ELSEVIER

Remote Sensing of Environment 84 (2003) 283–294

Remote Sensing of Environment

www.elsevier.com/locate/rse

Steady-state chlorophyll *a* fluorescence detection from canopy derivative reflectance and *double-peak* red-edge effects

P.J. Zarco-Tejada [a,*], J.C. Pushnik [b], S. Dobrowski [a], S.L. Ustin [a]

[a] *Center for Spatial Technologies and Remote Sensing (CSTARS), Department of Land, Air and Water Resources (LAWR), Veihmeyer Hall, University of California at Davis, Davis, CA 95616, USA*

[b] *Department of Biological Sciences, California State University, Chico, 400 West First Street, Chico, CA 95929, USA*

Received 4 April 2002; received in revised form 19 July 2002; accepted 27 July 2002

Abstract

A series of experiments carried out in a controlled environment facility to induce steady-state chlorophyll *a* fluorescence variation demonstrate that natural fluorescence emission is observable on the derivative reflectance spectra as a *double-peak* feature in the 690–710 nm spectral region. This work describes that the unexplained *double-peak* feature previously seen on canopy derivative reflectance is due entirely to chlorophyll fluorescence (CF) effects, demonstrating the importance of derivative methods for fluorescence detection in vegetation. Measurements were made in a controlled environmental chamber where temperature and humidity were varied through the time course of the experiments in both short- and long-term trials using *Acer negundo* ssp. californium canopies. Continuous canopy reflectance measurements were made with a spectrometer on healthy and stressed vegetation, along with leaf-level steady-state fluorescence measurements with the PAM-2000 Fluorometer during both temperature–stress induction and recovery stages. In 9-h trials, temperatures were ramped from 10 to 35 °C and relative humidity adjusted from 92% to 42% during stress induction, returning gradually to initial conditions during the recovery stage. Canopy reflectance difference calculations and derivative analysis of reflectance spectra demonstrate that a *double-peak* feature created between 688, 697 and 710 nm on the derivative reflectance is a function of natural steady-state fluorescence emission, which gradually diminished with induction of maximum stress. Derivative reflectance indices based on this *double-peak* feature are demonstrated to track natural steady-state fluorescence emission as quantified by two indices, the *double-peak* index (DPi) and the area of the *double peak* (A_{dp}). Results obtained employing these *double-peak* indices from canopy derivative reflectance suggest a potential for natural steady-state fluorescence detection with hyperspectral data. Short- and long-term stress effects on the observed *double-peak* derivative indices due to pigment degradation and canopy structure changes were studied, showing that both indices are capable of tracking steady-state fluorescence changes from canopy remote sensing reflectance.
© 2002 Elsevier Science Inc. All rights reserved.

1. Introduction

Natural chlorophyll fluorescence (CF) occurs when red and far-red light is emitted from photosynthetic green plant tissues in response to photosynthetically active radiation. In the chloroplast, light energy is harvested and processed by two photosystems, which produce oxygen and energy through a series of oxidation–reduction reactions (see Ke, 2001). The importance of CF has been the subject of several reviews detailing theory, measurement methods and interpretation, its relationship with photosynthesis, and plant physiological status and photosynthetic functioning (e.g., Krause & Weis, 1984; Larcher, 1994; Lichtenthaler, 1992; Lichtenthaler & Rinderle, 1988; Papageorgiou, 1975; Schreiber & Bilger, 1987; Schreiber, Bileger, & Neubauer, 1994). When plants are exposed to excess light, CF is a protective process by which plant chloroplasts dissipate light energy that exceeds photosynthetic demands, thereby minimizing light-induced oxidative damage (see, e.g., Gilmore & Govindjee, 1999; and several chapters in Frank, Young, Britton, & Cogdell, 1999).

In general, steady-state CF and photosynthetic rates are inversely related, such that CF is low when photosynthesis is high. However, CF can also decline when photosynthesis is low, because of an intensified protective quenching action on CF production. Under increasing stress, plant tissues

* Corresponding author. Escuela Tecnica Superior de Ingenierias Agrarias, Campus de la Yutera, Universidad de Valladolid, Avda. de Madrid, 44, Palencia 34004, Spain.

E-mail address: pzarco@cstars.ucdavis.edu (P.J. Zarco-Tejada).

0034-4257/02/$ - see front matter © 2002 Elsevier Science Inc. All rights reserved.
PII: S0034-4257(02)00113-X

shift toward increasing heat production to dissipate excess energy, and this tends to have the effect of reducing CF production, at least in the initial and intermediate stages of stress (Yahyaoui, Harnois, & Carpentier, 1998). The interdependence of photosynthesis and CF, and the various mechanisms of CF quenching have been the subject of much research into the photobiology of a wide range of plant species (Govindjee, 1995; Larcher, 1994; Lichtenthaler, 1992; Mohammed, Binder, & Gillies, 1995; Schreiber & Bilger, 1993).

The total amount of CF emitted by photosystems I (PS-I) and II (PS-II) is typically less than 5% of total light absorbed. Nevertheless, CF can be quantified with sensitive instrumentation in direct contact with the leaf tissue (Bolhar-Nordenkampf et al., 1989; Mohammed et al., 1995), such as the family of instruments known as modulating fluorometers.

Accurate remote detection of natural CF would enable the measurement of canopy-level CF without being in direct contact with the vegetation canopy. Measurement of solar-induced natural fluorescence in vegetation canopies were reported by McFarlane et al. (1980), who measured solar-induced fluorescence in citrus canopies using the H-α Fraunhofer line at 656 nm, and Carter, Theisen, and Mitchell (1999) and Carter, Jones, Mitchell, and Brewer (1996) using the H-α and O_2-B lines in leaf measurements. Buschmann and Lichtenthaler (1988) provided experimental evidence of a solar-induced fluorescence signal superimposed on leaf reflectance signatures. Additional studies suggested the effect of fluorescence on apparent reflectance (Gamon & Surfus, 1999), although the effect of the fluorescence signal on the apparent reflectance spectra of leaves was not quantified. Zarco-Tejada, Miller, Mohammed, and Noland (2000) demonstrated on a theoretical basis the CF effects on leaf-level reflectance and transmittance. These effects could be quantified using radiative transfer theory and a newly developed Fluorescence–Reflectance–Transmittance (FRT) leaf-level radiative transfer model. Subsequent studies demonstrated quantitatively that CF effects could be detected at the canopy level in the laboratory using light induction with cut-off filters, time-decay experiments, reflectance difference calculations, and optical indices from the reflectance spectra collected with the Compact Airborne Spectrographic Imager (CASI) (Zarco-Tejada, Miller, Mohammed, Noland, & Sampson, 2000; Zarco-Tejada, Miller, Mohammed, Noland, & Sampson, 2001; Zarco-Tejada, Miller, Mohammed, Noland, & Sampson, 2002). These authors suggested that a *double-peak* feature at 690–710 nm spectral region seen in the derivative reflectance was possibly due to the combined effects of fluorescence emission and low chlorophyll $a+b$ (C_{a+b}) content on stressed vegetation, providing potentially important applications in vegetation stress detection using passive hyperspectral remote sensing methods. Although promising results were found on natural CF detection through canopy reflectance and derivative analysis, further research is needed under controlled conditions to demonstrate that the *double-peak* feature seen on the canopy derivative reflectance is due solely to steady-state CF effects, and not to other confounding effects. Such derivative canopy reflectance methods would enable the remote sensing of small (about 3–5% of absorbed radiation) yet important natural CF at the canopy level, therefore facilitating the remote monitoring of photosynthetic functioning using passive hyperspectral sensors.

The experiments described here with small canopies in controlled conditions study the effects of steady-state chlorophyll fluorescence variation on canopy derivative reflectance, focusing on the *double-peak* feature described above. Changes in the natural steady-state CF were induced in a series of experiments using a controlled temperature and humidity-regulated chamber with stable light conditions. These experiments enabled the study of reflectance changes as function of the variable natural fluorescence, monitoring the derivative reflectance measured with a spectrometer. This paper describes that the unexplained *double-peak* feature previously seen on the canopy derivative reflectance is due entirely to chlorophyll fluorescence effects, demonstrating the importance of derivative methods for fluorescence detection in vegetation.

2. Experimental methods and materials

Two small canopies of 2-year-old potted trees of *Acer negundo* spp. californium of 1 m^2 in size were used in the experiments carried out in a controlled environment facility. Temperature and humidity were varied in the controlled facility in order to test whether canopy reflectance and derivative analysis methods were able to track induced variations of steady-state CF. Below we describe the experimental procedures for the experiments and the methodology employed.

2.1. Data collection

The two potted canopies of *A. negundo* used in these experiments varied in their stress levels as assessed through C_{a+b} concentration and CF steady-state (Ft) measurement (healthy canopy, $C_{a+b}=35$ μg/cm^2 and Ft = 0.64; chlorotic canopy, $C_{a+b}=21$ μg/cm^2 and Ft = 0.47). The plants were grown in a potting mixture containing 40% composed fir bark, 30% peat, 15% pumice, and 15% sand at an outdoor growth facility under 70% shade cloth. Plants were irrigated, and fertilized only at the beginning of the growth season.

A Conviron PGV36 (Controlled Environments, Winnipeg, MN, Canada) growth chamber (CEF) was used in the experiments in order to induce a stress condition in the canopies as function of temperature and humidity variation with constant light conditions. The CEF growing area was 3.3 m^2, with dimensions of 223 cm wide, 246 cm depth, and 203 cm height. Temperature (t) was programmed to vary from 10

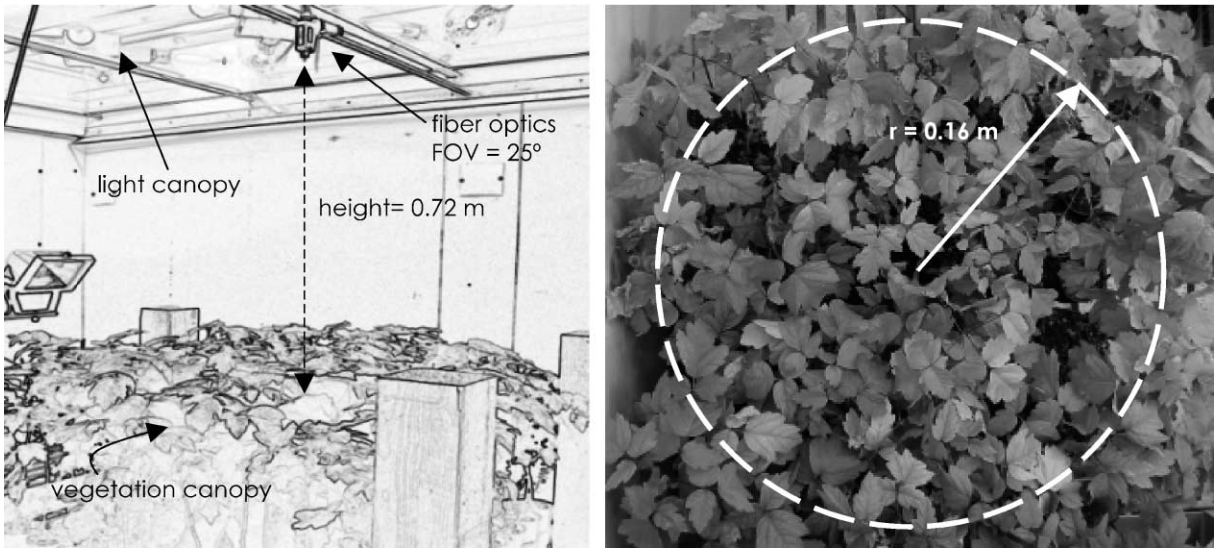


Fig. 1. Schematic view of the controlled environment facility used for reflectance data collection and experiments (left), and nadir view of the canopy used showing the area covered by the field of view of the instrument (right).

to 35 °C, while relative humidity (r_h) varied between 42% and 92%. Photosynthetic active radiation (PAR) was measured in the CEF with a Li-Cor LI-250 light meter coupled to a LI-190SA quantum sensor (Li-Cor, Lincoln, NE, USA), obtaining an average of 750 μmol quanta m^{-2} s^{-1} at the canopy surface. Light was provided using four types of light sources in the chamber: metal halide, high-pressure sodium, mercury vapor, and incandescent lamps.

The CEF was equipped with an ASD FieldSpec Pro spectrometer (Analytical Spectral Devices, Boulder, CO, USA) collecting data in the 350–2500 nm spectral region with spectral resolution of 3 nm at 700 nm, 10 nm at 1400–2500 nm, and with a sampling interval of 1.4 nm between 350 and 1050 nm, and 2 nm between 1050 and 2500 nm. A 200-μm-diameter single mode fiber optics was used for data collection with a field of view of 25° placed at 0.72 m from the vegetation canopy, therefore covering an area of 0.16 m radius of the vegetation canopy (Fig. 1). A 70×70 cm $BaSO_4$ calibration panel was used for calculation of reflectance. Vegetation and panel radiance measurements were taken by averaging 50 scans at 100 ms

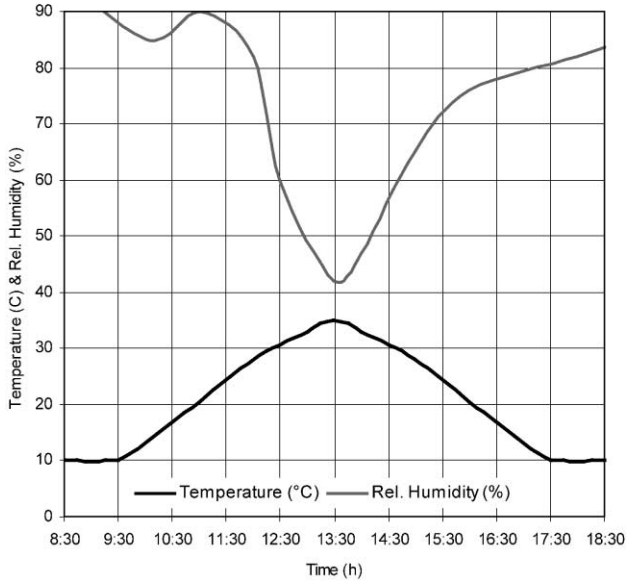


Fig. 2. Temperature and relative humidity variation in the controlled environment facility used for the experiments. Note the induced stress and recovery periods set for fluorescence variation detection.

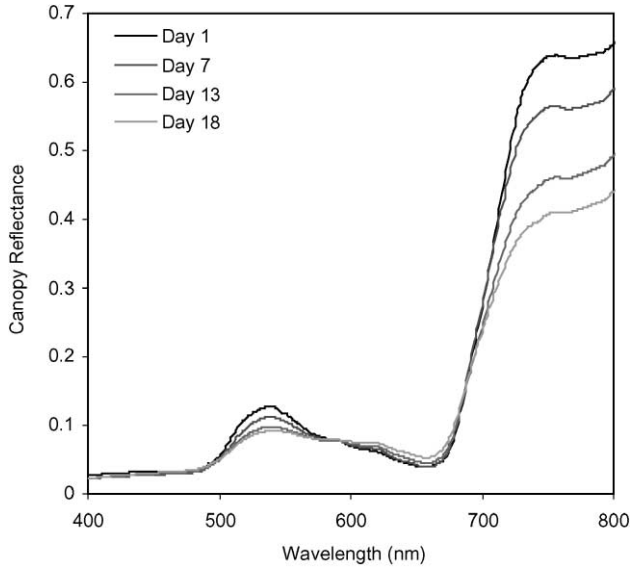
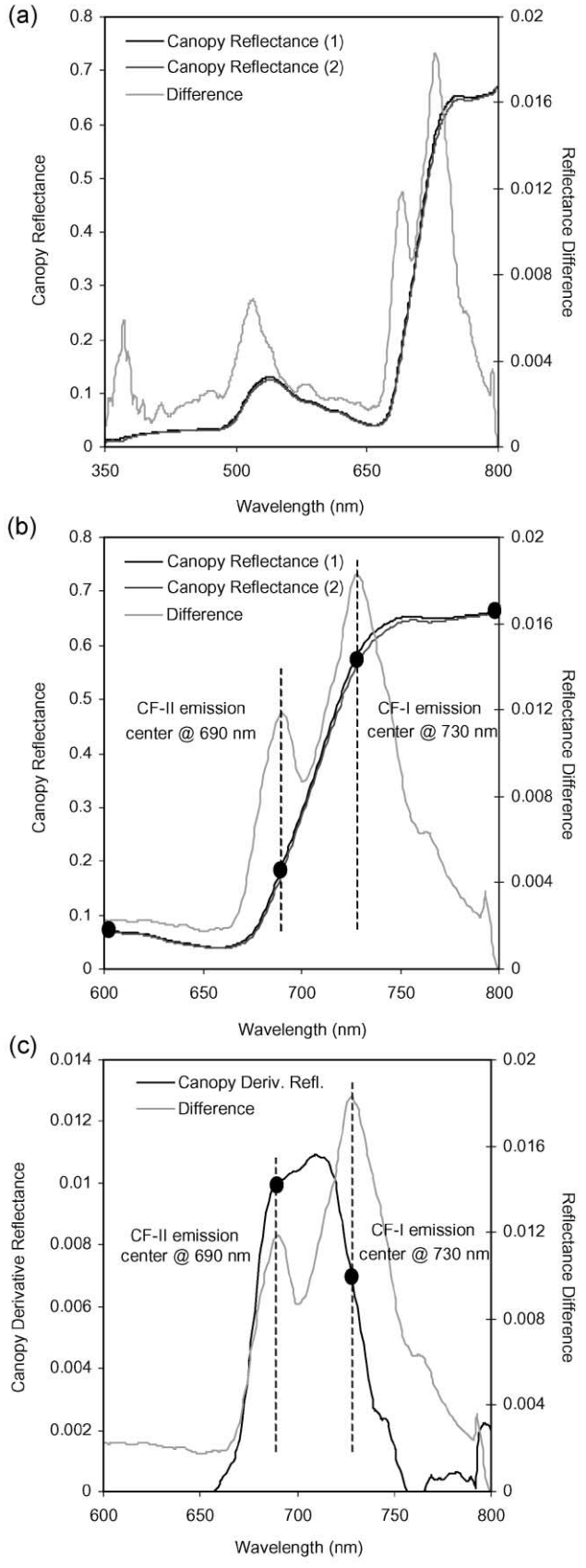


Fig. 3. Canopy reflectance collected from the healthy and chlorotic vegetation canopy using spectrometer in the 400–800 nm spectral range.



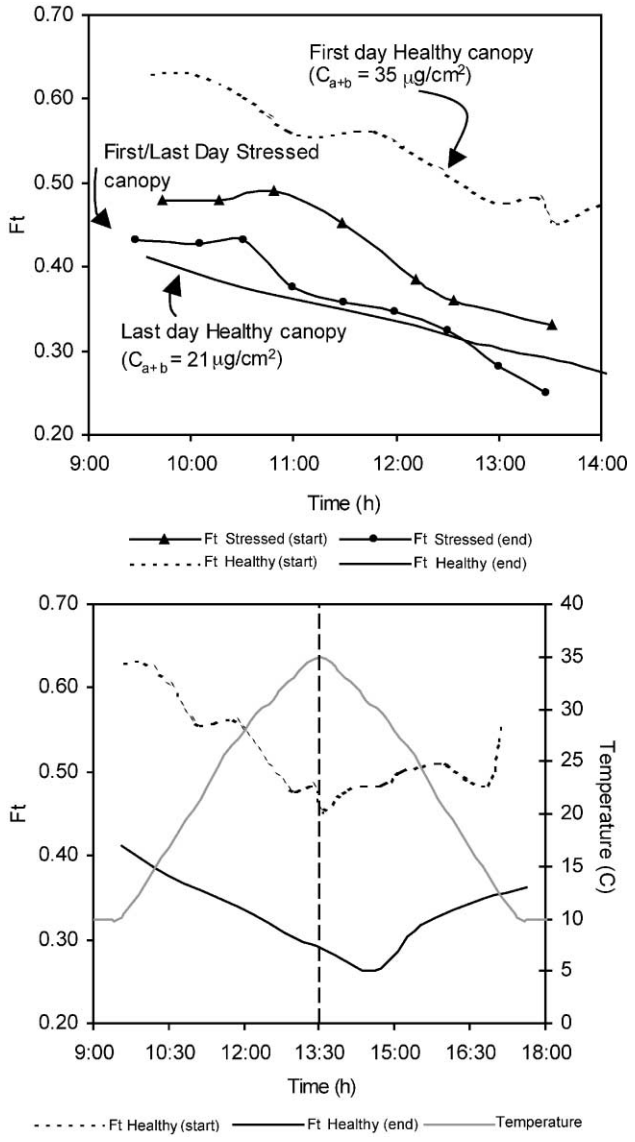


Fig. 5. Long-term variation of steady-state Ft fluorescence measured from the healthy and stressed canopies at the beginning and end of the experiments (top). Short-term fluorescence variation due to temperature and relative humidity change in the controlled environment facility (bottom).

integration time, and dark current correction at every spectral measurement. A Savitzky–Golay second order polynomial least-square function of 25 and 40 nm bandwidth were used to smooth the signal between 350–1050 and 1050–2500 nm spectral regions, respectively (Savitzky & Golay, 1964).

Single leaf reflectance and transmittance measurements were acquired on leaf samples using a Li-Cor 1800-12 Integrating Sphere apparatus (Li-Cor) coupled to the 200-

Fig. 4. (a) Calculation of reflectance difference to extract the fluorescence signal from the reflectance, (b) calculation of fluorescence-sensitive optical indices from reflectance spectrum, and (c) canopy derivative reflectance and fluorescence emission bands.

μm-diameter fiber of the spectrometer. Single leaf reflectance (ρ) and transmittance (τ) measurements were acquired as described in the manual of the Li-Cor 1800-12 system (Li-Cor, 1983) and in Zarco-Tejada, Miller, Mohammed, and Noland (2000) in which six signal measurements are required, including transmittance signal, reflectance signal, reflectance internal standard, reflectance external reference, and dark measurements.

Chlorophyll fluorescence was analyzed with a Pulse Amplitude Modulation (PAM-2000) Fluorometer (Heinz-Walz, Effeltrich, Germany). Procedures used for measuring chlorophyll fluorescence were based on standard procedures as described in the PAM-2000 manual (Heinz-Walz, 1993). Effective quantum yield, which denotes the actual efficiency of PS-II photon capture of light by closed PS-II reaction centers, was determined as $\Delta F/Fm' = (Fm' - Ft)/Fm'$, where Fm' is the maximal fluorescence of a pre-illuminated sample with PS-II centers closed, and Ft is the fluorescence at steady state (Genty, Briantais, & Baker, 1989; Van Kooten & Snel, 1990). Fluorescence measurements were made in the CEF facility by randomly sampling 50 leaves from the canopy using the PAM-2000 leaf clip holder, which exposes a 1-cm-diameter sample area to the fiberoptic light emitter and detector array. Steady-state fluorescence features were measured under the ambient light conditions of the CEF (750 μmol quanta m^{-2} s^{-1}).

Extraction of chlorophyll from leaves was carried out at different stages during the experiment. Sampled leaves were used to excise 2.3-cm circles, which were subsequently ground in liquid N_2, weighed, and placed in a 15-ml centrifuge tube. Ten milliliters of *N*,*N*-dimethylformamide (Spectral-analysis grade, Fisher) was added to the tube. Tubes were placed horizontally in a darkened 4 °C orbital shaker set to 100 rpm for 2 h to extract pigments, then centrifuged at 5 °C and 5000 × *g* for 20 min. Samples were then placed in a dark, 4 °C refrigerator for 20 min and removed from the refrigerator, placing 3 ml of supernatant in a cuvette and the absorbance measured at 663.8, 646.8, and 480 nm with a Hewlett Packard Diode Array 8452A spectrophotometer. Chlorophyll *a*, chlorophyll *b*, and total carotenoid concentrations were calculated using the extinction coefficients derived by Wellburn (1994).

2.2. Chlorophyll fluorescence induction and measurement protocol

Chlorophyll fluorescence variation on potted canopies was induced in the CEF chamber by ramping temperature

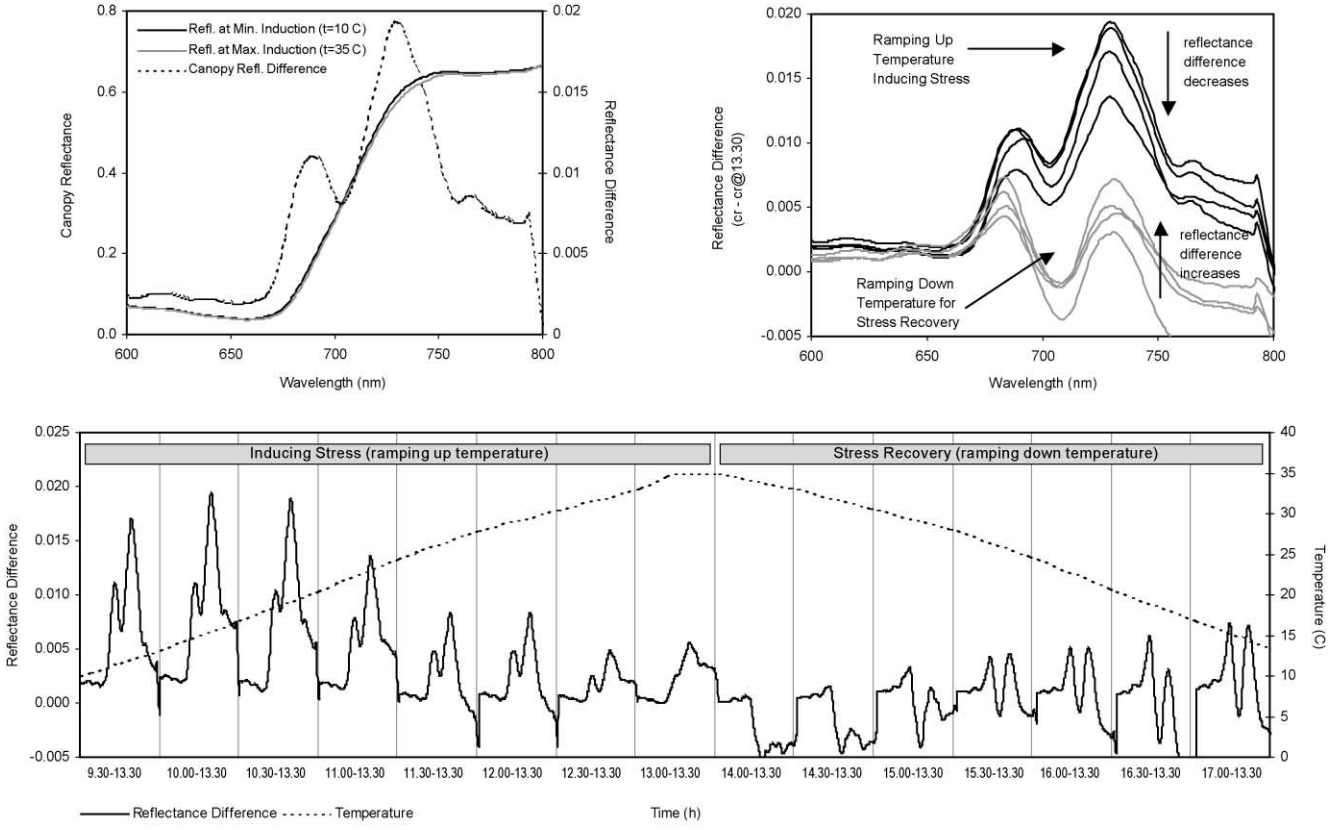


Fig. 6. Fluorescence emission extraction subtracting the canopy reflectance measured at two stress stages (top left). Spectral reflectance difference calculated between all measurements collected every 30 min during the course of a 9-h experiment, and the one collected at the maximum stress induction ($t = 35$ °C, $r_h = 42\%$), enabling the extraction of the fluorescence signature at any given time (top right and bottom).

and relative humidity in 9-h periods (Fig. 2). Temperature was varied from 10 °C at the start of the experiment ($t=0$ h) to 35 °C in the middle ($t=4.5$ h), followed by a recovery to initial conditions of 10 °C at the end of the experiment ($t=9$ h). Relative humidity varied from 92% at initial conditions down to 42% at the middle of the experiment ($t=4.5$ h), the time of the maximum temperature (35 °C) and, thus, the period of the highest induced stress of the 9-h experiment. The described variation of environmental conditions was chosen to induce fluorescence emission reduction from PS-II and PS-I while enabling recovery during the second half of the trial. Reflectance changes detected during stress induction were expected to occur during both induction and recovery periods, therefore uncoupling fluorescence effects on reflectance from structural changes and pigment degradation occurring during the course of the 9-h experiment sets.

Vegetation canopies were watered at field capacity at the start of each series of experiments, with no irrigation afterwards, thus inducing long-term stress over the duration of the trial. The chlorotic canopy ($C_{a+b}=21$ μg/cm^2) was used for data collection during a 7-day period of 9-h trials inducing stress conditions as previously explained. The healthy canopy ($C_{a+b}=35$ μg/cm^2) was used for stress induction and data collection in an 18-day period of 9-h trials. Reflectance spectra were collected every 30 min during the 9-h experiments and without touching or moving the vegetation canopy. Although the CEF chamber assured constant illumination during the 9-h periods, radiance data were calibrated to reflectance using the $BaSO_4$ panel at every measurement. This protocol ensured accurate reflectance calculations at every measurement, independent of the temperature change occurring inside the growth chamber, and therefore the possible effects on the instrument calibration and dark current characterization. Fig. 3 shows canopy reflectance measurements at different stages of the trial.

Chlorophyll fluorescence measurements were carried out at the start, middle, and end of the multi-day experiment every 30 min during each 9-h period. Steady-state and fluorescence yield were measured from 50 leaves sampled at random, and the average and standard deviation was calculated to characterize fluorescence variation during induction and recovery periods. Changes found in the canopy reflectance measured during the stress induction and recovery period could then be related to actual measurements of fluorescence measured during the same periods. Methods for extracting the fluorescence signal from the canopy-level reflectance spectra measured in these series of experiments are described in the next section.

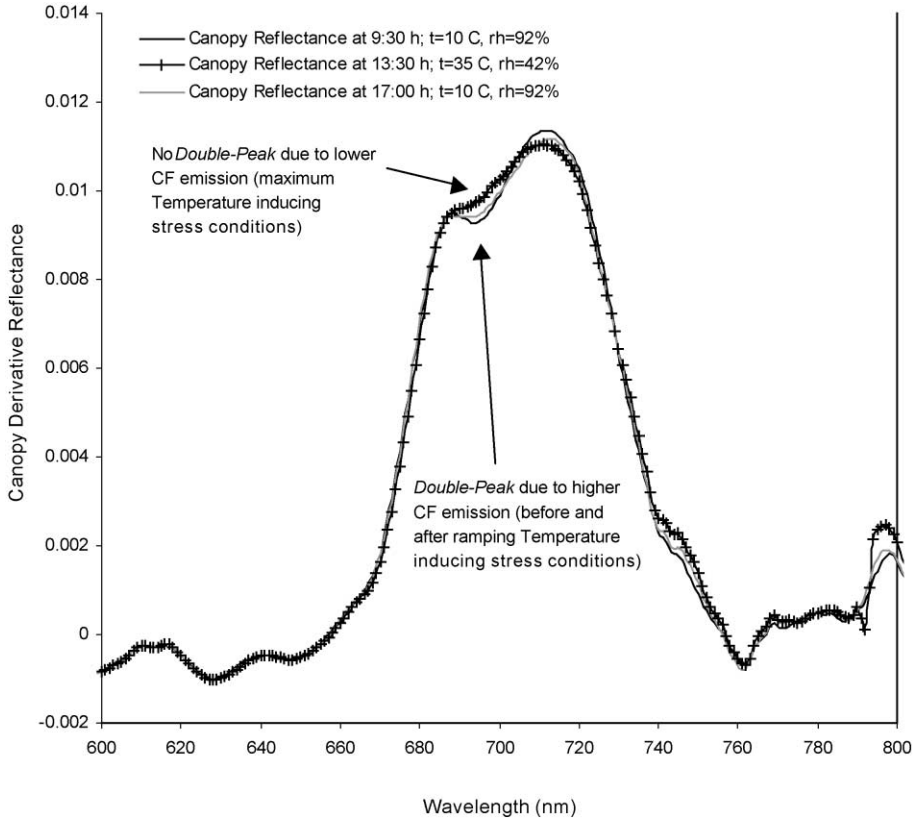


Fig. 7. The *double-peak* feature gets removed from the derivative reflectance at times of low fluorescence emission, appearing at initial and end conditions when fluorescence is higher through temperature and humidity induction.

2.2.1. Optical indices sensitive to chlorophyll fluorescence variation

Three different methods were used to extract the fluorescence signal from canopy-level reflectance. The first method consisted in the calculation of the reflectance difference between two times at which fluorescence emissions were different due to the induced effects described above. This method "extracts" the fluorescence change between the two times at which reflectance is collected (Zarco-Tejada, Miller, Mohammed, & Noland, 2000) (Fig. 4a). It also shows the changes in reflectance; see effects in the blue region at 370 nm, in the green region at 530 nm, and at 690 and 730 nm due to the CF emission. The second method is based on optical indices calculated from reflectance bands affected by fluorescence emission (i.e., bands in the spectral region between 670 and 770 nm with center emission at 690 and 730 nm) and then normalizing these to bands not affected by CF emission (e.g., 600 and 800 nm) (Fig. 4b). Several optical indices calculated within the red-edge spectral region from reflectance have been used in the literature (Zarco-Tejada, Miller, Mohammed, & Noland, 2000; Zarco-Tejada, Miller, Mohammed, Noland, & Sampson, 2000; Zarco-Tejada et al., 2002), such as the curvature index $(R_{675}R_{690})/R_{683}^2$, and ratio indices such as R_{750}/R_{800}, R_{685}/R_{655}, R_{690}/R_{655}. In the third method, the calculation of derivative reflectance enables the detection of subtle changes due to fluorescence emission in the red-edge region while minimizing other confounding effects. Derivative indices used are D_{705}/D_{722}, D_{730}/D_{706}, DP22 (D_{λ_p}/D_{720}), DPR1 $(D_{\lambda_p}/D_{\lambda_p}+12)$, where λ_p is the inflexion point of the reflectance spectrum in the red-edge spectral region. Additional indices indirectly related to chlorophyll fluorescence can be calculated from the 530 to 550 nm region and have been found to be responsive to changes in photosynthetic radiation use efficiency (Gamon, Serrano, & Surfus, 1997), such as the photochemical reflectance index (PRI), calculated as $(R_{531}-R_{570})/(R_{531}+R_{570})$.

The third method described here is the focus of the analysis carried out on the canopy-level reflectance spectra. Canopy derivative reflectance spectra were calculated as $\partial\rho_i=(\rho_{i+1}-\rho_{i-1})/(\lambda_{i+1}-\lambda_{i-1})$, and optical indices computed. The effects of the induced stress on these described indices and spectral derivative signatures were studied in order to account for the effects of chlorophyll fluorescence variation during stress induction and recovery.

3. Experimental results and discussion

Results of the data collection and analyses of fluorescence and canopy reflectance measurements are described

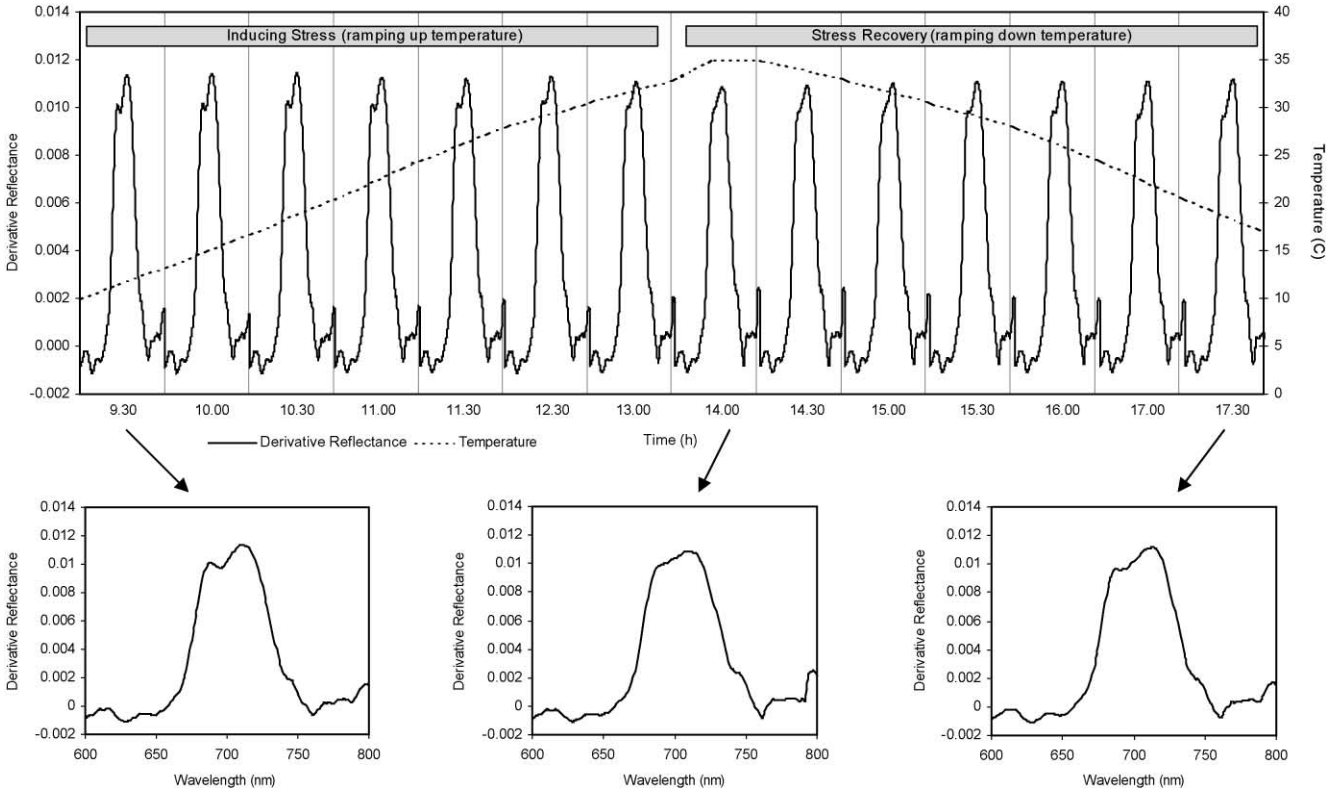


Fig. 8. Derivative reflectance calculated from canopy reflectance every 30 min during the course of a 9-h stress induction showing the effect of the fluorescence emission on the *double-peak* feature.

here, showing the relationships found between derivative reflectance spectra and chlorophyll fluorescence measurements.

3.1. Chlorophyll fluorescence results

Steady-state fluorescence measurements using the PAM-2000 instrument were demonstrated to track the stress status of the canopies used in these series of experiments (Fig. 5). Measurements taken at the beginning of the 7-day and 18-day experiment on the chlorotic and healthy canopies showed that lower Ft values were observed at the end of the trials. These results are consistent with the expectation of long-term stress due to the induced water stress over the 7-day and 18-day experiments. Moreover, these results demonstrate that CF measurements were able to differentiate stress status from steady-state fluorescence measurements collected at the beginning and at the end of the long-term stress induction (Fig. 5, top).

Short-term stress was shown to induce Ft variation through changes in temperature and relative humidity (Fig. 3) in the controlled environment facility (Fig. 5, bottom). Short-term stress induction affected steady-state fluorescence such that fluorescence varied inversely with temperature and positively varied with relative humidity. This decrease in CF associated with increasing temperature likely reflects the shift toward the increasing contribution of heat production as a mechanism of dissipating energy. These results also demonstrate that steady-state fluorescence values returned to initial (less stressful conditions) as the temperature and humidity returned to the initial conditions. Induced short- and long-term effects on steady-state fluorescence were therefore successful, enabling the study of the effects of fluorescence variation on canopy reflectance measurements.

3.2. Canopy derivative reflectance results

Short- and long-term stress effects due to pigment degradation, fluorescence emission variation, and canopy structural changes were captured by the canopy reflectance collected in the series of experiments. Short-term effects due to fluorescence induction, through temperature and relative humidity variation during the course of the 9-h trials, could also be detected on the canopy reflectance.

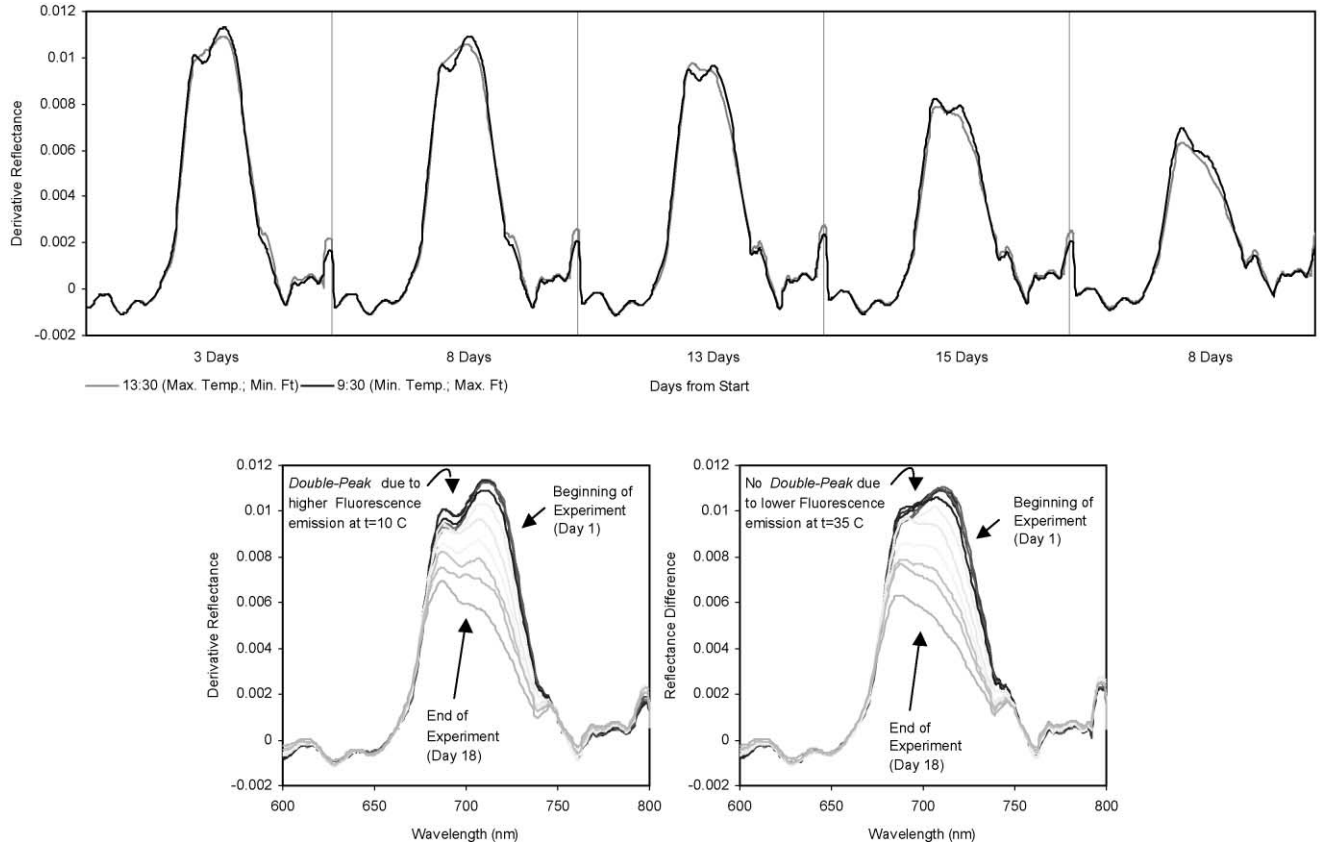


Fig. 9. Long-term stress effects of fluorescence on canopy derivative reflectance studied through an 18-day trial at times of maximum ($t = 10$ °C, $r_h = 92\%$) and minimum fluorescence emission ($t = 35$ °C, $r_h = 42\%$). It shows the effects on the derivative reflectance at times of higher fluorescence emission (bottom left) disappearing at the time of lowest fluorescence emission (bottom right).

The two fluorescence emission bands centered at 690 and 730 nm due to PS-II and PS-I photosystems, respectively (see Govindjee, Amesz, & Fork, 1986), could be extracted by subtracting the canopy reflectance measured at high stress conditions (lower fluorescence emission, $t=35$ °C, $r_h=42\%$) from the reflectance measured at low stress conditions (higher fluorescence emission, $t=10$ °C, $r_h=92\%$) (Fig. 6, top left). This procedure was conducted for all measurements during the course of the 9-h experiment, enabling the extraction of the fluorescence signature at any given time (Fig. 6, top right and bottom). It can be seen that reflectance changes induced by temperature and relative humidity were associated with the variation of the fluorescence emission. More importantly, it was also demonstrated that recovery occurred when temperature and humidity returned to initial conditions, therefore increasing the steady-state fluorescence emission and its effects on the canopy reflectance.

Derivative reflectance calculations were carried out from canopy reflectance measurements to test whether the *double-peak* feature previously observed (Zarco-Tejada et al., 2002) could be solely explained as a function of steady-state fluorescence variation. No canopy structural effects or extreme pigment degradation occurred during the course of a 9-h experiment. Therefore, canopy reflectance changes were solely attributed to fluorescence variation, thus enabling the test of these effects at both induction and recovery stages. Corroborating results for this were found by demonstrating that the canopy derivative reflectance at times of higher fluorescence emission (higher Ft, $t=10$ °C, $r_h=92\%$) clearly show a *double-peak* feature in the derivative reflectance and that this feature diminishes with increasing stress induction (lower Ft, $t=35$ °C, $r_h=42\%$) (Fig. 7). As demonstrated in Fig. 7, the *double-peak* feature is absent from the derivative reflectance at times of low fluorescence emission, but does appear under conditions when fluorescence is higher. The main potential of this method is that the *double peak* seen on the derivative reflectance is a direct indicator of fluorescence emission, and not a relative measurement of the difference existing between two canopy reflectance spectra, as shown in Fig. 6. Derivative reflectance values calculated from canopy reflectance at 30-min intervals show the effect of the fluorescence emission during the course of the experiment (Fig. 8).

Long-term stress effects of steady-state fluorescence on canopy derivative reflectance were also studied (Fig. 9). Canopy derivative reflectance calculated through the 18-day trial at times of maximum (09:30 h, $t=10$ °C, $r_h=92\%$) and minimum fluorescence emission (13:30 h, $t=35$ °C, $r_h=42\%$) showed that canopy structural changes and pigment degradation occurred during the course of the experiment (C_{a+b} varied from 35 to 23 μg/cm^2) but did not prevent the detection of fluorescence emission using the described *double-peak* feature. The maximum of the first derivative (the inflexion point of the red-edge reflectance spectrum) shifted to shorter wavelengths due to pigment degradation, as expected and previously reported (Horler, Barber, & Barringer, 1980; Horler, Dockray, & Barber, 1983; Rock, Hoshizaki, & Miller, 1988; Vogelmann, Rock, & Moss, 1993). Despite these described changes in the canopy, it can be seen that the *double-peak* indicator of fluorescence emission could be detected during the course of the 18-day experiment at times of higher fluorescence emission (Fig. 9, bottom left) and disappearing during the times of lowest fluorescence emission (Fig. 9, bottom right).

The next section describes the results obtained that quantitatively demonstrate the relationship between optical indices calculated from the *double-peak* feature in the derivative reflectance and steady-state Ft fluorescence measurements.

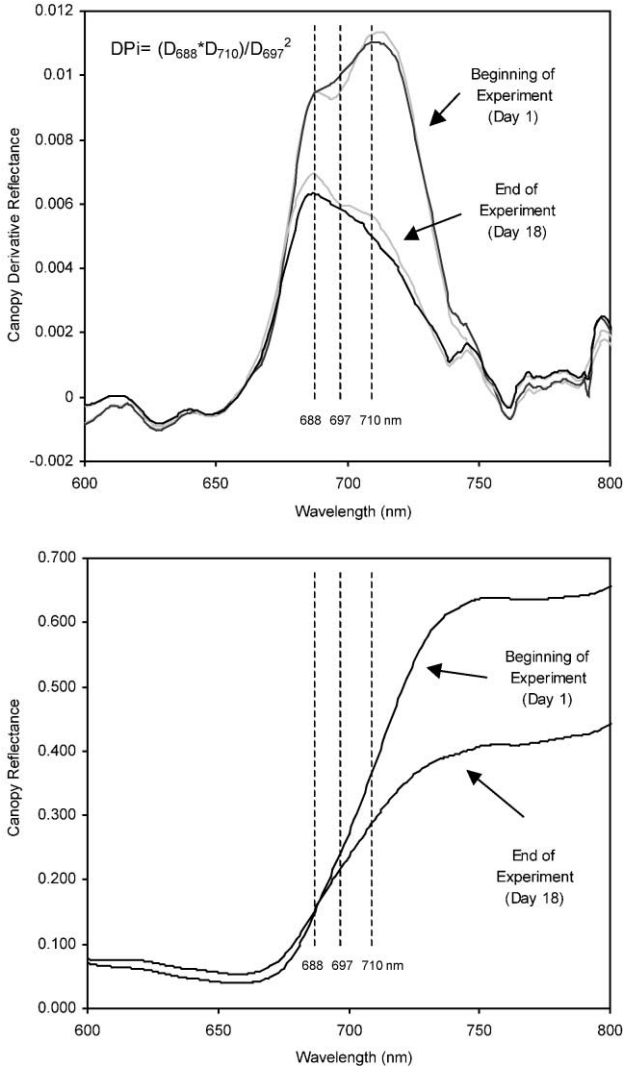


Fig. 10. Derivative reflectance bands at 688, 697, and 710 nm used for calculation of the *double-peak* optical index DPi at times of low and high induced fluorescence (top); canopy reflectance measured at the beginning and end of experiment 18 days later (bottom).

3.3. The double-peak indices DPi and A_{dp} to track steady-state chlorophyll fluorescence variation

The *double-peak* optical index (DPi) was calculated from the derivative reflectance bands 688, 697, and 710 nm as shown in Fig. 10 (top), showing the canopy reflectance measured at the beginning and end of the experiment (Fig. 10, bottom). The DPi index is calculated as $(D_{688} \cdot D_{710})/D_{697}^2$ to increase its sensitivity to the 697 nm band that generates the peak of the fluorescence signal. Derivative reflectance spectra show the peak feature due to the fluorescence effects at both the beginning (day 1) and end of the experiment (day 18), when both pigment and canopy structural changes occurred.

The short-term variation in Ft and the derivative index DPi for the beginning and end of the 18-day experiment are seen in Fig. 11. The behavior of Ft and DPi during the diurnal course of the trial for the first (Fig. 11, top left) and last day (Fig. 11, top right) of the experiment demonstrate their close relationship. It is seen that the *double-peak* index DPi closely tracks steady-state fluorescence when temperature and humidity changes are induced. The variation of Ft (Fig. 11, bottom left) and DPi (Fig. 11, bottom right) at the beginning and end of the experiment are consistent with expectations, showing that both Ft and DPi decrease due to the long-term stress effects induced during the 18-day experiment. The relationship found between DPi and Ft (Fig. 12, top) at the beginning ($r^2 = 0.97$) and the end of the trial ($r^2 = 0.88$) demonstrate that the suggested index based on the *double-peak* feature closely tracks steady-state Ft variation.

The effects of structural changes in the canopy, due to induced stress on the proposed *double-peak* index, were also considered. The area of the *double-peak* feature (A_{dp}) between 688 and 710 nm was calculated from derivative reflectance. Fig. 12 (bottom) shows the relationship between Ft and DPi ($r^2 = 0.67$), and A_{dp} ($r^2 = 0.77$) for the measurements before and after any long-term structural

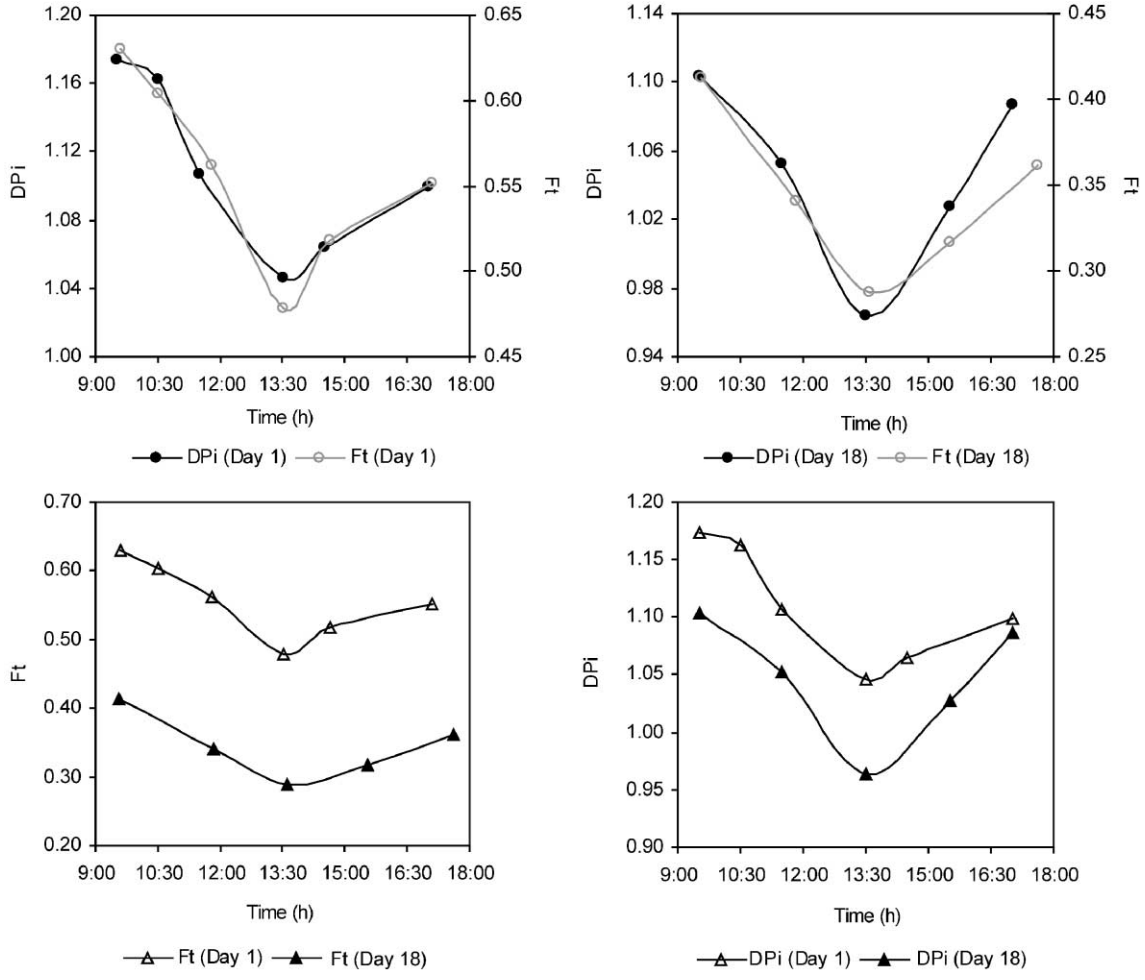


Fig. 11. Variation of Ft and DPi during the course of the induced stress by temperature and humidity during the first (top left) and last day (top right) of the 18-day trial. Variation of Ft (bottom left) and DPi (bottom right) at the beginning and end of the experiment showing that both Ft and DPi decrease their values due to the long-term effects induced during the 18-day experiment.

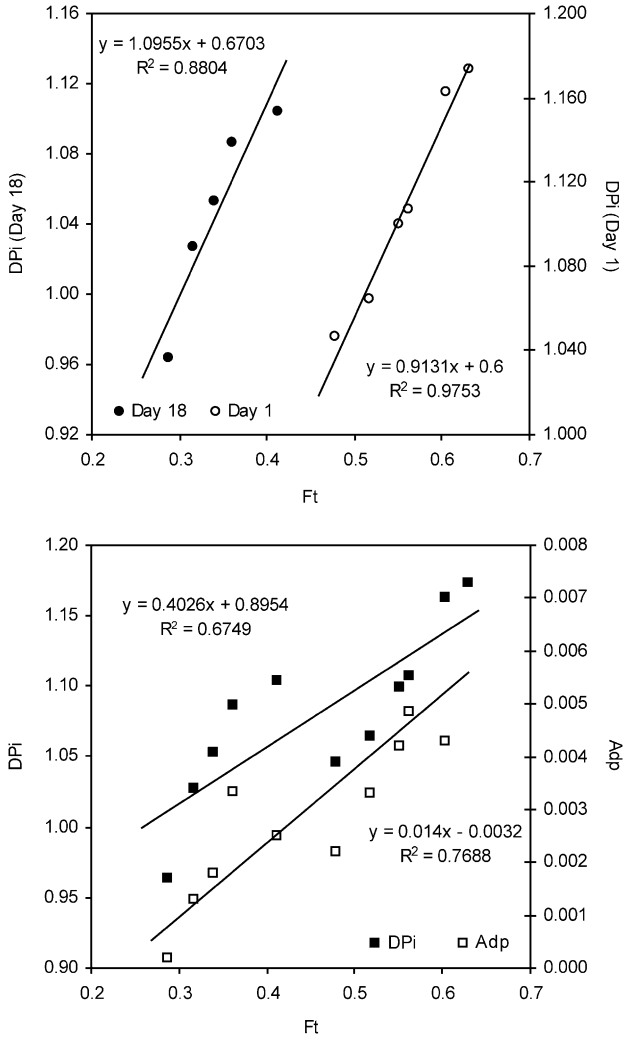


Fig. 12. Relationship found between DPi and Ft (top) at the beginning ($r^2 = 0.97$) and the end of the trial ($r^2 = 0.88$). Relationship between Ft and DPi ($r^2 = 0.67$) and A_{dp} ($r^2 = 0.77$) for the measurements before and after long-term structural changes occurred in the canopy (bottom).

changes occurred in the canopy in the 18-day experiment. These results suggest that both indices based on the derivative reflectance and *double-peak* feature are capable of tracking Ft variations for both short- and long-term stress conditions.

4. Conclusions

Experiments reported in this paper demonstrate that steady-state chlorophyll fluorescence effects are observable in canopy derivative reflectance through a *double-peak* feature that is created in the 690–710 nm spectral region. Experiments were carried out on small canopies of *A. negundo* in a controlled environment facility to induce stress through temperature and humidity variation. The experimental temperature ranged from 10 to 35 °C, with relative humidity varying inversely from 92% to 42%. This variation in conditions induced changes in natural steady-state fluorescence that were captured by both fluorometer measurements at the leaf level, and fiber optic spectrometer reflectance measurements at the canopy level. These results are consistent with previous theoretical descriptions of the chlorophyll fluorescence effects on apparent reflectance, and provide substantiating evidence that the *double peak* observed on the canopy derivative reflectance is a function of the steady-state natural fluorescence emission bands centered at 690 and 730 nm.

Short-term and long-term stress induction through temperature and humidity variation were studied through canopy derivative reflectance analysis, demonstrating that the *double-peak* feature at 690–710 nm in the canopy derivative reflectance is directly related to the natural fluorescence. Two optical indices calculated from canopy derivative reflectance are suggested as they demonstrate to be directly related to the steady-state fluorescence emission in vegetation. DPi, calculated as $(D_{688} \cdot D_{710})/D_{697}{}^2$, and the area of the *double-peak* feature, A_{dp}, have been shown to track Ft variation during both short- and long-term stress induction stages. Moreover, the reflectance difference calculation, the *double-peak* feature, and the proposed new optical indices were able to track fluorescence recovery after depression induced by temperature and humidity.

Relationships found between steady-state fluorescence measured with a fluorometer and the *double-peak* optical indices DPi ($r^2 = 0.67$) and A_{dp} ($r^2 = 0.77$) during the course of an 18-day trial demonstrated the validity of these indices for tracking fluorescence variation. The relationship found between DPi and Ft at the beginning ($r^2 = 0.97$) and the end of the trial ($r^2 = 0.88$) demonstrated that the index closely tracks steady-state Ft variation during depression and subsequent recovery.

The above results demonstrate the potential application of hyperspectral remote sensing and derivative reflectance analysis for natural steady-state chlorophyll fluorescence detection in vegetation. The conclusions in this paper have a direct bearing on the ongoing research efforts by the scientific community to utilize passive remote sensing methods for photosynthesis monitoring and photosynthetic efficiency estimation through quantification of chlorophyll fluorescence. Canopy structure and bidirectional reflectance effects on the described *double-peak* feature in the derivative reflectance due to fluorescence emission need to be further studied to successfully apply the methods described here to natural conditions.

Acknowledgements

The authors gratefully acknowledge the detailed revision of this paper and useful comments by Govindjee, J.R. Miller, S. Jacquemoud, and G.H. Mohammed. Financial support provided for this research to P.J. Zarco-Tejada through the California Space Institute and NASA Space Grant Program,

and sabbatical support for J.C. Pushnik from California State University, Chico is gratefully acknowledged.

References

Bolhar-Nordenkampf, H. R., Long, S. P., Baker, N. R., Oquist, G., Schreiber, U., & Lechner, E. G. (1989). Chlorophyll fluorescence as a probe of the photosynthetic competence of leaves in the field: a review of current instrumentation. *Functional Ecology*, *3*, 497–514.

Buschmann, C., & Lichtenthaler, H. K. (1988). Reflectance and chlorophyll fluorescence signatures in leaves. In H. K. Lichetnthaler (Ed.), *Applications of chlorophyll fluorescence* (pp. 325–332). Dordrecht: Kluwer Academic Publishing.

Carter, G. A., Jones, J. H., Mitchell, R. J., & Brewer, C. H. (1996). Detection of solar-excited chlorophyll *a* fluorescence and leaf photosynthetic capacity using a Fraunhofer line radiometer. *Remote Sensing of Environment*, *55*, 89–92.

Carter, G. A., Theisen, A. F., & Mitchell, R. J. (1990). Chlorophyll fluorescence measured using the Fraunhofer line-depth principle and relationship to photosynthetic rate in the field. *Plant, Cell and Environment*, *13*, 79–83.

Frank, H. A., Young, A. J., Britton, G., & Cogdell, R. J. (Eds.) (1999). *The photochemistry of carotenoids*. Dordrecht, The Netherlands: Kluwer Academic Publishing, 399 pp.

Gamon, J. A., Serrano, L., & Surfus, J. S. (1997). The photochemical reflectance index: an optical indicator of photosynthetic radiation-use efficiency across species, functional types, and nutrient levels. *Oecologia*, *112*, 492–501.

Gamon, J. A., & Surfus, J. S. (1999). Assessing leaf pigment content and activity with a reflectometer. *New Phytologist*, *143*, 105–117.

Genty, B., Briantais, J.-M., & Baker, N. R. (1989). The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta*, *990, 87–92.*

Gilmore, A., & Govindjee (1999). How higher plants respond to excess light: energy dissipation in photosystem II. In G. S. Singhal, G. Renger, S. K. Sopry, K.-D. Irrgang, & Govindjee (Eds.), *Concepts in photobiology: photosynthesis and photomorphogenesis* (pp. 513–548). Dordrecht, The Netherlands: Kluwer Academic Publishing/New Delhi, India: Narosa Publishers.

Govindjee (1995). Sixty-three years since Kautsky: chlorophyll *a* fluorescence. *Australian Journal of Plant Physiology*, *22*, 131–160.

Govindjee, Amesz, J., & Fork, D. C. (Eds.) (1986). *Light emission by plants and bacteria*. New York: Academic Press, 638 pp.

Heinz-Walz (1993). *Portable Fluorometer PAM-2000 and Data Acquisition Software DA-2000*, Effeltrich, Germany.

Horler, D. N. H., Barber, J., & Barringer, A. R. (1980). Effects of heavy metals on the absorbance and reflectance spectra of plants. *International Journal of Remote Sensing*, *1*, 121–136.

Horler, D. N. H., Dockray, M., & Barber, J. (1983). The red edge of plant leaf reflectance. *International Journal of Remote Sensing*, *4* (2), 273–288.

Ke, B. (2001). *Photosynthesis: photobiochemistry and photobiophysics*. Dordrecht, The Netherlands: Kluwer Academic Publishing, 763 pp.

Krause, G. H., & Weis, E. (1984). Chlorophyll fluorescence as a tool in plant physiology: II. Interpretation of fluorescence signals. *Photosynthesis Research*, *5*, 139–157.

Larcher, W. (1994). Photosynthesis as a tool for indicating temperature stress events. In E. D. Schulze, & M. M. Caldwell (Eds.), *Ecophysiology of photosynthesis* (pp. 261–277). Berlin: Springer.

Lichtenthaler, H. K. (1992). The Kautsky effect: 60 years of chlorophyll fluorescence induction kinetics. *Photosynthetica*, *27*, 45–55.

Lichtenthaler, H. K., & Rinderle, U. (1988). The role of chlorophyll fluorescence in the detection of stress conditions in plants. *CRC Critical Reviews in Analytical Chemistry*, *19* (Suppl. 1), 529–585.

Li-Cor. (1983). 1800-12 Integrating Sphere Instruction Manual. Publication Number 8305-0034.

McFarlane, J. C., Watson, R. D., Theisen, A. F., Jackson, R. D., Ehrler, W. L., Pinter Jr., P. J., Idso, S. B., & Reginato, R. J. (1980). Plant stress detection by remote measurement of fluorescence. *Applied Optics*, *19*, 3287–3289.

Mohammed, G. H., Binder, W. D., & Gillies, S. L. (1995). Chlorophyll fluorescence: a review of its practical forestry applications and instrumentation. *Scandinavian Journal of Forest Research*, *10*, 383–410.

Papageorgiou, G. (1975). Chlorophyll fluorescence: an intrinsic probe of photosynthesis. In Govindjee (Ed.), *Bioenergetics of photosynthesis* (pp. 319–371). New York: Academic Press.

Rock, B. N., Hoshizaki, T., & Miller, J. R. (1988). Comparison of in situ and airborne spectral measurements of the blue shift associated with forest decline. *Remote Sensing of Environment*, *24*, 109–127.

Savitzky, A., & Golay, M. J. E. (1964). Smoothing and differentiation of data by simplified least squares procedures. *Analytical Chemistry*, *36*, 1627–1639.

Schreiber, U., & Bilger, W. (1987). Rapid assessment of stress effects on plant leaves by chlorophyll fluorescence measurements. In J. D. Tenhunen, & E. M. Catarino (Eds.), *Plant response to stress* (pp. 27–53). Berlin, Germany: Springer-Verlag.

Schreiber, U., & Bilger, W. (1993). Progress in chlorophyll fluorescence research: major development during the past years in retrospect. *Progress in Botany*, *54*, 151–173.

Schreiber, U., Bilger, W., & Neubauer, C. (1994). Chlorophyll fluorescence as a non-destructive indicator for rapid assessment of in vivo photosynthesis. *Ecological Studies*, *100*, 49–70.

Van Kooten, O., & Snel, J. F. H. (1990). The use of chlorophyll fluorescence nomenclature in plant stress physiology. *Photosynthesis Research*, *25*, 147–150.

Vogelmann, J. E., Rock, B. N., & Moss, D. M. (1993). Red edge spectral measurements from sugar maple leaves. *International Journal of Remote Sensing*, *14*, 1563–1575.

Wellburn, A. R. (1994). The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids using various solvents with spectrophotometers of different resolutions. *Journal of Plant Physiology*, *144*, 307–313.

Yahyaoui, W., Harnois, R., & Carpentier, R. (1998). Demonstration of thermal dissipation of absorbed quanta during energy-dependent quenching of chlorophyll fluorescence in photosynthetic membranes. *FEBS Letters*, *440*, 59–63.

Zarco-Tejada, P. J., Miller, J. R., Mohammed, G. H., & Noland, T. L. (2000). Chlorophyll fluorescence effects on vegetation apparent reflectance: I. Leaf-level measurements and model simulation. *Remote Sensing of Environment*, *74* (3), 582–595.

Zarco-Tejada, P. J., Miller, J. R., Mohammed, G. H., Noland, T. L., & Sampson, P. H. (2000). Chlorophyll fluorescence effects on vegetation apparent reflectance: II. Laboratory and airborne canopy-level measurements with hyperspectral data. *Remote Sensing of Environment*, *74* (3), 596–608.

Zarco-Tejada, P. J., Miller, J. R., Mohammed, G. H., Noland, T. L., & Sampson, P. H. (2001). Estimation of chlorophyll fluorescence under natural illumination from hyperspectral data. *International Journal of Applied Earth Observation and Geoinformation*, *3* (4), 321–327.

Zarco-Tejada, P. J., Miller, J. R., Mohammed, G. H., Noland, T. L., & Sampson, P. H. (2002). Vegetation stress detection through chlorophyll $a+b$ estimation and fluorescence effects on hyperspectral imagery. *Journal of Environmental Quality*, *31* (5), 1433–1441.