm than the other N rates. Leaf reflectance was computed from 3 mature leaves detached and measured immediately from 5 different N rates.

Furthermore, research conducted by Mississippi State University concluded that leaf N had a better correlation with red and NIR channels than with blue and green (Sui and Thomasson., 2004). Nevertheless, the combination of blue bands with other bands, improved the correlation of leaf N with those channels. Also, the model that had the strongest relationship with leaf N content ($R^2 = 0.50$, $p < 0.05$) included blue, green, red bands, NIR, and plant height. An active sensor was designed to measure reflectance from a canopy and mounted on a four-wheel vehicle, 0.46 meters above the canopy. The spectral readings were taken continuously at the early square growth stage from four N treatments.

In addition to the spectral indices already discussed, such as NDVI, a number of researchers developed and studied the effectiveness of reflectance ratios in detecting N deficiency (Tarpley et al., 2000; Zhao et al., 2005; Sui et al., 2005; Zhao et al., 2004). A recent study reported good accuracy and precision in estimating leaf N concentration using wavelength ratios (Tarpley et al., 2000). The ratios were computed using a red-edge band (700 or 716) divided by a waveband in the near infrared region (755-920 and 1000nm). The cotton for this experiment was grown in growth chambers with different N rates. For instance, the correlation between the predicted N based on the ratio

716/808nm and leaf N was r = 0.74 (good precision) and the calibration curve had a slope of 0.94 (good accuracy). The reflectance data were collected at mid-fruiting stage from three to six detached leaves in each chamber. Leaves from each chamber were also analyzed for leaf N. Reflectance ratios (two waveband ratios) and leaf N concentrations were used to construct calibration curves. Field-grown cotton was used to compare predicted N values to actual leaf N concentrations.

Other research supported the use of simple reflectance ratios to increase precision of leaf N concentration (Zhao et al., 2005). The reflectance measurements were taken weekly or biweekly, from detached fully expanded leaves of four N treatments of 0 to 168 kg N ha^{-1}. Simple ratios of 517 (green) or 701 (red) nm were tested since in this study those wavelengths' reflectance best related to leaf N. The results showed that leaf N concentration had the best linear relationship with the reflectance ratio 517/413 nm (green/purple) ($R^2 = 0.83$).

Most recently, an Index, PNI ((Blue + Green)/NIR), was created to evaluate the spectral readings (Sui et al., 2005). The readings were taken with an active sensor developed with four channels (blue, green, red, and near infrared) to measure reflectance from the canopy. The field test consisted of five nitrogen rates. There were 5 scans taken per plot at the early square growth stage, 0.46 meters above the canopy. The results showed great correlation between PNI and the N rates ($R^2 = 0.74$) and yield ($R^2 = 0.82$).

Zhao et al. (2004) also attempted to identify the vegetation indices better capable of showing differences between N treatments. Their field experiment in China consisted of four N treatments, and three collection dates for the reflectance measurements, early (Jul.15, early stage with soil partially covered by the canopy), mid (Aug.14, when canopy covered the soil almost completely), and late season (Oct.1, when leaf became senescent). Spectral data were acquired with a spectroradiometer held 2.3 m above canopy. There were a total of seven indices out of 24 on July 15th that showed significant differences between the N rates, eight spectral indices on August 14th, and seventeen indices on October 1st. The increased number of indices later in the season that show a difference between treatments can be explained by the greater effect of N in the cotton canopy later in the season and by the fact that soil is less visible. Their findings suggested that the following four indices may be able to sense N status throughout the growing season: **mSR705** = (R750 - R445)/(R705 - R445) nm, **mND705** = (R750 - R705)/(R750 + R705 - 2 * R445) nm, **D720** = absorbed depth at R720nm, and **dRE** = value of the first derivate at red edge (680 to 730 nm). However, they reported that the accuracy rate to differentiate N rates of cotton canopies for all the spectral indices used alone were below 48% for early and mid season and below 61% for late season. Using a combination of six vegetation indices on July 15th, and seven indices on August 14th, and five indices on October 1st improved accuracies to 74.4%, 83.1%, and 89.6 respectively. The only indices present in all combinations were **mND705** and **PRI** = (R570 - R531)/(R570 + R531) nm. Nevertheless, it would be difficult to manage N in a field using so many combinations of spectral indices.

TIMING

The appropriate time to take the spectral readings to obtain accurate information about the N status is also an important issue. A field experiment showed that both early and late season differences between N treatments were significant in the visible range (Zhao et al., 2004). They also reported that early, mid, and late season reflectance in the near infrared region showed significant difference among the four N rates. Spectral data were acquired with a spectroradiometer held 2.3 meter above canopy.

In consistency with the previous results, a field study demonstrated that spectral readings had strong relationship to N rate ($R^2 > 0.80$) for mid-early square, late pinhead, and early flower growth stages (Wilkerson et al., 1998). Yield had a large response to the five N rates. A spectral index (NIR + Blue)/(Green + Amber) was used, where blue ranged from 460 to 490 nm, green ranged from 540 to 565 nm, amber ranged from 600 to 610 nm, and NIR ranged from 740 to 770 nm. Although the spectral index had generally lower correlation with N rate than did the petiole nitrate test, it had good relationships with petiole nitrate ($R^2 > 0.77$), and yield ($R^2 > 0.88$) in all three growth stages.

However, Buscaglia and Varco (2002) reported that reflectance measurements at the second week of flowering stage (SWF) had better capability of differentiating the N rates than the readings at second week of squaring (SWS). At SWS only the lowest N rate had constantly higher reflection in the visible range than the other rates. On the other hand, the SWF reflectance measurements were capable of differentiating all the N rates throughout the visible range. SWF N rates were inversely related to reflection in the visible range. Leaf reflectance was recorded on the most recently expanded leaf of four different N treatments using a spectroradiometer. The fact that the data collected at SWF was better for differentiating N rates than at SWS can be explained by the greater effect of N in the cotton canopy later in the season and by the fact that soil is less visible.

An important issue when considering the use of reflectance sensor to make N recommendations is the effect of N timing on yield. There is some evidence in the literature that when adequate residual N is present, waiting to sidedress N later in the season may not result in yield losses. In the Mississippi Delta region, Ebelhar and Spurgeon (1987) concluded that nitrogen applied as late as mid-bloom did not reduce yield. Gardner and Tucker (1967) found no yield difference for experiments that had split application of N (N at planting + N at early flower) when compared to treatments that received all N at planting.

FACTORS THAT CAN AFFECT SPECTRAL READINGS

External Factors Influencing Reflectance Measurements

Row orientation and sun elevation are determinants of wheat (*Triticum aestivum* L.) spectral reflectance collected with a passive reflectance sensor (Jackson et al., 1979). This work indicated that for north-south rows, visible reflectance increased with solar elevation, whereas the east-west plots did not change much with solar elevation except at solar noon. Row orientation did not affect infrared reflectance as much, but there was a significant decrease in IR reflectance for the north-south rows near solar noon. Souza et al. (2004) found comparable results to Jackson using a passive reflectance sensor on potted corn plants placed on a research field. The reflectance at 550 (green) nm was more variable during the day in north-south rows than in east-west rows. Subsequently, correction equations were developed for the reflectance values as a function of row direction, sun angle, and time of day. The regression model explained 90% of the variation in 550nm reflectance and 86% in green normalized difference vegetation index (Green NDVI = [NIR – green] /[NIR + green]) variation. Reflectance in the north-south rows is more variable because shadows cross behind the row at solar noon and also early and late in the day.

Sticksel et al. (2004), similar to Souza et al. (2004), concluded that time of day affects reflectance values with passive sensors. The vegetation indices used in this research were a combination of visible, red-edge, or near-infrared wavebands. The indices for the noon measurements were always significantly lower than the morning and afternoon time intervals. This was credited to the fact that at noon more visible light bounces back from the bare soil and senescent leaves and there is a decrease in reflectance for near-infrared.

Dew on leaves increased reflectance in the visible range by as much as 60% for wheat, but had no effect on near-infrared reflectance (Pinter, 1986). Higher dew density resulted in larger changes in reflectance for visible wavebands. Plants with water droplets on them had increased visible reflectance due to an increase in specular reflectance. Another study also reported that dew increased reflectance in the visible range 50% for bentgrass, and 4% for bluegrass (Madeira et al., 2000). For the NIR region, dew decreased the reflectance by 2 to 8% on bentgrass and 9% on the bluegrass canopy.

An increase in cloud cover was found to decrease reflectance of corn (Tumbo et al., 2002) and dew-covered bentgrass (Madeira et al., 2000). Wind may also affect reflectance of canopies by changing the canopy geometry (Lord et al., 1985). For this study the sensor was placed on a stationary mount 3 meters above the canopy and the readings were taken for 312 s at a time. It was reported as much as 60% of variability in the barley red reflectance measurements during windy period when compared to 12% of variability in the reflectance measurements during non-windy periods. This increased variation during windy periods was attributed to stem bending and leaf fluttering.

Conversely, windy conditions caused little variation in the reflectance measurements of alfalfa because of its short and dense canopy structure.

Elevation can also contribute to change in cotton canopy reflectance within a field (Li et al., 2001). They found that as site elevation decreased, reflectance in the red range decreased, and near-infrared reflection increased. This effect on reflectance was credited to higher plant densities and plant fresh biomass at lower elevations. Higher plant density and plant biomass decreased soil exposure and caused a decrease in red reflectance and an increase in NIR reflectance. Additionally, soil type can also have a significant effect on reflectance measurements (Plant et al., 2000). The NDVI in loamy areas was considerably higher than in sandy areas. The lower NDVI in the sandier areas was attributed to the reduced water holding capacity that limits growth and causes earlier senescence.

Another factor that can affect reflectance measurements is potassium deficiency (Fridgen and Varco, 2004). Cotton with an adequate amount of K was found to have a better correlation between reflectance and leaf N concentration at both early flower and peak bloom when compared to cotton that was K deficient. Potassium deficiency, like nitrogen deficiency, causes leaves to be light-green or yellow-green, and it may be difficult to distinguish these two deficiencies based on reflectance data. However, these results are from detached leaves. In whole plants, potassium deficiency may have less impact on spectral measurements because symptoms are often more visible on lower leaves than for N deficiency. Nonetheless, it is clear that to get good N recommendations from spectral properties, cotton producers should ensure adequate K levels.

Internal Factors Influencing Reflectance Measurements

Physiological changes in plants can affect spectral measurements. Chloroplast orientation can change at different light irradiances, and consequently affect chlorophyll meter (SPAD) readings (Hoel and Solhaug, 1998). This study conducted on winter wheat and *Oxalis acetosella* L. reported that high irradiance decreased SPAD values by about 8% and 15 % respectively, while low irradiance early in the day increased SPAD values by 6 % and 9 % respectively. It is reasonable to suppose that passive sensor values would be affected by irradiance to a greater extent than the chlorophyll meter since the SPAD meter is designed to block outside light when taking readings and the sensors are not. Brugnoli and Bjorkman (1992) reported that blue-light induced chloroplast movement resulted in a change in the spectral properties of detached leaves of western wild cucumber (*Marah fabaceus* Greene) and redwood sorrel (*Oxalis oregana* Nutt.). They found that leaf absorbance decreased and reflectance and transmittance increased in the visible spectrum, but not in the red-edge.

Changes on canopy structure because of water stress can also have a significant effect on reflectance measurements (Plant et al., 2000). In this study conducted in California, false color infrared aerial photographs were taken 6 times during the growing season. There were four different irrigation treatments that consisted of the number of times a field was irrigated, from zero to four and the same rate of N was applied to the three irrigation experiments. The results indicated that as water stress increases, NDVI decreases. The NDVI changes were attributed to changes on canopy structure, reduced LAI, and leaf optical properties. Carlson et al. (1971) reported that reflectance of detached leaves related well to relative leaf water content (RWC) for corn *(Zea mays* L.), soybean (*Glycine max* L.), and sorghum (*Sorghum bicolor* L.) ($R^2 > 0.59$) at four wavelengths in the NIR portion of the spectrum. Reduced leaf water content increased reflectance. Non-plant factors may also be involved.

Similar results were obtained from a two year cotton study in Texas. The results indicated that an increase in soil water content caused a decrease in reflectance in the visible range and an increase in reflectance in the NIR bands. Plants reflect NIR light to avoid over-heating, especially in water-stress conditions. These results can also be caused by an increase in N uptake when there is an increase in soil water content. This research reported that soil water content had a significant linear correlation ($\alpha = 0.01$) with blue ($r = -0.63$), green ($r = -0.70$), red ($r = -0.70$), NIR ($r = 0.40$), Mid-infrared (MIR) ($r = -0.67$) bands, and with NDVI ($r = 0.69$), and NIR/RED ($r = 0.68$) (Li et al., 2001). The experiment consisted of two irrigation treatments at 50 and 75% of calculated evapotranspiration. Reflectance measurements were taken throughout the growing season twice a week per plot using a portable Cropscan radiometer. Sensor was placed 2 m above the canopy and soil water content was monitored monthly.

An additional issue that can change reflectance in the visible range is the use of plant growth regulator. Recently, Zhao et al. (2005) reported that mepiquat chloride (MC) application decreased reflectance around 556 and 710 nm. This decrease was explained by an increase in chlorophyll concentration and leaf N in plants that where treated with mepiquat chloride. Chlorophyll is concentrated in smaller plants treated with MC. This study included one N rate for all plots and 4 rates of MC from 0, to 2.34 L MC ha^{-1}. The reflectance measurements were taken weekly or biweekly, from detached fully expanded leaves using spectroradiometer.

USING SENSORS TO MAKE N MANAGEMENT DECISIONS

So far, few studies have used sensors to actually make N management decisions for cotton. Recently, Chua et al. (2003) and Bronson et al. (2003) tested a method of using spectral reflectance and chlorophyll meter readings to make in-season N decisions. In these associated studies, there were five N treatments: 0 N, well-fertilized N (202 kg N ha^{-1}), and soil test-based N, chlorophyll meter-based N, and reflectance-based N. The soil test plots, chlorophyll meter plots, and reflectance plots received 34 kg N ha^{-1} after emergence. Additional amounts of N were applied to these plots, in increments of 34 kg N ha^{-1}, if they met certain conditions at early square, early flower, and peak bloom. Soil-test plots received increments based on a yield goal of 1400 kg lint ha^{-1}, which was equivalent to 168 kg N ha^{-1} minus the results from the spring soil NO_3-N test. Chlorophyll meter (SPAD) and reflectance plots received increments when the sufficiency index was < 0.95 relative to the well-fertilized plots. The chlorophyll meter (CM) sufficiency index was obtained by dividing the CM reading per plot by the average CM reading for the well-fertilized plots. The reflectance plots' sufficiency index consisted of the Green Vegetation Index, GVI (820/550nm), of a reflectance plot divided by the average GVI of the well-fertilized plots. The reflectance and chlorophyll meter plots had the same amount of N applied in 9 of the 12 cases. Additionally, in 9 of the 12 cases, less N was applied to the reflectance and chlorophyll meter-based treatments than to the soil test treatments and the yields were similar. On average an extra 62 kg N ha^{-1} was applied to the soil test plots, and an extra 125 lbs N/acre was applied to the well-fertilized plots compared to both the reflectance and chlorophyll meter-based plots. The well-fertilized plots yielded an average 56 kg ha^{-1} of lint more than the reflectance treatment, 58 kg ha^{-1}

more than the chlorophyll meter plots and 8 kg ha^{-1} more than the soil test treatment. Generally, both chlorophyll meter readings and GVI had good correlations with N rate (5 out of 9 cases), leaf N (7 out of 9 cases), and leaf N accumulation (7 out of 9 cases). Nitrogen management based on sufficiency indices was successful both in low and high-yielding experiments, an important goal that may be difficult to achieve with some N recommendation systems.

SUMMARY

There is great spatial variability of N within a field and between fields. Cotton fields that receive a blanket rate of nitrogen, ignoring the variability, will have areas of excessive growth. This unnecessary growth raises production cost by delaying crop maturity, increasing the need for growth regulator, defoliant, and increasing insect pressure. A variable-rate application of nitrogen based of non-destructive diagnostic tools will resolve that problem and may also reduce the amount of nitrogen applied, which will save producers money. However, current N diagnostic tools, such as petiole nitrate test, are not suited for varying N rate within a field.

Recently, newer methods of N status detection have been developed. These tools are based on the spectral properties of a single leaf or a canopy, and have the potential to sense N status. Numerous studies demonstrated the capability of these sensors to detect N status, while others questioned its capability. Several studies have also focused on the light reflectance wavelengths that can better diagnose N status and a number of researchers developed and studied the effectiveness of reflectance ratios or indices in detecting N deficiency. The results indicated that green, red edge, and NIR bands are the most affected by changes in the N status. Ratios containing blue, green, red edge, and NIR bands were also very effective in sensing N differences.

Diagnosing and applying N at the right time is also an important issue. The results showed that N deficiency could be diagnosed as early as early square. However some studies seemed to point out that spectral data at later stages could better predict N status. It is also important to take in consideration that there are several factors other than N status that can affect reflectance. Factors such as water stress, soil type, elevation, other nutrient deficiencies, and plant growth regulator can decrease the accuracy and precision of spectral readings. These factors should be managed to the best of the producer's ability to reduce their effect.

Although there is a need for rapid and easy methods of nitrogen status detection in cotton, little research has been done to use sensors for application of N in cotton. One of the studies tested a method of using the sensor's data to make N management decisions. This nitrogen management based on sufficiency indices was successful both in low and high-yielding experiments. More research is needed to develop on-the-go N diagnosis and application.

RESEARCH OBJECTIVES

The goal of this research is to develop on-the-go variable nitrogen sidedressing for cotton based on interpretation of reflectance measurements. In order to achieve this goal we must consider the following sub-objectives: (I). Determine the best growth stage for sensor-based sidedressing. (II). Determine the height, sensor model, and wavelength that most accurately predict nitrogen need. (III). Determine the how time of day affects the sensor's accuracy.

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CHAPTER II

CROP SENSORS FOR VARIABLE RATE MID-SEASON NITROGEN APPLICATION ON COTTON

ABSTRACT

Nitrogen (N) is an essential nutrient for cotton (*Gossypium hirsutum* L.) production; consequently there is a tendency to over-apply nitrogen. High N can cause excessive vegetative growth and delayed crop maturity, which result in increased cost in pesticides, growth regulator, and defoliant**.** Currently, soil and plant tissue tests are the most common methods for obtaining information about the nitrogen needs of cotton. Unfortunately, the labor-intensive and time-consuming nature of these N diagnostic tools limits their use.

Reflectance sensors offer the potential to diagnose N needs immediately in a spatially intensive manner, and to translate this diagnosis into a variable-rate application of N in real time. The objective of this study was to develop on-the-go sidedress N rate recommendations based on reflectance measurements.

Three nitrogen rate experiments were conducted on silt-loam, fine sandy loam, and clay soils in 2006 and 2007. Reflectance was measured with three sensors (Crop Circle, GreenSeeker, and Cropscan) at three growth stages (early square, mid square and early flower) and at three heights above the cotton canopy (25, 50, and 100 cm). Optimal N rate based on cotton yields for these six experiments ranged from 0 to 220 kg N ha^{-1}. Results indicated that all three sensors have potential for accurate prediction of optimal N rate. Prediction accuracy was low at the early square stage but acceptable at mid square or early flower. Recommendation equations were not significantly different from mid square to early flower for any sensor at any height, suggesting that a single equation can

be used to translate reflectance measurements to N rates over the period from mid square to early flower. All three sensors gave good predictions of optimal N rate from 50 cm above the canopy, while only the Cropscan sensor gave good predictions from 25 cm and only the GreenSeeker gave good predictions from 100 cm. These results suggest that variable-rate N applications to cotton based on real-time reflectance measurements are feasible for the mid-square to early flower growth stages.

INTRODUCTION

Nitrogen is an essential nutrient for cotton production. Cotton plants need a total of 200 to 220 kg ha^{-1}of plant available N (soil NO_3-N plus applied N) to achieve 1500 kg of lint ha^{-1} (Hutmacher et al., 2004). However, determining the amount of nitrogen fertilizer that is needed by cotton is a difficult task because the amount of N available from soil varies from field to field, and also varies spatially within a field. Coefficient of variation for soil nitrate was high in a 5.3 ha cotton field reported by Elms et al. (2001). Similarly, the economically optimal N rate (EONR) within a field varied from 0 to 280 kg N ha^{-1} in five of eight corn (*Zea mays* L.) fields studied by Scharf et al. (2005). When a uniform rate of nitrogen is applied throughout a field, some areas will probably have excess nitrogen, while others will have too little N to fully meet the crop's needs.

When the N supply is insufficient or exceeds the N requirement of cotton, a number of production problems may occur. Nitrogen deficiency causes reduced vegetative growth, reduced number of bolls and seed, and low fiber yields (Malavolta et al., 2004). On the other hand, over-application of N leads to excessive vegetative growth (McConnell et al., 1993). Excessive vegetative growth increases production cost since it increases the need for growth regulator (McConnell et al., 1992), defoliant (McConnell et al., 1993), and insecticide due to higher insect pressure (Cisneros and Godfrey, 2001). Over-application of N can also delay crop maturity (McConnell et al., 1993). Delayed crop maturity can reduce profit when rainy weather at the end of the season that might causes boll rot. Bourland et al. (2001) showed that increased nitrogen rate corresponded to delayed maturity.

Accurately diagnosing N status will help to avoid both nitrogen deficiency and excess N application, and therefore help to minimize production cost and maximize yield and quality. The current diagnostic tools available to the cotton producers are soil testing for nitrate and ammonium, petiole nitrate concentration, and leaf total N concentration. These tools are not well-suited for variable N diagnosis and application since they are labor intensive and time consuming. Also, they are used mainly to troubleshoot problems, not to routinely select N fertilizer rate. There is a need both to calibrate diagnostic tools to predict how much N fertilizer is needed, and to develop tools that can be used to diagnose N need in a spatially variable manner.

Recently, with the advance of precision agriculture and remote sensing, newer methods of N-status detection have been developed. These tools are based on the spectral properties (absorption or reflectance) of a single leaf or a canopy. Canopy reflectance sensors have the potential to sense N status and guide N application in a spatially variable manner.

Only a few studies have directly related yield response to nitrogen to spectral data. Wilkerson et al. (1998) first showed that spectral data at early square were highly related to yield response to N. The spectral index [(NIR + blue)/(green + amber)], relative to high N plots, was related to the size of the yield response to N. Conversely, the size of the yield response was only weakly related to the relative spectral index 'NDVI-days' (the integral of NDVI over time) in a group of five N response experiments with low and moderate yield response to N (Plant et al., 2000). The location with the largest yield response to N was only intermediate in terms of spectral differences between N-rate treatments.

Some wavelengths are more related to leaf chlorophyll and consequently are more sensitive to a change in N status. Green and red-edge reflectance had the best correlations with leaf N in the field (Zhao et al., 2005) and greenhouse conditions (Buscaglia and Varco, 2002). Sui and Thomasson (2004) found that when the sensors were driven across the plots at the early square growth stage, leaf N had a better correlation with red and NIR reflectance than with blue and green. Other studies supported the use of simple reflectance ratios, such as red-edge/near-infrared (Tarpley et al., 2000) or green/blue (Zhao et al., 2005), to estimate leaf N concentration. Sui et al. (2005) proposed a new index, PNI (Blue + Green)/NIR, which they found was strongly related to N rates (R^2 = 0.74) and yield (R^2 = 0.82).

The appropriate time to take spectral readings to obtain accurate information about crop N status is also an important issue. Buscaglia and Varco (2002) reported that reflectance measurements at the second week of flowering stage (SWF) had better capability of differentiating the N rates than the readings at second week of squaring (SWS) under greenhouse conditions. The fact that the data at SWF was better at differentiating N rates than at SWS can be justified by the greater effect of N in the cotton canopy later in the season. Nonetheless, reflectance measurements in the near-infrared region showed significant difference between the four N rates in a field study at early, mid, and late season (Zhao et al., 2004). Early and late season differences between N treatments were also significant in the visible range. Early season in their study was defined by a rapid growth stage, when soil was not totally covered by cotton, whereas mid season was the full green coverage period, and late season was the senescent period.

Even though there is a need for rapid and easy methods of nitrogen status diagnosis in cotton, little has been done to use sensors to make N management decisions. The goal of this research was to calibrate reflectance measurements to predict optimal N rate in support of on-the-go variable-rate nitrogen sidedressing. Sub-objectives include: (I). Determine the best growth stage for sensor-based sidedressing. (II). Determine the sensor height, model, and wavelengths that most accurately predict nitrogen need.

MATERIALS AND METHODS

Two fields at the University of Missouri Delta Research Center Lee Farm near Portageville and one at the Rhodes Farm near Clarkton were chosen for this study. These trials were conducted in 2006 and 2007 and the soils types in these fields were Bosket fine sandy loam (fine-loamy, mixed, thermic Mollic Hapludalf) at Rhodes and Tiptonville silt loam (fine-silty, mixed, thermic, Typic Argiudoll) and Portageville clay (fine, smectitic, calcareous, thermic Vertic Endoaquolls) at Lee. Cotton was planted using a ridge-till production system at each location, with winter wheat (*Triticum aestivum* L.) planted between the rows at the Rhodes (sandy loam) experiment. The cotton cultivar 'DP 117 B2RF' (Delta Pine, Memphis, TN) was planted in all fields, except for the clay soil field in 2006 which was planted with 'DP 444 BG/RR'. For the sandy loam and silt loam plots planting rate was approximately 11 seeds per meter, for the clay experiments planting rate was 10 seeds per meter. For both years, the silt loam and sandy loam plots were 4 rows wide by 15 m in length. The clay plots were 4 rows wide by 10 m long. The row spacing was 97 cm for all plots. The silt loam and clay studies had furrow irrigation while the sandy loam study had sprinkler irrigation.

University of Missouri extension recommendations were followed for weed and insect management. All fields received mepiquat chloride as needed for control of plant height. The silt loam and sandy loam fields were defoliated with a mixture of thidiazuron, and ethephon, while the clay field received a combination of thidiazuron, ethephon, and tribufos.

Treatments and Experimental Design

The experiments were conducted using a randomized complete block design with four replications. The silt loam and sandy loam studies included twelve nitrogen treatments ranging from 0 to 224 kg N ha^{-1} (Table 1). All treatments received ammonium nitrate as the N source. The check plots received no N, while a high N rate was applied to one of the treatments at planting to be used as a reference for the reflectance measurements. Trials were designed to determine the best N rate and to relate optimal N rate to reflectance measurements.

There were four replications and twelve treatments for the silt loam and sandy loam experiments (Table 1). The clay experiment was part of a separate study that was added to our research. It also had four replications and twelve treatments (Table 2). The University of Missouri Extension recommends a N rate of 90 kg ha^{-1} for irrigated cotton on sandy loam or silt loam soil in Missouri and an extra 30 kg N ha^{-1} for clay soils. For the purpose of this study we assumed a producer rate of 100 kg N ha^{-1} since the three types of soil were present in this research.

Reflectance Measurements

Three sensors were used to take our reflectance measurements: (1) Cropscan™ (passive) (Cropscan, Inc., Rochester, MN), (2) Crop Circle™ (active) (Holland Scientific, Lincoln, NE), and (3) GreenSeeker™ (active) (N-Tech Industries, Ukiah, CA). Passive sensors measure the sunlight reflected from the plants, and active sensors measure the reflected light emitted by their own light source. The Crop Circle sensor emits yellow (590 nm) and near-infrared (NIR) (880 nm) wavelelngths, while the Greenseeker sensor emits red (656 nm) and NIR (774 nm). For the Cropscan sensor we used the green (560 nm) and NIR (810 nm) wavelengths. The sensors were mounted on a tool bar behind a tractor and were connected to a computer in the cab. The computer had custom software that constantly recorded the readings sent by the sensors and also recorded position information from a global positioning system (GPS) receiver while the tractor was driven through the field.

Reflectance measurements were taken at early square, mid square and early flower growth stages. The readings were also taken at three different heights above the canopy: 25, 50, and 100 cm. The three different heights were achieved by moving the tool bar up and down. In 2007, the early flower readings were taken with the sensors mounted on a sprayer. In 2006, the software for integrating GPS data with Cropscan readings was not available by the early square growth stage; thus we were not able to associate that data with the appropriate plots.

Breaking reflectance measurements into plots

Files containing reflectance sensor data were processed to allow us to compute the average value for each sensor parameter for each plot. Each data file was sorted to create derivative files containing GPS data and reflectance data for only one sensor. Each derivative file was then projected spatially using ArcGIS software (ESRI, Redlands, CA; version 8.5 in 2006 and 9.1 in 2007) and a polygon was used to isolate the average data from each plot. Alleys between plots contained little or no vegetation and were easily seen in the spatially projected data, providing useful feedback on the accuracy of the polygon location and size. Polygons were then moved or resized to match the plots. Cropscan data, recorded in millivolts, were converted to reflectance using the POSTPROC procedure in the MSR (Multispectral Radiometer) software supplied with the Cropscan and a calibration performed after the early flower growth stage.

Statistical Analyses

The statistical analysis to produce a recommendation system was a two step process. The first step was describing yield response to N rate mathematically and calculating economically optimal N fertilizer rate (EONR) for each experiment. The second step was regressing sensor reflectance values against optimal N rates in order to check which variables were the best predictors of EONR.

Yield response to nitrogen rate was modeled for each experiment as a quadratic-plateau function using PROC NLIN in SAS. The quadratic plateau function was found to be the best function for describing yield response to nitrogen rate for corn (Scharf et al., 2005). Cotton, like corn, would have the biggest yield increase with the first increment of N (curved or quadratic shape), and would not likely have any yield decrease with excessive N (yield plateau). Residuals from this model were entered on plot maps for each experiment, and spatial patterns in the residuals were usually apparent. Nearest neighbor estimates of position effect were calculated for each plot as the mean of residuals of neighboring plots. These estimates of position effect were used in analysis of covariance for yield with N rate as a class variable and calculations done using Proc GLM in SAS. In all cases, position effect was a significant ($p < 0.0001$) covariate. Position-adjusted yields were estimated in this way and then used to calculate a second quadratic plateau yield response function for each experiment. These functions were used to calculate economically optimal N rate for each experiment, using the cotton loan price of \$ 1.15 kg^{-1} and a nitrogen price of \$ 1.10 kg^{-1}, which was the approximate average nitrogen price during the period that this experiment was conducted.

Relative reflectance values were calculated for each location/year for treatments receiving 0, 28, and 56 kg N ha^{-1} preplant for the silt loam and sandy loam experiments and 0, 56, and 112 kg N ha^{-1} preplant for the clay experiment. These values were calculated in Excel 2003 (Microsoft Corporation, Redmond, WA) by averaging the reflectance values for the four replications of each N rate treatment and then dividing by the average reflectance measurements of the high N treatment (treatment 12 in table 1 and treatment 10 in table 2).Data were pooled across all six experiments for each sensor, growth stage, and sensor height combination. Finally, the relative reflectance sensor values were regressed against the EONR using PROC REG in SAS. We arbitrarily selected an R^2 value of 0.5 as the value above which we felt there was potential for acceptably accurate prediction of EONR.

RESULTS AND DISCUSSION

Economically Optimal Nitrogen Rates

The EONR for our experiments ranged from 0 to 220 kg N ha^{-1} (Figure 1). For both 2006 and 2007, the silt loam and sandy loam soils needed less nitrogen for optimum cotton yields than the assumed normal producer rate of 100 kg N ha^{-1}. For those experiments the EONR varied from 0 to 90 kg N ha^{-1}. More nitrogen was needed for the clay experiments: 220 kg N ha^{-1} in 2006 and 196 kg N ha^{-1} in 2007. The wide variation in optimal N rate between fields reinforces the need for an accurate method of in-season diagnosis of how much N to apply. It is also ideal for calibrating a diagnostic test which must be able to predict when N needed is low, intermediate, or high.

Predicting Economically Optimal Nitrogen Rate from Reflectance Measurements

For the early square growth stage some sensor variables for Crop Circle and GreenSeeker were significant predictors of EONR at $\alpha = 0.05$ for the 25 and 50 cm heights, but not for 100 cm. However, the highest R^2 between EONR and any sensor parameter at early square was 0.47. We felt that this was not a strong enough relationship to serve as a basis for N rate recommendations and therefore early square is too early to sidedress N based on the sensors. In Missouri, most in-season nitrogen is applied at early square and it would be desirable to be able to use sensors to control variable-rate N at this stage, but our results do not provide adequate support for this practice. In 2007, we were unable to collect reflectance measurements at the early square stage for the clay soil experiment because wet field conditions made it impossible to drive through the field. In addition, the Cropscan readings (i.e., passive sensor) at the sandy loam field in 2007 for all the heights at early square had many ‘out of range’ values for unknown reasons, so the data were discarded.

However, later in the season, we found stronger linear relationships between the reflectance measurements and the optimal N rate. At mid square a total of 18 variables out of 66 were related to EONR with $R^2 > 0.5$ describing from 0.519 to 0.684 of the EONR variability (Table 3). At early flower the number of predictors increased to 28 and the R^2 values ranged from 0.517 to 0.910. Each of these 46 variables (18 mid-square, 28 early flower) was significant at the $\alpha = 0.01$ level. Therefore, our results suggest that waiting until mid square or early flower to apply N will increase the accuracy of N rate prediction using reflectance sensors. The planting date for the clay soil experiment in 2007 was earlier than for the other two experiments, so when we took mid square measurements in those two experiments, the clay experiment was already at late square. Similarly, when we took early flower reading in the other two experiments, the clay plots were slightly past that growth stage. However, data from the clay experiment seemed to fit well with the data from the other experiments despite being a slight more advanced growth stage. In 2007, problems were encountered with the Cropscan readings at the sandy-loam site for all the heights at early flower and at the silt loam site at 25 cm above the canopy at mid square, so those data were discarded. Many negative values were observed among these data, but their reason is not known.

Buscaglia and Varco (2002) found that crop stage affected the relationship between N status and reflectance. They reported that reflectance measurements at the second week of flowering stage (SWF) had better ability to differentiate between N fertilizer rates than readings at second week of squaring (SWS).

All three sensor types appeared to be potentially useful for predicting optimal N rates during the season. The Visible/NIR ratio, expressed relative to high N plots and measured from a height of 50 cm, was able to predict EONR with $R^2 > 0.5$ for all sensors at both mid square and early flower. For the Cropscan sensor this was true when the visible band used was green (560nm), but data were missing for one site-year at mid-square stage in 2006, and two site-years at early flower stage in 2007. Coefficients of determination for this relationship ranged from 0.580 to 0.705 (Table 3).

Our results are in agreement with Zhao et al. (2005), who reported that a visible/NIR ratio (551nm/915nm) was a good estimator of cotton leaf chlorophyll concentration. Relative NDVI values were equally good predictors of EONR for mid square and early flower stages. They described from 0.557 to 0.737 of the EONR variability when measured 50 cm above the canopy.

Relative NIR alone was also a reasonably good predictor of EONR in the two sensors that reported this measurement (Crop Circle and Cropscan). From a 50 cm height, both sensors gave $R^2 > 0.5$ at mid-square, and the Cropscan also gave $R^2 > 0.5$ at early flower.

Cropscan also had good relationships between EONR and the relative blue-green (510 nm), green (560nm), yellow (610nm), red (660nm), and red-edge (710nm) reflectance at early flower from a 100 cm height above the cotton canopy. Red-edge (710nm) at 25 cm height also related well to EONR at mid-square and early flower. However, we are hesitant to put confidence in these relationships with two site-years missing Cropscan data for this particular growth stage/height combination.

Many sensor variables at all three heights (25, 50, and 100 cm) had high correlations with optimal N rate. Overall, the most reliable height was 50 cm above the canopy. Out of the 46 total variables that predicted EONR with $R^2 > 0.5$, 21 were taken at 50 cm above the canopy, 12 were taken at 1m, and 13 were taken at 25 cm. The 50 cm height was better than the other two heights particularly for the Crop Circle sensor (Figure 2). For the other two sensors, the advantage of the 50 cm height was not as strong. The manufacturers of Crop Circle suggest that the sensor can be used from 30 to 240 cm above the canopy (Holland Scientific, Inc., 2008). We found that its ability to predict EONR for cotton was very height-sensitive within this range, and suggest that 50 cm is optimal for this application. The GreenSeeker manufacturer states that the sensor should be used between 80 and 120 cm above the canopy (Ntech Industries, Inc., 2007). The GreenSeeker predicted EONR acceptably at the middle of this range, but if anything performed better at 50 cm height. There are no guidelines for height from the Cropscan sensor manufacturer. However, it is more susceptible to interference from background soil reflectance properties than active sensors, which are very distance-sensitive and therefore receive much stronger reflection of pulsed light from elevated targets (plants) than from the soil. This may explain why Cropscan measurements predicted EONR best from 25 cm where soil in the field of view was minimized.

Comparison of Mid Square and Early flower Recommendation Equations

Linear regression lines relating EONR to sensor values were quite similar at the mid square and early flower growth stages (Figure 3). Although quadratic terms for the relationships between EONR and the reflectance measurements were also tested, they were not found to be significant ($\alpha = 0.05$) for any of the sensors.

The interaction between the Visible/NIR ratio and growth stage was not a significant predictor of EONR for the Crop Circle ($p = 0.92$) or the GreenSeeker ($p = 0.60$) sensors. This indicates that the slopes of the equations for mid-square and early flower are equal and that these can be combined into a single equation. There was more evidence that the lines for mid-square and early flower stages are different for the Cropscan sensor ($p = 0.11$), suggesting greater risk of bad decisions if the same equation is used for both growth stages.

With relative NDVI as a predictor of EONR, equations for the Cropscan sensor were the same at the two stages ($p = 0.3$). There was weak evidence of a difference between stages for Crop Circle ($p = 0.09$) and GreenSeeker ($p = 0.16$) sensors. Nonetheless, the R^2's for combined mid square and early flower data were all above 0.6 (Figure 4).

Although the linear correlations between the reflectance measurements and EONR improved as the season progressed, the equations for mid square and early flower were not different at $\alpha = 0.05$ for any of the three sensors. This suggests that it may be reasonable to use a single equation (Figure 4) to translate reflectance measurements to N rates anytime between mid square and early flower, especially for Crop Circle and GreenSeeker Vis/NIR measurements.

Implications for N Management

We suggest that visible/NIR or NDVI (relative to a high-N reference) measured from a height of 50 cm above the canopy can be used to predict the N needs of cotton for sidedressing from mid square through early flower (Figure 4). These two variables at a height of 50 cm worked consistently for all three sensors, and the two latter growth stages.

For all three sensors and both variables, EONR = 20 to 30 kg N ha^{-1} when relative sensor value = 1 (i.e. target plants have same value as high-N reference plants). This is consistent with previous research (Scharf et al., 2005) and indicates that on average there is no difference in appearance between plants that do not need N and plants that need low rates of N. The fact that N will always be recommended by these equations influences the potential to save on N fertilizer expense by using sensors. Minimizing pre-plant N applications helps to preserve the potential for N savings, but the yield effects of this strategy need to be studied more thoroughly.

N timing and Yield Effect

One weakness in our study is that N rates used to determine EONR values were applied at the early square growth stage, but we found that sensor values at this stage were not accurate predictors of EONR. Sensor values at mid-square and early flower stages were good predictors of EONR applied at early square. Probably EONR applied at early square would be similar to EONR applied at mid-square or early flower, though further research between N rates and sensor values at mid square and early flower would be desirable. The bigger question is whether delaying N application to mid square or early flower would cause a yield loss.

Nitrogen timing treatments in our experiments provided some evidence in this regard. In the silt loam and sandy loam experiments, early-split (56 kg N ha^{-1} preplant + 56 kg N ha^{-1} early square) and late-split (56 kg N ha^{-1} preplant + 56 kg N ha^{-1} early flower), treatments gave the same yield ($\alpha = 0.05$). However, only one of these four site-years needed substantially more N than the 56 kg N ha^{-1} applied at planting.

The clay soil experiment had a different design. Nitrogen application timings were: (1) all N preplant, (2) ½ preplant and ½ at early square (2-split), and (3) 1/3 preplant, 1/3 at early square and 1/3 at early flower (3-split). For clay 2006, the 3-split timing was higher yielding ($\alpha = 0.05$) than the preplant and 2-split timing. Nitrogen timing did not affect yield ($\alpha = 0.05$) in 2007.

These experiments provide a small amount of evidence that delaying part of N until early flower either increases or does not affect yield. There is also some evidence in the literature that waiting until later in the season to sidedress N will result in no yield penalty. Ebelhar and Spurgeon (1987) concluded that delaying nitrogen application as late as mid-bloom did not reduce yield. Gardner and Tucker (1967) found no yield difference for experiments that had split application of N (N at planting + N at early flower) when compared to treatments that received all N at planting. From the available evidence, using sensors to diagnose EONR and control N application at mid square or early flower should not reduce yield due to nitrogen stress experienced before these stages. However additional research on the effect of delaying N application would be justified before suggesting widespread use of sensor-guided N sidedressing at these stages.

Another timing issue is the possibility of an unplanned delay in N applications. The excessive rain that precluded taking reflectance measurements at early square for the clay experiment in 2007 is an example. A producer using reflectance measurements to control sidedress N rate starting at mid-square could be delayed by rain, possibly resulting in N applications well after early flower and a possible negative impact on yield.

CONCLUSIONS

The wide variation in optimal N rate between our fields reinforces the need for an accurate method of in-season diagnosis of how much N to apply. The goal of this research was to develop on-the-go variable-rate nitrogen sidedressing based on reflectance measurements. Our results suggest that the sensors can accurately predict the amount of nitrogen needed by the cotton plants.

Best Growth Stage

At the early square growth stage, sensor values and ratios relative to the high-N plots were weak predictors ($R^2 < 0.5$) of EONR. However, the relationships between the reflectance measurements and the optimal N rate at mid square and early flower were strong enough to be useful in making N rate recommendations. A total of 46 total variables for mid square and early flower were good predictors of EONR ($R^2 > 0.5$) and were significant at $\alpha = 0.01$ level.

Best Sensor Model

All three sensor types appeared to be potentially useful for in-season N prediction. The relative visible/NIR ratio and relative NDVI were consistently good predictors of EONR for all sensors at both mid square and early flower with R^2 values ranging from 0.542 to 0.705.

Height above Canopy

The reflectance measurements at all three heights (25, 50, and 100cm) had high correlations with optimal N rate. The most reliable height for all three sensors was 50cm above the canopy. Out of the 38 variables that predicted of EONR with $R^2 > 0.5$, 17 were taken at 50 cm above the canopy.

Best Reflectance Variable

The Visible/NIR ratio and NDVI measured at a height of 50 cm above the canopy were the only reflectance variables to predict EONR with $R^2 > 0.5$ for all three sensors. We suggest that these relationships should be used to predict the N needs of cotton for sidedressing at mid square or early flower growth stages.

Comparison of Mid Square and Early flower Recommendation Equation

The equations for mid square and early flower growth stages were similar enough that they could be combined to predict the amount of N needed anytime between these two growth stages for both Visible/NIR and NDVI relative values.

N timing and Yield Effect

Our results suggest that waiting to sidedress N at early flower would not reduce the cotton yield when compared to early square. There was also no evidence that split N application will have a smaller yield than preplant N application.

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Table 1. N treatments for the silt loam and sandy loam experiments in 2006 and 2007.

Preplant (kg N ha $^{-1}$)	**Sidedress (kg N ha $^{-1}$) †**	**Late Sidedress (kg N ha $^{-1}$) ††**
0	0	0
28	0	0
56	0	0
56	28	0
56	56	0
56	84	0
56	112	0
56	140	0
56	168	0
56	0	56
56	0	CM †††
168	0	0

† Sidedress applications were made at early square growth stage on approximately June 23rd in 2006 and June 29th in 2007

†† Late Sidedress applications were made at early flower growth stage on approximately July 18th in 2006 and 2007.

††† N rate in this treatment was based on chlorophyll meter measurements at early flower interpreted according to a previous study that has not been published

Table 2. N treatments for the clay experiment in 2006 and 2007.

Preplant (kg N ha $^{-1}$)	**Sidedress (kg N ha $^{-1}$) †**	**Late Sidedress (kg N ha $^{-1}$) ††**
0	0	0
56	0	0
0	56	0
112	0	0
56	56	0
37	37	37
168	0	0
84	84	0
56	56	56
224	0	0
112	112	0
74	74	74

† Sidedress applications were made at early square growth stage on approximately June 13th in 2006 and June 18th in 2007

†† Sidedress applications were made at early flower growth stage on approximately July 19th in 2006 and 2007.

Table 3. Coefficients of determination for sensor reflectance variables predicting EONR at Mid Square and Early flower. Measurements with $R^2 > 0.5$ are in bold font with a shaded background.

Sensor	Parameter	Height (cm)	Mid Square	Early flower
Crop Circle	NDVI	25	0.2800	0.1084
Crop Circle	NDVI	50	**0.5572**	**0.7248**
Crop Circle	NDVI	100	0.0155	0.0154
Crop Circle	VIS/NIR	25	0.3043	0.1493
Crop Circle	VIS/NIR	50	**0.5806**	**0.6931**
Crop Circle	VIS/NIR	100	0.3442	0.3358
Crop Circle	VIS	25	0.2726	0.4600
Crop Circle	VIS	50	0.2036	0.1415
Crop Circle	VIS	100	**0.6839**	0.1794
Crop Circle	NIR	25	0.3326	0.4757
Crop Circle	NIR	50	**0.5591**	0.4267
Crop Circle	NIR	100	0.2601	0.2516
GreenSeeker	NDVI	25	0.3647	0.2540
GreenSeeker	NDVI	50	**0.6048**	**0.6247**
GreenSeeker	NDVI	100	**0.6394**	**0.6305**
GreenSeeker	VIS/NIR	25	0.4036	**0.5222**
GreenSeeker	VIS/NIR	50	**0.6038**	**0.6030**
GreenSeeker	VIS/NIR	100	0.3844	0.4133
Cropscan	510	25	0.1604	0.1487
Cropscan	510	50	0.3537	0.0948
Cropscan	510	100	0.3431	**0.7451**
Cropscan	560	25	0.1552	0.4330
Cropscan	560	50	0.2345	0.1496
Cropscan	560	100	0.3022	**0.8099**
Cropscan	610	25	----------------†	0.3713
Cropscan	610	50	0.1418	0.2197
Cropscan	610	100	0.3845	**0.9106**
Cropscan	660	25	----------------†	0.3414
Cropscan	660	50	0.1178	0.3406
Cropscan	660	100	0.3889	**0.8218**
Cropscan	460	25	----------------†	**0.5401**
Cropscan	460	50	0.0584	----------------†
Cropscan	460	100	----------------†	----------------†
Cropscan	710	25	**0.5398**	**0.5401**
Cropscan	710	50	0.0857	0.1386
Cropscan	710	100	0.2799	**0.7808**
Cropscan	760	25	0.4430	0.0263
Cropscan	760	50	0.0861	----------------†
Cropscan	760	100	0.0555	**0.7410**
Cropscan	810	25	0.3203	0.2216
Cropscan	810	50	**0.5711**	**0.7538**
Cropscan	810	100	0.0095	**0.5983**
Cropscan	Green NDVI	25	**0.5711**	**0.6653**
Cropscan	Green NDVI	50	**0.5740**	**0.7375**
Cropscan	Green NDVI	100	0.3588	0.0845

Cropscan	NDVI 810	25	0.1660	**0.6440**
Cropscan	NDVI 810	50	0.3436	**0.6750**
Cropscan	NDVI 810	100	0.4959	0.2765
Cropscan	NDRE	25	0.3655	0.4964
Cropscan	NDRE	50	0.4301	**0.6787**
Cropscan	NDRE	100	**0.5193**	0.2358
Cropscan	G560/NIR810	25	**0.6371**	**0.5578**
Cropscan	G560/NIR810	50	**0.6218**	**0.7049**
Cropscan	G560/NIR810	100	0.4990	0.1469
Cropscan	G560/R760	25	**0.6757**	0.2601
Cropscan	G560/R760	50	**0.5984**	----------------†
Cropscan	G560/R760	100	0.4457	**0.5173**
Cropscan	Y610/NIR810	25	**0.5627**	0.4971
Cropscan	Y610/NIR810	50	0.4029	**0.6621**
Cropscan	Y610/NIR810	100	0.4901	0.2744
Cropscan	R660/NIR810	25	0.4115	0.4484
Cropscan	R660/NIR810	50	0.3387	**0.6520**
Cropscan	R660/NIR810	100	0.4599	0.2498
Cropscan	R710/NIR810	25	**0.5537**	**0.6532**
Cropscan	R710/NIR810	50	0.2001	**0.6971**
Cropscan	R710/NIR810	100	0.4671	0.2299

†Data not available due to problems with this wavelength at some locations.

Abbreviations:
NDVI = Normalized Difference Vegetation Index (NIR - RED)/(NIR + RED)
NDRE = Normalized Difference Red Edge (NIR - RED EDGE)/(NIR + RED EDGE)
VIS = Visible light
G = Green wavelength
Y = Yellow wavelength
R = Red wavelength
NIR = Near-infrared

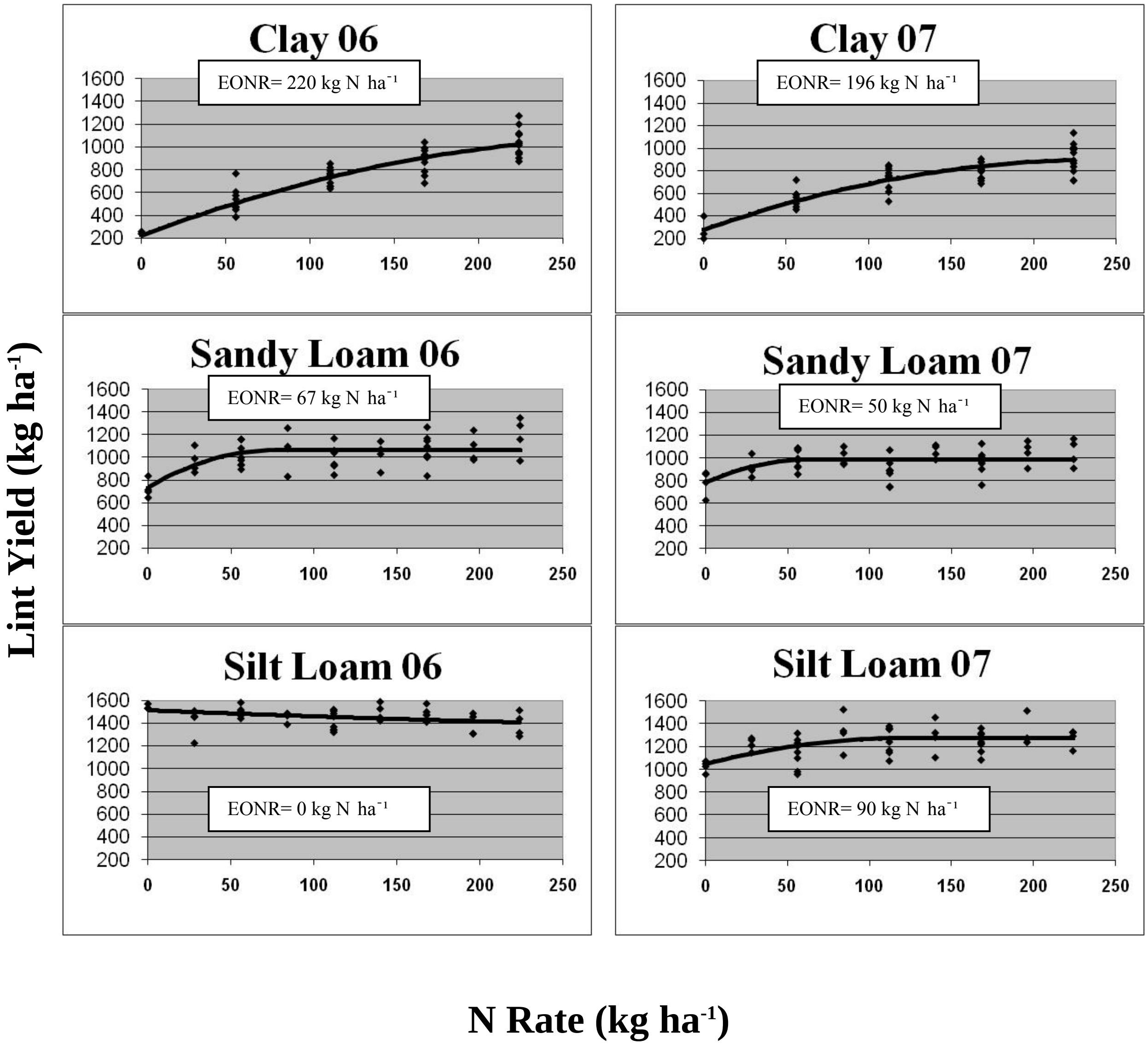

Figure 1. Economically Optimal N Rates (EONR) of the three experimental sites in 2006 and 2007. Lines are best-fitting quadratic-plateau functions.

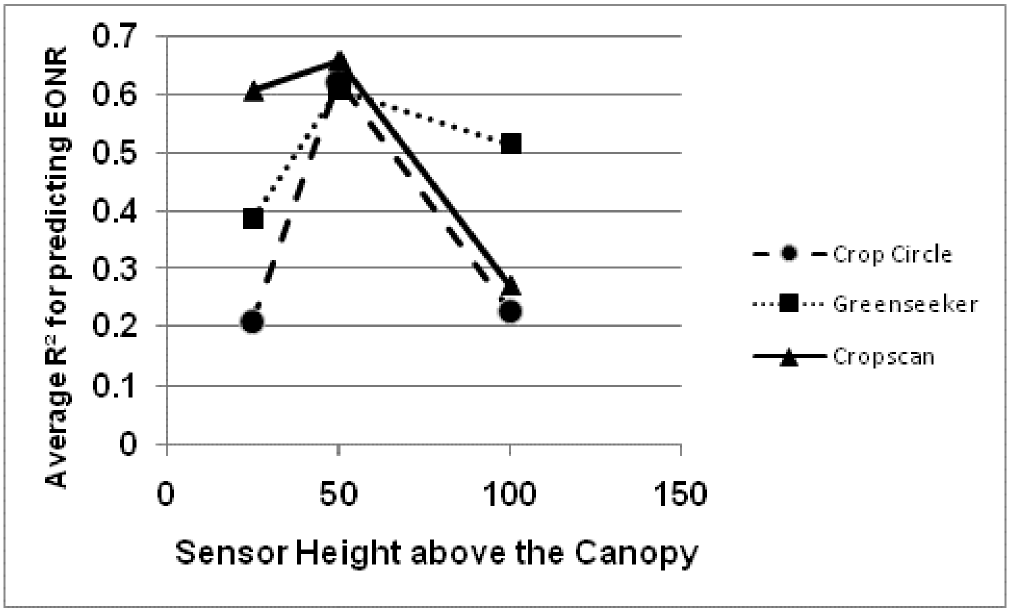

Figure 2. Average R^2 for predicting EONR from NDVI and VIS/NIR as a function of sensor height above the canopy for the combined mid square and early flower data.

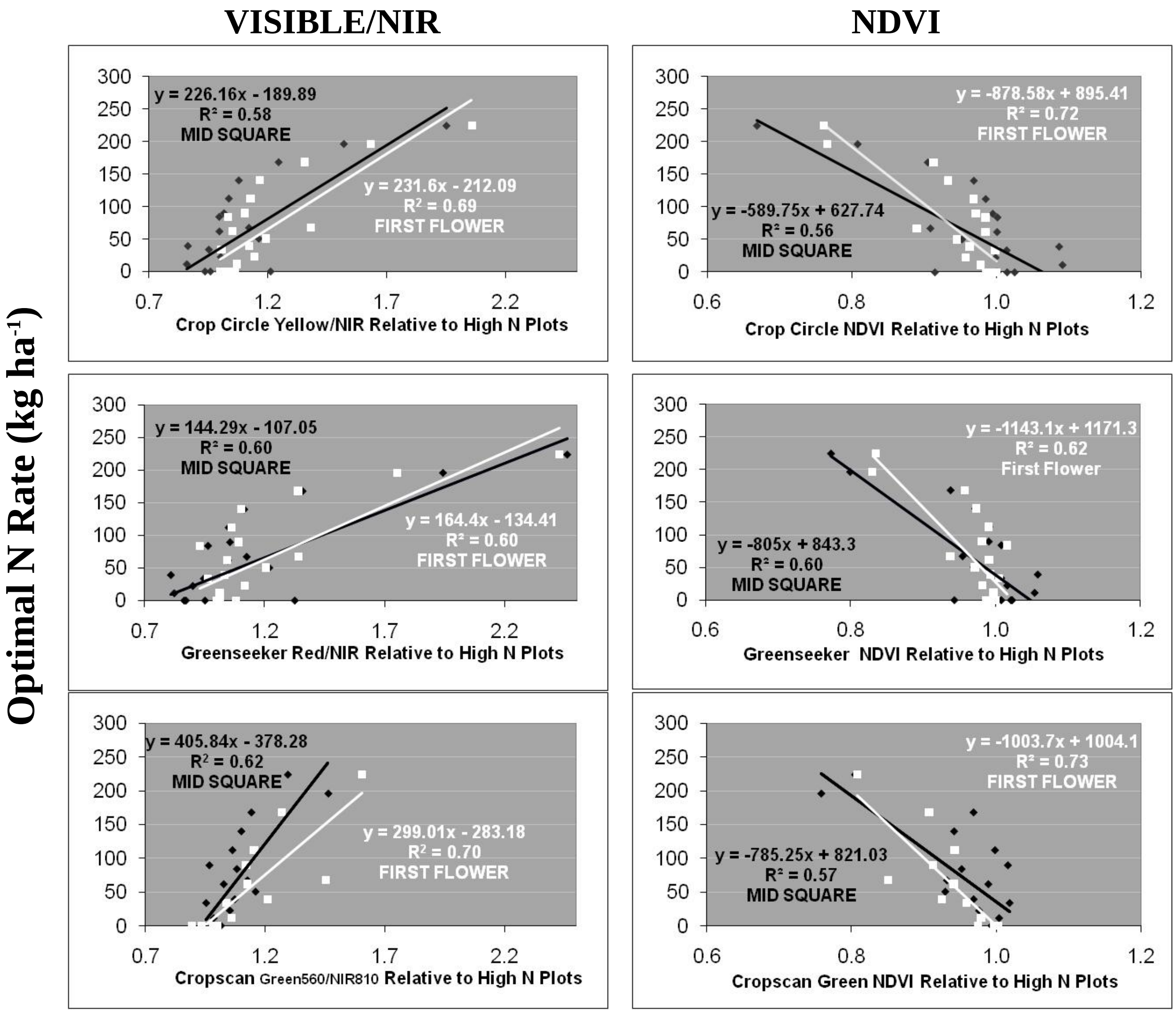

Figure 3. Linear regression between Optimal N Rate and the reflectance measurements relative to the high N plots for mid square and early flower growth stages. Reflectance measurements were a combination between the 2006 and 2007 data and were taken 50 cm above the canopy.

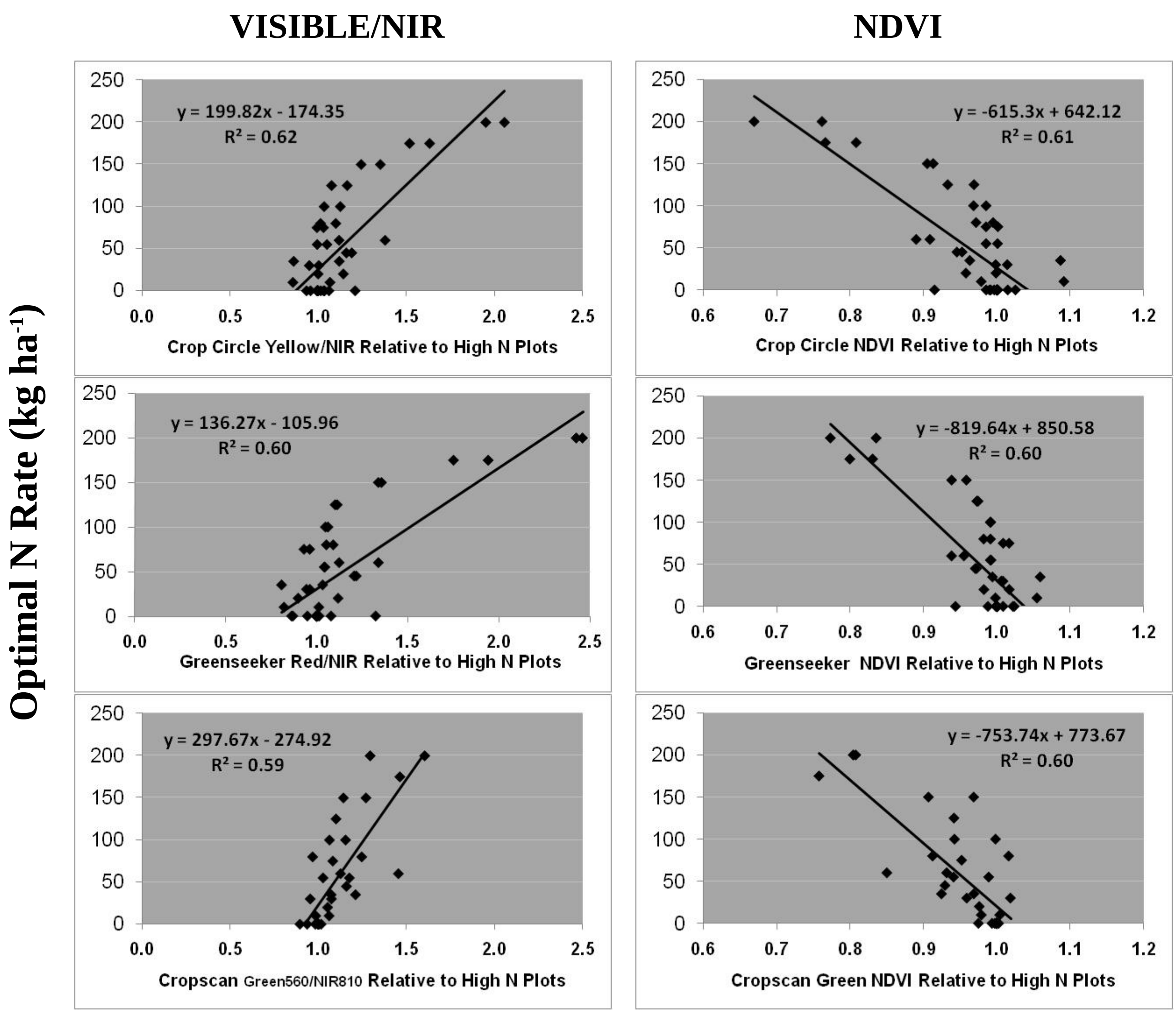

Figure 4. Linear regression between Optimal N Rate and the reflectance measurements relative to the high N plots for the combined equations for mid square and early flower growth stages. Reflectance measurements were a combination between the 2006 and 2007 data and were taken 50 cm above the canopy.

CHAPTER III

VARIABILITY OF REFLECTANCE MEASUREMENTS DURING THE DAY

ABSTRACT

The variability during the day of passive reflectance reflectance measurements on crop plants has been documented by several studies. Active sensors are now commercially available to diagnose and control N rate. Diurnal variability in reflectance measurements could introduce substantial error in sensor-based N rates, but we are not aware of any published reports looking at variability in active sensors. Our objective was to quantify variability during the day for both passive and active sensors mounted above cotton (*Gossypium hirsutum* L.) plants. The reflectance measurements were taken in 2006 and 2007 from 6:00 to 20:00 at early square, mid square and early flower growth stages. Three sensors were used to take reflectance measurements: Cropscan (passive) (Cropscan, Inc., Rochester, MN), Crop Circle (active) (Holland Scientific, Lincoln, NE), and GreenSeeker (active) (N-Tech Industries, Ukiah, CA). Variability in reflectance values during the day was relatively large for all three sensors. For GreenSeeker Vis/NIR (Vis = 656 nm & NIR = 774 nm), an equation based on temperature, solar radiation, and solar time explained about 50% of this variability and could potentially be used to correct GreenSeeker Vis/NIR during field use. Similar equations for Cropscan and Crop Circle sensors explained ≤10% of the variability in Vis/NIR. For both Vis/NIR and NDVI, the standard deviations for Crop Circle (Vis = 590 nm & NIR = 880 nm) and Cropscan (Vis = 560 nm & NIR = 810 nm) in the east-west row directions were not significantly different from each other. However the variability for Cropscan in north-south rows was always significantly larger than all the sensors indicating that passive sensors are inapt to

some of the field conditions. For Vis/NIR, the GreenSeeker SDs were not significantly (α = 0.05) different than Crop Circle, but the Crop Circle SDs for NDVI were significantly smaller than the GreenSeeker sensor. Standard deviations for N rate based on equations from chapter 2 showed the opposite pattern; the GreenSeeker sensor (SD = 24) was worse than Crop Circle (SD = 11.80) for Vis/NIR but no significant difference was found between the two sensors for NDVI (Crop Circle SD = 13.82 and GreenSeeker SD = 19.92). Standard deviations of reflectance parameters or calculated N rates were not influenced due to growth stage for any of the three sensors at α = 0.05. Mid-day was the time with the least error introduced into N rates by drift in reflectance measurements. Increasing the duration over which readings were taken resulted in greater error for predicted N rate. Spraying water on the plants resulted in slightly lower N rate predictions for active-light sensors (Crop Circle and GreenSeeker) and higher N rate predictions for the passive sensor (Cropscan).

INTRODUCTION

Recently, with the advance of precision agriculture and remote sensing, newer methods of nitrogen (N) status detection have been developed. These tools are based on the spectral properties (absorption or reflectance) of a single leaf or a canopy, and have the potential to sense N status and guide N application. Spectral reflectance is affected by N deficiency. Wilkerson et al. (1998) found that cotton reflectance at early square (early square) is highly related to yield response to N. Chua et al. (2003) and Bronson et al. (2003) found that using reflectance measurements to trigger small in-season N applications to irrigated cotton saved nitrogen fertilizer with no yield reduction in most of the cases. In Chapter II of this thesis, Visible/Near Infrared ratio (Vis/NIR) and normalized difference vegetation index (NDVI = [NIR - red]/[NIR + red]) measured at a height of 50 cm above the canopy were good predictors of EONR with $R^2 > 0.5$ at mid-square and early flower growth stages for Cropscan (passive), Crop Circle (active), and GreenSeeker (active) sensors. Passive sensors measure the sunlight reflected from the plants, and active sensors measure the reflected light emitted by their own light source.

However, it is known that there are several factors other than N status that can affect reflectance of passive sensors (e.g. cloud cover). These would introduce error when diagnosing crop N status or need. There are no published reports examining diurnal variation of reflectance measurements from active sensors. When diurnal variation in reflectance measurements exists, there are possible sources for this variation: (1) External optical factors that affect reflectance measurements even though leaf properties have not changed. These include sun angle, cloud cover, dew or rain on leaves, and wind (changing leaf angle). (2) Internal physiological factors that change actual reflectance properties of leaves. These include changes in pigment concentration or arrangement, changes in cell shape as turgor pressure changes, and changes in leaf angle, shape, or orientation. (3) Sensor properties that may be sensitive to environmental changes, especially temperature but perhaps humidity as well. We did not find published examples of this source of variability but also did not find evidence that ruled out this source.

External Factors Influencing Reflectance Measurements

Row orientation and sun elevation are determinants of wheat (*Triticum aestivum* L.) spectral reflectance collected with a passive reflectance sensor (Jackson et al., 1979).

Their work indicated that for north-south rows, visible reflectance increased with solar elevation, whereas the east-west plots did not change much with solar elevation except at solar noon. Row orientation did not affect infrared reflectance as much, but there was a significant decrease in IR reflectance for the north-south rows near solar noon. Souza et al. (2004) found comparable results using a passive reflectance sensor on potted corn (*Zea mays* L.) plants placed in a research field. The reflectance at 550 nm was more variable during the day in north-south rows than in east-west rows. Subsequently, correction equations were developed for the reflectance values as a function of row direction, sun angle, and time of day. The regression model explained 90% of the variation in 550nm reflectance and 86% in green normalized difference vegetation index (Green NDVI) variation. Reflectance in the north-south rows is more variable because shadows cross behind the row at solar noon and also early and late in the day.

Sticksel et al. (2004), similar to Souza et al. (2004), concluded that time of day affected reflectance values. The vegetation indices used in their research were a combination of visible, red-edge, or near-infrared wavebands. The indices for the noon measurements were always significantly lower than the morning and afternoon time intervals. This was credited to the fact that at noon more sunlight visible reflectance bounces back from the bare soil and senescent leaves and there is a decrease in reflectance for near-infrared.

Dew increased wheat reflectance in the visible range by as much as 60%, but had no effect on near-infrared reflectance (Pinter, 1986). Higher dew density resulted in larger changes in reflectance for visible wavebands. Plants with water droplets on them had increased visible reflectance due to an increase in specular reflectance. Another research also reported that dew increased visible 50% for bentgrass, and 4% for bluegrass (Madeira et al., 2000). For the NIR region, dew decreased the reflectance by 2 to 8% on bentgrass and 9% on the bluegrass canopy.

An increase in cloud cover was found to decrease reflectance of corn (Tumbo et al., 2002) and dew-covered bentgrass (Madeira et al., 2000). Wind may also affect reflectance of canopies by changing the canopy geometry (Lord et al., 1985). For this study the sensor was placed on a stationary mount 3 meters above the canopy and the readings were taken for 312 s at a time. It was reported as much as 60% of variability in the barley red reflectance measurements during windy period when compared to 12% of variability in the reflectance measurements during non-windy periods. This variation was attributed to stem bending and leaf fluttering. Conversely, windy conditions caused little variation in the reflectance measurements of alfalfa because of its short and dense canopy structure.

Internal Factors Influencing Reflectance Measurements

Physiological changes in plants can also affect spectral measurements. Chloroplast orientation can change at different light irradiances, and consequently affect chlorophyll meter (SPAD) readings (Hoel and Solhaug, 1998). Their study conducted on winter wheat and *Oxalis acetosella* L. reported that high irradiance decreased SPAD values by about 8 % and 15 % respectively, while low irradiance early in the day increased SPAD values by 6 % and 9 % respectively. It is reasonable to suppose that the sensor values would be affected by irradiance to a greater extent than the chlorophyll meter since the SPAD meter is designed to block outside light when taking readings and the sensors are not. Brugnoli and Bjorkman (1992) reported that blue-light-induced chloroplast movement resulted in a change in the spectral properties of detached leaves of western wild cucumber (*Marah fabaceus* Greene) and redwood sorrel (*Oxalis oregana* Nutt.). They found that leaf absorbance decreased and reflectance and transmittance increased in the visible spectrum, but not in the red-edge.

Water stress can have a significant effect on reflectance measurements. In a healthy non-stress plant, light is absorbed in the red wavelengths and reflected in the NIR range to avoid over-heating Plant et al. (2000) found that as water stress increases, NDVI in aerial photos decreases. In addition to reduced NIR reflectance from individual leaves, water stress effects on canopy structure and LAI may also be involved. Similarly, Li et al. (2001) found that an increase in soil water content caused a decrease in cotton reflectance in the visible range and an increase in reflectance in the NIR bands. Carlson et al. (1971) reported that reflectance of detached leaves was significantly correlated with relative leaf water content (RWC) for corn *(Zea Mays* L.), soybean *(Glycine max* L.), and sorghum (*Sorghum bicolor* L.) ($R^2 > 0.59$) at four wavelengths in the NIR portion of the spectrum. Reduced leaf water content increased reflectance. Non-plant factors may also be involved.

The objectives of this research are to: (I). Quantify variability during the day for both passive and active sensors mounted above cotton plants. (II). Assess the potential impact that this variability has on errors in diagnosing N need based on sensors; and (III). Assess the possibility of mathematically correcting any consistent diurnal patterns in reflectance.

MATERIALS AND METHODS

Trials were located at University of Missouri Bradford Research and Extension Center in Columbia. The experiments were conducted during 2006 and 2007, with two planting dates each year. Ammonium nitrate was broadcast at planting to supply 220 kg N ha^{-1} each year. The soil type in the trials was Putnam silt loam (description) and the cotton cultivar 'DP 444 BG/RR' (Deltapine, Memphis, TN) was planted in all fields for both years. Weed control was done by application of glyphosate to cotton that was 45 cm tall. No insecticide was required during the trials.

Reflectance measurements

Three sensors were used to take reflectance measurements: Cropscan (passive) (Cropscan, Inc., Rochester, MN), Crop Circle (active) (Holland Scientific, Lincoln, NE) and GreenSeeker (active) (N-Tech Industries, Ukiah, CA). The Crop Circle sensor emits yellow (590 nm) and Near Infrared (880 nm) wavelengths, while the GreenSeeker sensor emits red (656 nm) and Near Infrared (774 nm). The Cropscan sensor wavelengths used for this study were green (560 nm) and Near Infrared (810 nm). Each sensor was positioned on a stationary mount 50 cm above the canopy. A computer with custom software was used to collect 92 readings from each sensor for 10 seconds every two minutes.

The reflectance measurements were taken from 6:00 to 20:00 (CST) at early square, mid-square and early flower growth stages. In 2006, the reflectance measurements were taken in the east-west rows for all three growth stages, and also in the north-south rows at early flower. In 2007, readings were taken in the north-south rows for the three growth stages and also in east-west rows at early flower. Water was sprayed on the cotton at early flower growth stage on the north-south rows at 12:20 in 2006.

Processing Reflectance measurements

ASCII files containing reflectance sensor data were sorted by sensor using Excel 2003 (Microsoft Corporation, Redmond, WA). For the Crop Circle and GreenSeeker sensors, PROC MEANS in SAS (SAS Institute Inc., Cary, NC; version 9.1) was used to calculate the mean value for each sensor variable for each two minute period. All the data were normalized using PROC MEANS in SAS to divide each observed sensor reading by the average value between 11:00 and 15:00.

Equations for predicting necessary N rate from chapter 2 were used to calculate the effect of sensor drift on N rate recommendations for mid square and early flower growth stages only, since sensor values at the early square stage were not found to be useful predictors of N rate in that study. Equations used to predict the N rate were specific for each sensor and for the form in which visible and NIR were combined (Visible/NIR or NDVI). Nitrogen rates were calculated using the each data reading collected as the high-N reference reflectance (i.e. the denominator in the equation). The 'target' reflectance was held constant throughout the day and was chosen arbitrarily to be the value that would give an N recommendation of 80 kg N ha^{-1} when experimental data averaged 13:00 to 13:10 (i.e. the numerator in the equation). This was done to simulate how much error in N rate would be introduced by drift in the high-N reference that was not accounted for, for example, if the high-N reference was measured only once at the beginning and then the whole field was fertilized without re-measuring the high N-reference.

The water effect on N recommendations was calculated in a slightly different way. For this experiment the goal was to find out how N rate recommendations would change on a uniform 'target' area over a short time due to leaf wetting then drying. Observed sensor values between 12:00 and 13:00 were used as the 'target' area (i.e. numerator) in calculating N rate. The high-N reference value was chosen arbitrarily to give an average N rate of 80 kg N ha^{-1} at 13:00 to 13:10.

The Cropscan data required additional steps to consolidate the readings before processing them the same way as data from the other two sensors. The Cropscan data were averaged for each two-minute period, temperature-corrected using custom software supplied by Cropscan, and formatted appropriately for POSTPROC software. Reflectance was calculated using the POSTPROC procedure in the Multispectral Radiometer (MSR) software supplied with the Cropscan radiometer.

Statistical Analyses

Standard deviation (SD), calculated using PROC MEANS in SAS, was used as our main measure of data consistency from sensors. For each of our eight experiments, SD was calculated for each sensor and each predictor (Vis/NIR and NDVI forms). We observed that Cropscan values were unstable in the early morning and late evening, as would be expected for passive sensors dependent on sunlight. Thus we calculated SD for several time restricted datasets with this sensor: 8:00 to 18:00 and 9:00 to 17:00 as well as the full 6:00 to 20:00. Standard deviations of N rates based on sensor values were also calculated. Analysis of variance (computed using PROC GLM in SAS) was used to test the effect of growth stage, row direction, and sensor on average SD of reflectance parameters and reflectance-based N rates.

Polynomial equations that modeled Visible/NIR and NDVI-based N rate as a function of temperature, solar radiation, and solar time were calculated for each of the sensors using PROC REG in SAS. The temperature and solar radiation data were obtained from the weather station at Bradford Research Center. Six different polynomial equations were calculated for each sensor/N rate combinations by increasing the solar time term from linear to 6^{th} order. Solar time was expressed in hours as difference from solar noon.

Standard deviations for different time intervals were calculated for sensor-based N rates. This was done to determine the effect of starting time and time since measuring the high-N reference on variability (error) in N rate recommendation. Starting time ranged from 6:00 to 16:00 in 1-hour increments, and measurement duration ranged from 1 to 4 hours in 1-hour increments. The final step was using PROC GLM in SAS to calculate the mean comparisons for each sensor/predictor combination to determine whether starting time, duration, or their interaction affected SD of N rate. When significance at $\alpha = 0.05$ was found, t-tests were used to determine which starting times and durations were different from each other at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Stability of Reflectance measurements During the Day

Variability in reflectance values during the day was relatively large for all the sensors (Figure 1). If reflectance sensors are used to control variable-rate N fertilizer applications, changes in sensor values over time could lead to errors in diagnosis of N needs. Figure 2 suggests that the scale of these errors is large, and that failure to correct them would be a substantial obstacle to successful use of sensors to control N rate.

Standard deviations (SD) for the reflectance parameters and for the calculated N rates were used to compare the variability associated with different sensors, parameters, and times. Cropscan reflectance values and N rates were extremely unstable early and late in the day (Figure 1). Since the Cropscan does not have its own light source like the active sensors, its readings are more affected by the changes in sunlight. This instability suggests that Cropscan cannot be used early morning and late evening, but may be used for a smaller time window. In order to determine the best time window for the Cropscan readings, the average standard deviations for Vis/NIR and NDVI for three different time intervals were compared: 6:00-20:00, 8:00-18:00, and 9:00-17:00. The 6:00-20:00 time intervals had the highest SDs, while 8:00-18:00 and 9:00-17:00 had equal standard deviations (Table 1) that were approximately equal to SDs observed with the GreenSeeker. High SDs from 6:00-20:00 were mainly associated with north-south rows (Table 1) which have shadows from adjacent rows crossing then from 6:00-8:00 and 18:00-20:00. Limiting Cropscan use to the period 8:00-18:00 minimizes variability while maximizing the available time window and is the Cropscan data subset used in all further analyses.

The average standard deviation for the Crop Circle was the lowest of the three sensors, 0.009 for Visible/NIR and 0.013 for NDVI (Table 1). Variability with the GreenSeeker was somewhat higher with SD of 0.015 for Visible/NIR and 0.026 for NDVI. One possible reason for the higher variability with GreenSeeker is its physically narrow band of active light that may be sensitive to slight movement of plants. Any variability due to this effect should decrease in real-time N application because the sensor would not be affected by the plant movements when driving. The Cropscan sensor, like GreenSeeker, might be affected by plant movements given that its bands are spatially separated and as a result they take readings of slightly different but overlapping targets.

There was no significant ($\alpha = 0.05$) difference in the standard deviations of reflectance parameters due to growth stage or row direction for any of the three sensors. The mean SD of NDVI for the Cropscan sensor was probably higher ($p = 0.07$) for the north-south row direction than the east-west row direction. This conclusion agrees with Souza et al. (2004), who also found that time of day affected reflectance in the north-south row direction more than the east-west direction for a passive sensor (Crop Circle passive). This is probably due to shadows crossing the north-south rows as the sun moves east-west.

Analysis of variance on standard deviation suggests differences between the sensors for NDVI and Visible/NIR at the $\alpha = 0.05$ level. For NDVI, the standard deviations for Crop Circle and Cropscan east-west row direction are equal but significantly smaller than the GreenSeeker, while SD for Cropscan north-south row direction is significantly larger than all the others. For Vis/NIR, Crop Circle, Cropscan east-west row direction, and GreenSeeker SDs are not significantly different from each other, but they are significantly smaller than Cropscan north-south. Mean SD for the north-south row direction was 5 times higher than for the east-west row direction for Cropscan. For mean separations, Cropscan east-west row data were considered as separate mean from Cropscan north-south row data.

Standard Deviations for Recommended N rates for Crop Circle and GreenSeeker

The Crop Circle sensor had the least variability in N rate recommended. Average day-long standard deviation was 11.8 kg N ha^{-1} for the NDVI-based N rate (Table 2) and 13.8 kg N ha^{-1} for Visible/NIR-based N rate. The differences between the GreenSeeker NDVI and Visible/NIR N rates were even more pronounced than the Crop Circle, with standards deviations of 24.2 kg N ha^{-1} and 53.4 kg N ha^{-1} respectively. These numbers suggest that GreenSeeker N rates had more variability than Crop Circle. The Cropscan sensor seemed to have more variability than the Crop Circle but less than the GreenSeeker sensor. Averaged SDs were 16.8 kg N ha^{-1} for NDVI and 52 kg N ha^{-1} for Vis/NIR, suggesting again difference between the NDVI and Vis/NIR N rates.

As with reflectance parameters, standard deviations for predicted N rate were not significantly different between growth stages or row directions for either sensor at $\alpha = 0.05$. Mean standard deviations were not significantly different between the Vis/NIR- and NDVI-based N rate for the Crop Circle sensor. The NDVI-based N rate was significantly better than the Vis/NIR-based N rate for the GreenSeeker sensor. The Cropscan was again divided into two groups, east-west and north-south row directions. The SDs were not different between the sensors for the NDVI-based N rate, except for the Cropscan east-west row direction SDs which were significantly smaller that the Greenseeker. The standard deviations for GreenSeeker and Cropscan north-south Vis/NIR-based N rate were significantly greater than the SDs for Crop Circle and Cropscan east-west Vis/NIR-based N rate. The GreenSeeker and Cropscan north-south Vis/NIR-based N rate would be undesirable for controlling field-scale N applications.

Correction Functions for Visible/NIR

GreenSeeker Vis/NIR measurements tended to start low in the morning, increase until mid-day, and then decreased in the afternoon (Figure 1), leading to the opposite pattern in N rates (Figure 2) when the data from figure 1 were used as the high-N reference values (denominator). This type of systematic variation, observed repeatedly over the eight days we collected data, lends itself to description with a correction function. A function with solar time, temperature, and solar radiation as the independent variables described 0.5 of the variability on GreenSeeker Vis/NIR:

= 1.01712 + (0.01323 * solar time) hours + (-0.00395 * temperature) °C + (0.00039335 * solar radiation) Watts/M^2

All parameters of this equation are significant with $p < 0.001$. Although the data in Figure 2 appear to be at least 2nd order with respect to solar time, adding polynomial (2nd through 6th order) solar time to the model increased R^2 by less than 0.01. The temperature and solar irradiance variables, which tend to peak mid-day, apparently described the curvature adequately. This does not necessarily prove that changing temperature or solar irradiance were the true causes of the systematic changes in reflectance measured with the GreenSeeker.

When the central tendency described by equation 1 was removed from the data (i.e. data were residuals from equation 1), the systematic pattern in N rate recommended through the day was removed and variability was greatly reduced (Figure 3). Standard deviation for the Vis/NIR N was reduced from 53.3 to 24.1 (Table 2). This greatly reduces the amount of error that would be expected if using the GreenSeeker Vis/NIR-based N rate to fertilizer fields, but a simpler approach with the GreenSeeker is to use the NDVI-based N rate.

The systematic variation was also observer for Greenseeker NDVI and a similar linear function was used to describe its variability:

= 0.967925 - (0.00333 * solar time) hours (0.001083 * temperature) °C - (0.0000781 * solar radiation) Watts/M^2

This function described 0.45 of the Greenseeker NDVI variability and it was highly significant ($p < 0.001$). Higher-order term for solar time only increased the R^2 to 0.47. The standard deviation for NDVI was reduced from 19.2 to 13.1 (Table 2) and the systematic pattern was reduced (Figure 3).

Regression functions describing Visible/NIR and NDVI reflectance for Crop Circle and Cropscan were also highly significant ($p < 0.0001$) for solar time, solar radiation, and temperature, but with low coefficients of determination: 0.10 for Crop Circle Vis/NIR, 0.09 for Crop Circle NDVI, 0.04 for Cropscan Vis/NIR, and 0.037 for Cropscan NDVI. Increasing the solar time term as high as 6th order did not change R^2 value for the Cropscan sensor, and only increased R^2 for the Crop Circle sensor to 0.14 for both Vis/NIR and NDVI. Using a higher-order term for solar time to describe complex patterns through time did not appear to be justified for any of the three sensors. These low coefficients of determination suggest that little improvement would be made by using these equations to correct Visible/NIR for Crop Circle or Cropscan sensors.

Effect of Time of the Day on Standard Deviation of N Rate

There were no significant interactions between start time and duration. Effect of start time was thus evaluated averaged over duration (1, 2, 3, and 4 hours). This analysis was only carried out for active-light sensors (Crop Circle and GreenSeeker).

Standard deviation for N rate appeared to be lower in mid-day for both sensors and for both Visible/NIR and NDVI based N rates (Figure 4). Thus, mid-day would be the time with the least error introduced to N rates by drift in reflectance measurements. Nitrogen rates based on the Crop Circle sensor had SD values at both the beginning and end of the day that were significantly ($\alpha = 0.005$) higher than the lowest standard deviation in mid-day (Figure 4). GreenSeeker also had higher SD values for corrected N rate at the last start time considered (16:00) and in the morning, but the significantly higher values in the morning did not extend to the earliest start time. In general, the pattern in SD for N rate was similar and consistent for both sensors and for both parameters (Vis/NIR and NDVI) for start time from 12:00 to 16:00, and less consistent from 6:00 to 11:00. We do not know why patterns of SD over time were more variable in the morning. In addition, N rates based on uncorrected GreenSeeker Visible/NIR have the same SD as the corrected N rates in the middle of the day but much larger SD in the morning and afternoon.

The number of consecutive hours with SDs not significantly different ($\alpha = 0.05$) from the lowest value can also be used as an indicator of times with lower levels of error. For both sensors the period extended from 9:00 to 15:00 for N rates based on NDVI (Figure 4). When N rates were based on Vis/NIR, this period was shorter, ranging from 11:00 or 12:00 to 15:00. Mid-day is the time when the standard deviations for N rate are the smallest for all sensors. Errors in N rate due to drift in reflectance values would be smaller at this time than early morning or late afternoon. It also appears possible that N rate based on NDVI has a bigger window of time with less introduced error than N rates based on Visible/NIR for both sensors.

Time Interval for Consecutive Readings without updating the High-N Reference Value

An important management issue is how long the sensors can be used to control variable-rate N fertilizer applications before updating the reflectance values from the high-N area. We examined standard deviation for N rates with measurement durations of 1, 2, 3, and 4 hours. Since there was no interaction between start time and duration, SDs for each duration were averaged over 11 start times from 6:00 to 16:00. Not surprisingly, the longer the duration over which readings were taken, the greater the error introduced for predicted N rate (Figure 5).

Once more, Crop Circle N rates had much less error than GreenSeeker N rates associated with changes in sensor values over time. The Crop Circle can be used to estimate N rates for 4 consecutive hours and still not reach the amount of error that GreenSeeker N rates have in the first hour. That problem is even more apparent with the uncorrected GreenSeeker Vis/NIR-based N rates. It is difficult to point out how long the readings can be taken before updating the high-N reference value, but it is evident that the Crop Circle can be used for a longer time interval than the GreenSeeker sensor.

Water Effect on the Sensor N Rate

This experiment was used to simulate how N rate would be affected by light precipitation. For active-light sensors (Crop Circle and GreenSeeker), spraying water on the plants resulted in lower N rate recommendations (Figure 6). The magnitude of this change was small, no more than 7 kg N ha^{-1}, indicating that this is a minor source of error compared to other changes in reflectance over time. This can be seen particularly in the GreenSeeker data in Figure 6, where the change in N rate just prior to spraying is greater than the rate of change after spraying. As the leaves were drying off, it took around 20 minutes for the sensor N rates to return to pre-spraying values for Crop Circle and GreenSeeker. The amount of water sprayed on leaves was small. Greater wetting (of crop and soil) might influence N rates more. Further study to better understand how much error in N rate would be introduced by leaf wetting and drying is needed.

Previous research with passive reflectance sensors reported that dew increased visible reflectance in wheat by 40 to 60% (Pinter, 1986), 50% for bentgrass, and 4% for bluegrass (Madeira et al., 2000). For the NIR region, dew decreased the reflectance by 9% on the bluegrass canopy (Madeira et al., 2000), and had no effect on the wheat reflectance (Pinter, 1986). An increase in visible reflectance and a small decrease in NIR reflectance will lead to higher Visible/NIR values and lower NDVI values. This would cause the sensors to recommend higher N rates. This is what was observed with the (passive) Cropscan sensor: N rates increased after the water was sprayed (Figure 6). We cannot say whether this was really an effect of the water sprayed on the leaves, since this trend was observed both before spraying and after the period (20 minutes) that appeared to correspond with leaf drying.

The fact that the effect of leaf wetting was to decrease N rate based on the active sensors is puzzling, since it is the opposite of what would be expected based on the literature of studies done with passive sensors.

CONCLUSIONS

With both the passive and active sensors, we observed substantial variability in cotton reflectance during the day, before and after solar noon. Cropscan was highly variable early (6:00-8:00) and late (18:00-20:00) in the day, and clearly would not be useful for making N rate decisions during these times. However, when Cropscan reflectance measurements were restricted to the period 8:00-18:00, standard deviation of reflectance was similar to the GreenSeeker. Standard deviations for the Crop Circle were less than or equal to the standard deviations of other sensors.

For the six experiments conducted at mid square or early flower growth stages, average standard deviation for N rate (predicted from equations from Chapter II) ranged from 12 kg N ha^{-1} (Crop Circle Vis/NIR) to 53 kg N ha^{-1} (GreenSeeker Vis/NIR). Errors in N rate with the Crop Circle sensor (< 23 kg N ha^{-1} 90% of the time) would probably be acceptable to producers. With the GreenSeeker, error associated with NDVI-based N rates (< 32 kg N ha^{-1} 90% of the time) were much smaller than with Vis/NIR-based N rates (< 88 kg N ha^{-1} 90% of the time). Errors in Vis/ NIR-based N rates could be greatly reduced (< 40 kg N ha^{-1} 90% of the time) by correcting for time of day, temperature, and solar radiation. However, using NDVI-based N rates seem like a better solution even though the quality of prediction was slightly lower in the previous experiment.

Our data do not support the idea that active light sensors have eliminated the diurnal variability previously reported for passive sensors. However, active light sensors have extended the time when useful observations can be made by at least two hours in the morning and two hours at night (and quite possibly farther).

Sensor-guided variable-rate N applications will more accurately meet crop needs if appropriate strategies are developed to minimize the effects of diurnal variation in reflectance reflectance measurements.

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Table 1. Standard deviations for Visible/NIR and NDVI for the Crop Circle, GreenSeeker, and Cropscan sensors.

Sensor	Year	Time	Row Direction	Growth Stage	NDVI SD	Average SD NDVI	VIS/NIR SD	Average SD VIS/NIR
Crop Circle	2006	6-20	N-S	Mid-Square	0.010	0.013 **a**	0.006	0.009 **a**
Crop Circle	2007	6-20	E-W	Mid-Square	0.008		0.005	
Crop Circle	2006	6-20	E-W	Early flower	0.017		0.012	
Crop Circle	2007	6-20	E-W	Early flower	0.005		0.003	
Crop Circle	2006	6-20	N-S	Early flower	0.023		0.016	
Crop Circle	2007	6-20	N-S	Early flower	0.014		0.009	
GreenSeeker	2006	6-20	N-S	Mid-Square	0.028	0.026 **b**	0.016	0.015 **a**
GreenSeeker	2007	6-20	E-W	Mid-Square	0.024		0.014	
GreenSeeker	2006	6-20	E-W	Early flower	0.033		0.020	
GreenSeeker	2007	6-20	E-W	Early flower	0.019		0.011	
GreenSeeker	2006	6-20	N-S	Early flower	0.023		0.013	
GreenSeeker	2007	6-20	N-S	Early flower	0.027		0.015	
Cropscan	2006	8-18	N-S	Mid-Square	0.034	0.044 **c**	0.023	0.037 **b**
Cropscan	2006	8-18	N-S	Early flower	0.054		0.051	
Cropscan	2007	8-18	E-W	Mid-Square	0.008	0.008 **a**	0.005	0.005 **a**
Cropscan	2007	8-18	E-W	Early flower	0.008		0.005	
Cropscan	2006	6-20	N-S	Mid-Square	0.132	0.077	0.056	0.035
Cropscan	2007	6-20	E-W	Mid-Square	0.017		0.013	
Cropscan	2006	6-20	N-S	Early flower	0.147		0.063	
Cropscan	2007	6-20	E-W	Early flower	0.014		0.007	
Cropscan	2006	8-18	N-S	Mid-Square	0.034	0.026	0.023	0.021
Cropscan	2007	8-18	E-W	Mid-Square	0.008		0.005	
Cropscan	2006	8-18	N-S	Early flower	0.054		0.051	
Cropscan	2007	8-18	E-W	Early flower	0.008		0.005	
Cropscan	2006	9-17	N-S	Mid-Square	0.029	0.025	0.020	0.021
Cropscan	2007	9-17	E-W	Mid-Square	0.007		0.005	
Cropscan	2006	9-17	N-S	Early flower	0.058		0.054	
Cropscan	2007	9-17	E-W	Early flower	0.005		0.003	

Table 2. Standard deviations for the N rates based on Visible/NIR and NDVI for the Crop Circle and GreenSeeker sensors.

Sensor	Year	Time	Row Direction	Growth Stage	NDVI N Rate SD	Average SD N Rate NDVI	VIS/NIR N Rate SD	Average SD N Rate VIS/NIR
Crop Circle	2006	6-20	N-S	Mid-Square	10.7	13.8	7.3	11.8
Crop Circle	2007	6-20	E-W	Mid-Square	11.1		7.7	
Crop Circle	2006	6-20	E-W	Early flower	18.9		14.6	
Crop Circle	2007	6-20	E-W	Early flower	9.7		8.7	
Crop Circle	2006	6-20	N-S	Early flower	21.5		17.4	
Crop Circle	2007	6-20	N-S	Early flower	10.8		15.1	
GreenSeeker	2006	6-20	N-S	Mid-Square	12.2	19.2	41.4	53.3
GreenSeeker	2007	6-20	E-W	Mid-Square	15.8		46.2	
GreenSeeker	2006	6-20	E-W	Early flower	30.1		69.5	
GreenSeeker	2007	6-20	E-W	Early flower	17.7		42.3	
GreenSeeker	2006	6-20	N-S	Early flower	17.1		37.9	
GreenSeeker	2007	6-20	N-S	Early flower	22.4		82.7	
GreenSeeker*	2006	6-20	N-S	Mid-Square	13.2*	13.1*	25.3*	24.1*
GreenSeeker*	2007	6-20	E-W	Mid-Square	9.3*		22.7*	
GreenSeeker*	2006	6-20	E-W	Early flower	20.6*		30.0*	
GreenSeeker*	2007	6-20	E-W	Early flower	8.2 *		19.3*	
GreenSeeker*	2006	6-20	N-S	Early flower	14.8*		20.4*	
GreenSeeker*	2007	6-20	N-S	Early flower	12.4*		26.6*	
Cropscan	2006	8-18	N-S	Mid-Square	21.0	16.8	79.3	52.0
Cropscan	2006	8-18	N-S	Early flower	12.7		24.7	
Cropscan	2007	8-18	E-W	Mid-Square	4.6	5.7	16.8	14.5
Cropscan	2007	8-18	E-W	Early flower	6.8		12.3	
Cropscan	2006	8-18	N-S	Mid-Square	21.0	11.3	79.3	33.3
Cropscan	2007	8-18	E-W	Mid-Square	4.6		16.8	
Cropscan	2006	8-18	N-S	Early flower	12.7		24.7	
Cropscan	2007	8-18	E-W	Early flower	6.8		12.3	

*This symbol indicates the GreenSeeker data that was corrected with the polynomial function.

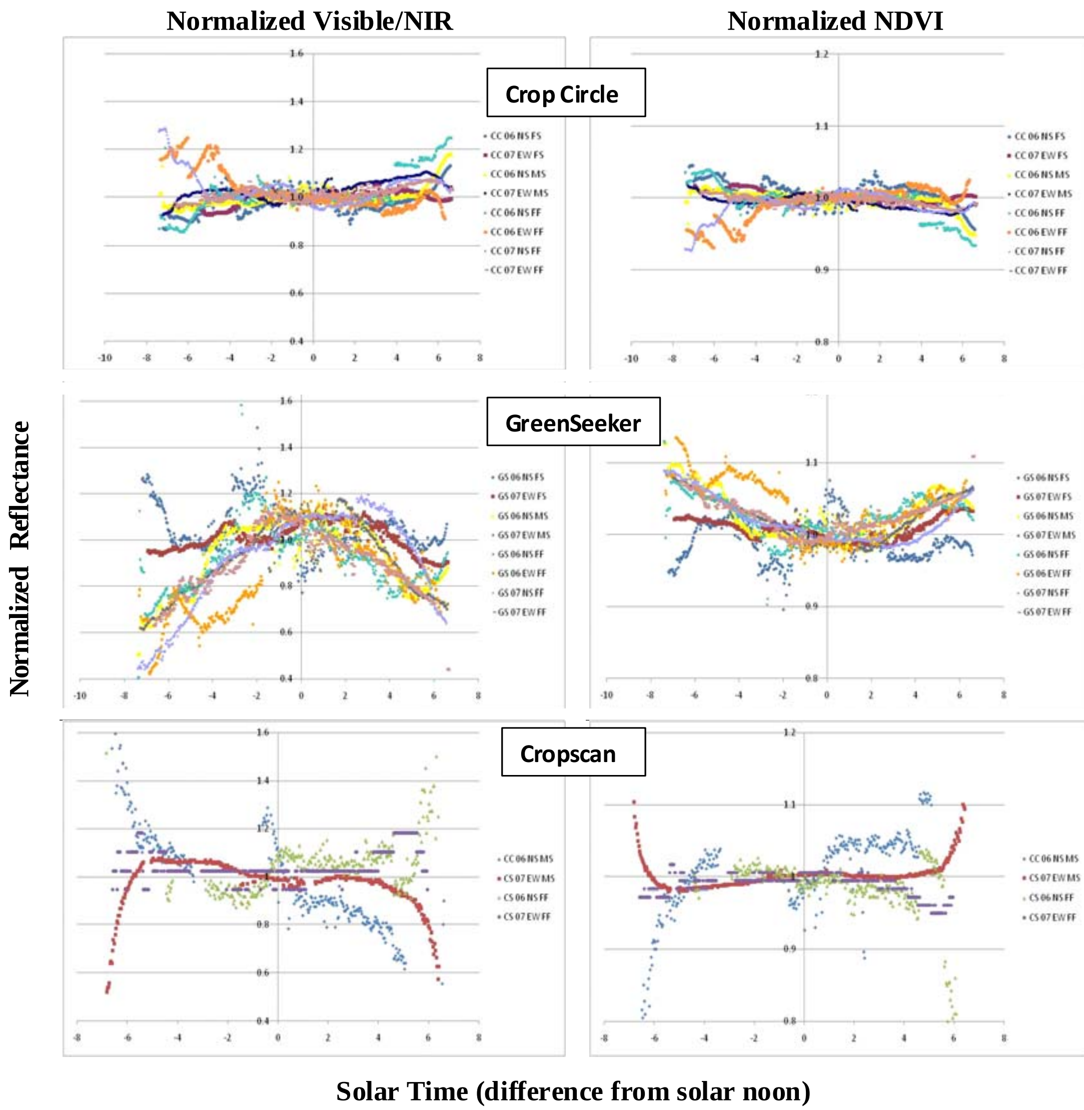


Figure 1. Changes in normalized visible/NIR and NDVI of Crop Circle (CC), GreenSeeker (GS), and Cropscan (CS) throughout the day. Growth Stage, year, and row direction did not significantly affect standard deviation of normalized reflectance variables, so we present all data together even though it is difficult to distinguish between these groups.

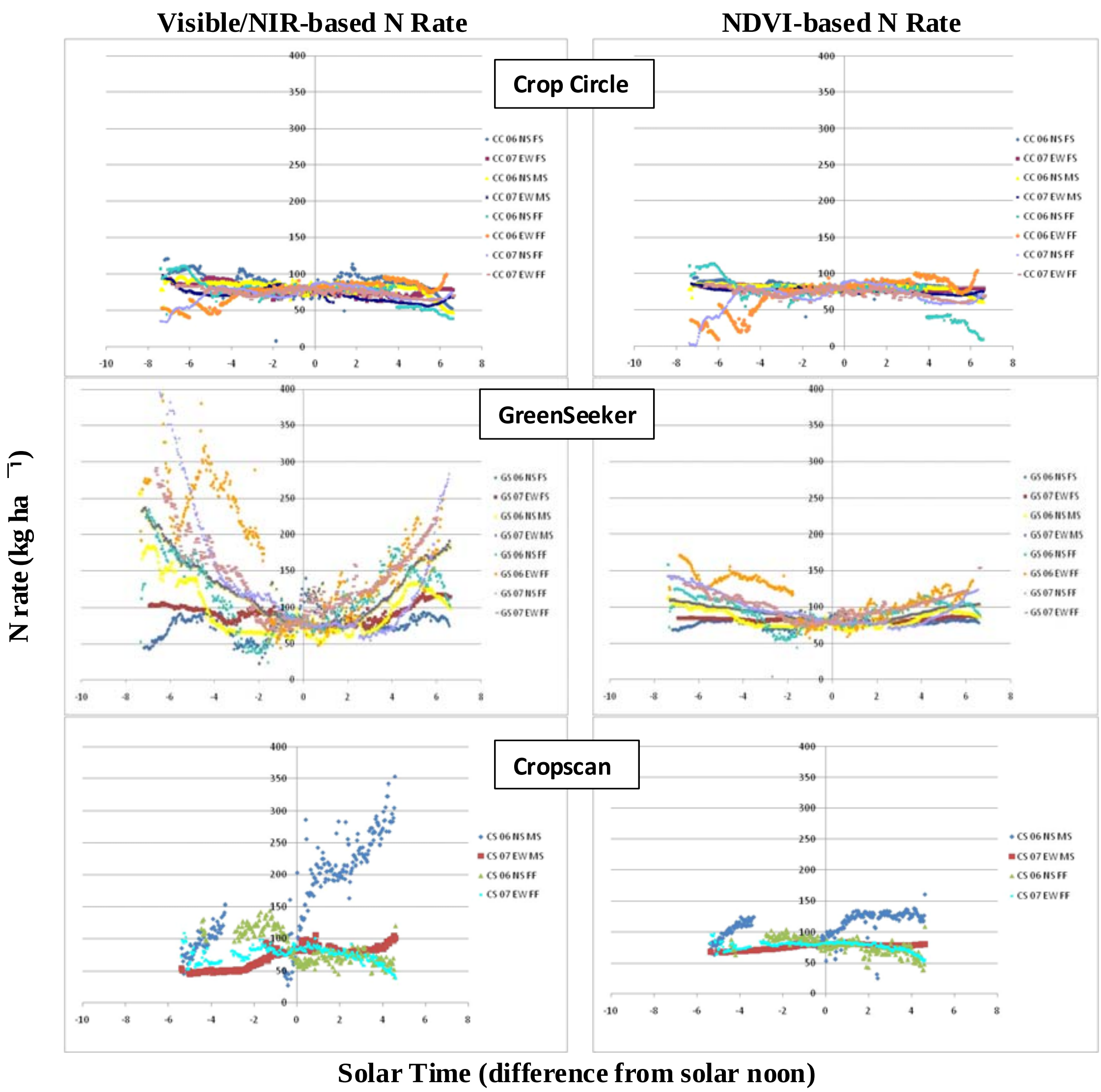


Figure 2. Changes in N rate recommendations based on visible/NIR and NDVI of Crop Circle (CC), GreenSeeker (GS), and Cropscan (CS) throughout the day.

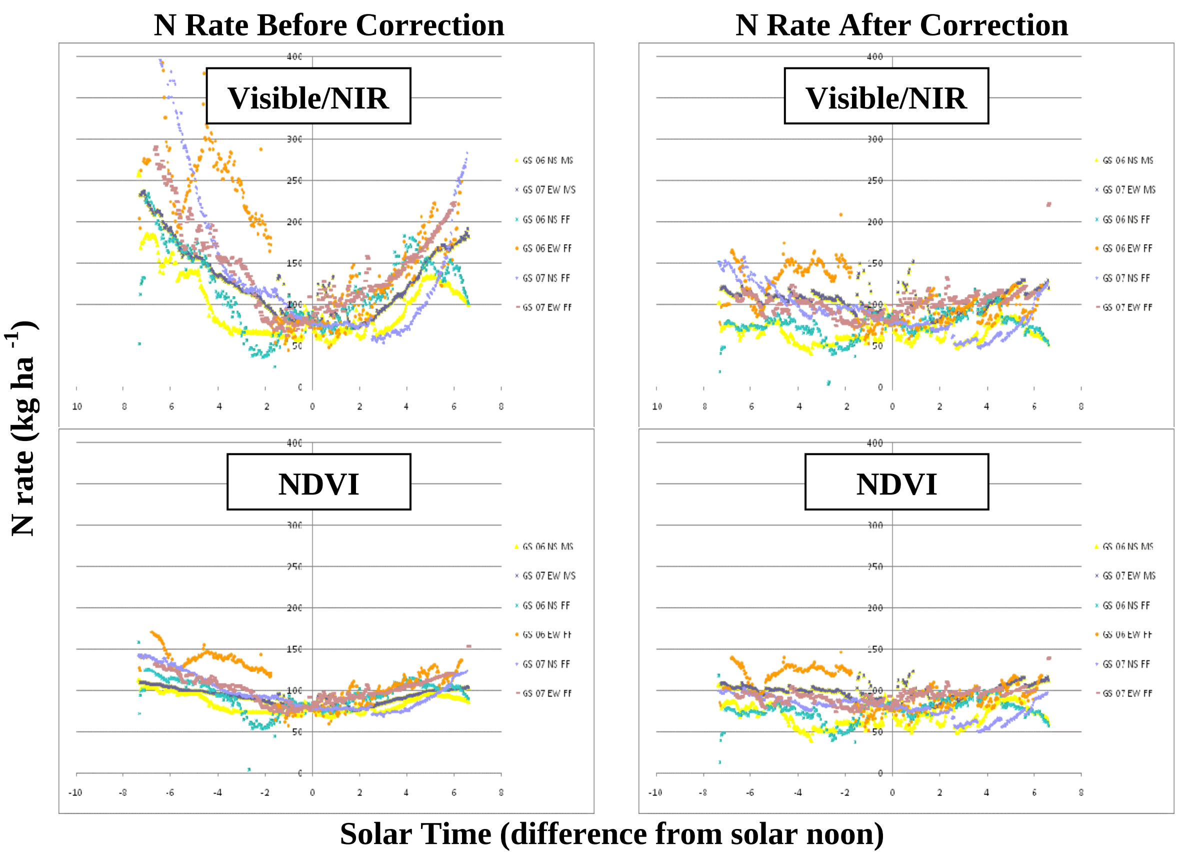


Figure 3. Uncorrected (left) and corrected (right) visible/NIR and NDVI-based N rate from the GreenSeeker sensor.

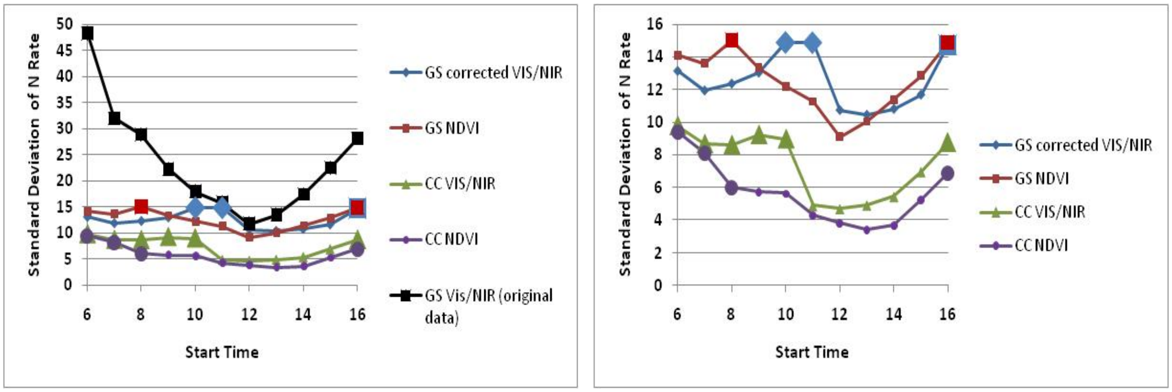


Figure 4. Standard deviations of sensor-based N rate vs. the starting time of reflectance measurements. The graph on the right is the same as the graph on the left except that GreenSeeker uncorrected data have been removed and scale has been expanded to make the data easier to see. The larger symbols on the graph represent starting times for which standard deviation is significantly higher than the lowest value for that sensor/parameter combination at $\alpha = 0.05$. Data for each start time are averaged over four durations (1, 2, 3, and 4).

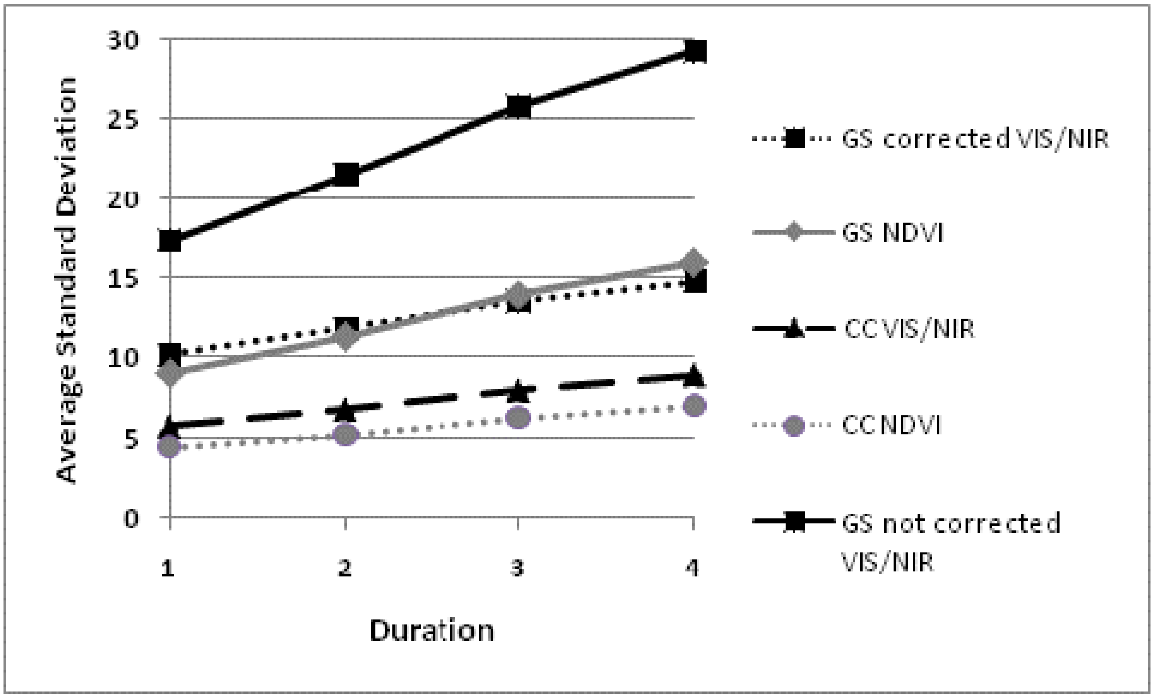


Figure 5. Standard deviations of sensor-based N rate vs. the time interval of reflectance measurements.

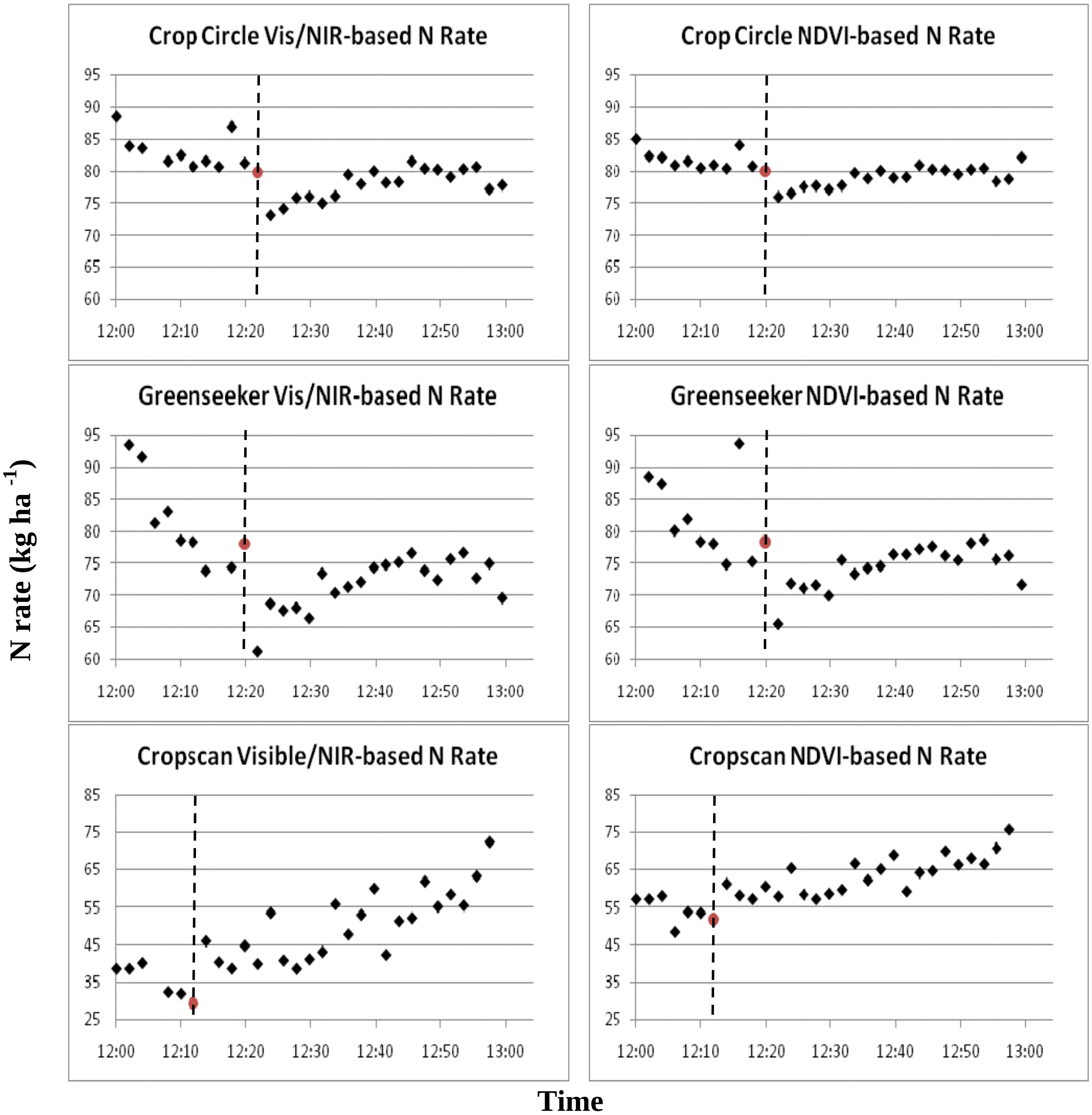


Figure 6. Leaf wetting effect on the Visible/NIR and NDVI-based N rate recommendations for the three reflectance sensors: Crop Circle, GreenSeeker, and Cropscan.

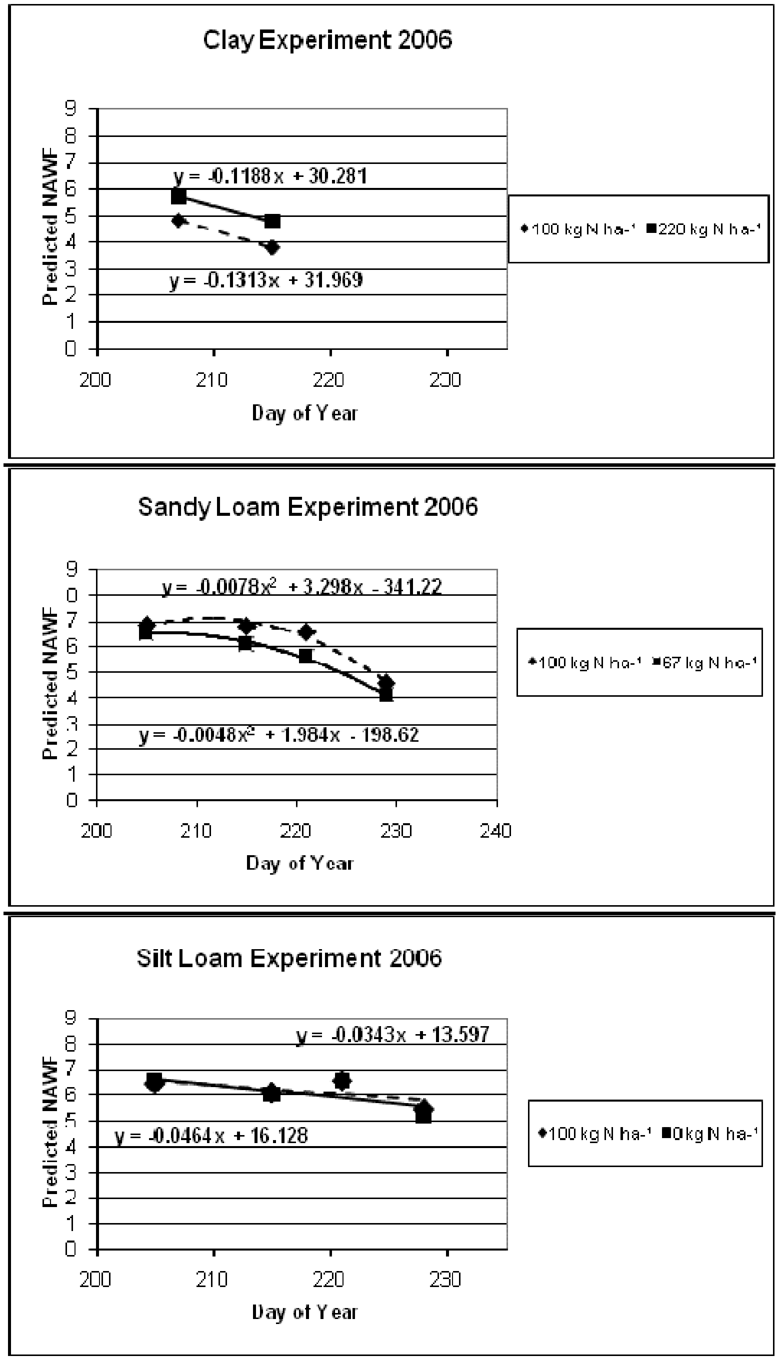
APPENDIX

1. Quadratic-plateau functions for yield response to N rate.

Silt loam 2006	lint yield kg ha^{-1} = 1370 - (1.55 * N rate) + (0.00699 * N rate * N rate); up to N rate = 0 kg ha^{-1} when yield plateaus at 1530 kg ha^{-1} .
Sandy loam 2006	lint yield kg ha^{-1} = 655 + (8.61 * N rate) - (0.063 * N rate * N rate); up to N rate = 67 kg ha^{-1} when yield plateaus at 1060 kg ha^{-1} .
Clay 2006	lint yield kg ha^{-1} = 200 + (5.59 * N rate) - (0.0101 * N rate * N rate); up to N rate = 220 kg ha^{-1} when yield plateaus at 1020 kg ha^{-1} .
Silt loam 2007	lint yield kg ha^{-1} = 983 + (1.56 * N rate) - (0.0032 * N rate * N rate); up to N rate = 90 kg ha^{-1} when yield plateaus at 1260 kg ha^{-1} .
Sandy loam 2007	lint yield kg ha^{-1} = 700 + (6.45 * N rate) - (0.0578 * N rate * N rate); up to N rate = 50 kg ha^{-1} when yield plateaus at 984 kg ha^{-1} .
Clay 2007	lint yield kg ha^{-1} = 247 + (5.14 * N rate) - (0.0119 * N rate * N rate); up to N rate = 196 kg ha^{-1} when yield plateaus at 875 kg ha^{-1} .

2. NAWF data for EONR and 100 kg N ha^{-1} (typical for producers) interpolated from data at discrete N rates.

Field	Year	Date	EONR kg ha^{-1}	NAWF EONR	NAWF N rate 100 kg ha^{-1}	Difference
sandy-loam	**2006**	**24-Jul-06**	**67**	6.50	6.83	0.33
sandy-loam	**2006**	**3-Aug-06**	**67**	6.08	6.78	0.70
sandy-loam	**2006**	**9-Aug-06**	**67**	5.62	6.56	0.94
sandy-loam	**2006**	**17-Aug-06**	**67**	4.10	4.56	0.46
silt-loam	**2006**	**24-Jul-06**	**0**	6.52	6.46	-0.06
silt-loam	**2006**	**3-Aug-06**	**0**	5.98	6.09	0.11
silt-loam	**2006**	**9-Aug-06**	**0**	6.53	6.57	0.04
silt-loam	**2006**	**16-Aug-06**	**0**	5.18	5.44	0.26
clay	**2006**	**26-Jul-06**	**224**	5.70	4.80	-0.90
clay	**2006**	**3-Aug-06**	**224**	4.75	3.75	-1.00
sandy-loam	**2007**	**1-Aug-07**	**50**	3.30	3.79	0.49
silt-loam	**2007**	**1-Aug-07**	**90**	3.12	3.20	0.08
clay	**2007**	**27-Jul-07**	**196**	5.94	5.02	-0.92



3. Effects of N rate on maturity using Nodes Above White Flower (NAWF) as a proxy for maturity. NAWF is a measurement of cotton maturity, delayed maturity will lead to a higher number of nodes above the white flower.