

# “Red Edge” Optical Properties of Corn Leaves from Different Nitrogen Regimes

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**Abstract-** High resolution (< 2 nm) optical spectra and biophysical measurements were acquired from corn leaves from field plots having four nitrogen fertilizer application rates: 20%, 50%, 100% and 150% of optimal levels. Reflectance (R), transmittance (T), and absorbance (A) spectra were obtained for both adaxial and abaxial leaf surfaces. The strongest relationships between foliar chemistry and optical properties were demonstrated for C/N content and two optical parameters associated with the “red edge inflection point” (REIP): 1) a normalized first derivative maximum (Dmax) occurring between 695 and 730 nm (Dmax/D744); and 2) the wavelength associated with Dmax (WL of REIP). A non-linear increase in the Dmax/D744 ratio as a function of C/N content was observed for all optical properties ( $r^2 = 0.90-0.95$ ). Similarly, a non-linear decrease in the WL of REIP as a function of C/N content was observed for all optical properties (RT, RB, TT, and AT) ( $r^2 = 0.85-0.96$ ). The Dmax/D744 ratio increased as the WL of REIP declined from ~730 to 700 nm, with curves per optical property expressing different degrees of non-linearity.

## I. INTRODUCTION

Vegetation exhibits characteristic spectral features that indicate foliage constituents and physiological vigor. Although interesting spectral features occur throughout the optical and middle infrared spectra (350-2400 nm), the region associated with the red and far-red spectrum (e.g., 650-750 nm) shows the greatest dynamic change, typically attributed to chlorophyll content and photosynthetic function. In this region, healthy green foliage has an absorption feature centered at ~665-670 nm associated with minimum reflectance (R) and transmittance (T), with a steep increase in R and T from the “red edge” (~680 nm) to the far-red to near-infrared (NIR) plateau achieved by ~775 nm. In vegetation expressing responses to chronic stress or senescence, the dynamic range is dampened as the red minimum increases and the far-red/NIR plateau decreases. These characteristic responses are the basis for the use of spectral “vegetation indices” that combine information from the red and either the far-red or NIR (plus other visible) wavelengths (WL) [1-3]. In addition to a decrease in the magnitude of the NIR plateau, many investigators examining relatively high resolution spectra ( $\leq 10$  nm) have observed a blueward shift in the WL position of the far-red shoulder, often referred to as the “blue shift of the red edge”, as a function of chlorophyll content or environmentally stressful conditions [4-5]. This shift is most often expressed in terms of the “red edge inflection point” (REIP), where the first derivative of the optical spectrum is maximum in this region [6-7]. The magnitude of the maximum derivative (Dmax) provides the slope of the original spectrum,

and the WL where Dmax occurs is the “WL of REIP”. However, unraveling the complex spectral behavior in the red edge has been difficult to achieve.

We studied the spectral dynamics at the red edge in leaves of corn, a monocot species, taken from plants growing under controlled nitrogen (N) regimes. We hypothesized that either Dmax or WL of REIP were primarily influenced by the physiologically active, and temporally dynamic, fraction of vegetation associated with the relative foliar C and N content, or C/N ratio.

## II. METHODS AND MATERIALS

### A. Plant Material

In 2001, leaves were obtained from corn (*Zea mays* L.) grown on the USDA Agricultural Research Center, Beltsville, MD, as part of a larger project, “Optimizing Production Inputs for Economic and Environmental Enhancement (OPE<sup>3</sup>)”. The four N fertilizer application rates on field plots were 210, 140, 70, and 28 kg N/ha, which provided 150%, 100%, 50% and 20% of the optimal recommended N level. Leaf-level measurements were obtained from the third from terminal leaf on five dates over a two week period at the grain fill (R3) reproductive stage near the end of the growing season.

### B. Leaf Level Biophysical Measurements

The following biophysical data were collected on each fresh leaf: 1) pigment concentration (chlorophyll *a* and *b*, total carotenoid content,  $\mu\text{g}/\text{cm}^2$ ); 2) specific leaf mass ( $\text{g}/\text{m}^2$ ); and 3) photosynthetic capacity ( $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ ) determined with a Li-Cor 6400 Photosynthetic System (Li-Cor, Inc., Lincoln, NE). Pigments were determined on leaf discs extracted in 3.5 ml dimethyl sulfoxide (DMSO) using standard equations and absorption coefficients at 4 wavelengths (470, 640, 648, and 750 nm) obtained with a dual-beam Lambda 4 spectrophotometer (Perkin-Elmer, Norwalk, CN). Elemental C-H-N composition was determined from dried and ground leaf material (CHN-600 Elemental Analyzer System 785-500, LECO Corp., St. Joseph, MO) at the University of Maryland (College Park, MD). Additional optical [8] and fluorescence spectral measurements were also acquired.

### C. Leaf Level Optical Property Measurements

Leaf spectral optical properties were determined on both adaxial (top) and abaxial (bottom) leaf surfaces. Spectral measurements were acquired by using an integrating sphere (Li-Cor 1800, Li-Cor, Inc.) attached to an ASD spectroradiometer (ASD-FR FieldSpec®Pro), with a 1 nm sampling interval and an effective spectral resolution of < 2 nm (3 nm FWHM) in the 350-2500 nm range (Analytical Spectral Devices, Inc., Boulder, CO). Reflectance, transmittance, and absorption spectra were produced for both leaf surfaces. Optical spectra are described for: adaxial and abaxial reflectance (RT and RB); adaxial transmittance (TT ≈ TB); and absorptance determined from the adaxial surface (AT). Twenty published spectral indices were calculated from each spectrum. On each leaf RT, RB, TT, and AT spectra, the first derivative spectrum was computed and several red edge derivative parameters were determined, including the maximum derivative (Dmax) between 690 and 730 nm, the wavelength at which Dmax occurred (WL of REIP), and derivatives at 704, 714, and 744 nm (D704, D714, D744). Several derivative indices were calculated, including Dmax/D744. Reflectance (RT, RB), transmittance (TT, TB), and absorptance (AT, AB) spectra and their calculated indices were examined for N treatment responses and correlation with leaf chemistry. The data sets were analyzed with a statistical package, Systat 9 (SPSS Inc., Chicago, IL).

### III. RESULTS AND DISCUSSION

The portion from the spectrum examined (350-2500 nm) that exhibited the most change over time and over N treatments was the region between 500-800 nm, with TT values having the greatest dynamic range relative to RT and RB (Fig. 1). The first derivative spectra over this spectral range (for the same date) for RT, RB, TT, and AT demonstrate a blueward shift of Dmax with decreasing available N is apparent (Fig. 2). The data set covers a two week period during grain filling when leaf chemistry changed considerably, for C/N content (Fig. 3) and chlorophyll (not shown).

The C/N content increased over time with leaf maturation in each of the four N treatment groups, with significantly higher values obtained on the last two days. The foliar C/N ratio of leaves in the 100% and 150% soil N groups ranged between 8-18, with higher values (13-22) in the 50% soil N group, and the highest values (17-27) were observed in leaves from the 20% soil N group. By the last date, statistically significant ( $P < 0.01$ ) increases in the foliar C/N ratio were observed in leaves from each of these three N treatment groups (Fig. 3).

The most successful spectral indices in relationship to leaf physiological parameters were two red edge derivative parameters: the WL of REIP (Fig. 4) and Dmax/D744. The WL of REIP showed significant differences among optical properties within the higher N treatments: the WL of REIP for AT > TT for the 50, 100, and 150% N groups, and for AT < RT and RB in the 100 and 150% N groups (Fig. 4).

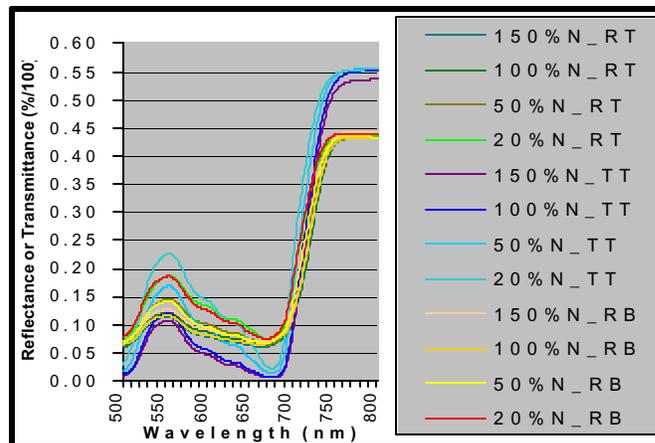


Fig. 1. Optical spectra (500 - 800 nm) obtained from green corn leaves during grain fill, near the end of the growing season (9/18/01). Plants were grown in four levels of soil N treatments -- 20, 50, 100 and 150% of optimal N levels. Optical properties are: RT, reflectance from the dorsal (adaxial) surface; transmittance (TT); and RB, reflectance of the lower (abaxial) surface.

The WL of REIP had a non-linear and inverse relationship to  $\log(C/N)$  content for all optical properties (Fig. 5), with the strongest associations obtained for AT and TT ( $r^2 = 0.96$ ), whereas lower associations were obtained for RT ( $r^2 = 0.91$ ) and RB ( $r^2 = 0.85$ ). Even stronger, direct associations were demonstrated for Dmax/D744 as a function of  $\log(C/N)$  content: TT ( $r^2 = 0.95$ ), AT ( $r^2 = 0.93$ ), RB ( $r^2 = 0.92$ ), and RT ( $r^2 = 0.90$ ). For comparison, the associations of this ratio with chlorophyll *a* content were: TT ( $r^2 = 0.88$ ), AT ( $r^2 = 0.88$ ), RB ( $r^2 = 0.84$ ), and RT ( $r^2 = 0.80$ ).

The Dmax/D744 ratio was inversely related to the WL of REIP (Fig. 6) ( $r^2 = 0.95$  over all optical properties), after removal of statistical outliers at the ~700 nm boundary. The relationship was linear for RB, but increasingly more curvilinear for RT, TT and AT.

### IV. CONCLUSIONS

We found strong relationships between two red edge spectral parameters and the foliar C/N content of corn leaves. Similar relationships of these parameters and other spectral indices with chlorophyll were weaker. Significant differences in the WL of REIP were observed among optical properties, with higher average WL of REIP obtained from reflectance (RT and RB) spectra, as compared to transmittance and absorptance spectra (AT and TT). Furthermore, the normalized Dmax ratio, Dmax/D744, was inversely related to the WL of REIP for each optical property. This relationship was strongly linear for RB but increasingly curvilinear for RT, AT, and TT. These results demonstrate the potential of high spectral resolution for remote determination of foliar N, using derivative parameters such as Dmax/D744. They also increase our understanding of the dynamics of the red edge in relation to leaf chemistry.

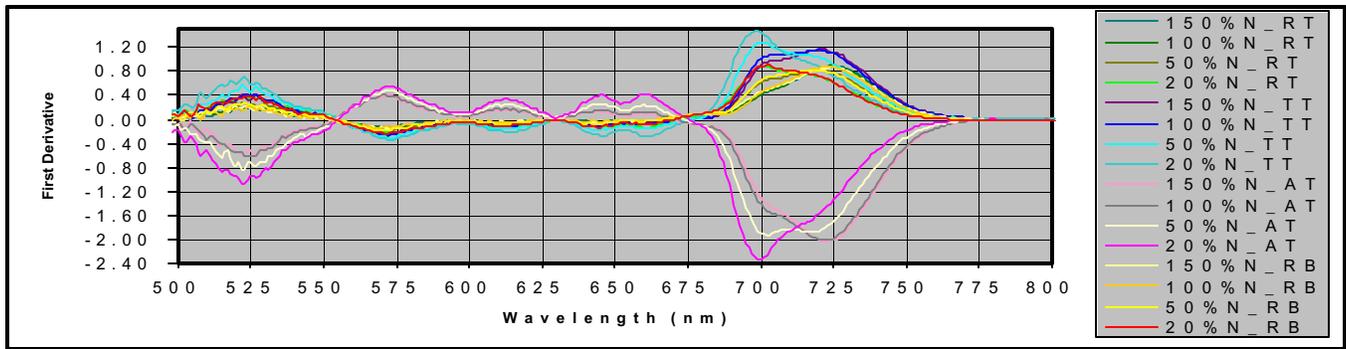


Fig. 2. The First Derivative spectra between 500 and 800 nm for leaf optical properties [RT, TT, RB, and AT] and N treatments for data shown in Fig. 1.

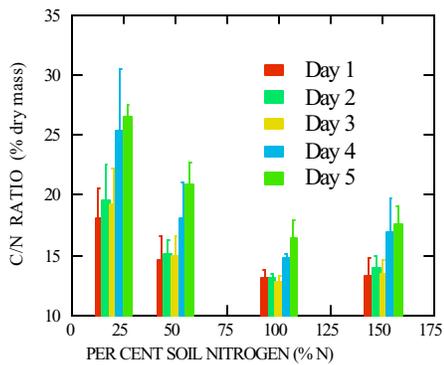


Fig. 3. The change in foliar C/N content is shown for leaves from four N treatments measured on five days over a two week period near the end of the growing season during grain development.

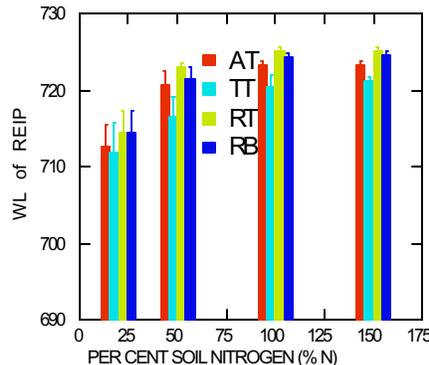


Fig. 4. The wavelengths (WL) per N group for the position of the REIP, averaged ( $\pm$  SE) over a two week period, are shown for AT, RB, RT, and TT.

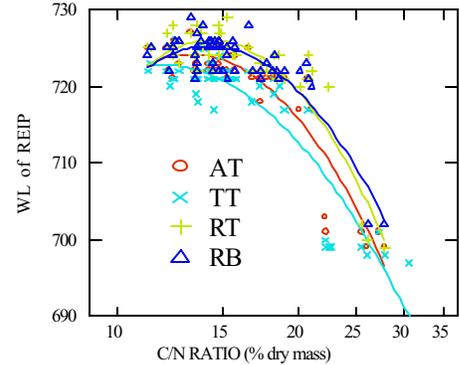


Fig. 5. For data acquired on all days, the non-linear relationships for the WL of REIP to the foliar C/N content is shown for AT, TT, RT, and RB.  $r^2 \geq 0.85$ .

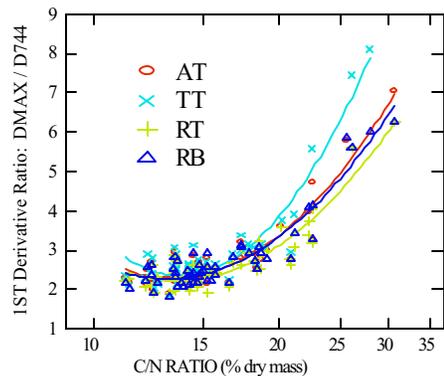
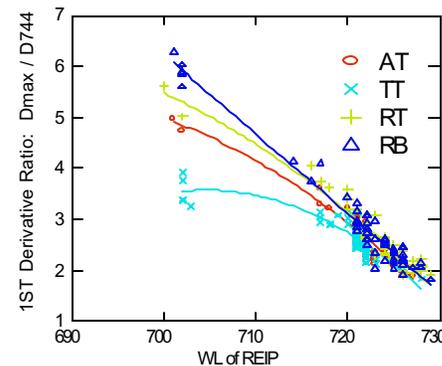


Fig. 6 [Left]. The non-linear relationships for the 1st Derivative Ratio, Dmax/D744, to the foliar C/N content is shown for all data for AT, TT, RT, and RB.  $r^2 \geq 0.92$ .

Fig. 7 [Right]. The relationship of Dmax/D744 to the WL of REIP, which is linear for RB, and increasingly curvilinear for RT, AT, and TT.  $r^2 = 0.94$  overall groups.



## V. REFERENCES

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