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Chlorophyll Fluorescence Effects on Vegetation Apparent Reflectance: II. Laboratory and Airborne Canopy-Level Measurements with Hyperspectral Data

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Relationships found between Compact Airborne Spectrographic Imager (CASI) hyperspectral canopy reflectance measurements at laboratory and field levels with PAM-2000 chlorophyll fluorescence data are presented. This is a continuation of the paper where relationships at the leaf level between leaf reflectance and chlorophyll fluorescence were found and demonstrated to be consistent with theory using the Fluorescence-Reflectance-Transmittance (FRT) model. Experiments using the hyperspectral CASI sensor in the laboratory to observe a canopy of maple seedlings are performed as an intermediate step to demonstrate the link between the results at leaf-level and the CASI field canopy levels. Scene observations of the seedlings utilizing a long-pass blocking filter showed that apparent canopy reflectance in the laboratory is affected by changes in fluorescence emissions. A laboratory experiment on seedlings subjected to diurnally induced change shows the strong link between CASI canopy reflectance optical indices in the 680–690-nm region and Fv/Fm dark-adapted chlorophyll fluorescence. Stressed and healthy maple seedlings are used to demonstrate the use of optical indices calculated from the 680–690-nm spectral region to track changes in steady-state fluorescence: the curvature index $R683^2/(R675 \cdot R691)$ and the R685/R655 ratio calculated from the canopy reflectance are related to leaf-measured Ft, Fm′ and ΔF/Fm′ steady-state features, and are in agreement with theoretical simulations using the leaf Fluorescence-Reflectance-Transmittance model. To test these findings in a field setting, airborne field hyperspectral CASI data of 2-m spatial resolution, 7.5-nm spectral resolution, and 72 channels was used, collected in deployments over 12 sites of Acer saccharum *M. in the Algoma Region, Ontario (Canada) in 1997 and 1998. A field sampling campaign was carried out for biochemical contents of leaf chlorophyll and carotenoids, chlorophyll fluorescence, and leaf reflectance and transmittance. Leaf-level relationships obtained between optical indices and physiological indicators were scaled up to canopy level through canopy reflectance models using input model parameters related to the canopy structure and viewing geometry at the time of data acquisition. Results show that scaled-up optical indices in the 680–690-nm region are related to Fv/Fm chlorophyll fluorescence measured in the 20×20-m study sites. Consistency between leaf, laboratory, and field canopy hyperspectral data is shown in this and the previous paper, demonstrating the effect of fluorescence on observations of apparent vegetation reflectance.* ©*Elsevier Science Inc., 2000*

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INTRODUCTION

The development of remote sensing methods to measure chlorophyll fluorescence is currently receiving much attention [e.g., see *Rem. Sens. Environ.*, Vol. 47 (Jan.

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1994), special issue] due to its potential to yield insight into plant physiological functioning (e.g., Schreiber, 1983; Lichtenthaler and Rinderle, 1988; Rosema et al., 1998), and as a potential previsual indicator of plant stress (e.g., Chappelle et al., 1984). Measurement techniques have focused on active methods encompassing well-established, near-contact, pulse amplitude modulation (PAM) Fluorometers (Schreiber et al., 1986), Light Detection and Ranging (LIDAR)s in which measurements of fluorescence signal ratios are used to deduce fluorescence yield (Lichtenthaler and Rinderle, 1988; Gunther et al., 1991; Goulas et al., 1997), laser-spectrometer devices to provide diagnostic fluorescence excitation-emission signatures as introduced by Dudelzak et al. (1991), and τ-LIDARs, which measure fluorescence lifetime (Cerovic et al., 1996). In a number of recent investigations (e.g., McMurtrey et al., 1994) combined active and passive experiments are reported in which canopy reflectance signatures are collected along with the fluorescence signals, where the former is expected to yield information about plant canopy pigment or foliar status and the latter about plant physiological functioning.

For aquatic vegetation, observations of solar-induced natural fluorescence using passive remote sensing techniques have been reported for some time in the literature using spectrometers (Neville and Gower, 1977; Topliss and Platt, 1986), and more recently, a Fraunhofer line discriminator (Hu and Voss, 1997). Similar measurements for terrestrial vegetation canopies are more difficult due to the significant reabsorption of fluorescence emission by chlorophyll pigment, especially near the 680-nm level. Nevertheless, measurement of solar-induced natural fluorescence in terrestrial vegetation canopies has been reported by McFarlane et al. (1980), who measured solar-induced fluorescence in citrus canopies using the H-α Fraunhofer line at 656 nm. Similar measurements for leaves using the H-α and O_2-B lines were reported by Carter et al. (1990, 1996). On the other hand, experimental evidence of an observable solar-induced fluorescence signal superimposed on a terrestrial vegetation reflectance signature has remained speculative.

In the companion paper Zarco-Tejada et al. (2000) report leaf-level measurements and model simulation of the effects of chlorophyll fluorescence on leaf apparent spectral reflectance and transmittance. Those experiments primarily examined fluorescence effects for constant leaf chlorophyll pigment levels and suggested, through model simulation, optical indices that might be used to track fluorescence signals independent of pigment levels. In this paper we extend these findings to the canopy level where the bidirectional reflectance effects of canopy architecture and combined variations in leaf pigment and fluorescence are possible, as well as potentially confounding issues related to atmospheric correction. The scaling up from leaf level to canopy level is approached in two steps: first, in laboratory experiments using the Compact Airborne Spectrographic Imager (CASI) hyperspectral sensor and a canopy of maple seedlings, and second, through airborne CASI hyperspectral data combined with leaf-level chlorophyll fluorescence (Fv/Fm using the PAM-2000 Fluorometer) and chlorophyll content data obtained as part of the Bioindicators of Forest Sustainability Project (Mohammed et al., 1997; Sampson et al., 1998). Preliminary analysis of data from the latter field project, which involved 12 test sites of *Acer saccharaum* M.(sugar maple), show high correlation between CASI-derived canopy red edge reflectance indices and leaf fluorescence (Zarco-Tejada et al., 1999a; Zarco-Tejada et al., 1999b). The efficacy of an interpretation of these results as evidence for the remote observation of solar-induced fluorescence is explored in this paper.

OPTICAL INDICES FOR BIOINDICATORS OF FOREST CANOPY STATUS AND FUNCTION: CHLOROPHYLL CONTENT AND CHLOROPHYLL FLUORESCENCE

In the Bioindicators of Forest Sustainability Project (Mohammed et al., 1997; Sampson, et al., 1998) the primary objective is to develop links between physiologically based bioindicators (e.g., pigment concentrations, chlorophyll fluorescence) from field and laboratory data and optical indices from hyperspectral remote sensing for assessing forest condition.

Optical indices are based on observed relationships between reflectance at a specific wavelength and leaf pigments or photosynthetic functioning that are related directly or indirectly to conditions of stress.

A passive remote sensing approach is being used in this study in which observed canopy reflectance is primarily influenced by canopy pigment levels in conjunction with canopy architecture and viewing-illumination geometry. The investigation of fluorescence contributions to the observed signature requires attention to optical indices related specifically to fluorescence emission since indices related to pigment content have been the subject of extensive research, as summarized briefly below.

The chlorophyll content in leaves is potentially one of the most important indicators of vegetation strain. The total chlorophyll content in leaves decreases in stressed vegetation, changing the proportion of light-absorbing pigments and leading to less overall absorption. The absorption of electromagnetic radiation by this pigment varies with the wavelength, with strong absorption in the blue (400–500 nm) and red (600–700 nm) portions of the visible spectrum and relatively less absorption in the green (500–600 nm) portion. Differences in reflectance between healthy and stressed vegetation due to changes in pigment levels may be detected in the green peak and

along the red edge (690–750 nm; Rock et al., 1988; Vogelmann et al., 1993; Carter, 1994; Gitelson and Merzlyak, 1996).

Chlorophyll fluorescence (CF) has been shown to be a useful tool in identifying previsual strain. Specifically, changes in chlorophyll function frequently precede changes in chlorophyll content, hence changes in CF can be observed long before leaves become chlorotic. The technique also possesses the advantage of being rapid, nondestructive, and noninvasive (Mohammed et al., 1995). In particular, CF has often been used to investigate stress effects and recovery in plant tissues and in comparing the stress resistance of different populations. In the last 15 to 20 years, it has been used increasingly with forest tree species in studies of dormancy induction, cold hardiness, light acclimation, heat damage, water stress, disease effects, nutrient deficiencies, and forest decline (Mohammed et al., 1995).

Most studies related to optical indices for vegetation functioning are based on measurements made at the leaf level rather than at the canopy level, where correlation between chlorophyll fluorescence and spectral reflectance can be readily observed (Peñuelas et al., 1998; Gamon et al., 1997; Peñuelas et al., 1997; Gitelson et al., 1999; Gamon and Surfus, 1999). For example, the effective quantum yield of Photosystem II (PS-II) in the light, denoted $\Delta F/Fm'$, was shown to be related linearly to the Photochemical Reflectance Index, derived as PRI= (R531−R570)/(R531+ R570), in top canopy leaves of a wide range of species. Gamon et al. (1997) suggested that PRI could be used as an interspecific index of photosynthetic radiation-use efficiency for leaves and canopies in full sun, but not across wide ranges in illumination from deep shade to full sun. Further, they suggested that relative photosynthetic rates could be derived remotely if issues of canopy and stand structure could be resolved. Gitelson et al. (1999) showed that the inverse reflectance $(R_{700})^{-1}$ was an excellent predictor of leaf chlorophyll content, but that the apparent reflectance value was also significantly affected by fluorescence emission. Boochs et al. (1990) proposed the use of the inverse of the derivative ratio $D\lambda_p$/D703 as a plant vitality indicator.

Furthermore, indices related to fluorescence maxima at 685 nm and 740 nm are considered potentially useful, in addition to the newly identified spectral curvature index (Zarco-Tejada et al., 2000) to study the relationship of canopy reflectance with chlorophyll fluorescence [i.e., R685/R655, $R683^2$/(R675·R691), D730/D706], where D represents derivative spectra.

A summary of such potentially valuable optical indices from reflectance and derivative spectra, grouped into four categories based on the spectral region and the type of parameter used, is provided in Zarco-Tejada et al. (1999a, 1999b). A selection of those reflectance indices considered directly related to chlorophyll fluorescence is applied at leaf, CASI laboratory, and CASI canopy levels in this study.

LABORATORY EXPERIMENT METHODS AND MATERIALS

Laboratory Bidirectional Reflectance Factor Facility

CASI hyperspectral canopy reflectance measurements in the laboratory were made using a bidirectional reflectance factor (BRF) facility (Soffer, 1996), which is comprised of four subsystems: the CASI sensor, the illumination system, the canopy created by maple seedlings, and the mechanical system. The CASI sensor head unit was installed in the system sensor support arms at the altitude of 2.5 m from the canopy of plant material. The CASI was operated in a hyperspectral mode at maximum spectral resolution with 288 channels, spectral spacing of 1.8 nm, and nominal bandwidth of 2.5 nm, with f/2.0 aperture. Collimated illumination at 45° inclination was provided by a regulated 1,000-W halogen light source and a collimating lens. The raw 12-bit CASI data were calibrated to spectral radiance using radiance sensitivity factors (Gray et al., 1997) derived from the calibration methodology designed at York University and CRESTech (Miller et al., 1995). A translation table under the plant canopy attached to an electric motor with user-controlled speed enabled the collection of above-canopy spatial imagery with the CASI viewing a line oriented perpendicular to platform motion. A Spectralon reflectance panel placed on the moveable platform with the plant material so as to be viewed at the end of each CASI scene permitted nonuniformities between across-track pixels to be normalized by scaling each canopy pixel by the relative response of the Spectralon panel in the same position. Thus pixel responses were scaled to reflectance values by applying the known reference panel bidirectional reflectance factor values.

Measurement Protocols

A filter holder was custom-designed to permit a Schott RG695 (Melles Griot, Irvine, California) high-pass filter to be placed in front of the 1,000-W halogen light source to restrict incident radiant energy on the scene to $\lambda>705$ nm. This facilitated the collection of canopy reflectance measurements with CASI in the absence of fluorescence-generating radiation, similar to measurement protocols at the leaf level, as described in the companion paper (Zarco-Tejada et al., 2000). Canopy scene reflectance measurements were collected using the CASI first with the blocking filter and then without the filter to study canopy apparent reflectance signatures as the plant material made a transition from dark-adapted to steady-state illumination conditions.

Plant Material

Acer saccharum M. seed was collected from a single tree in Sault Ste. Marie, ON, in September, 1998. In November 1998, the seed was soaked in aerated deionized water for 24 hours and placed in stratification (at 2°C) for 60 days. On January 15, 1999 the seeds were sown in seven multipot 6-45 containers with 2:1 (peat:vermiculite) soil mix and placed in +2°C cooler and kept moist. The containers were placed in an outdoor shade house (50% of full sun) on April 6 and germination was complete in about 5 weeks. Seedlings were grown in the shade house at the Ontario Forest Research Institute until July 30. The containerized seedlings formed a vegetation canopy of 100×50 cm used for the CASI laboratory hyperspectral data collection carried out in the BRF facility.

Experiment Description

Fluorometer Fluorescence Measurements

Chlorophyll fluorescence measurements were carried out with a Pulse Amplitude Modulation (PAM-2000) Fluorometer as described in the companion paper.

Canopy Measurements of Apparent Reflectance

Hyperspectral CASI data were collected from the plant material using the Schott RG695 filter with dark-adapted plant material. The scene reflectance was reacquired without the blocking filter, allowing red light to reach the plant material, generating steady-state fluorescence.

Time-Decay Fluorescence in Apparent Canopy Reflectance

A time-decay fluorescence experiment was carried out to study the canopy reflectance change with time. Dark-adapted plant material was fixed motionless under the CASI sensor and consecutive hyperspectral data frames were collected over the same area of plant canopy for a full 3 minutes. A study of the variation in apparent reflectance during the 3-minute experiment was designed to track changes in fluorescence from the same plant material during this time period.

Diurnal Variation of Apparent Reflectance and Fluorescence

Fv/Fm measurements were collected with the PAM-2000 Fluorometer during a 1-day period to study relationships between changes in Fv/Fm and CASI optical indices. Maple seedlings were moved outside the laboratory to get direct solar illumination and then moved inside the laboratory to make measurements of Fv/Fm and execute a CASI scene data collection, and then moved back outside again. Eight CASI hyperspectral measurements were carried out during the day, between 8:30 A.M. and 9:30 P.M. Plants were dark-adapted for 15 minutes before each set of Fv/Fm readings.

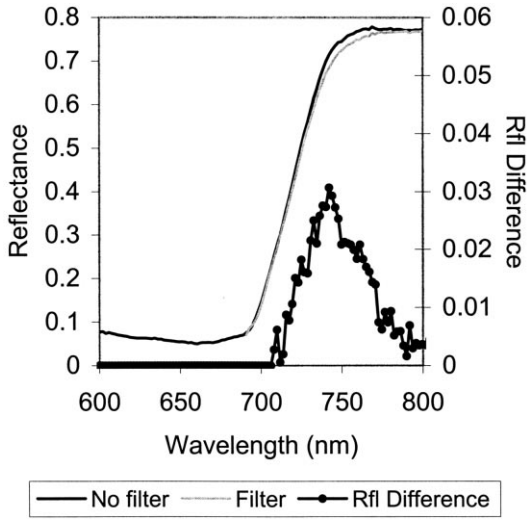


Figure 1. CASI canopy reflectance measurements of *Acer saccharum* M. seedlings in laboratory. Data were collected from the plant material using the Schott RG695 filter with dark-adapted plant material and then without the filter, thereby allowing red light to reach the plant material. A reflectance change in the 730–750-nm range can be detected due to the photosystem excitation by red light. The maximum reflectance difference of 3% is observed at 742 nm.

Reflectance Measurements of Steady-State Fluorescence in Healthy and Stressed Plant Material

The last laboratory experiment was intended to study differences in steady-state fluorescence between two trays of maple seedlings, one healthy and the other one in severe drought stress. CASI images and steady-state readings were collected for 3 hours, and optical indices from CASI data were studied for consistency with PAM-2000 steady-state readings and with the FRT leaf simulation model (Zarco-Tejada et al., 2000). Canopy reflectance from the plant material was determined by selecting pixels with the 30% highest values in the near-infrared (NIR; 850 nm), thereby minimizing effects of shadows and canopy openings, as performed in field CASI canopy reflectance extraction from *Acer saccharum* M. study sites (Zarco-Tejada et al., 1999a; Zarco-Tejada et al., 1999b).

LABORATORY EXPERIMENTAL RESULTS

Canopy Measurements of Apparent Reflectance and Fluorescence

Results in Fig. 1 show that changes in canopy apparent the reflectance from targeted plant material are observed when the Schott 695-nm blocking filter is used. This effect is evident at 730 nm to 750 nm, and is most pronounced at 742 nm. These findings are generally consistent with results at leaf level, indicating that canopy ap-

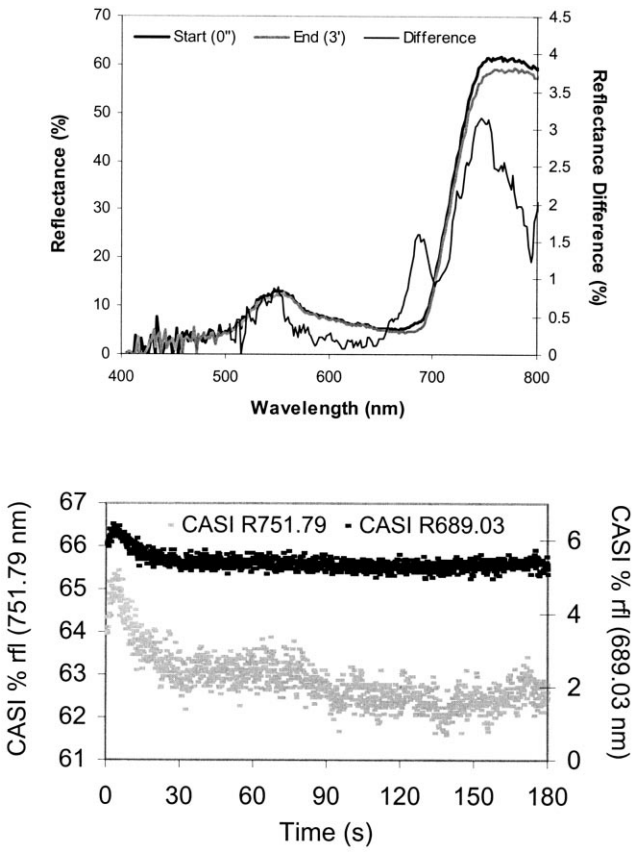


Figure 2. Time-decay fluorescence in apparent canopy reflectance. Top plot shows the CASI reflectance measurements from *Acer Saccharum* M. seedlings in the laboratory taken after dark adaptation and after 3 minutes of illumination. Differences in reflectances at 680–690 nm and 730–750 nm are observed due to changes in chlorophyll fluorescence. Changes at 530–550 nm can also be detected, consistent with photosynthetic radiation use efficiency changes as described by Gamon et al. (1997). Bottom plot shows the variation of CASI bands 751.8 nm and 689 nm over the same target during the 3-minute period.

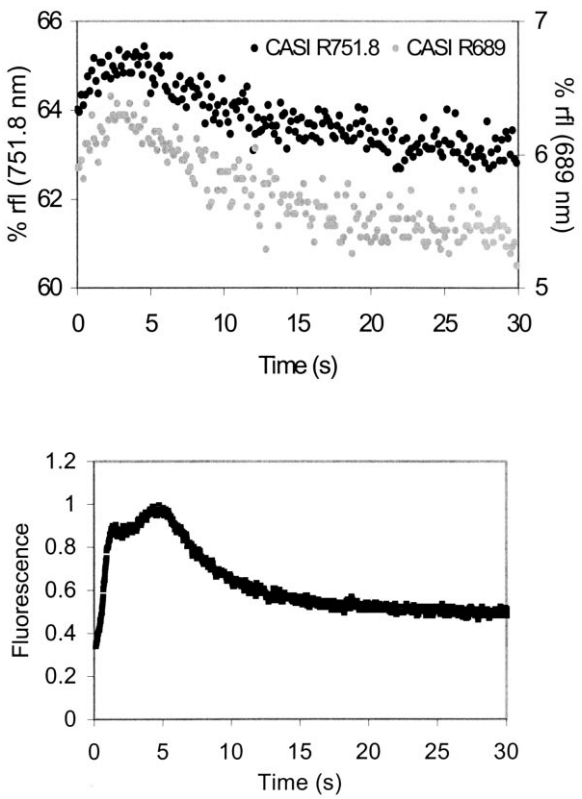


Figure 3. Time-decay fluorescence in apparent canopy reflectance during the first 30 seconds of the study. Top plot shows the CASI reflectance bands 751.8 nm and 689 nm from the canopy of *Acer Saccharum* M. seedlings in laboratory taken after dark adaptation. Differences in the reflectance bands are associated to changes in chlorophyll fluorescence. Bottom plot shows the Kautsky curve from one leaf of the canopy measured with PAM-200 Fluorometer. Both CASI apparent reflectance and Kautsky curve show similar behavior.

parent reflectance is affected by chlorophyll fluorescence. The apparent lack of effect at 690 nm is due to the Schott RG695 cut-off filter, which does not allow a comparison of reflectance at wavelengths less than 695 nm.

Time-Decay Fluorescence in Apparent Canopy Reflectance

The maple seedling canopy was kept in a fixed position during 3 minutes of CASI data acquisition in the 72-channel (7.5-nm bandwidth) mode of operation. Changes in the CASI reflectance bands affected by chlorophyll fluorescence in this time-decay experiment can be seen in Fig. 2. Changes at 680–690-nm and 730–750-nm spectral regions can be seen clearly if we compare the reflectance measurement at the start of and at the end of 3 minutes of illumination. Changes of reflectance bands affected by chlorophyll fluorescence are also shown for R751.8 and R689 nm.

The first 30 seconds of reflectance variation (Fig. 3) show a temporal decay of CASI reflectance at 751.8 nm and 689 nm similar to the behavior of the Kautsky curve measured (using the PAM-2000 Fluorometer) from the same leaf. No changes in reflectance were found during the 3-minute experiment in bands that are not associated with fluorescence emissions, such as at R553 nm.

Diurnal Variation of Apparent Reflectance and Fluorescence

Results in this diurnal study show that optical indices in the 680–690-nm region track changes in Fv/Fm, R680/R630, R685/R630, R687/R630, and R690/R630, achieve determination coefficients r^2=0.93, r^2=0.94, r^2=0.92, and r^2=0.91, respectively. Indices sensitive to changes in the reflectance curvature in the 675–690-nm region that were observed previously (Zarco-Tejada et al., 2000) also

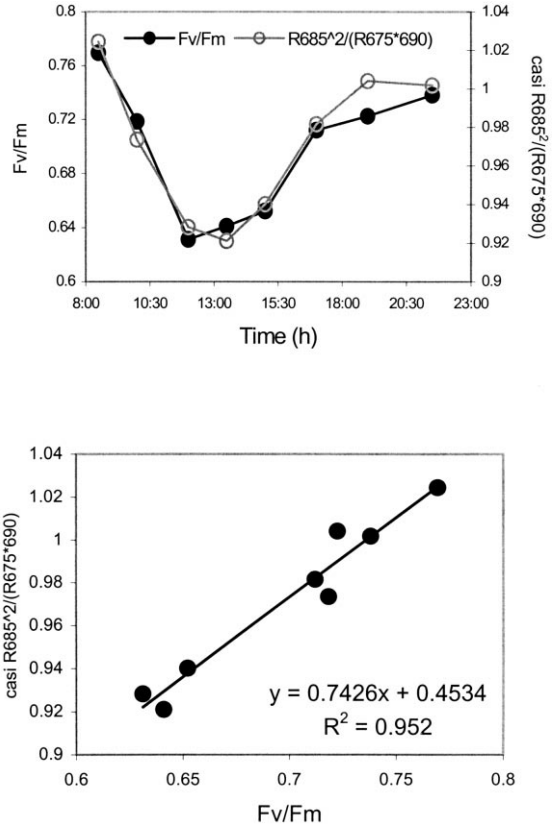


Figure 4. Diurnal variations of Fv/Fm and the optical index $R685^2/(R675 \cdot 690)$ calculated from CASI canopy reflectance in laboratory using *Acer sacharum* M. seedlings. The behavior of CF during the day is tracked by the optical index derived from CASI reflectance, achieving $r^2=0.95$. Maple seedlings were moved inside the laboratory to make measurements of Fv/Fm and CASI reflectance, keeping the seedlings outside between measurements. Eight measurements were carried out from 8.30 A.M. to 9:30 P.M. with plants dark-adapted for 15 minutes prior to readings of Fv/Fm.

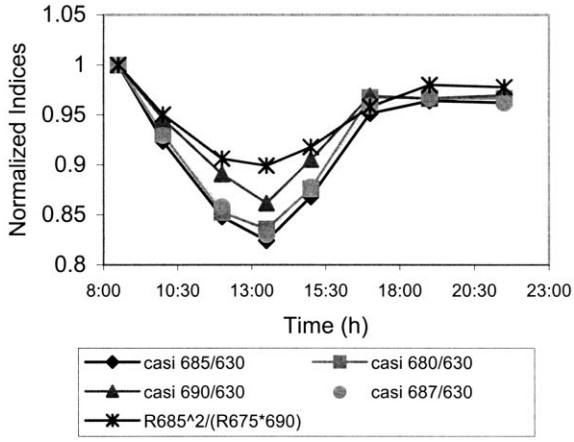


Figure 5. Comparison of R685/R630, R690/R630, R680/R630, R687/R630, and $R685^2/(R675 \cdot 690)$ normalized to the first measurement for comparison purposes. R685/R630 varies up to 17% during the day, while the percentage variation of $R685^2/(R675 \cdot 690)$ is 10%. All indices show similar behavior as expected for changes in the 680–690-nm region due to fluorescence.

show correlation with diurnal changes in Fv/Fm [i.e., $R685^2/(R675 \cdot 690)$] (Fig. 4) yields $r^2=0.95$.

The diurnal variation range of each one of the indices was also studied. Figure 5 shows the four indices normalized to the first image so that their ranges of variation can be compared. The plot shows that R685/R630 varies up to 17% during the day, while the percentage variation of $(R685^2)/(R675 \cdot 690)$ is 10%. All the indices tested in the 680–690-nm region vary similarly, with comparable behavior and showing a direct relationship with Fv/Fm as shown in Fig. 4.

The behavior of the indices is consistent with the theoretically expected variation: in the morning we get high values of Fv/Fm, therefore high values of F685–F690. R690/R630 should therefore decrease during the day and then recover at night.

Potential variations in these optical indices due to changes in the NIR or inconsistencies between the collected CASI images were examined relative to the behavior of normalized Fv/Fm (Fig. 6). It is shown that variations in the R680–R690-nm region (R690/R630) are independent of changes in the NIR (R850) and R650/R500, where no response to chlorophyll fluorescence is expected.

A study of the optical index R690/R630 using the FRT model (see companion paper) shows that R690/R630 behaves as expected by theory. Figure 7 shows the predicted R690/R630 by the FRT model and the index calculated from CASI data, yielding a high determination coefficient ($r^2=0.93$) in the relationship between the predicted and calculated index.

No relationship was found between fluorescence and the CASI optical index R750/R710, also in agreement with simulation based on the leaf FRT model that will be discussed in next section.

Reflectance Measurements of Steady-State Fluorescence in Healthy and Stressed Plant Material

CASI reflectance measurements over healthy and stressed plant material were collected in the laboratory (Fig. 8), where PAM-2000 derived $\Delta F/Fm'$, Ft, and Fm' data and optical indices can be compared. Direct relationship between steady-state fluorescence and dark-adapted Fv/Fm should be found, as reported in the companion paper, where diurnal variations of Fv/Fm were directly related to $\Delta F/Fm'$, Ft, and Fm'. Optical indices $R683^2/(R675 \cdot R691)$ and R685/R655, sensitive to changes

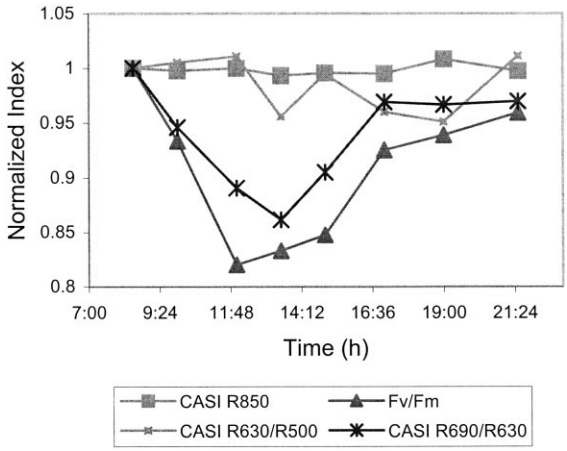


Figure 6. Variations in R680–R690-nm region (R690/R630) are independent of changes in the NIR (R850) and R650/R500, where no response to chlorophyll fluorescence is expected. Variations in these optical indices are not due to changes in the NIR or inconsistencies between the collected CASI images. The diurnal behavior of R690/R630 is similar to Fv/Fm.

in chlorophyll fluorescence, were studied at this intermediate level at the laboratory between leaf and field canopy.

Results show that the two trays with maple seedlings with different stress status show differences in $\Delta F/Fm'$, Fm', and Ft steady-state features. The indices behave consistently with the model and with previous experiments: indices calculated from the 680–690-nm region, such as R685/R655 and $R683^2/R675 \cdot R691$, show consistent behavior with the other two experiments in this paper. Indices show lower values in the stressed than in the healthy plant material. This is also consistent with results predicted by the FRT model (Table 1).

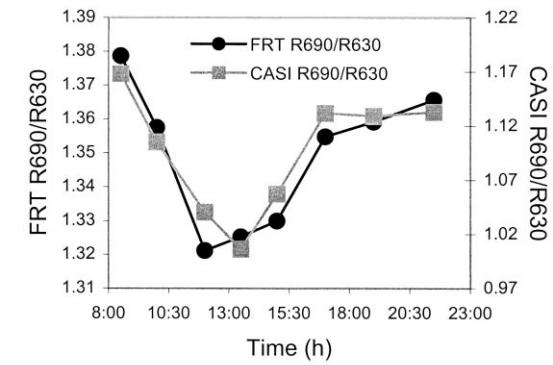
Table 1 shows four different stress situations, two of them at the extremes: stressed (low photochemical efficiency Fv/Fm, low chl $a+b$), healthy (high photosynthetic efficiency Fv/Fm, high chl $a+b$). The other two are intermediate stress. This simulation helps us to understand the experimental results obtained and demonstrate that they are consistent with theory. When the plant is stressed (photosynthetic efficiency, Fv/Fm=0.5, chl $a+b$=15) we expect lower R685/R655 values than when the plant is healthy (Fv/Fm=0.8, chl $a+b$=50). This is confirmed in the experimental results. R750/R710 is shown in the FRT simulation as less sensitive to changes in CF. Model simulation of R750/R710 predicts a change from 1.6 (chl $a+b$=15, Fv/Fm=0.5, Fv/Fm=0.8) to 1.9 (chl $a+b$=50, Fv/Fm=0.5, Fv/Fm=0.8). That is, R750/R710 is primarily affected by changes in chl $a+b$, and to a smaller degree, by changes in CF. Results in this experiment show that R750/R710 is able to differentiate between the two stress conditions, due to their different values of chl $a+b$ (SPAD=20→≈15 chl $a+b$ $\mu g/cm^2$; SPAD=35→≈35 chl $a+b$ $\mu g/cm^2$). According to the FRT model simulation, the CASI R750/R710 from the stressed plants shows lower values than the healthy plants, with the effect due to both changes in chl $a+b$ and CF.

MATERIALS AND METHODS FOR FIELD CANOPY STUDY

Field Experiment Using Hyperspectral Airborne Data

CASI data were collected in deployments over twelve sites of *Acer saccharum* M. in the Algoma Region, Ontario (Canada), in 1997 and 1998. A field sampling campaign was carried out for biochemical analysis of leaf chlorophyll and carotenoid concentrations, and fluorescence along with leaf reflectance and transmittance within the same period of the field data acquisition (Zarco-Tejada et al., 1999a; Zarco-Tejada et al., 1999b). Mean reflectance values per plot were calculated from the imagery in each *Acer saccharum* M. study site of

Figure 7. Study of the optical index R690/R630 modeled by the FRT model (companion paper) and calculated from CASI canopy reflectance. Plot shows that predicted R690/R630 by the FRT model and the index calculated from CASI data behave consistently. The linear relationship between the predicted and calculated index is shown, achieving r^2=0.93.



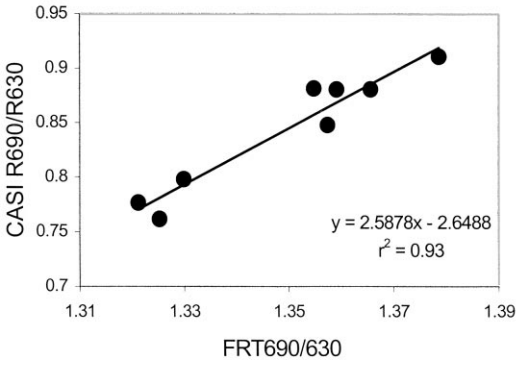


Table 1. FRT Model Simulation of R750/R710 and R685/R655 for Four Different Chlorophyll and Fluorescence Values, with Two Considered the Stress Extremes: (i) Stressed (Low Photochemical Effeciency Fv/Fm, Low Chl $a+b$); (ii) Healthy (High Photochemical Efficiency Fv/Fm, High Chl $a+b$)

FRT Simulation	*Efficiency Fv/Fm*	*Chl a+b*	*R750/R710*	*R685/R655*
Stressed	0.5	15	1.64	1.19
	0.8	15	1.63	1.24
	0.5	50	1.91	1.24
Healthy	0.8	50	1.90	1.35

In stressed conditions (photochemical efficiency=0.5, chl $a+b$=15), results show lower R685/R655 values than when the plant is healthy (photochemical efficiency Fv/Fm=0.8, chl $a+b$=50). In the FRT simulation R750/R710 is not sensitive to changes in photochemical efficiency: when efficiency changes, R750/R710 varies from 1.6 (chl $a+b$=15, Fv/Fm=0.5, Fv/Fm=0.08) to 1.9 (chl $a+b$=50, Fv/Fm=0.5, Fv/Fm=0.8). This shows that R685/R655 is affected by both changes in chl $a+b$ and CF, and it is sensitive to variations in CF when chl $a+b$ is constant.

20×20 m. CASI data were acquired in the hyperspectral reflectance mode, with 2-m spatial resolution and 72 spectral channels (7.5-nm spectral bandwidth). The mean reflectance per plot was calculated selecting the 25% of pixels with highest reflectances in the NIR, therefore targeting crowns while minimizing the influence of shadows, canopy openings, and the direct understory reflectance.

LAI measurements were acquired for all the plots using a PCA Li-Cor 2000 instrument. A total of 440 single leaf samples were collected at 12 *Acer saccharum* M. sites for biochemical analysis and measurement of leaf chlorophyll, carotenoid concentrations, and fluorescence. The ratio of variable to maximum chlorophyll fluorescence (Fv/Fm), a measure of photosynthetic efficiency (Mohammed et al., 1995) was measured in all leaf samples. Single leaf reflectance and transmittance measurements were acquired on all leaf samples using a Li-Cor 1800-12 Sphere (LI-COR, Inc., Lincoln, NE, USA) apparatus with an Ocean Optics fiber spectrometer (Ocean Optics Inc., Dunedin, FL, USA) with 0.5-nm spacing and 7.5-nm spectral resolution in the 340–860-nm range.

Study Sites Description

Twelve study sites of *Acer saccharum* M. (sugar maple) were selected in 1997 from existing provincial plot networks in the Algoma Region, Ontario (Sault Ste. Marie and the surrounding area). The sites were selected to represent a range of productivity and decline. In particular, six permanent sample plots from the provincial Growth & Yield Program (Anonymous, 1993; Hayden et al., 1995) were chosen to investigate the effects of stand productivity in maple. Another six plots were selected from the provincial Hardwood Forest Health network (McLaughlin et al., 1992; McLaughlin et al., 1999) to represent a gradient in maple forest decline. Detailed stand records exist and these sites are considered representative of tolerant hardwood forests in the Algoma Region.

Leaf Sampling Scheme

Two sampling collections were carried out in June and July 1998, collecting from the top of the crowns at each one of the twelve 30×30-m Sugar Maple study sites. Four leaves per tree with five trees per study site were sampled for measurements of chlorophyll pigments $a+b$, total carotenoids, chlorophyll fluorescence, and spectral measurements of reflectance and transmittance. The methodologies for measurement of leaf pigments and fluorescence derived from the PAM-2000 Fluorometer are described in our companion paper (Zarco-Tejada et al., 2000). Leaf reflectance and transmittance measurements were acquired from the same leaf samples to be able to use ρ and τ as inputs in the canopy reflectance models. Scaling up from leaf level to canopy level requires leaf reflectance and transmittance data as input to canopy reflectance models. Reflectance and transmittance measurement techniques are also described in detail in the companion paper.

Remote Sensing Data Acquisition

The above-canopy data acquisition using the CASI sensor was divided into three missions each with a specific sensor mode of operation: the mapping mission, with 0.5-m spatial resolution and 5 spectral bands; the hyperspectral mission, with 2-m spatial resolution, 72 channels, and 7.5-nm spectral resolution; and the full-spectral hyperspectral mission, with 288 channels and 2.5-nm spectral resolution. The 12-bit radiometric resolution data collected by CASI was processed to at-sensor radiance using calibration coefficients derived in the laboratory by CRESTech. Aerosol optical depth data at 550 nm were collected using a Micro-Tops II sunphotometer (Solar Light Co. Inc., Philadelphia, USA) in the study area at the time of data acquisition to process image data to ground reflectance using the CAM5S atmospheric correction model (O'Neill et al., 1997). Reflectance data were georeferenced using GPS data collected onboard the aircraft. Final registration of the hyperspectral mode

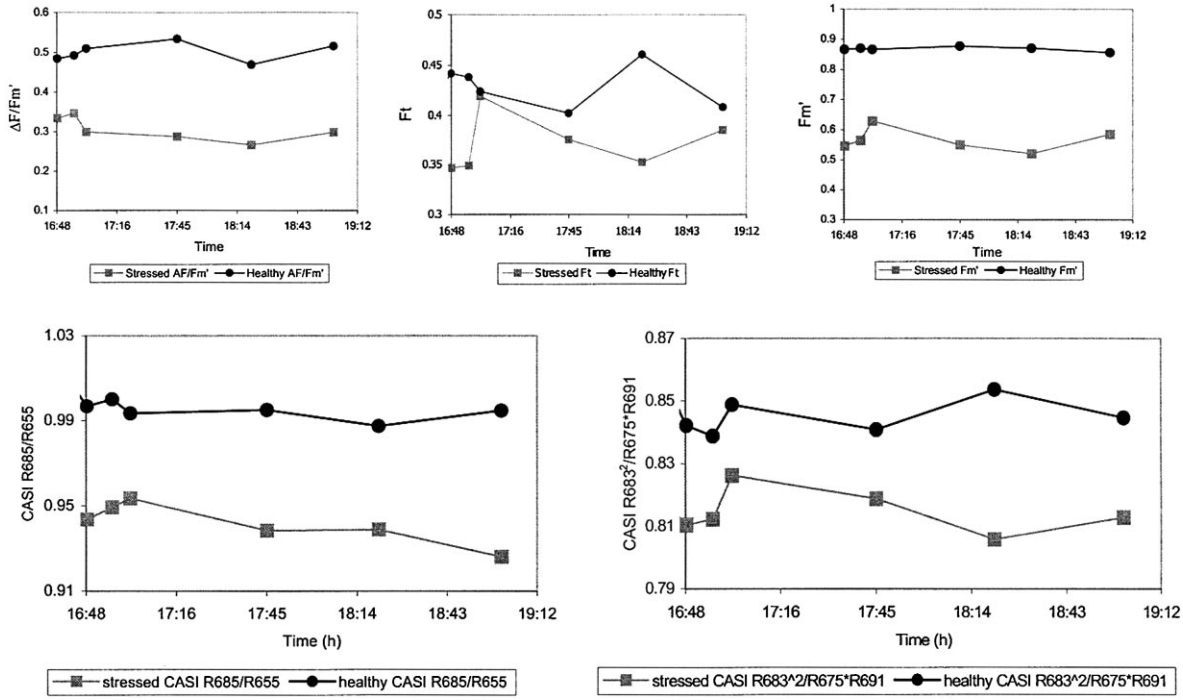


Figure 8. CASI canopy reflectance measurement over healthy and stressed seedlings of *Acer Saccharum* M. in laboratory, where PAM-2000 fluorescence measures ΔF/Fm′, Ft, and Fm′ (top) and reflectance optical indices (below) are compared. The two trays with maple seedlings with different stress status show differences in ΔF/Fm′, Fm′, and Ft steady-state features. The indices behave consistently with the model and with previous experiments: indices calculated in the 680–690-nm region, R685/R655 (bottom left) and $R683^2/R675{\cdot}R691$ (bottom right) show consistency with the other two experiments in this paper. The two indices show lower values in the stressed than in the healthy plant material.

imagery was achieved by registration to the CASI mapping-mission imagery using visual identification of ground-referenced 1-m white targets, which served to accurately identify the location of the sites.

Scaling Up Leaf-Level Relationships to Above-Canopy Level

The laboratory measurements of leaf reflectance and transmittance (440 samples) and the corresponding bioindicator data (pigment content and fluorescence Fv/Fm) permit a selection of candidate optical indices with strong correlation to the leaf-level bioindicators (Zarco-Tejada et al., 1999a; Zarco-Tejada et al., 1999b). However, for airborne optical spectral data to be used to predict bioindicator values at the study sites, the leaf level relationships need to be scaled up to the canopy level. The approach adopted (Zarco-Tejada et al., 1999a; Zarco-Tejada et al., 1999b) was to use leaf-level reflectance and transmittance data to calculate the corresponding above-canopy reflectance through optically thick vegetation (infinite reflectance R_∞) formulae or through canopy reflectance models (Kuusk and SAIL). Thereby, the leaf-level-measured bioindicators can be associated with the corresponding simulated canopy-level optical indices, providing prediction algorithms for leaf chlorophyll content and fluorescence Fv/Fm. This prediction algorithm, when applied to CASI-observed above-canopy reflectance spectra (where the high spatial resolution airborne data is screened to preferentially select tree crown pixels to increase the applicability of such simple formulae or models), yield estimates of site-averaged values of leaf chlorophyll content and fluorescence Fv/Fm. An accuracy assessment is now possible by comparing the in-field measured bioindicators at each site with the CASI-derived estimates.

A number of infinite reflectance (R_∞) formulae may be useful to represent the tree crown reflectance spectral content with respect to retrieving optical indices. The infinite reflectances $R_{\infty 1}$ and $R_{\infty 2}$ correspond to optically thick stacks of leaves in which multiple reflectance between leaves is ignored ($R_{\infty 1}$) (Lillestaeter, 1982) and in which multiple scattering is included ($R_{\infty 2}$), derived using the matrix formulation of Fujimura (Yamada and Fujimura, 1991; Miller et al., 1992). Infinite reflectance ($R_{\infty 3}$) characterizes the optically thick medium with the single-leaf absorption and scattering properties and assumes isotropic scattering (Hapke, 1993).

The formulae for these thick canopy reflectances are shown in Eq. (1), Eq. (2), and Eq. (3):

$$R_{\infty 1} \text{ approximate leaf stack} \rightarrow R_\infty = \frac{r}{1-t^2} \qquad (1)$$

Table 2. Determination Coefficients Obtained in Chl *a+b* and Fv/Fm Estimations Applying Relationships from SAIL and Kuusk CR Models to Hyperspectral CASI Data Collected over *Acer saccharum* M. Study Sites, Algoma, Ontario, Canada

Optical Index	r^2 (*Chl* a+b/*cm*^{2})	r^2 (*CF Fv/Fm*)
D730/D706	0.49	0.81
DP21 ($D_{\lambda p}$/D703)[a]	0.4	0.83
Curvature R683^2/(R675·R691)	0.18	0.7
R685/R655	–	0.52
PRI (R570−R539)/(R570+R539)	–	0.4

[a] DP21 ($D_{\lambda p}$/D703) is defined as the derivative of the reflectance at the inflection point in the red edge spectral region ($D_{\lambda p}$) over the derivative at 703 nm (D703).

$$R_{\infty 2} \text{ leaf stack} \rightarrow R_\infty = \frac{r}{1-\frac{2t^2}{1+(1-4t^2)^{1/2}}} \qquad (2)$$

$$R_{\infty 3} \text{ thick leaf} \rightarrow R_\infty = \frac{1-a^{1/2}}{1+a^{1/2}} \qquad (3)$$

In a more comprehensive approach, the single leaf reflectance and transmittance data collected from the ground-truth deployment were used to derive above-canopy level reflectances through SAIL (Verhoef, 1984) and Kuusk (Kuusk, 1996) canopy reflectance models. Optical indices calculated from the simulated above-canopy reflectance were therefore a function of the canopy structure and viewing geometry. Nominal model input parameters derived from the study areas were adopted: leaf area index (LAI)=3.5, plagiophile leaf angle distribution function (LADF), soil reflectance data derived from CASI imagery, and model-estimated skylight irradiance fraction based on conditions during airborne acquisitions. Additional parameters needed in the Kuusk model were n=1.4, sl=0.007, and θ^*=40°, and ε=0.95 and θ_m=45° for the LADF for the assumed plagiophile leaf distribution function. The relative insensitivity of the selected optical indices to departure of site characteristics from nominal values was confirmed in a sensitivity study (Zarco-Tejada et al., 1999b).

FIELD CANOPY STUDY RESULTS

Results Using Hyperspectral Airborne Data

The determination coefficients, shown in Table 2, between measured chlorophyll *a+b* and leaf fluorescence Fv/Fm values and the corresponding estimated values are derived by applying leaf optical index relationships to the canopy-level CASI spectral reflectance data for the study sites through simulation with infinite reflectance R_∞ models and the SAIL and Kuusk canopy reflectance models. The results are arranged according to decreasing success of specific optical indices to predict site averages of leaf fluorescence Fv/Fm, but also show the corresponding determination coefficients for site average leaf chlorophyll *a+b* pigment content to address the primary hypothesis of these two papers. A more detailed description of the experimental results from this field study with a wide range of optical indices will appear in a subsequent publication. From Table 2 it can be seen that several optical indices were found to exhibit a strong determination coefficient r^2 with Fv/Fm at the canopy level with CASI data. These include red edge spectral derivative indices D730/D706 and DP21, with r^2 = 0.81 and 0.83, respectively (which, however, also show correlation with chlorophyll *a+b* content at r^2 = 0.49 and 0.4, respectively). These indices appear to be responding to both changes in Chl *a+b* and in Fv/Fm, which were found to covary to some extent (r^2=0.52) for these 12 sites. However, the curvature index R683^2/R675·R691, R685/R655, and PRI determination coefficients for Chl *a+b* pigment are very low, although their relationships to Fv/Fm appears to have some predictive basis, particularly for the spectral curvature index. These results appear to be consistent with results reported earlier in this paper.

Estimation of chl *a+b*/cm^2 and Fv/Fm from an optical index progressively improves as indices are scaled up using $R_{\infty 1}$, $R_{\infty 2}$, and $R_{\infty 3}$, through to the SAIL and Kuusk CR models (Zarco-Tejada et al., 1999a). For all indices used, the estimations improve (linear regression slope progressively approaches unity) when the optical indices are calculated using first R_∞ and then canopy reflectance (CR) models. Figure 9 shows the relationship between the estimation of Fv/Fm using the CASI curvature index R683^2/(R675·R691) and Fv/Fm. Figure 10 shows the relationship between the estimation of Fv/Fm using the index DP21 ($D_{\lambda p}/D_{703}$) and Fv/Fm. It can be seen that R683^2/(R675·R691) is not affected by simulations that take into account the canopy structure or the viewing geometry, while the simulation using a derivative index such as DP21 ($D_{\lambda p}/D_{703}$) improves when canopy structure and viewing geometry are considered. Derivative indices were shown not to be so sensitive to canopy structure and viewing geometry as structural VIS/NIR indices (Zarco-Tejada et al., 1999b): the comparison between R683^2/(R675·R691) and DP21 ($D_{\lambda p}/D_{703}$) indicates that the curvature index is not influenced when structure or viewing geometry are accounted for by the canopy reflectance models. The 680–690-nm spectral region, with maximum chlorophyll absorption, is relatively unaffected

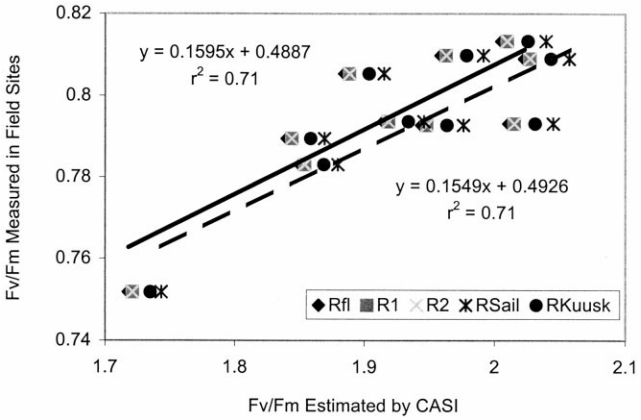


Figure 9. Estimation of Fv/Fm from CASI data using the $R683^2/(R675 \cdot R691)$ algorithm developed at leaf level (Rfl) through R_∞ (R1 and R2) and CR (SAIL and Kuusk) simulation models (RSAIL and RKuusk). The data correspond to *Acer saccharum* M. study sites. Estimations are not affected by the simulation approach whether the optical index is calculated using R_∞ (dashed line) or the canopy reflectance (CR) models (solid line).

by the structure of the canopy and viewing geometry; therefore, canopy models are not superior to infinite reflectance models or to the relationships obtained directly from leaf reflectance without simulation of canopy density or architecture effects.

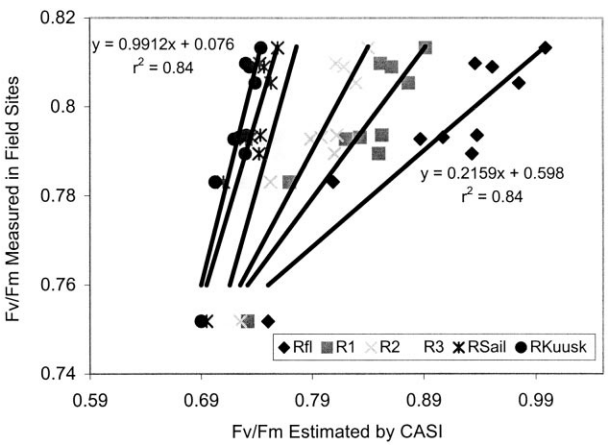
CONCLUSIONS

Canopy reflectance optical indices from hyperspectral data related to chlorophyll content and chlorophyll fluorescence have been studied at three different levels in these two papers. The effect of chlorophyll fluorescence on leaf-level spectral reflectance and transmittance measurements has been confirmed and measured and is shown to be in agreement with theory through a FRT simulation model (described in the companion paper). Laboratory hyperspectral reflectance measurements from a canopy of maple seedlings verify a quantitative link between canopy reflectance and chlorophyll fluorescence when optical indices in the 680–690-nm region are used. Laboratory experiments, using alternately a Schott blocking filter that cuts off the red light and a halogen lamp, demonstrate that canopy reflectance is affected by photosystem II excitation. Diurnal variation of Fv/Fm in maple plant material was shown to be strongly correlated with indices in the 680–690-nm region, such as R685/R655 and the curvature index $R683^2/(R675 \cdot R691)$, among others. A time-decay experiment also showed at laboratory level that canopy reflectance is affected by changes in chlorophyll fluorescence when dark-adapted plant material is illuminated with light over a 3-minute period. Consistency with previous experiments at leaf level and with theory using the FRT model is found when canopy optical indices are calculated from stressed and healthy plant material at the laboratory level. It shows that indices from the 680–690-nm region are directly related to steady-state fluorescence Ft, Fm′, and ΔF/Fm′ measured with a PAM-2000 Fluorometer.

Results at field canopy level from airborne CASI hyperspectral sensor demonstrate consistency with leaf and laboratory levels, as well as with theory. These results further suggest that leaf-level measurements of pigments and fluorescence along with leaf reflectance and transmittance can be used to produce algorithms to estimate these variables from above-canopy spectral reflectance. The studies at three scales, which progressively more closely represent the observed above-canopy reflectance spectra from the sites, show improvements in the estimation of leaf-based physiological indicators, such as chlorophyll *a*, chlorophyll *a*+*b*, carotenoids, and Fv/Fm chlorophyll fluorescence.

These results provide some evidence that hyperspectral sensors may offer a means to track changes in solar-induced fluorescence in vegetation canopies, as an added capability to its maturing role as a sensor to determine canopy pigment content levels. Further research will be needed to determine whether inference of natural chlorophyll fluorescence using such methods can be done with useful accuracy and the extent to which confounding factors that plague passive optical measurements can be adequately minimized.

Figure 10. Estimation of Fv/Fm from CASI data using the DP21 ($D_{\lambda i}/D_{703}$) algorithm developed at leaf level (Rfl) through R_∞ (R1, R2, and R3) and CR (SAIL and Kuusk) simulation models (RSAIL and RKuusk). The data corresponds to *Acer saccharum* M. study sites. It can be seen that estimations improve (linear regression slope progressively approaches unity) when the optical indices are calculated using first R_∞ and then canopy reflectance (CR) models.



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