



## Correlating Plant Nitrogen Status in Cotton with UAV based Multispectral Imagery

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**Abstract.** Cotton is an indeterminate crop; therefore, fertility management has a major impact on the growth pattern and subsequent yield. Remote sensing has become a promising method of assessing in-season cotton N status in recent years with the adoption of reliable low-cost unmanned aerial vehicles (UAVs), high-resolution sensors and availability of advanced image processing software into the precision agriculture field. This study was conducted on a UGA Tifton campus farm located in Tifton, GA. The main goal of this study was to correlate in-season cotton N status with multispectral imagery acquired with a UAV. For this study, six N treatments consisting of 0, 34, 67, 101, 135 and 168 kg/ha rates were applied to attain varying levels of plant N status within the same field. Cotton tissue samples were collected during crop growth stages (first, third, fifth, and seventh week of bloom) to quantify plant N status during these stages. Tissue analysis results provided leaf blade N (%) and petiole N (ppm) for each N treatment implemented in the study. Crop multispectral imagery in the spectral wavelengths of 550 nm (green), 660 nm (red), 735 nm (red-edge) and 790 nm (near infrared) was acquired during these crop growth stages by utilizing a commercially available quadcopter equipped with a high-resolution multispectral camera. Different vegetation indices (VIs) were selected and calculated based on potential correlation with plant N status and were calculated from the data acquired from the multispectral aerial imagery. Correlations between the indices and leaf blade N (%) and petiole N (ppm) as obtained from plant tissue analysis were compared. Regression equations correlating the VIs to actual N levels were generated to evaluate the use of different VIs for accurately measuring N levels in the crop at the selected growth stages. Initial data analysis indicated that NDVI was strongly correlated to leaf blade N (%) and petiole N (ppm) from the first week of bloom samples, whereas, NDRE had stronger correlation for the samples that were taken in the third, fifth, and seventh week of bloom. These correlations may provide promise for using multispectral imaging to detect in-season N variability in cotton.

**Keywords.** Cotton, Remote Sensing, Vegetative Index, Tissue Sampling, Nitrogen Detection.

## Introduction

Upland Cotton (*Gossypium hirsutum* L.) is the most widely grown row crop in the state of Georgia with over 1.2 million acres planted in 2017 (USDA, 2017). As with other crops, proper management of cotton is critical to produce high quality fiber and optimize profitability. Cotton yield can vary spatially within a field and that variability can often be a function of soil properties and nutrient availability, especially Nitrogen (N).

Georgia soils, especially in the Coastal Plain region where sandy soils are prevalent, are relatively infertile. Georgia soil lab tests normally do not report N content because of its mobility in these soils. For cotton, and other crops in Georgia, N rates are determined by yield goal, soil type, and field history. N is considered the most limiting nutrient (Mullins et al., 2003) used on cotton, and it is the most challenging to manage (Whitaker et al., 2017). Insufficient N can reduce yield and quality, while an excess supply of N can negatively affect plant growth and development, boll retention, and lint yield (Zhao et al., 2010; Porter et al., 2010; Whitaker et al., 2017). The recommendation from the University of Georgia is to apply N in split applications with one-fourth to one-third of the recommended N at planting and the remainder at sidedress (Whitaker et al., 2017). Currently, the use of variable rate N applications in Georgia are commonly based on field history, yield data, and soil maps. The status of the cotton crop is not usually a factor in the decision process of those variable rate N applications. Precision agriculture allows for variable rate N application based on the crop's current growth conditions without reducing final yield (Arnall et al., 2016). Over application of N, especially in combination with high late-season moisture availability, can delay maturity, impact harvestability, and cause boll shedding, disease, and damage (Boquet and Breitenbeck, 2000). A more comprehensive understanding of the current N status of a cotton crop would lead to better management decisions about variable rate N applications and increased profitability.

N requirements change throughout the growth and development of cotton; therefore, the most efficient way to supply N is to have the optimal amount available for the plant to take up only when it needs it (Arnall et al., 2016; Porter et al., 2010; Torbett et al., 2008). The maximum daily uptake of N occurred between 49 and 71 days after planting (DAP) per the findings of Boquet and Breitenbeck (2000). Daily uptake of N from planting until 28 DAP is low (Boquet and Breitenbeck, 2000); therefore, N applied at planting should not be excessive so that N loss is mitigated, especially in sandy soils. The University of Georgia recommendation for sidedress N application timing is between first square and first bloom depending on crop growth and color. N should not be applied to cotton after the third week of bloom (WOB) because studies have shown that uptake of N by cotton roots is ineffective after this critical point (Whitaker et al., 2017). Peak N demand by cotton occurs between early bloom to peak-bloom with two-thirds of the total N taken up after early bloom (Mullins et al., 2006). Timeliness of N application is an important consideration when providing the optimal amount of N for the given growth stage. Research has shown that split applications of N increase the nitrogen-use efficiency (NUE), which often leads to maintaining yields with fewer inputs, thus, increasing profitability (Arnall et al., 2016; Mullins et al., 2006; Zhao et al., 2010). Remote sensing can provide useful information about cotton N status that can be used to determine timing of split N applications.

Remote sensing uses a combination of sensing technologies including photography, multispectral scanning, and infrared imaging. Studies have shown that remote sensing can be a useful tool to detect crop growth, environmental stresses, and yields (Zhao et al., 2010). N status can vary spatially within a field; however, detecting that variability by ground based data collection methods has many limitations, including spatial resolution. Unmanned aerial systems (UAS) have the capability to provide a better understanding of where variability exists in the field in a timely manner and with good spatial resolution (Huang et al., 2013). UASs can be equipped with many different sensor types that can record large amounts of data quickly. A UAS's ability to record a dense amount of data that is georeferenced provides the capability of analyzing data with geographic information systems (GIS) software which can then be correlated to ground sampling data (Vellidis et al., 2004).

Multispectral imagery is used to record the reflectance of different bands of the electromagnetic spectrum. Generally, most of the spectral bands that multispectral sensors capture are between 400 nm and 1000 nm (Huang et al., 2013; Raper and Varco, 2014; and Zhao et al., 2010). Visible light is between 380 nm and 750 nm, and plants use the light between 400 nm and 700 nm for photosynthesis. The most common multispectral bands used for remote sensing are blue ( $\approx$  460 nm), green ( $\approx$  550 nm), red ( $\approx$  660 nm), red edge ( $\approx$  735 nm), and near infrared ( $\approx$  790 nm). The bandwidths of each of these spectra captured by multispectral sensors can vary depending on the capability and resolution of the sensor and by the calibration.

Spectral data recorded from plants have been used to create different ratios or vegetative indices. A popular vegetative index is the normalized difference vegetation index (NDVI) which has been used to predict mid-season N requirements for cotton (Arnall et al., 2016; Porter et al., 2010). NDVI uses the NIR and red spectra, and the formula is:  $NDVI = (NIR - red) / (NIR + red)$  (Arnall et al., 2016; Porter et al., 2010; Zhao et al., 2010; and Huang et al., 2013). The strongest wavelength correlations with leaf N concentration, lint yield, and plant total N content were noted near 700 nm by Raper and Varco, (2015). N deficiency causes a decrease in leaf chlorophyll content, resulting in an increase in spectral reflectance between 550 nm and 710 nm (Zhao et al., 2010). One of the most sensitive regions to changes in chlorophyll content is the red edge, and Raper and Varco (2015) found that other vegetative indices that include the red edge showed stronger correlation to N status than NDVI.

## Objectives

The main objective of this study was to determine the feasibility of utilizing a UAS to determine the mid-season tissue N content of cotton as it relates to vegetative indices. The secondary objectives of this study were to determine the correlations between popular VIs and the N content of leaf and petiole tissue during critical N uptake growth stages, to track the changes of plant tissue N during the season by N rate treatment, and to determine the optimal timing for collecting multispectral data for making in-season fertility decisions.

## Materials and Methods

Upland Cotton cultivar Deltapine DPL 1646 B2XF was planted on 02 May 2017 on 76.2 cm row centers in 12.2 m long plots under conventional tillage in Tifton, GA. Preliminary soil samples were collected and on 17 May 2017, base fertilizer rates were applied based on the sample results and by using University of Georgia extension cotton production guide fertilizer recommendations (Whitaker et al., 2017). The N treatments were based on total N applied and were, 0, 34, 67, 101, 135 and 168 kg/ha. One-third of each N treatment was applied to the plots using ammonium nitrate (34-0-0) at 15 DAP. The remaining two-thirds of the N for each treatment was applied using ammonium nitrate (34-0-0) at 43 DAP. Plant tissue samples were collected during five growth stages, first square, 1<sup>st</sup> WOB, 3<sup>rd</sup> WOB, 5<sup>th</sup> WOB, and 7<sup>th</sup> WOB. The fourth leaf down from the terminal that is larger than 24 mm wide and not attached to a square was sampled. For the first square sampling date, only leaf blades were removed from the plant. For the 1<sup>st</sup> WOB, 3<sup>rd</sup> WOB, 5<sup>th</sup> WOB, and 7<sup>th</sup> WOB samples, the leaves and the petioles were removed from the plant and were separated within one hour of pulling the samples. The tissue samples were dried in paper bags in a forced air heated drier for a minimum of 48 hours and sent to a private lab for nutrient analysis. Rows two and three from each plot were harvested for yield using a two-row cotton picker modified with a bagging attachment on 29 September 2018. The seed cotton was bagged, weighed and weight recorded for each plot. The bagged seed cotton from each plot was ginned at the University of Georgia MicroGin and lint turnout was calculated. Lint yield was calculated by using the plot area, seed cotton weight, and lint turnout.

Multispectral data were collected using a Sequoia multispectral sensor (Sequoia, Micasense Inc., Seattle, WA, USA) mounted on a 3DR Solo UAV (3D Robotics, Inc, Berkeley, CA). The four discrete spectral bands collected by the Sequoia were Green (550 nm, 40 nm wide), Red (660

nm, 40 nm wide), Red Edge (735 nm, 10 nm wide), and Near Infrared (790 nm, 40 nm wide). An autonomous flight plan was developed and utilized for every flight for the entire season by using the Tower app associated with the 3DR system on an android tablet. The flight altitude was set at 45.72 m and the speed was set to 4.47 m/s. Images were collected with 80% frontlap and 80% sidelap. Multispectral images were collected of a radiometric calibration panel provided with the Sequoia pre-flight and post-flight for proper calibration. The images were processed by utilizing Agisoft Photoscan Pro (Agisoft LLC, St. Petersburg, Russia) and Pix4Dmapper Pro (Pix4D SA, Switzerland) software.

The calibration panel images were used to normalize the reflectance values in the images of the multispectral data. All individual images for each band were stitched together to create an accurate orthomosaic image. The orthomosaic image for each band was imported into ArcGIS Version 10.4.1 (ESRI, Redlands, CA) and then used to create VIs. The two VIs that were analyzed for this study were NDVI and NDRE. A shape file was created around each plot that placed a polygon over all four rows, but slightly shorter to prevent data analysis outside of the plot. The VI data was clipped to each plot, averaged by plot, and output in tabular format.

## **Results and Discussion**

The tissue sample results (Figure 1) showed little to no differences in N% levels in the leaf blade N% at the first square sampling date which was 43 DAP. There were differences observed in the leaf blade N% and petiole N ppm tissue samples based on N treatment at the 1<sup>st</sup> WOB sampling date which was 66 DAP. These differences existed throughout the season, however, were not as prominent as the season progressed.

The first square sampling date was not available for the petiole tissue analysis, however, similar trends to the leaf N% were observed (Figure 2), where the 1<sup>st</sup> WOB had the highest level of treatment separation of N% concentration. However, a decrease in petiole N% was observed as the season progressed, and similar to the leaf N% concentration the separation between treatments was reduced as the season progressed. The main difference between the leaf and petiole N% was that the leaf N% dropped up until the 3<sup>rd</sup> WOB and then increased, where the petiole N% continually dropped throughout the season.

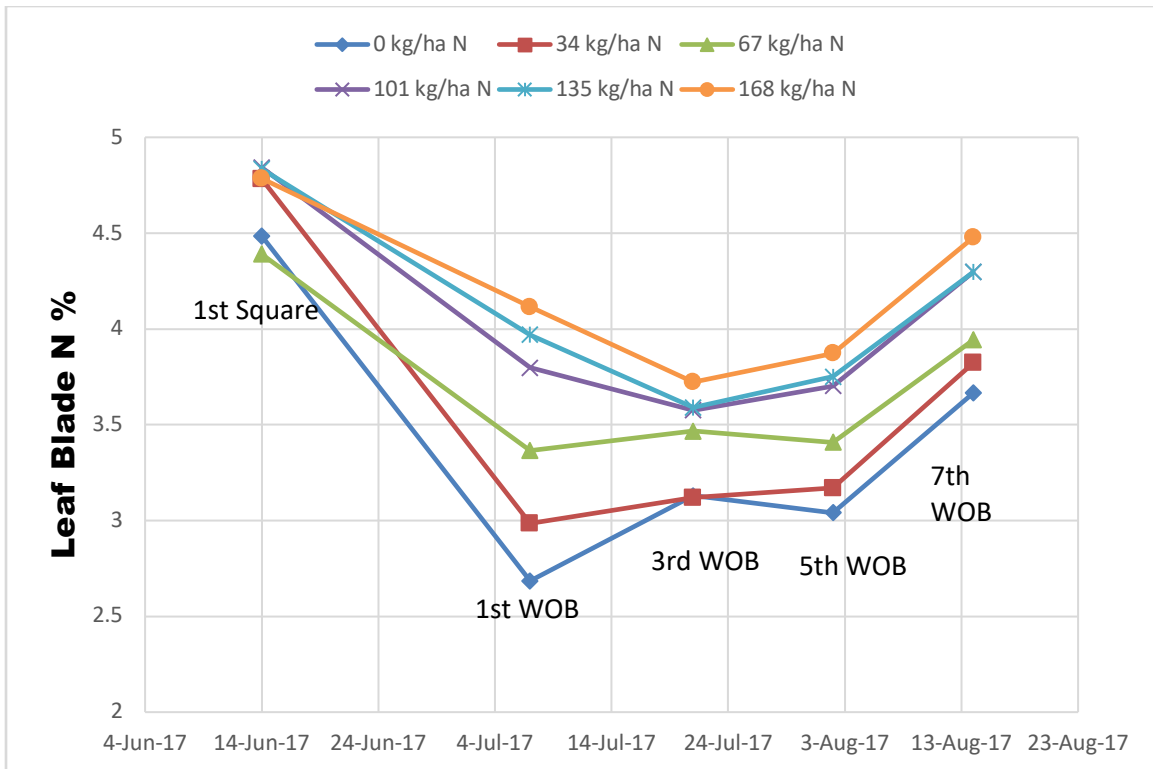


Figure 1. Leaf blade tissue N% from each of the N treatments as reported from tissue analysis over time.

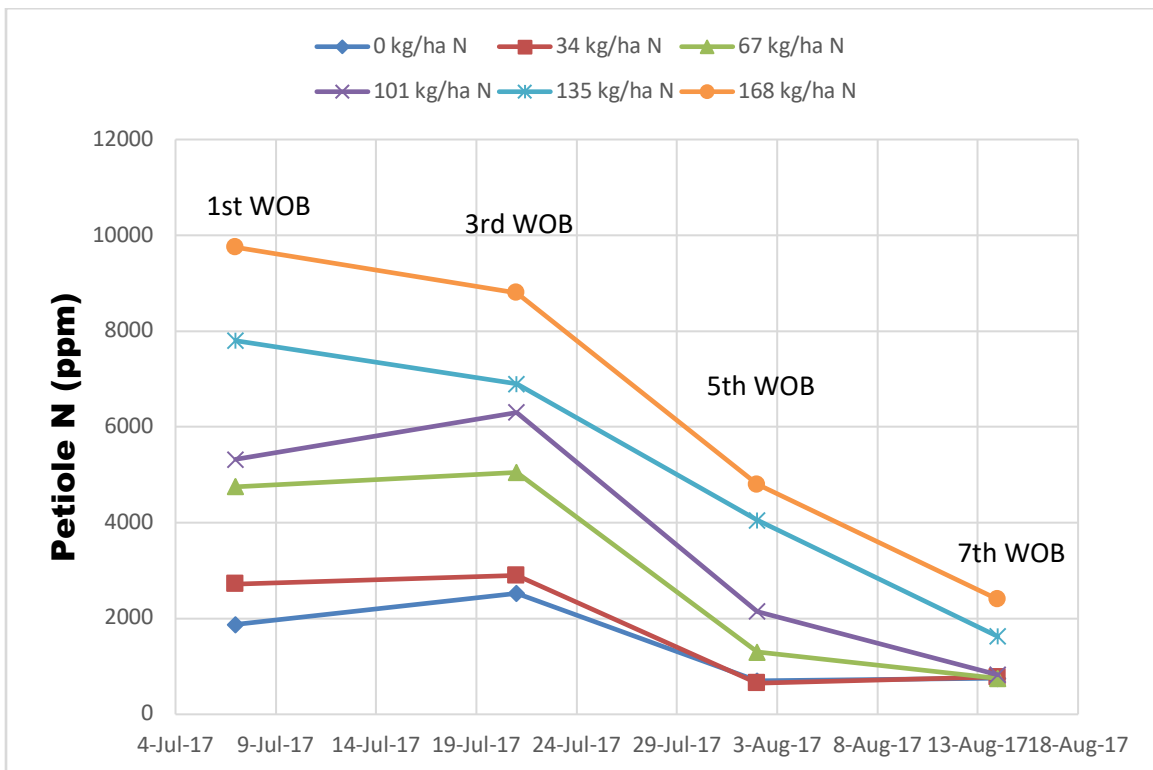


Figure 2. Petiole tissue N% from each of the N treatments as reported from tissue analysis over time.

Tissue data show that the levels of N applied can affect the levels of leaf blade and petiole N. The 1<sup>st</sup> WOB sampling date had the greatest separation in N% which was likely due to the

application of the remainder of the total N rate 23 days prior to that sampling date. The data from the tissue results represented in Figures 1 and 2 suggest that the optimal separation in N% in cotton tissue occurs during the 1<sup>st</sup> WOB. This suggested that data collected during the 1<sup>st</sup> WOB has the highest potential for determining when cotton is N deficient. Thus, the 1<sup>st</sup> WOB could be an optimal time to collect nutrient data on cotton to make in-season decisions.

Lint yield increased as N rate increased; however, the 135 kg/ha and the 168 kg/ha N rates returned very little increase in lint yield when compared to the 101 kg/ha rate. The 168 kg/ha rate yielded slightly less than the 135 kg/ha rate which is consistent with diminishing returns and the nature of cotton when excess N is applied (Arnall et al., 2016; Porter et al., 2010; Zhao et al., 2010). Interestingly, the 0 kg/ha rate yielded 1170 kg/ha of lint; this is likely due to following peanut which, per University of Georgia recommendations, may account for approximately 34 kg/ha of residual N and the ability of cotton to translocate N to optimize boll development.

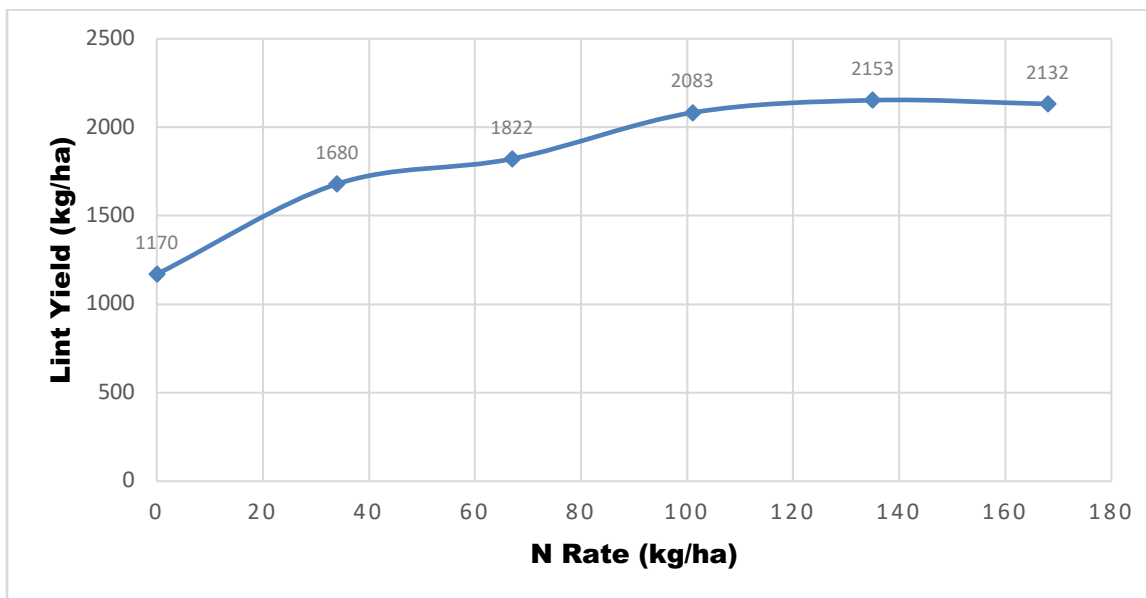


Figure 3. Lint yield as it is correlated to nitrogen treatment.

The separation in tissue N% was correlated to the final lint yield. The lower tissue test levels had lower yields while the higher tissue levels had higher yields. This suggests that the ground sampling method is a good prediction for the subsequent yield.

Multispectral data were processed in Agisoft and Pix4D. Radiometric calibration panel images were not used to normalize the multispectral data in Agisoft; however, the radiometric calibration panel images were used in Pix4D. Data were averaged by treatment. Linear regression for leaf blade N% versus each VI were graphed as well as petiole N versus each VI. This process was completed for each sampling date and R<sup>2</sup> values were plotted over time.

The correlation of NDVI and leaf N% at the first square sampling date was much lower than the other sampling dates as seen in Figure 4 and Figure 5. Prior to that date, only one-third of the total N for each treatment was applied and the cotton was still young and had not utilized all of the N that was present from the first application. The differences in leaf blade N% at that time were not separated in order by treatment; therefore, the multispectral data and the leaf blade N% data were not linearly correlated as well as they were during the other sampling dates. The differences in the Agisoft data and the Pix4D data follow a similar trend, however, they differ due to the normalization of the data from the radiometric calibration used in Pix4D and not in Agisoft.

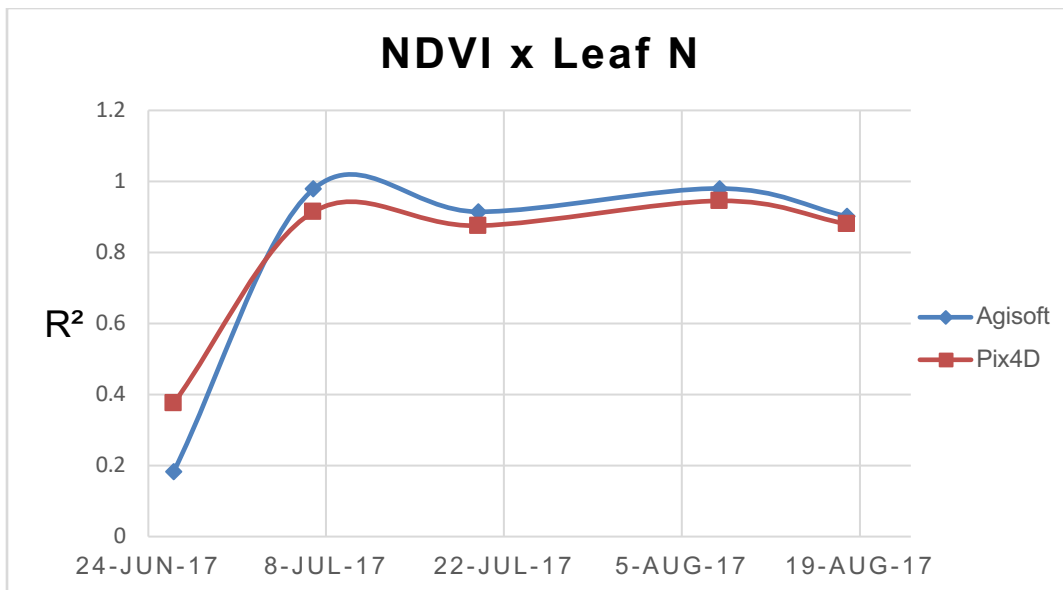


Figure 4. Coefficients of determination for the N% in the leaves by NDVI plotted for the sampling dates.

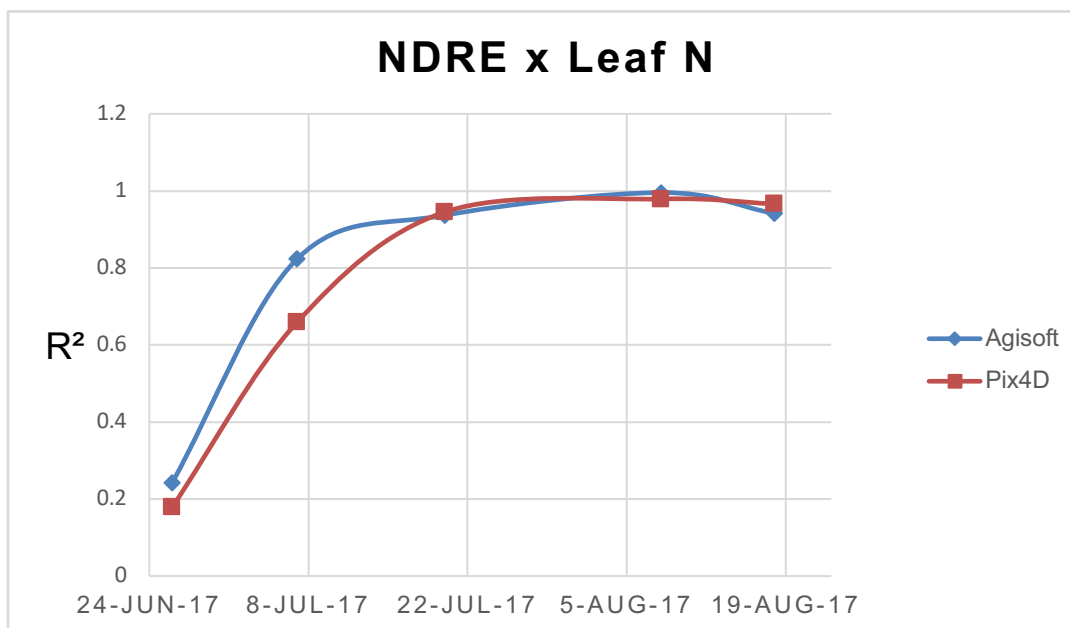


Figure 5. Coefficients of determination for the N% in the leaves by NDRE plotted for the sampling dates.

Petiole N samples were collected beginning at the 1<sup>st</sup> WOB. At this time the separation by treatment was similar to that found in leaf blade N. Figure 6 and Figure 7 show that the strongest correlation was at the 3<sup>rd</sup> WOB sampling date. Similar trends were observed from Agisoft data and Pix4D data.

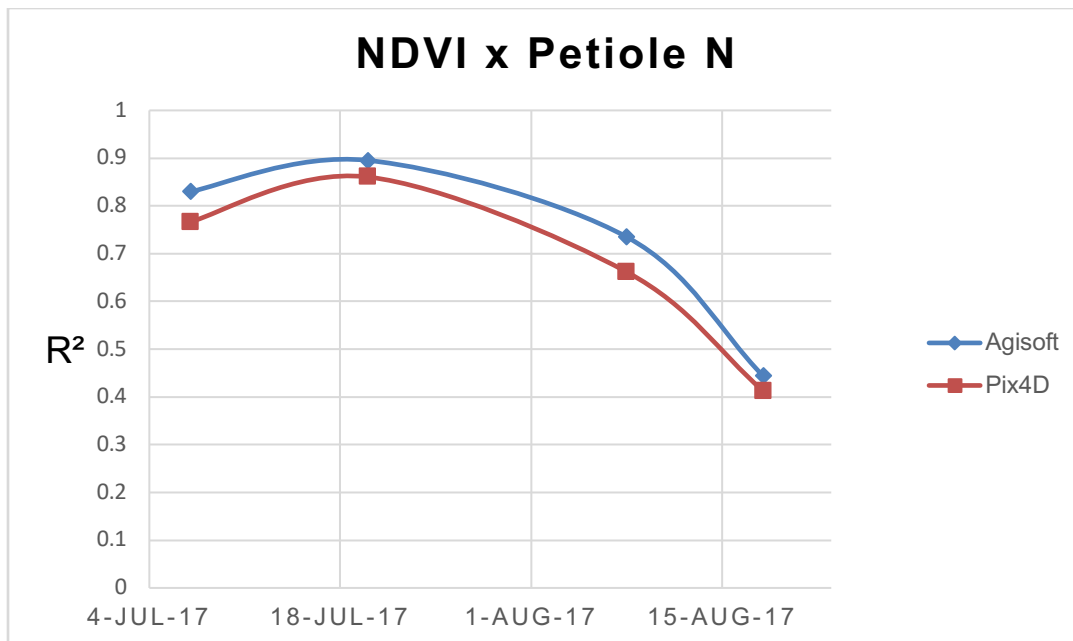


Figure 6. Coefficients of determination for the N in the petioles by NDVI plotted for the sampling dates.

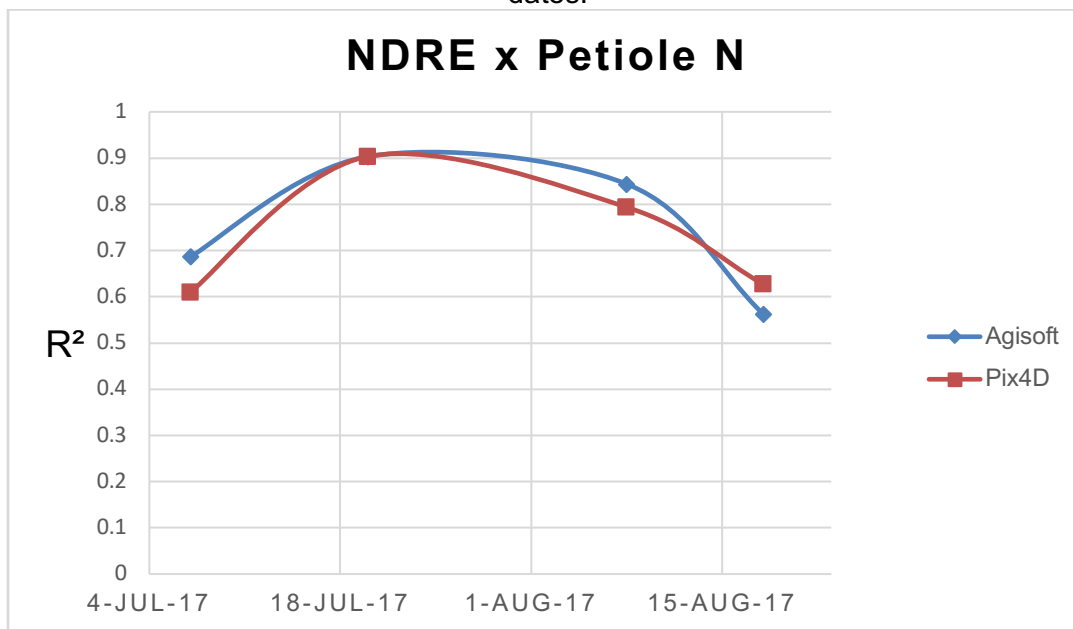


Figure 7. Coefficients of determination for the N in the petioles by NDRE plotted for the sampling dates.

As can be seen in figures 4 through 7 the difference in the multispectral data processed by the two software packages shows that the radiometric calibration data can affect the values of the multispectral data. Thus, the authors strongly suggest utilizing the calibration panel to ensure the data quality has the highest integrity possible.

## Summary

In conclusion, leaf and petiole tissue sample N results have shown to have the strongest potential of determining plant N deficiencies during the 1<sup>st</sup> WOB. Future research and analyses of these data may provide a basis for determining the optimal time for collecting multispectral data for predicting plant N content. Preliminary analysis of multispectral data show that varying levels of N in the tissue provide a strong correlation to NDVI beginning at the 1<sup>st</sup> WOB, whereas NDRE



has a stronger correlation beginning at the 3<sup>rd</sup> WOB. This may indicate that for the 1<sup>st</sup> WOB, NDVI may be used to predict the levels of N in the plant and for the 3<sup>rd</sup> WOB, the combined use of NDVI and NDRE may be used to predict the levels of N in the plant. These data may provide insight to aid in making corrective applications of N prior to the 3<sup>rd</sup> WOB if the N levels are less than sufficient. Further research will be conducted to explore the utility of other VIs and the correlation to N levels in cotton. The findings of this study indicate that multispectral data may be a viable method for detecting N levels in cotton.

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