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# Contribution of Chlorophyll Fluorescence to the Reflectance of Corn Foliage

P.K. Entcheva Campbell<sup>1</sup>, E.M. Middleton<sup>1</sup>, L.A. Corp<sup>1</sup>, J.E. McMurtrey<sup>2</sup>,  
M.S. Kim<sup>3</sup>, E.W. Chappelle<sup>1</sup> and L.M. Butcher<sup>1</sup>

<sup>1</sup> Biospheric Sciences Branch, Laboratory for Terrestrial Physics, NASA/GSFC, Greenbelt, MD 20771

<sup>2</sup> Hydrology & Remote Sensing Laboratory, Agricultural Research Service, USDA, Beltsville, MD 20705

<sup>3</sup> Instrumentation and Sensing Laboratory, Agricultural Research Service, USDA, Beltsville, MD 20705

**Abstract-** To assess the contribution of chlorophyll fluorescence (ChlF) to apparent reflectance (Ra) in the red/far-red, spectra were collected on a C<sub>4</sub> agricultural species (corn, *Zea Mays* L.) under conditions ranging from nitrogen deficiency to excess. A significant contribution of ChlF to Ra was observed, with on average 10-25% at 685nm and 2-6% at 740nm of Ra being due to ChlF. Higher ChlF was consistently measured from the abaxial leaf surface as compared to the adaxial. Using 350-665nm excitation, the study confirms the trends in three ChlF ratios established previously by active F technology, suggesting that the ChlF utility this technology has developed for monitoring vegetation physiological status is likely applicable also under natural solar illumination.

## I. INTRODUCTION

Current remote sensing techniques for monitoring the status of terrestrial vegetation rely on reflectance data to estimate chlorophyll content and stand structure in order to infer vegetation vigor. However, reflectance observations are not able to directly assess vegetation photosynthetic function and physiological status. Improvements in satellite-based assessments of the terrestrial vegetation's carbon budget require additional information on vegetation physiological condition, which can be obtained *directly* by fluorescence (F) emissions. Considering the rapid developments in the technology: 1. signal amplification and sensor miniaturization, 2. laser induced fluorescence technologies [1, 2, 3, 13] and 3. passive chlorophyll F sensing technologies, utilizing the Fraunhofer line depth approach [4, 5], F has strong practical potential for monitoring vegetation status.

Assuming that the solar radiation incident on a leaf surface results in a reflected, transmitted or absorbed irradiation, the F contribution to R is commonly ignored. However, in the red/far-red region the apparent vegetative reflectance (Ra) typically includes the contribution of both the reflected (R) and F, emitted from the foliage surface. A portion of the absorbed solar energy is utilized in the process of photosynthesis via biochemical reactions, while the absorbed energy not utilized in the photosynthesis is either emitted as F at longer wavelengths or dissipated as heat. Vegetation F is emitted from the foliage throughout the ultraviolet to visible regions of the spectrum, with peaks occurring at 320, 445, 530, 685, and 740 nm [1, 2, 3, 6, 13]. While it has been assumed that F is a very small portion of the apparent vegetation reflectance, further research is needed to quantify the contribution of F to Ra, especially in the red

(685nm) and far-red (740nm) where the ChF emissions are maximal.

Fluorescence spectral indices, derived using active ChlF in the red and far-red, have successfully detected various types of vegetation stress [4, 6, 7, 8, 9, 13], and quantified the amount of crop residue covering agricultural soil surfaces [10]. Active F technologies apply a strong narrow excitation beam of illumination, while a broad radiation spectrum is present under the natural solar illumination. The amount of the emitted F strongly depends on the spectral composition and amount of the excitation energy. The photosynthetic pigments absorb radiation primarily in the 350-700 nm region, while their emittance occurs in broad peaks in the red (F685) and far-red (F740) portions of the spectrum. While active F techniques have established ChlF as a reliable approach for diagnosis of vegetation physiological condition, very few studies [4, 5] validate the potential of ChlF, using as excitation the full solar spectrum.

The goal of this investigation is to evaluate the relative R and ChlF fractions contributing to the cumulative vegetation irradiance at 685 and 740 nm. The objectives of the study are to evaluate the relationship between F and R at the foliar level, quantify the contribution of ChlF to Ra, and determine the trends in ChlF occurring under nitrogen (N) deficiency, using simulated solar excitation.

## II. MATERIALS AND METHODS

Experimental data were collected on a C<sub>4</sub> agricultural species (corn, *Zea Mays* L.) under a gradient of inorganic N fertilization levels. The N treatments provided plant growth conditions ranging from N deficiency to N excess. The N applications, in percentage of the recommended level, were: 0% (28kg/ha), 50% (70kg/ha), 100% (140kg/ha), 150% (210kg/ha). Leaf samples were acquired from the OPE<sup>3</sup> sites (Optimizing Production for Economic and Environmental Enhancement), at USDA-ARSL, Beltsville, MD. Measurements were obtained from the third leaf from the terminal at the grain fill (R3) reproductive stage. Foliar spectra were acquired in conjunction with measurements of photosynthesis, pigment concentration, carbon and N contents.

Spectral measurements were conducted using procedures established at leaf level by Kim [1] and further developed at foliar and canopy scale by Zarco-Tejada [11]. High spectral resolution measurements were acquired using an ASD-FR

FieldSpec® Pro, with 3nm FWHM and 1.4nm sampling interval in the 350-800nm region (Analytical Spectral Devices, Inc.; Boulder, CO). The sensor foreoptic view angle was 8 degrees and the view area was approximately 2cm<sup>2</sup>. A halogen panel was used as a reference standard. A 300W xenon arc lamp and a set of neutral density filters were used to simulate constant solar radiation spectrum. A Schott RG 665 long pass filter was used to prevent fluorescence induction from below 665nm. The illumination setup was used with the RG 665 filter blocking the light source to measure **R**, and without the filter to measure **Ra** (**R**+**F**). Samples were dark adapted for 5 minutes immediately before initiating the **Ra** measurements. Recording data at 0.01s for 25 seconds, spectra were obtained across the 650-800nm region. **ChlF** was calculated as the difference between the **R** and **Ra** amounts. Estimates of the attained maximum **ChlF** (**Fmax**) and the **ChlF** at steady state (**Fs**), as a percentage of the incoming radiation are given in Table 1. The differences in **ChlF** recovery trends from **Fmax** to **Fs** were assessed by computing **Fslope** (Table 1). **Fslope** is the first derivative maximum of the spectra obtained at F685 and F740 nm during the time of consecutive scanning.

To test for significant differences in **Fmax** and **Fs** occurring among treatment levels associated with **ChlF** (at 685nm and 740nm), general linear model analysis of variances was conducted (GLM, ANOVA, SYSTAT 8.0; SPSS Inc., 1997). The significance of the differences was determined by Tukey-Kramer test (Table 1).

### III. RESULTS AND DISCUSSION

In the red and far-red region the **Ra** (Fig. 1, dashed lines) measured without the long pass filter of the corn leaf was consistently higher than the reflectance measured with filter (Fig.1, solid lines). The relative contribution of **ChF** to the **Ra** at each wavelength was determined by expressing **Fs** as a proportion of **Ra** (Fig. 2). In the red edge region vegetation has relatively low **R**, due to strong chlorophyll *a* absorption. Although the absolute **ChlF** amounts are relatively small, **Fs** contributes significantly to **Ra** (Fig. 2). On average 10-25%, at

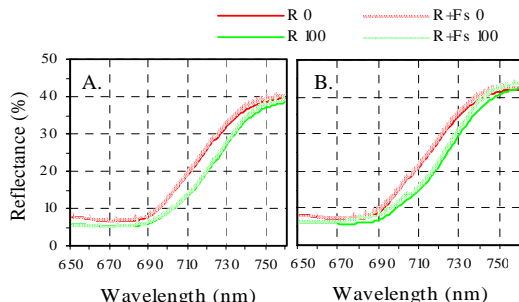


Fig.1. Reflectance (**R**) and apparent reflectance (**Ra**=**R**+**F**) from the top (A) and back (B) of healthy (100) and nitrogen deficient (**N**=0%) corn foliage (ANOVA LS Means). To measure **R** was used a 665nm long pass filter, while the apparent reflectance (**Ra**=**F**+**R**, no filter) includes both **R** and **F** contributions.

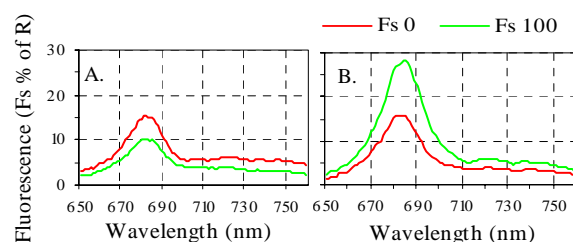


Fig.2. Contribution of steady state fluorescence (**Fs**, for **N**=0% and **N**=100%) to the apparent vegetation reflectance (**Ra**) measured from the top (A.) and back (B.) of the leaf. **Fs** is expressed as percentages of **Ra**.

685nm and 2-6% at 740nm of **Ra** was due to **ChlF**. The **ChlF** values measured at the adaxial surface were lower than the abaxial measurements (Table 1, Fig. 1 and 2). The amount of **F** varied in association to **N** treatment, significantly affecting the relationship between foliar **ChlF** and **R**. Comparing **Fs** data from the adaxial (Fig. 2A) and abaxial (Fig.2B) leaf surfaces, opposite trends occurring under **N** deficiency were established: for the adaxial surface the relative **Fs** fraction increased in concert with the nutrient stress levels, whereas the opposite was shown for the abaxial surface (Fig. 2). However, a decrease in **Fmax** associated with **N** availability was observed for both leaf surfaces (Table 1). Optimal separation of the **N** treatments was achieved for the leaf adaxial surface by **F685s**, while for the abaxial surface performed best **F740max** (Table 1). The strongest treatment differences were produced by the abaxial surfaces for three parameters: **F740max**, **F740slope**, and **F685slope** (Table 1).

Using active **F** techniques the red /far-red **ChlF** ratio has been shown to increase as chlorophyll content decreases or as photosynthetic rate declines in association with vegetation stress [2, 3, 4, 7, 8]. The trends in the **F685max/F740max** ratio from the abaxial leaf surface (Fig. 3), comply with these suggested in the literature with advancement of vegetation stress (**N** deficiency or excess), but do not for the adaxial surfaces. However, the **F685/F740s** from the leaf adaxial surface

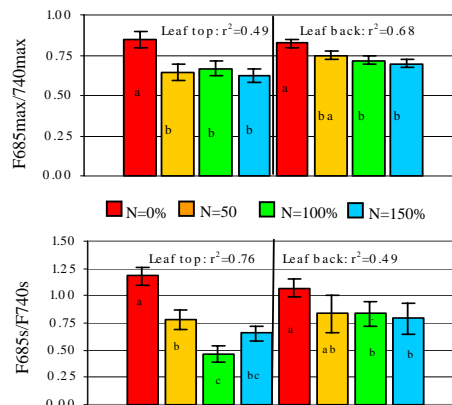


Fig.3. Differences in the **Fred/Ffar-red** ratio associated with nitrogen treatments (ANOVA, LS Means and Std. Errors).

TABLE 1  
CHANGES IN FLUORESCENCE PARAMETERS IN ASSOCIATION TO NITROGEN TREATMENT\*

Fluorescence parameter (% of incoming radiation)	Nitrogen treatment level (N in % of optimum, ANOVA LS Means and $r^2$ )									
	Leaf adaxial (top) surface					Leaf abaxial (back) surface				
	0	50	100	150	$r^2$	0	50	100	150	$r^2$
F685max (%)	2.98ns	2.65ns	2.51ns	2.65ns	0.35	3.53a	3.76ab	3.98b	3.48a	0.64
F685s (%)	<b>1.07a</b>	<b>0.79b</b>	<b>0.41c</b>	<b>0.67b</b>	<b>0.74</b>	1.08ab	1.21ab	1.36b	0.99a	0.51
F685slope	-0.076a	-0.06ab	-0.05b	-0.05ba	0.69	<b>-0.19a</b>	<b>-0.12b</b>	<b>-0.06c</b>	<b>-0.06c</b>	<b>0.79</b>
F740max (%)	<b>3.48a</b>	<b>4.08bc</b>	<b>3.91b</b>	<b>4.40c</b>	<b>0.61</b>	<b>4.29a</b>	<b>5.15b</b>	<b>5.71c</b>	<b>4.71b</b>	<b>0.89</b>
F740s (%)	1.02ab	1.04a	0.78b	1.03a	0.62	1.28a	1.30ab	1.61b	1.29ab	0.45
F740slope	-0.11 ns	-0.10 ns	-0.09 ns	-0.10ns	0.46	<b>-0.24a</b>	<b>-0.20b</b>	<b>-0.13c</b>	<b>-0.12c</b>	<b>0.83</b>

\*LS means are compared within row per leaf surface. Significant differences among treatments are indicated with different letters.

provided optimal treatment separation (Fig. 3). The **Fvs** and **Fvm** ratios [Fig. 4] have been suggested as indicators of overall photosynthetic function [12]. Our finding of **Fvs** values of ~2 for N=0% (Fig. 4) could be indicative of an inefficiency in the carbon dioxide assimilation associated with N deficiency [8].

#### IV. CONCLUSIONS

This investigation confirms the significant contribution of **ChlF** to the apparent vegetation reflectance in the red and far-red regions. On average, 10-25% of apparent reflectance at 685 nm (**Fs**) and 2-6% (**Fs**) at 740nm was actually due to **ChlF**. At 685nm **Fmax** was ~2-4% and **Fs** 0.5-1.5% of the incoming radiation, while at 740nm the **ChlF** values were higher: **Fmax** ~4-6%, **Fs** ~1-2%. The relationship between foliar **F** and **R** was significantly affected by the bio-physiological status of the vegetation. The relative steady **ChlF** fraction at 685nm increased in concert with the nutrient stress levels, based on leaf adaxial (but not abaxial) measurements. Using a simulated solar excitation spectrum, this study confirms the trends in **F685/F740s**, **Fvs** and **Fvm** ratios associated with vegetation stress, previously established using narrow spectral excitation band and. This finding suggests that the **ChlF** utility for monitoring the physiological status of vegetation, established by the active **ChlF** technology is likely applicable under natural solar illumination.

Further analysis of the spectra in relationship to the vegetation bio-physiological parameters, and future investigations of

similar data sets from different vegetation species are needed to expand and validate the present findings.

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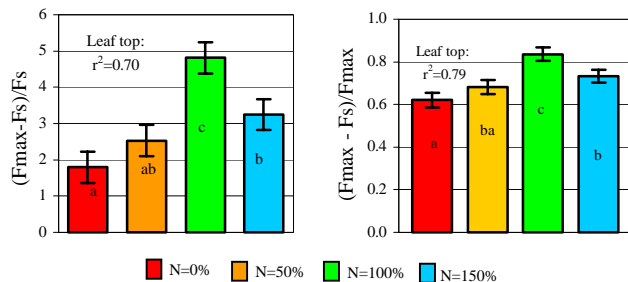


Fig.4. Differences in the F685vs [F685vs=(Fmax-Fs)/Fs] and F685vm [F685vm=(Fmax-Fs)/Fmax] parameters in association with nitrogen treatment.