# In-crop nitrogen detection of cotton – turning passive into active with the Hydraspectra™

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**Abstract:**
Australian cotton yields and fibre quality are some of the highest in the world. Increasing the nitrogen use efficiency (NUE) in new varieties (Bollgard® 3 currently) is a priority for the industry to ensure minimal impact on our environment. The application rates and strategic timing of nitrogen is pivotal for better in-crop management to ensure that yields achieved are nearing the yield potential of new varieties. The Hydraspectra™ is a multi-headed sensor system developed by CSIRO that captures reflectance in the visible light spectrum (380 to 700 nm). The device was designed to capture spectra passively in a horizontal orientation. The aim of this research was to investigate a new emerging technology for crops (Hydraspectra™) and evaluate if it can be used in a vertical orientation using artificial lighting by installing a light source to penetrate the canopy. The concept was to research the possibility of mapping leaf colour changes (reflection of spectra) and establish a relationship with N. There was an established relationship between visible light spectrum and total nitrogen, equipping crop managers with the ability to detect deficiencies in the lower canopy. Many studies that assess the relationship of spectral reflectance data with canopy N typically ignore the stratification of N throughout the canopy. The translocation of nitrogen in cotton was detected, indicating that spectral reflectance could be used to relate nitrogen use within the plant. This would provide crop managers with a tool to be more proactive for addressing nitrogen deficiencies. The data also highlighted the need for further engineering development, converting the orientation and light source. The Hydraspectra™ system through further development and lighting modifications has the potential to assist in the reduction of mis-timed N application and therefore have a positive impact both economically and environmentally.

Keywords: Nitrogen, Detection, Efficiency, Technology & Sensing

## Introduction

Australia is the fourth largest exporter of cotton, representing 3% of the global cotton trade. The Australian cotton industry in recent years has come under public pressure to better utilise input resources. Resource inputs of cotton production are often subject to mismanagement and applied sub-optimally. The combination of inefficient resource (fertiliser) use and the need to increase fibre production are two reasons for investigating better methods for monitoring crop nutrient needs.

The current industry varieties rely heavily on nitrogen fertiliser to reach their optimum yield potential. The global awareness of greenhouse gas emissions has prompted investigation into better nitrogen fertiliser management across agriculture, highlighting major environmental impacts caused by nitrate leaching and runoff. Due to the negative externalities associated with over application of nitrogen fertiliser, there has been an increased demand for decision support tools to guide correct application rate and timing whilst maximising profitability (Gerik et al., 1998). Sensing devices capture the ratio of incidence radiation to the reflected radiation dependent upon the chlorophyll. The use of current commercial proximal sensing devices is most favourable to convey field conditions, whilst maintaining research level accuracy. These devices capture reflectance to relate to crop nitrogen content for decision support. The calibration of plant nutrient status against plant vegetation indices can inform management decisions in line with economic variables.

The rapid development of recent technologies in precision agriculture, including cloud modelling, has allowed nitrogen management research to mature through variable rate fertiliser application to align to site specific management (Beluhova and Dunchev, 2019). The current site-specific management research highlights that fertiliser use is spatially and temporally varied causing application decisions to incorporate multivariate analysis. This has led to decision support systems that are difficult to understand, limiting adoption. Therefore, the production of a simplified decision support matrix is needed to limit the negative effects of nitrogen mismanagement whilst maximising productivity and profitability. The creation of a decision support tool must therefore possess a combination of nitrogen status calculation, soil nitrogen sensing and yield prediction indices to ensure multivariate accounting is captured. Further, nitrogen use within the plant varies in proportion to the profile of light distribution within the canopy. This study aimed to identify which vegetation index is the optimal predictor of nitrogen status detection. The emerging tool, the Hydraspectra™ will highlight the commercial viability of crop sensing technologies whilst developing prediction models for plant N use, hence in-crop application. There has been a lot of research using sensor technologies mounted on drones to estimate N status of cotton, however, these do not address the issue of varying N content vertically throughout the canopy, as cotton pushes N to the top of canopy (Milroy *et al*., 2001; Marang *et al.,* 2021). This project utilises the Hydraspectra™ to try and overcome this limitation whilst building a robust understanding of plant nitrogen use.

**Methods**

The field experiment was located at the Australian Cotton Research Institute (ACRI) Myall Vale NSW and utilised two commercial Bollgard III® varieties (Sicot 714 B3F and Sicot 746 B3F), which are the main varieties sown in the Namoi Valley. These varieties selected contained both the *Bacillus thuringiensis (Bt)* insecticide protein stack and Roundup Ready Flex® glyphosate tolerance technology. The experiment was conducted in Field B2 and had been subject to a long wheat fallow rotation, which led to a consistent nitrogen background across the two plots. The crop experienced 300 mm of in-crop rainfall, predominately towards the end of the growing season, with five irrigations occurring across the 33-week growing season. Crop checks occurred fortnightly with pest populations never exceeding economic thresholds whilst in-crop growth regulation was also not needed. The final defoliant sprays consisted of Dropp® UltraMAX cotton defoliant, Prep® 720 growth regulator and Hasten® cotton spray adjuvant with average rates at 120 mL/ha, 1.5 L/ha and 500 mL/ha, respectively. This management was consistent with local commercial practices to ensure that economic variables could be considered in a decision support matrix.

The field experiment was subjected to a developing proximal sensing technology which was used to extract various vegetation indices to be compared to the actual nitrogen status of plant samples. The proximal sensing took place to align with common in-crop application periods during flowering. There were three separate recordings taken at ~600 degree days (DD), ~1200 DD and ~1400 DD (base temperature of 12˚C) (CSD, 2020). The sensing in field took place from 10 - 11 am on all occasions to ensure sunlight was consistent whilst all samples were taken at the same time. The HydraSpectra™ multi-headed, multi-spectral sensor was the proximal sensing device used in-field. The HydraSpectra™ was developed by Dr Stephen Gensemer (CSIRO Manufacturing) with the intention of tracking nitrogen translocation. The device was used in a horizontal and vertical frame of reference with the horizontal reading occurring on all three recordings whilst the vertical reading was limited to when plant height was greater than 30 cm. Using different frames of reference allowed for the calculation of nitrogen content through the length of the plant, highlighting the variation of nitrogen use through the plant. The device was secured to a trolley which allowed for the accurate positioning of the HydraSpectra™ 15 cm away from the plant for maximum reflectance accuracy. This device captured the spectral data of the cotton plants' reflectance that was then extracted through Python code to calculate Normalised Difference Vegetation Index (NDVI), Normalised Difference Red Edge (NDRE) and Canopy Chlorophyll Content Index (CCCI). The vegetation indices were contrasted to define the best prediction index relating to in-crop nitrogen content.

Physical plant samples were taken from the replicated plots, the leaf and petiole samples were assigned to the variety, treatment, and leaf position. These samples were dehydrated in a fan forced air heating chamber at 70˚C overnight. The dried samples were ground using a Rocklab Benchtop Ring Mill. The dried samples were weighed into two specific quantities, 0.200 g ±0.01 g and 0.100 g ±0.02 g, to be prepared for total nitrogen analysis using a LECO CN928 analyser. The weighted sample was placed in the LECO CN928 analyser where the Dumas combustion procedure separated the gaseous components of the combustion into carbon and nitrogen percentage. The exact nitrogen status of the samples was recorded, and vegetation indices were calculated from the spectra captured from the Hydraspectra™. The ability of the Hydraspectra™ to estimate N levels throughout the canopy was tested by using linear stepwise functions in the software R. This helped in identifying the optimal vegetation index, as well as the accuracy of the approach.

**Results and Discussion**

The chemical analysis using LECO CN928 analyser provided a leaf total nitrogen content ranging from 3.48% to 4.98% whilst petiole nitrogen content ranged from 1.59% to 3.91%. This nutrient analysis was used to model against the vegetation indices with linear models and the model relationships are shown in Figure 2 (predicted vs observed). The LECO CN928 analysed data showed clear statistical differences between the 0 N treatment and the 300 N treatment plots total leaf and petiole nitrogen content. When error bars do not overlap, the total nitrogen content (%) of the cotton leaf and petiole are different (Figure 1). This is shown on all occasions, with the exception of the Sicot 746 B3F petiole nitrogen content at ~1200 DD. The combination of both destructive chemical analyses provides a calibration for nitrogen prediction through both vegetation and yield estimation indices.

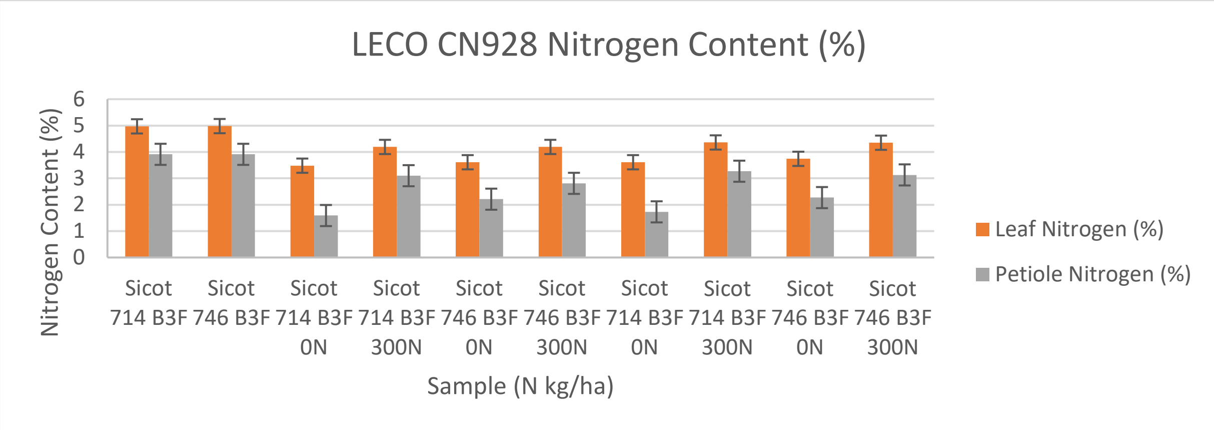


Figure 1. Chemical combustion analysis by LECO CN928 machine indicating statistical difference between treatments; Treatments align to 0 N kg/ha and 300 N. The first two recordings were taken at ~600 DD with the next four at ~1200 DD and the final four at ~1400DD.

The Hydraspectra™ was used in a horizontal and vertical orientation. The data taken from the multi-head multi-spectral device was plotted against nitrogen content (Figure 2). The spectral data was used to produce multiple vegetation indices. A linear model with a stepwise function was used to highlight that the best predictor was Canopy Chlorophyll Content Index (CCCI) for both leaf and petiole total N. This suggests that CCCI best described the variation in N content in both the vertical and horizontal orientation whilst showing the lowest RMSE, and the highest $R^2$.

Table 1. The $R^2$, Mean Square Error (MSE) and Root Mean Square Error (RMSE) from linear models

| **Proof** | $\mathbf{R^2}$ | **MSE** | **RMSE** |
|---|---|---|---|
| **Leaf Total Nitrogen** | 0.65 | 0.75 | 0.28 |
| **Leaf Nitrate** | 0.69 | 0.77 | 30.0 |
| **Petiole Total Nitrogen** | 0.36 | 0.54 | 0.55 |
| **Petiole Nitrate** | 0.45 | 0.61 | 61.36 |

The leaf nitrate model expressed the highest accuracy with an $R^2$ of 0.69 (Table 1). As expected, the petiole models expressed lower model accuracy with an $R^2$ 0.36 and 0.45, representing petiole nitrogen and petiole nitrate, respectively (Figure 2).

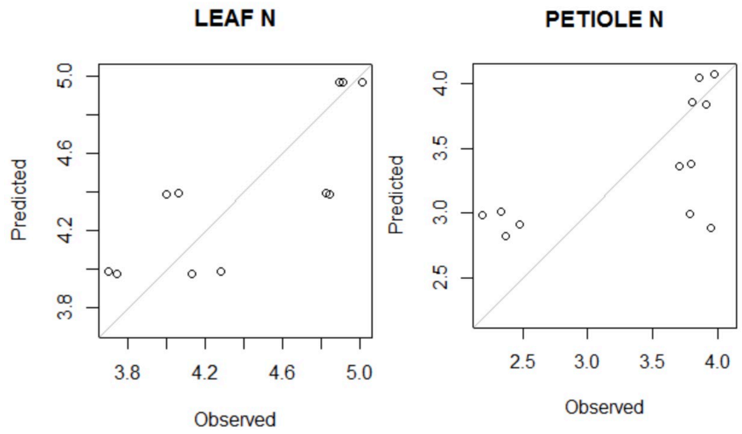


Figure 2. Predictions of leaf and petiole N content (%) with Canopy Chlorophyll Content Index (CCCI) as a predictor in linear models. Corresponding $R^2$ values are shown in Table 1.

Hence, a linear relationship between nitrogen status of cotton and spectral reflectance (CCCI) could be developed. This relationship is linked to chlorophyll activity and pigmentation change (Kume et al., 2018). The growing season showed that the nitrogen status was different between the two treatments after ~1200 day degrees (DD). This was supported through the vegetation indices calculation as the variance of predicting nitrogen content with NDVI, NDRE and CCCI decreased across the season. This relationship would play a pivotal role in the creation of a nitrogen decision support matrix as the greater the accuracy of using vegetation indices to predict N content, the greater the likelihood of predicting nitrogen use. The approximation of a decision support matrix is a multifactored task which would see yield data and vegetation indices combined. This was originally a goal of the project, although further analysis of previous studies would be needed to test the validity. The future direction of this project would see the first task being the creation of a decision support matrix. This matrix would need to be easy to use, similar to a cotton pest threshold matrix (Gonzalez and Wilson, 1982), to ensure grower adoption and minimise misuse.

## Conclusion

The testing of the two most widely used commercial cotton varieties with the Hydraspectra™ provides confirmation that CCCI was the optimal vegetation index for deriving plant nitrogen status. The performance of the vegetation indices was relatively limited, although two conclusions can be drawn; 1. Nitrogen content is directly related to the pigmentation of the cotton leaf, and 2. CCCI is paramount when predicting nitrogen content in cotton plant leaves and petioles. These data can provide the basis for future research directions which support the relevance of vegetation indices as a decision support tool.

## Acknowledgements

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