Canopy Level Solar Induced Fluorescence for Vegetation in Controlled Experiments

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***Abstract-* Solar induced chlorophyll fluorescence (SIF) was retrieved from high resolution canopy reflectance spectra acquired with an Analytical Spectral Devices (ASD) FieldSpec spectroradiometer one meter above tree sapling canopies. Three deciduous tree species were examined during four measurement periods, spanning the two growing seasons in 2005 and 2006: tulip poplar (*Liriodendron tulipifera* L.), red maple (*Acer rubrum* L.), and sweet gum (*Liquidambar styraciflua* L.). The Fraunhofer Line Depth (FLD) principal was applied to above-canopy spectra for SIF retrievals at the two atmospheric oxygen (O_2) absorption features that occur in the chlorophyll fluorescence (ChlF) region (660-780 nm). The broader and deeper telluric feature centered at 760 nm, $O_2\alpha$, enables retrievals of SIF760 on the shoulder of the far-red ChlF peak. $O_2\beta$, although a narrower feature, is well positioned for SIF retrieval at 688 nm (SIF688) near the red ChlF peak. Canopy-level SIF688 for tree saplings varied between 1.1 and 2.9 (± 0.3) mW m^{-2} nm^{-1} sr^{1}, while SIF760 varied between 0.5 and 1.3 (± 0.15) mW m^{-2} nm^{-1} sr^{-1}. Typical values for the SIF red/far-red ratio (SIF688 / SIF760) ranged between 1.45 and 6.9, with the highest values observed in autumn. Contemporary canopy and leaf level measurements (e.g., fluorescence, F; reflectance; photosynthesis) were obtained. Leaf-level ChlF obtained from laboratory measurements of actively induced and solar-corrected fluorescence (SCF) emissions at the ChlF peaks (SCF685, SCF740) was determined for leaves of these tree species and three other species (corn, *Zea mays* L; green pepper, *Capsicum annuum* L; and soybean, *Glycine max* L.). For the trees, leaf-level estimates were determined at the SIF wavelengths (SCF688, SCF760) and compared with canopy field retrievals (SIF688, SIF760). The red ChlF values from leaf and canopy (SCF688 vs. SIF688) were similar, but SCF760 was ~4 times higher than SIF760; consequently, considerably (~5X) higher red/far-red ratios (SIF688/760) resulted for tree canopies as compared to individual tree leaves. The 2006 tree leaf and canopy SIF measurements were also compared with leaf-level SIF estimates derived using FluorMOD, a radiative transfer model that simulates F properties of foliage, utilizing our supporting leaf and environmental measurements as model inputs. The preliminary FluorMOD red and far-red SIF estimates were higher than our field measurements and higher than the laboratory estimates (except for far-red F). SIF of tree foliage was also compared with similar estimates made over corn crops under N treatments. These results will assist in determining the expected SIF intensity of vegetation's near-surface signal associated with $O_2\alpha$ and $O_2\beta$.**

I. INTRODUCTION

In this study, we address the determination of solar induced fluorescence (SIF) of vegetation canopies in the ambient field environment and of individual leaves in the laboratory. SIF is expected to provide important information to complement reflectance properties, for describing the physiological dynamics of vegetation related to carbon uptake. Retrieval of steady state SIF from high spectral resolution observations is the goal of the Fluorescence Explorer (FLEX) mission concept under development by the European Space Agency [1]. The validity of this approach is supported by recent studies [2-9]. We examined the fluorescence (F) properties of foliage and canopies of several crop and deciduous tree species in the field and in the laboratory. Here, we present preliminary results of these experiments and compare laboratory and field results and include initial leaf-level SIF simulations using FluorMOD, a model developed for this purpose [10]. Our goal is to determine the magnitude of the SIF in the ambient environment, and relate it to laboratory estimates and model simulations.

II. METHODS

A. Plant Material

Plant material was obtained at the USDA Beltsville Agricultural Research Center, Beltsville, MD, USA, from experimental research plots and from plants grown in pots under controlled conditions. The primary experiments reported here were conducted over 5-day periods on tree saplings of deciduous trees that were planted in 2001 at USDA in blocks for N augmentation, which was applied each spring at four treatment levels (37.5, 75, 112.5, 150 kg N/ha). The results reported here were acquired in four experiments conducted in 2005 (August, October) and 2006 (May, August) when saplings were 10-11 years old (n ~90). The species were tulip poplar (*Liriodendron tulipifera* L.), red maple (*Acer rubrum* L.) and sweet gum (*Liquidambar styraciflua* L.).

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Table 1. Comparison of steady state ChlF at 688 and 760 nm, the positions of the $O_2\alpha$ and $O_2\beta$ atmospheric features, derived from leaf and canopy techniques.

Species	Month	Year	Solar Corrected Leaf F*			Passive FLD SIF*		
			SCF688	SCF760	SCF Ratio	SIF688	SIF760	SIF Ratio
Gum	Aug	2005	1.52 cd	3.34 ab	0.45 e	2.46 abc	0.99 abc	2.63 bcd
	Oct	2005	1.75 bcd	3.49 ab	0.47 cde	2.93 a	0.52 c	6.93 a
	May	2006	1.38 d	2.91 b	0.46 de	1.50 cde	0.84 abc	1.81 d
	Aug	2006	1.56 cd	3.09 b	0.51 cde	1.79 bcde	1.30 a	1.48 d
Maple	Aug	2005	1.38 d	3.05 b	0.45 e	1.93 abcd	0.79 bc	2.95 bcd
	Oct	2005	1.37 d	2.91 b	0.48 de	2.40 ab	0.66 c	4.69 ab
	May	2006	1.62 cd	2.98 b	0.54 bcd	1.11 e	0.84 bc	1.45 d
	Aug	2006	1.86 abcd	3.56 ab	0.53 bcde	1.62 cde	1.30 a	1.47 d
Poplar	Aug	2005	2.37 ab	4.31 a	0.55 bc	2.11 abcd	0.67 c	4.06 bc
	Oct	2005	2.05 abc	3.38 b	0.60 ab	2.48 ab	0.92 abc	3.41 bcd
	May	2006	2.51 a	3.81 ab	0.66 a	1.29 de	0.88 abc	1.69 cd
	Aug	2006	1.91 abcd	3.51 ab	0.54 bcd	2.00 abcd	1.10 ab	1.97 d
Means			1.77	3.35	0.52	1.87	0.81	2.88
$LSD_{.01}$(df=134-229)			0.62	1.05	0.08	0.91	0.47	2.37

Within group column wise means with the same letter are not separable by $ANOVA_{LSD.01}$.
*SIF intensities are reported in mW/m2/nm/sr.

We also determined SIF for green pepper (*Capsicum annuum* L., in 2006) and soybean (*Glycine max* L., in 2003) plants grown outside in one-gallon pots (30 pots, 2 plants/pot) with standard nutrient and water supply for optimal growth; the soybeans were provided high and low N treatments. We also compared the tree results with those from similar experiments conducted on corn (*Zea mays* L.) in August 2004 and July 2005 in a field site where N augmentation regimes were 28, 70, 140, and 280 kg N/ha (20%, 50%, 100% and 200% of the recommended levels). Measurements on corn were made when the crop had achieved a height of ~3 m at the grain filling reproductive (R3) growth stage.

Leaf level measurements were made on mature sun leaves (3^{rd} or 4^{th} leaf from terminal) for the dicot leaves (pepper, soybean, and tree species), and on the 13^{th} (ear) leaf for corn; *in situ* measurements included photosynthesis and steady state F (acquired with a photosynthesis and fluorimeter system, Li-Cor, Lincoln, NE, USA). Excised leaves were held in water-filled florist tubes during laboratory measurements for optical properties, ChlF kinetics derived from reflectance spectra, and steady state spectral F emissions (EM) induced at several excitation (EX) wavelengths, followed by determinations for chlorophyll content and specific leaf mass [11-12].

B. FLD determination of SIF in the atmospheric O_2 bands

Nadir canopy radiances per sample within a 22^o field of view were acquired at ~1 m above plant canopies around mid-day on clear days using an ASD spectroradiometer, paired with contemporaneous irradiance measurements acquired above a Spectralon reference panel normalized to a standard PAR intensity [4] of 1660 μmol m^{-2} s^{-1}. Reflectance and SIF were determined for the O_2 bands using the FLD principal [2,10,13-14] as: $R = (c - d) / (a - b)$; and $SIF = d - Rb = (ad - cb) / (a - b)$. Here, 'a' and 'b' represent the reference panel radiances within (for 688 and 760 nm) and outside (e.g., for 680 or 750 nm) each O_2 feature, respectively, while 'c' and 'd' represent the comparable vegetation radiances. SIF was determined in the tree plots (**Fig. 1**). SIF was also obtained in the cornfield plots [10-11], and for a pepper plant "canopy" that was artificially created by arranging pots into a solid circle (~0.5 m circumference) for SIF determinations [15]. Leaves for laboratory measurements were excised following *in situ* leaf chamber and SIF measurements.

C. Laboratory fluorescence measurements

Actively induced F spectra of adaxial (upper) leaf surfaces were obtained in the laboratory at selected discrete EX wavelengths using a spectrofluorometer (Fluorolog-II, Spex Industries, Edison NJ, USA). EX spectra were also obtained for EMs at the peak ChlF wavelengths, F680 and F740. The standard, calibrated excitation-emission matrices (EEMs) were produced at a spectral resolution of ≤5 nm (EX: 400-750 nm; EM: 600-800 nm). From the calibrated EEM, a solar-corrected EEM was developed (normalized for PAR at 1660 μmol m^{-2} s^{-1}), from which simulated leaf SIF intensities were derived through integration of monochromatically acquired EX spectra [2, 6,11,16]. Here, solar corrected leaf SIF (SCF) estimates of tree, corn, soybean, and pepper foliage at the 685 and 740 nm ChlF

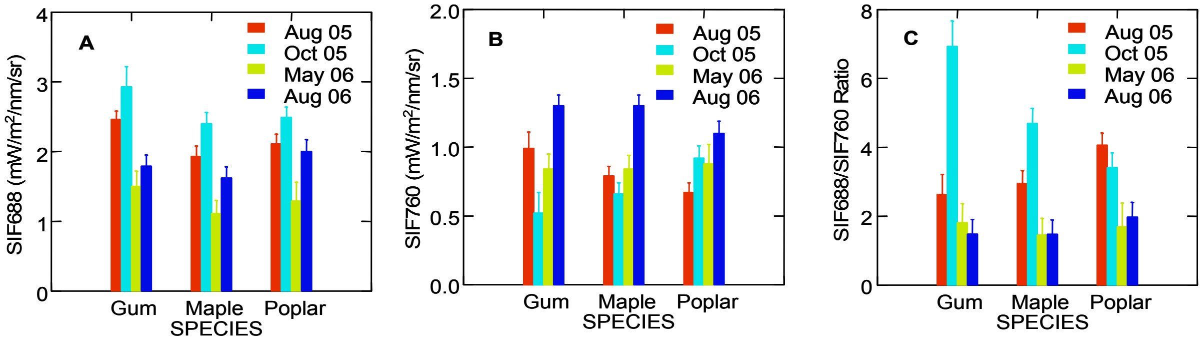

Fig. 1. The field SIF values obtained above tree saplings of three species during four experiments conducted in 2005 and 2006 in Beltsville, MD USA for: A] SIF688; B] SIF760; and C] the SIF688/SIF760 Ratio. The species are sweet gum, red maple, and poplar. The 4 experiments were conducted in August 2005 (red bar), October 2005 (aqua bar), May 2006 (green bar), and August 2006 (navy bar).

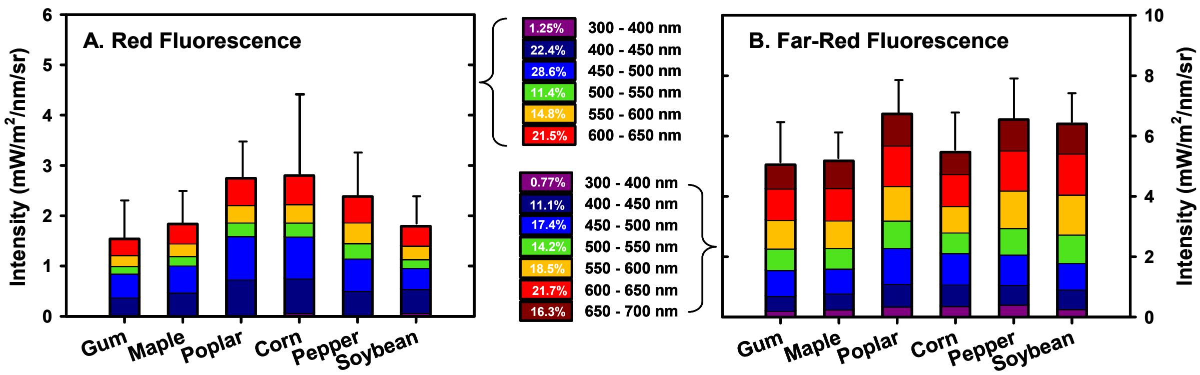

Fig. 2. Solar corrected fluorescence (SCF) emissions at the ChlF peaks (685 and 740 nm) are shown for six plant species. The segments of each bar represent the contribution in energy units (mW m^{-2} nm^{-1} sr^{-1}) induced by EX of different wavelength ranges of the solar spectrum. The wavelength ranges, and their average contribution to F685 (upper group, center) and F740 (lower group, center) are provided in the figure label: purple, UV (300-400 nm); navy, 400-450 nm; blue, 450-500 nm; green, 500-550 nm; yellow, 550-600 nm; and red, 600-650 nm.

peaks were produced from actively induced F spectra, and expressed in energy units (mW m^{-2} nm^{-1} sr^{-1}), for data averaged over all available samples (2003-2006) per species (**Fig. 2**). For the tree data, these SCF estimates were also determined at 688 and 760 nm to correspond with the SIF in the O_2 lines. Since the average SCF688 was 10% lower than the red SC685 peak and the average SCF760 was 40% lower than the far-red F740 peak, these factors were applied to the individual SCF leaf spectra to obtain values at 688 and 760, which are summarized in **Table 1**.

Table 1. Comparison of steady state ChlF at 688 and 760 nm, the positions of the $O_2\alpha$ and $O_2\beta$ atmospheric features, derived from leaf and canopy techniques.

Species	Month	Year	Cab	Cm	Cw	PARre
Gum	May	2006	36.0 b	0.008 a	0.031 a	0.009 b
	Aug	2006	64.2 a	0.011 a	0.018 bc	0.007 d
Maple	May	2006	27.9 c	0.008 a	0.029 ab	0.011 a
	Aug	2006	38.2 b	0.010 a	0.013 c	0.007cd
Poplar	May	2006	35.5 b	0.021 a	0.015 c	0.010 b
	Aug	2006	38.7 b	0.007 a	0.013 c	0.008 c
$LSD_{.01}$(df=124)			4.93	0.0275	0.0122	0.0013

Within group column wise means with the same letter are not separable by $ANOVA_{LSD.01}$.

D. FluorMOD estimates and data analyses

The FluorMOD V3.1with graphic user interface [10] combines plant F with leaf and canopy radiative transfer equations to predict the spectral responses of vegetation, including leaf-level and top-of-canopy SIF. We used FluorMOD to simulate leaf-level SIF for the three tree species during May. The FluorMOD leaf SIF estimates (**Table 2**) were compared with our leaf-level SCF and canopy SIF estimates (**Fig. 3**). Measurements were evaluated using Systat V.10 and SAS V.9. We did not address N treatment effects on SIF in this report.

III. RESULTS AND DISCUSSION

The SIF field values retrieved from ASD canopy-level spectra acquired above tree saplings during four measurement periods from August 2005 to August 2006 are presented in **Fig. 1 (A-C)**. SIF688 estimates in those two years ranged between 1.1 - 2.9 ± 0.3 mW m^{-2} nm^{-1} sr^{-1}, and were significantly lower ($P<0.01$) for gum and maple in 2006 as compared to 2005. The highest SIF688 was observed in October when the foliage was undergoing senescence, although still visually green. SIF688 was similar for maple and poplar leaves in each experiment, but gum leaves produced higher SIF688 in 2005 than the other two

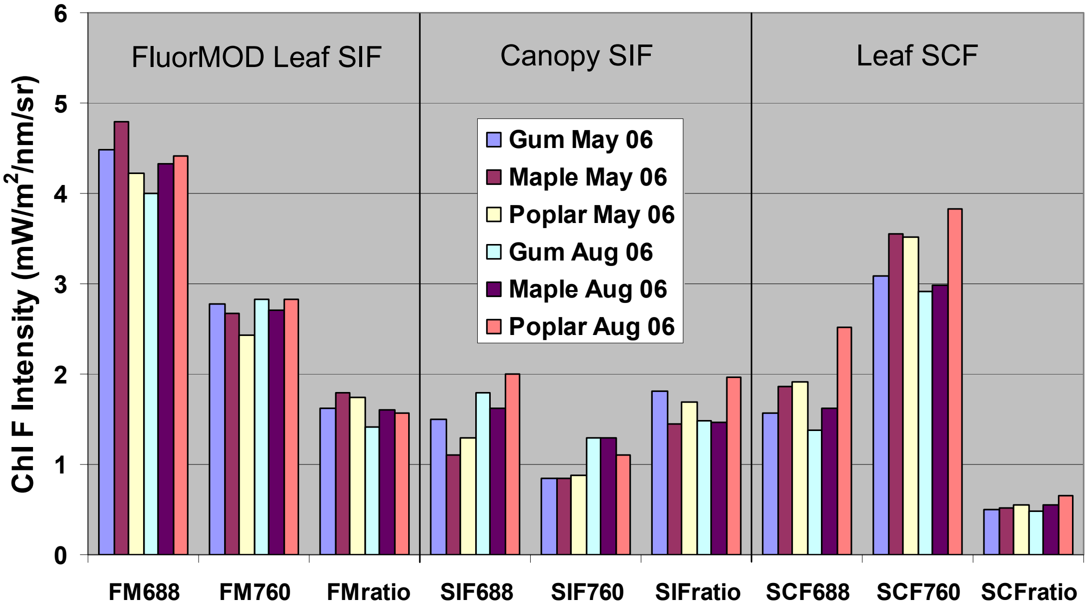

Fig. 3. The red, far-red, and red/far-red ratio for May and August 2006 data of 3 tree species (sweet gum, red maple, and tulip poplar) was computed at 688 and 760 nm, to match the wavelengths of the $O_2\alpha$ and $O_2\beta$ features.

species (**Fig. 1A**). SIF760 ranged between 0.5 - 1.3 ± 0.15 mW $m^{-2}nm^{-1}sr^{-1}$, with significantly higher values in August 2006 foliage. The SIF760 of poplar sapling canopies increased throughout the year, and was distinct from gum (but not maple) for 3 of 4 experiments. The red/far-red SIF ratio (SIFratio) ranged between 1.45 and 6.93 (± 0.75), for which values were significantly higher in 2005 for all species. The higher SIF values in 2005 compared to 2006 are consistent with greater environmental stress. 2006 was sunnier and drier than 2005: the mid-day seasonal PAR average (May-October) at USDA was 194 Wm^{-2} in 2005 and 202 Wm^{-2} in 2006; the total May-October precipitation was 566 mm (2005) vs. 548 mm (2006).

The leaf-level SCF laboratory estimates at the ChlF peaks obtained from solar corrected EEMs, averaged over N treatments for samples measured in 2004-2006, are shown (**Fig. 2**) for the six species examined: SCF685 (**Fig. 2A**) was between 1.5 – 3.0 (± 0.8-1.5) mW m^{-2} nm^{-1} sr^{-1}; whereas SCF760 (**Fig. 2B**) varied from 5-7 (± 1.0) mW m^{-2} nm^{-1} sr^{-1}. Averaged over the six species, ~52% of F685 was induced by solar wavelengths between 300-500 nm; these shorter wavelengths induced the largest F685 contributions in corn and poplar leaves (**Fig. 2A**). The contributions to F740 from EX wavelength intervals were more uniform for all species (**Fig. 2B**), and only ~30% was induced by wavelengths between 300-500 nm. Utilizing wavelengths on the shoulders of the ChlF peaks produces lower F values and different red/far-red ratios than at the ChlF peaks. Since on average, SCF688 was 10% lower than the red SC685 peak, and F760 was 40% lower than the far-red F740 peak, the red/far-red ratios computed for F688/F760 were 25% lower than the standard red/far-red ratio at the ChlF peaks (F6895/F740).

The 2005 and 2006 canopy-level SIF field values (SIF688, SIF760) for trees are compared with the comparable leaf-level SCF values (SCF688, SCF760) in **Table 1**. The average red F values are similar for leaf and canopy: 1.77 (SCF688) vs. 1.87 (SIF688) mW m^{-2} nm^{-1} sr^{-1}. The far-red values are much different in magnitude: 3.35 (SCF760) vs. 0.81 (SIF760) mW m^{-2} nm^{-1} sr^{-1}, with leaf laboratory values an average of ~4 times higher in intensity than field estimates. Therefore, the average field SIFratio is ~2.88 compared with 0.52 for the SCFratio. The similar information for 2006 data (only) is presented in **Fig. 3**, which also includes the estimates using FluorMOD-leaf. The average FluorMOD estimates for the tree leaves (May and August 2006 combined) were: red ChlF at 688 nm (FM688), 4.37 mW m^{-2} nm^{-1} sr^{-1}; far-red ChlF at 760 nm (FM760), 2.71 mW m^{-2} nm^{-1} sr^{-1}; and the red/far-red ratio (FMratio), 1.62. **Fig. 3** indicates that FluorMOD overestimated leaf red ChlF (FM688), as compared to SIF688 and SCF688. However, FluorMOD estimates of leaf far-red ChlF (FM760) and the red/far-red ratio (FMratio) were intermediate between those for SIF (SIF760, SIFratio) and SCF (SCF760, SCFratio), respectively. Further analysis will be pursued in the future with FluorMOD to attempt closer correspondence to field values, and also to examine the FluorMOD canopy SIF estimates. In additional experiments, we estimated average canopy SIF688 of 4.3 ± 1.0 mW m^{-2} nm^{-1} sr^{-1} and SIF760 of 2.0 ± 0.8 mW m^{-2} nm^{-1} sr^{-1} for a cornfield, under a relative PAR intensity of 1660 μmol m^{-2} s^{-1} [11-12,15-16]. These SIF values are higher than those obtained from the tree canopies, but this difference is likely due to the higher leaf area index and C_4 photosynthetic pathway of the corn crop.

Compared with measured or modeled leaf-level estimates, neither SIF values (SIF688, SIF760) appear to be overestimated, as is sometimes suggested for retrievals of ASD spectra with a 3.2 nm spectral resolution (and1.2 nm sampling interval). The lower canopy SIF760 and SIFratio values, as compared to leaf values, are due to many factors, including the effects of multiple scattering within tree crowns, the influence of the fluorescent leaf undersides [9], and the inclusion of non-foliar materials such as stems and grass background in the field-of-view. These lower SIF760 canopy retrievals relative to laboratory values produce higher red/far-red ratios (SIFratio vs. SCFratio). The SIFratio was the most successful for separating groups: gum and maple foliage exhibited statistically different SIFratios through the at 3 of 4 experiments; both maple and poplar had higher SIFratios in 2005 vs. 2006; and gum and maple foliage produced higher SIFratios in October than other times. The seasonal and interannual variations offer important functional information, to document that vegetation F is affected by phenology and environmental conditions.

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