NOVEL LEAF-LEVEL MEASUREMENTS OF CHLOROPHYLL FLUORESCENCE FOR PHOTOSYNTHETIC EFFICIENCY

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ABSTRACT

Solar induced chlorophyll fluorescence (SIF) from vegetation can now be obtained from satellites as well as ground-based field studies, at select wavelengths associated with atmospheric features. At the leaf level, full spectrum (650-800 nm) chlorophyll emissions (ChlF) can be measured using specialized instrumentation to support interpretation of these SIF observations. We found that ChlF spectra differ for leaf bottoms versus upper leaf surfaces, potentially affecting within-canopy radiative scattering. Our ChlF measurements for leaves of eight tree species (n≥125) obtained during fall 2013 senescence at the Duke Forest in North Carolina, USA and the 2014 growing season (n=72) at the USDA cornfield in Beltsville, MD, USA also demonstrate the benefit of acquiring measurements for both the Red and Far-Red emission peaks. The Red/Far-Red ChlF Ratio was strongly related to both the Photochemical Reflectance Index (PRI) for corn leaves (r≥0.76) and tree leaves (r≥0.89) and to a PSII Photosynthesis Efficiency parameter (r~0.90).

1. INTRODUCTION

Chlorophyll fluorescence (ChlF) measurements from terrestrial vegetation have become a new remote sensing capability from space [1]. ChlF serves as a unique probe for photosynthetic function. We report here the combined measurements of spectral reflectance, spectral emittance, and photosynthetic parameters for both the top and the bottom (transmitted emittance) of leaves, which has not been reported previously. We used a custom leaf clip, the FluoWat developed at the University of Valencia [2] and a spectrometer to obtain ChlF spectra from the tops and bottoms of foliage of eight tree species, and corn leaves. The tree leaves were measured during the 2013 FLuorescence EXplorer (FLEX) airborne campaign in the USA (FLEX-US) at the Duke Forest in North Carolina, USA. The corn leaves were measured during the 2014 summer growing season at the USDA research cornfield in Beltsville, MD. We show that the Photochemical Reflectance Index (PRI) was well-correlated (R = 0.75-0.79) with the Red/Far-Red Ratio of the ChlF peaks at 685 and 740 nm, respectively, for all foliage examined. We also show that the downward emissions are important to quantifying the total leaf ChlF, and that the Red/Far-Red ChlF Ratio was highly correlated to photosynthetic efficiency (R ~ 0.90), with significant species differences.

2. METHODS

We report results from two experiments. The tree foliage was measured in a field lab during late September/early October autumn senescence as part of the 2013 FLEX-US airborne campaign at the Duke Forest in North Carolina, USA. We acquired leaf-level measurements on leaves of 8 tree species – seven deciduous and one coniferous species. The seven deciduous species were: black oak (*Quercus velutina* Lam.), White Oak (*Quercus alba* L.), red oak (*Quercus rubra* L.), sweet gum (*Liquidambar styraciflua* L.), mixtures of pignut (*Carya glabra* Mill) and white-heart hickory (*Carya tomentosa* L. Nutt.), tulip poplar (*Liriodendron tulipifera* L.), and red maple (*Acer rubrum* L.); the coniferous species was loblolly pine (*Pinus tadea* L.). We obtained leaf-level measurements of spectral fluorescence with the custom FluoWat leaf clip coupled with a portable FieldSpec FR spectrometer (ASD Inc.) to obtain ChlF spectra from both the leaf tops and bottoms using an artificial light source. The top-of-canopy (TOC) corn (or maize) leaves (*Zea mays* L.) were similarly measured, but were made *in situ* with ambient solar radiation during the 2014 summer growing season at the USDA research cornfield in Beltsville, MD, USA. For both experiments, the FluoWat ChlF measurements were made in conjunction with traditional photosynthesis with a LI-6400 system (gas exchange plus fluorimeter chamber).

We computed various indices from the reflectance and emitted spectra, including the Photochemical Reflectance Index (PRI), in combination with photosynthetic parameters such as PSII Efficiency. The Red/Far-Red Fluorescence Ratio was computed from FluoWat peak ChlF values at 685 and 740 nm, and for the ratio pertinent to Solar Induced Fluorescence (SIF) using the 685 and 760 nm wavebands.

3. RESULTS

We show a typical dataset for the top, bottom, and total ChlF spectra obtained for corn leaves with the FluoWat/ASD spectrometer (**Fig. 1**). The PRI was well-correlated to the Red/Far-Red ChlF Ratio for these corn leaves (**Fig. 2**), in agreement with our previous results at the canopy level for corn plots [3, 4, 5]. Likewise, a similar linear result was obtained for the tree leaves (**Fig. 3**), with significantly different data ranges per species during senescence, and with different slopes. The ChlF Ratio obtained with FluoWat was highly correlated to leaf-level PSII Photosynthetic Efficiency represented by the light-induced variable to maximum ChlF, Fv'/Fm' (**Fig. 4 A,B,C**), obtained with the LI-6400 system for these leaves. A similar result was obtained for the corn leaves (not shown).

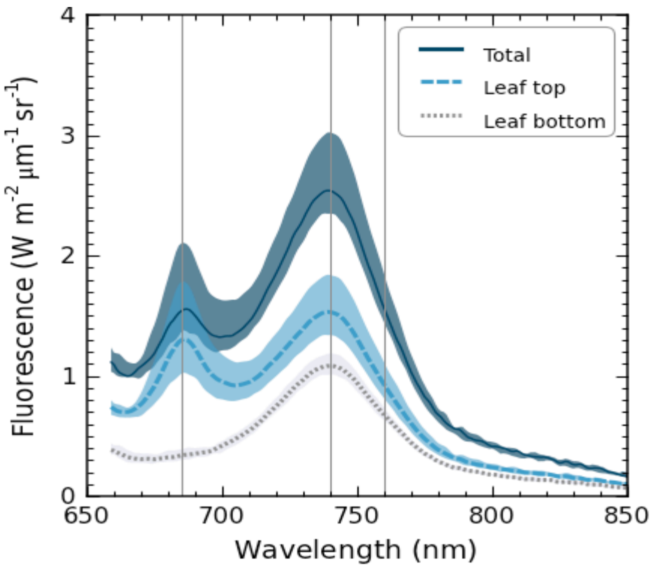

Fig. 1. Chlorophyll fluorescence emitted from corn leaves was measured using the FluoWat leaf clip and an ASD spectrometer July 30, 2014 in Beltsville, MD, USA. The values are the average of 4 leaves from an irrigated plot without supplemental nitrogen fertilization. The blue dashed line is the fluorescence emitted from the solar illuminated leaf top, the gray dashed line is the fluorescence emitted from the leaf bottom (transmitted away from the illumination), and the solid blue line is the total fluorescence emitted from the whole leaf (Top + Bottom). The vertical lines represent the two Red and Far-Red peak wavelengths 685 nm 740 nm, and at the position of the Far-Red SIF retrieval wavelength, centered at 760 mn.

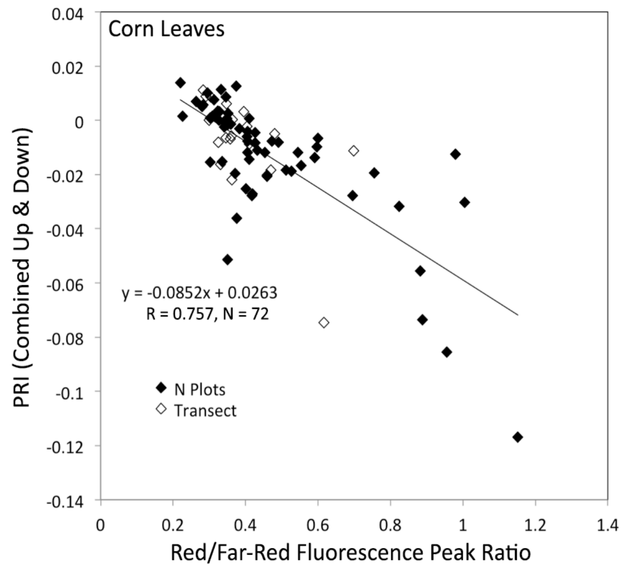

Fig. 2. The PRI was calculated using the combination of leaf reflectance and transmittance (Top and Bottom measurements) versus the ratio of the fluorescence emissions at the Red and Far-Red spectral peaks at 685 and 740 nm, respectively. Data were collected using the FluoWat for TOC leaves in the USDA research cornfield during the 2014 growing season. Solid black points are from nitrogen manipulation plots (corn plants fertilized at 0% to 150% of the recommended application); open points are values for leaves from a transect through the cornfield where the entire field was fertilized at 100%N. The correlation between these two leaf measurements was significant (R = 0.757, P≤0.05).

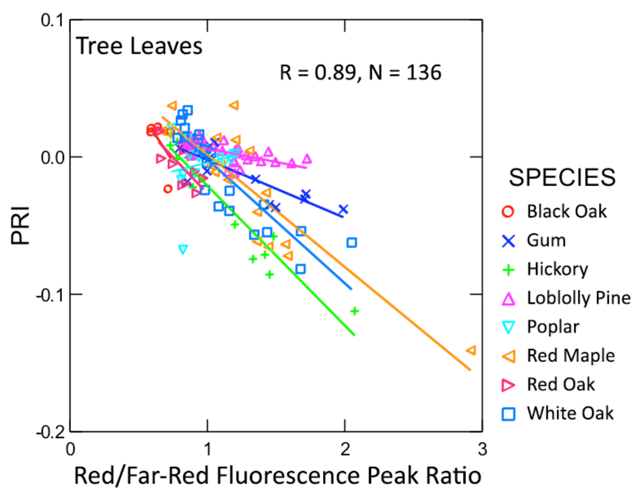

Fig. 3. The PRI was derived from the upward (Top of leaf) spectral radiances and compared with the corresponding upward Red/Far-Red fluorescence ratio computed from the Top of leaf emission spectra (R = 0.89, P≤ 0.000). These laboratory data (N =136) were acquired during early autumn senescence at the Duke Forest in Durham, NC during the 2013 FLEX-US airborne campaign. The seven deciduous species examined were: black oak, white oak, red oak, sweet gum, hickory, tulip poplar, red maple, and one coniferous species – loblolly pine. The species differences and slopes were highly significant (P≤ 0.000).

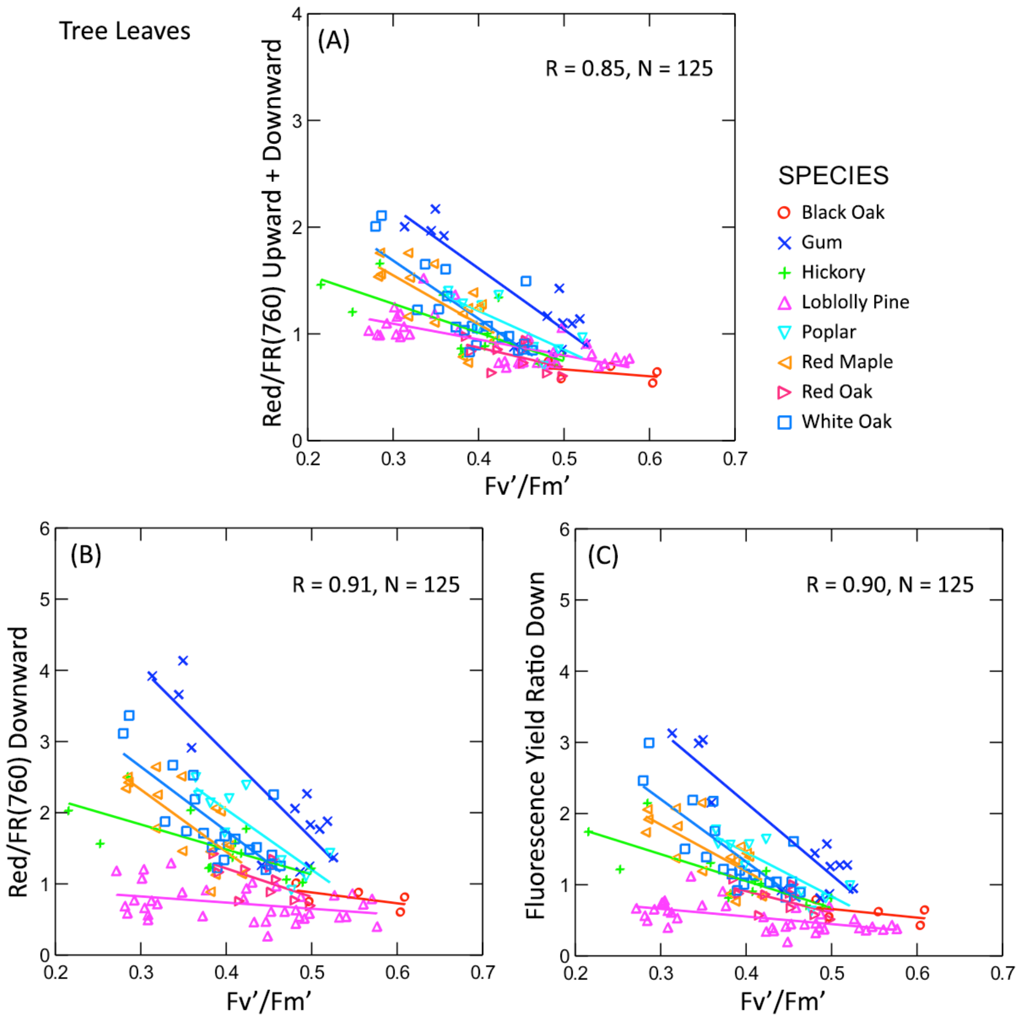

Fig. 4. For the 8 species examined at the Duke Forest as part of FLEX-US, the Red/Far-Red Fluorescence ChlF Ratio was determined from the leaf fluorescence emissions obtained using the FLuoWat leaf clip, and compared with the light induced PSII efficiency index, the ratio of variable to maximum fluorescence (Fv'/Fm'), obtained with the LI-6400 photosynthetic system with a fluorimeter chamber (N = 125). Three variations of this comparison are shown: A] The Red/Far-Red ChlF Ratio determined from the ***total*** (Top + Bottom) leaf fluorescence emissions (R = 0.85, P≤ 0.000); B] The Red/Far-Red ChlF Ratio determined from the leaf ***Bottoms***, where the Far-Red fluorescence is the value from the 2 nm region centered at 760 nm (R = 0.91, P≤ 0.000) ; and C] The Fluorescence Yield based on the Red/Far-Red ChlF Ratio from the leaf ***Bottoms***, normalized to incoming PAR (R = 0.90, P≤ 0.000). The species differences and slopes were highly significant (P≤ 0.000) in all cases.

4. CONCLUSIONS

Leaf level studies of ChlF provide important information for the interpretation of remotely sensed observations. The results of this study highlight the need for retrieving information from both the Red and Far Red emission peaks of ChlF. Further, we show ChlF emitted from leaf bottoms differs spectrally from the usually-measured leaf top emission and may be particularly useful for field observations of plant condition. Therefore, our results also show the importance of measuring the downward fluorescence flux in canopies. Differences between the leaf top and bottom ChlF emissions will affect the radiative transfer of the ChlF signal within canopies and affect scaling from leaf to canopy and larger-area ChlF measurements.

5. REFERENCES

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