Foliar Reflectance and Fluorescence Responses for Plants Under Nitrogen Stress Determined with Active and Passive Systems

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***Abstract*- Leaves of corn *(Zea mays* L.) and soybean (*Glycine max* L.) plants were examined in a set of four experiments conducted in the summer of 2001 at USDA in Beltsville, MD. Red edge reflectance spectra and red (R) and far-red (FR) fluorescence emissions were acquired for adaxial and abaxial leaf surfaces, in conjunction with foliar chlorophyll content, photosynthetic capacity (Amax), and C/N ratio. A red edge first derivative ratio was strongly related to chlorophyll content, Amax, and C/N ratio. R/FR fluorescence emission ratios also were related to Amax and C/N levels, and enabled species discrimination.**

I. INTRODUCTION

We are studying the effects of nitrogen (N) application on photosynthetic carbon (C) uptake and storage in several crop and tree species. Both passive and active spectral measurements acquired with a variety of instruments to detect and monitor plant photosynthetic function, photosynthetic pigment accumulation, and C and N status are being investigated by our project for remote sensing potential [1-8]. Many researchers have documented the use of fluorescence measurements, especially the red (R, 680 nm) and far-red (FR, 740 nm) fluorescence peaks, for assessing photosynthetic function and the use of reflectance spectra for assessing chlorophyll amounts [1-8]. In this set of four experiments, we examined the relative responses of corn and soybean leaves to N treatments using reflectance and fluorescence measurements in the red edge spectrum (650-750 nm) and fluorescence emissions centered at 680 and 740 nm.

II. PROCEDURES

A. Plant Material and Treatments

Corn *(Zea mays* L.) and soybean (*Glycine max* L.) were grown under applied inorganic N treatments at the USDA Agricultural Research Center in Beltsville MD, USA in 2001. The field corn was grown as part of a larger project; "Optimizing Production Inputs for Economic and Environmental Enhancement (OPE[3])" for which the N application rates (210, 140, 70, and 28 kg N/ ha) provided 150%, 100%, 50% and 20% of the optimal recommended N level for corn on soils in central Maryland. Leaf-level measurements were obtained on the third from terminal leaf on four days during early August (Experiment 1) when corn was in the vegetative (V2) stage, and on five days in September (Experiment 2) over a two week period at the grain fill (R3) reproductive stage near the end of the growing season. Two experimental sets of soybean were grown in perlite in pots in a whitewashed glasshouse under identical N fertilization regimes in June (Exp. 1) and again in September (Exp. 2). Both groups of plants received a full complement of nutrients and the same range of N fertilization; 0.004 M, 0.003, 0.002 M, 0.001 M and 0 M, which correspond to 100, 75, 50, 25 and 0% of recommended N for soybean. In Exp. 2, plants received full sun outdoors three times per week for 4-6 hours each. Measurements were collected on the first fully expanded trifoliate ten weeks after germination.

B. Biophysical Measurements

The following biophysical data are reported on each fresh leaf sample: 1) chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*) content ($\mu g\ cm^{-2}$) ; 2) photosynthetic capacity, Amax ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$) determined with a Li-Cor 6400 Photosynthetic System; and 3) C/N ratio. Amax was determined under controlled conditions of high photosynthetically active radiation (PAR, 1800 $\mu mol\ m^{-2}\ s^{-1}$), saturating CO_2 concentration (1000 ppm), leaf chamber temperature (25 ^{o} C), and relative humidity (~35%). Pigment concentrations 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, USA). 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, USA) at the University of Maryland (College Park MD, USA) using the Dumas combustion method for determination of C, N and H levels by percent dry mass.

C. Fluorescence Measurements

Fluorescence emission spectra and images were obtained from the adaxial leaf surfaces in all experiments, and from both abaxial and adaxial surfaces in Exps. 2 for both species. A spectrofluorometer (Fluorolog II, Spex Industries, Edison NJ, USA) was used to produce and collect fluorescence emission spectra at 5 nm resolution; two excitation wavelengths are reported here: 380 nm (380Ex) for corn or 350 nm (350Ex) for soybean, and 532 nm (532Ex) which was added in Exps. 2, with emission spectra recorded between 400-800 nm and 600-800 nm, respectively. From the 380Ex, 350Ex or 532Ex emission spectra, the red/far-red (R/FR) ratios are reported here as R/FR[350], R/FR[380], or R/FR[532]. A dark room fluorescence imaging system (FIS) was used to quantify leaf

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fluorescence emissions in 10 nm bands, from which the R/FR[FIS] ratio was obtained [2]. Leaf samples were placed on a black, non-fluorescent tray below the CCD. Integration times were adjusted per band each day to ensure adequate dynamic ranges for measurements and intensities are reported in digital counts per second (dcps). FIS images were processed using special software packages (ENVI and IDL, Research Systems Incorporated, Boulder CO, USA). Images for the spectral bands were co-registered to enable calculation of band ratios, and threshold values were used to constrain the region of interest before descriptive statistics were calculated.

D. Optical Properties

Reflectance properties were determined on both adaxial (RT) and abaxial (RB) leaf surfaces using an integrating sphere (Li-Cor 1800) attached to an ASD spectroradiometer (ASD-FR FieldSpec®Pro, Analytical Spectral Devices, Inc., Boulder CO, USA) having an effective spectral resolution of < 2 nm (3 nm FWHM) in the 350 - 2500 nm range. More than twenty published spectral indices were calculated from each spectrum [e.g., 16]. On each original leaf RT or RB spectrum, a first derivative spectrum was computed and several red edge derivative parameters were determined. We report results for the ratio of the maximum derivative (Dmax) between 690 and 730 nm to the derivative at 744 nm (D744), or Dmax/D744.

III. RESULTS AND DISCUSSION

The corn leaves developed under field conditions and were monitored by us at two growth and development stages, the V2 (Exp. 1) and R3 (Exp. 2). The soybean leaves developed in a whitewashed glasshouse (June, Exp. 1) or under a mixed regime of full sun and glasshouse conditions (September, Exp. 2). The Chl *a* and Chl *b* content and Amax of foliage differed between species and changed with N treatment and growing conditions (Fig. 1A, B). Pigment levels were higher (20-210%) in corn than soybean, except for similar Chl *b* levels in the high N group (Fig. 1A,B). In growing corn leaves (Exp. 1), N treatment did not significantly affect Chl *a* ($\sim 37 \pm 2$ µg cm^{-2}) or Chl *b* ($\sim 11 \pm 3$ µg cm^{-2}) content, although higher pigment levels were seen in the two lower N groups at the R3 stage (Exp. 2), due to earlier transport to reproduction in the high N treatments. However, soybean leaves accumulated Chl *b* (≤ 10 µg cm^{-2}) in concert with N availability during both Exp. 1 & 2, and Chl *a* (≤ 19 µg cm^{-2}) during Exp. 1 (Fig. 1A, B). Amax (Fig. 1C) increased with N application in Exp. 1 for both corn (45-55 µmol CO_2 m^{-2} s^{-1}) and soybean (10-23 µmol CO_2 m^{-2} s^{-1}). In soybean leaves during Exp. 2, Amax in the two high N treatments was lower than in Exp. 1 even though pigment content was higher (Fig. 1A,B,C). The result was that mature corn leaves (R3) had significantly higher C relative to N (C/N ratio) as compared to the V2 stage (Fig. 1D); at R3, the C/N ratio increased with N treatment. However, the C/N ratio declined with N treatment in soybean leaves during both Exp. 1 & 2 (Fig. 1D).

In this paper, we present the trends that shared consistency among these variables observed across these four data sets. The strongest associations of spectral measurements to foliar variables (Chl *a*, Chl *b*, Amax, and C/N ratio) were achieved with indices derived from hyperspectral reflectances. The Dmax/D744 spectral index was strongly related to chlorophyll content, as shown for Chl *b* (Fig. 2A), and soybean and corn leaves show virtually the same near-linear response(differences exaggerated with log-log plot). With the Dmax/D744 red-edge derivative index, soybean leaves (upper groups) are separated from corn leaves, exhibiting a linear relationship to C/N ratio (Fig. 2B). The relationships of Dmax/D744 and the fluorescence Red/FR[350Ex or 380Ex] ratio to Amax (Fig. 3A,B) show similar responses per species, as do results obtained for R/FR[FIS]($r^2 = 0.83$) and R/FR[532] (neither shown), although the trends are clearer in the reflectance index. This is because the reflectance and fluorescence ratios are capturing somewhat different foliar characteristics as shown by the correlations between Dmax/D744 and the R/FR[350Ex or 380Ex] and R/FR[532Ex] ratios (Figs. 4A,B). A comparison of these spectral indices for abaxial versus adaxial foliar surfaces (Figs. 5A,B) shows that the R/FR[532Ex] ratio is more successful than Dmax/D744 in separating species. More pronounced species separation was obtained by comparison of two fluorescence ratios: R/FR[350Ex or 380Ex] and R/FR[FIS] (Fig. 6).

IV. SUMMARY

To examine the status and vigor of agricultural crops using remote sensing technologies, we show that merging spectral information from passive red-edge reflectance measurements with those of active R and FR fluorescence emissions provides additional information about pigment content, photosynthetic performance, C/N content, and species differences. Although the DmaxD744 is superior to R/FR fluorescence ratios for pigment and Amax evaluations, the R/FR fluorescence ratios enable species separations based on abaxial/adaxial comparisons. These foliar measurements are basic to understanding the canopy-level observations needed for remote sensing approaches to crop monitoring.

V. REFERENCES

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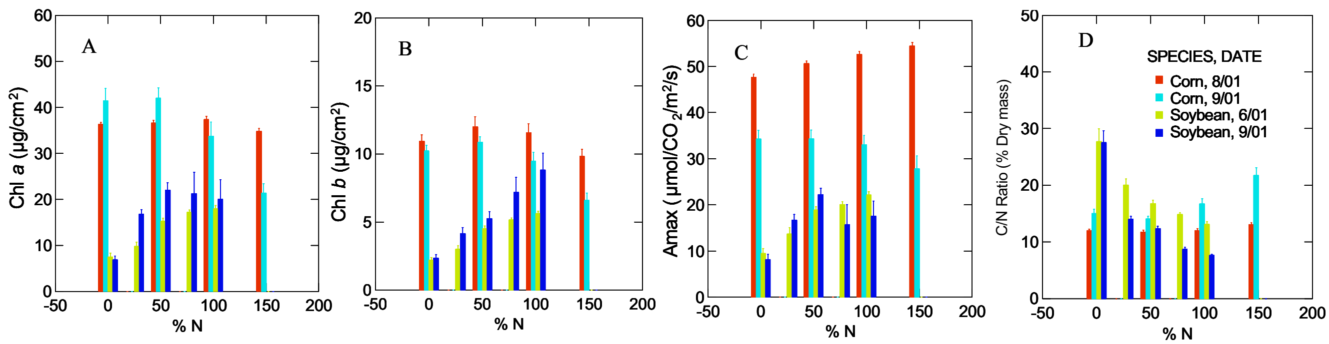

Figure 1. The means (± SE) for four foliar parameters, as a function of N treatment level in two Experiments, are given for corn (red, Exp. 1; teal, Exp. 2) and soybean (lime, Exp. 1; navy, Exp. 2): (A) Chl *a*; (B) Chl *b*; (C) Amax; and (D) C/N ratio. For soybean, an additional treatment group (no N) having all other essential nutrients was included.

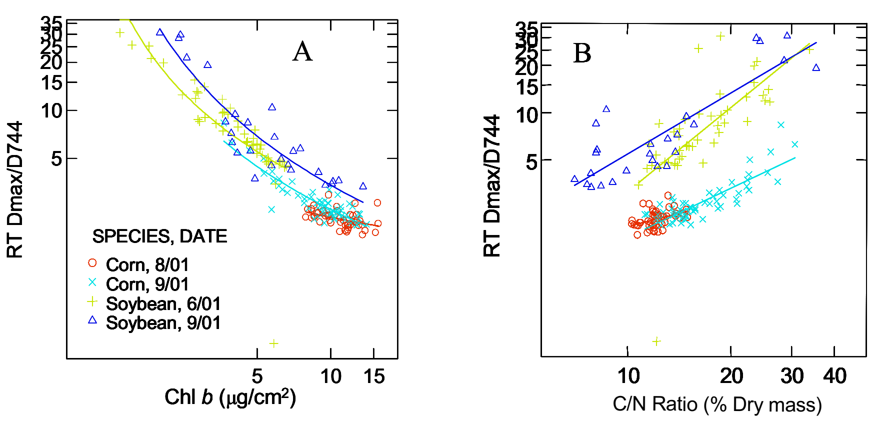

Figure 2. The relationships of Dmax/D744 for the adaxial surface (RT) as a function of leaf parameters ratio are given for corn and soybean leaves from two experiments: (A) Chl *b* content ($r^2 = 0.97$, n=169, $p \leq 0.01$); and (B) C/N ($r^2 = 0.96$, n=173, $p \leq 0.01$).

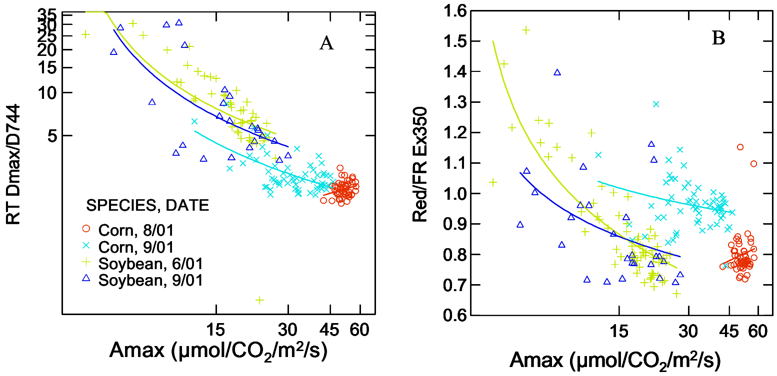

Figure 3. The relationships of two adaxial spectral indices to Amax are given for corn and soybean leaves from two experiments: (A) Dmax/D744 ($r^2 = 0.97$, n=176, $p \leq 0.01$); and (B) R/FR[350Ex] for soybean and R/FR[380Ex] for corn ($r^2 = 0.73$, n=194, $p \leq 0.04$).

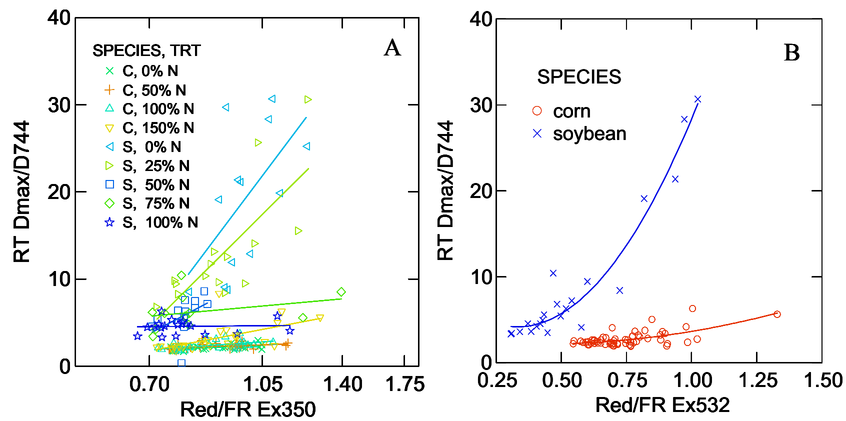

Figure 4. The correlation between adaxial measurements of Dmax/D744 and two R/FR indices for corn and soybean from two experiments is given: (A) R/FR[350Ex] for soybean and R/FR[380Ex] for corn, shown for 8 species*N treatment groups (r = 0.94, n = 195, $p \leq 0.07$); and (R/FR[532Ex], where blue symbol/line = soybean and red symbol/line = corn (r = 0.97, n = 82, $p \leq 0.05$).

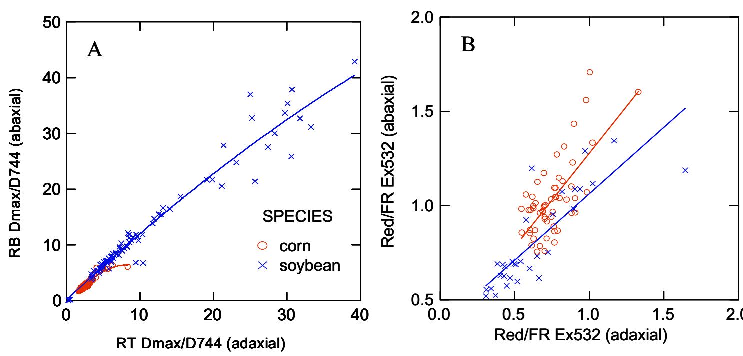

Figure 5. The correlation between the spectral indices obtained from *abaxial* (undersides) vs. *adaxial* (upper) surfaces of corn and soybean leaves in two experiments is shown for: (A) Dmax/D744 (r = 0.99, n = 196, $p \leq 0.02$); and (B) R/FR[532Ex]for soybean and R/FR[380Ex] for corn (r = 0.82, n = 88, $p \leq 0.05$). Blue symbol/line = soybean; Red symbol/line = Corn.

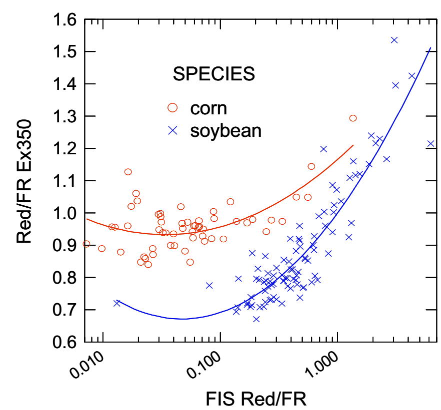

Figure 6. The correlation between two R/FR fluorescence ratios, either R/FR[350Ex] for soybean or R/FR[380Ex] for corn vs. R/FR[FIS], for adaxial leaf surfaces of corn and soybean leaves in two experiments is shown (r = 0.90, n = 147, $p \leq 0.002$). Blue symbol/line = soybean; Red symbol/line = Corn.