Detection of Water Stress in Orchard Trees With a High-Resolution Spectrometer Through Chlorophyll Fluorescence *In-Filling* of the O_2-A Band

Oscar Pérez-Priego, Pablo J. Zarco-Tejada, John R. Miller, Guadalupe Sepulcre-Cantó, and Elias Fereres

***Abstract*—A high spectral resolution spectrometer with 0.065-nm full-width half-maximum was used for collecting spectral measurements in an orchard field under three water stress treatments. The study was part of the FluorMOD project funded by the European Space Agency to develop a leaf-canopy reflectance model to simulate the effects of fluorescence. Water deficit protocols generated a gradient in solar-induced chlorophyll fluorescence emission and tree physiological measures. Diurnal steady-state chlorophyll fluorescence was measured from leaves in the field between June and November 2004 using the PAM-2100 fluorometer to study the effects of water stress on chlorophyll fluorescence. Spectral measurements of downwelling irradiance and upwelling crown radiance were conducted with the narrow-band spectrometer, enabling the canopy reflectance to be obtained at subnanometer spectral resolution and permitting the evaluation of the fluorescence *in-filling* effects on reflectance in trees under water stress conditions. Diurnal and seasonal measurements showed consistently lower steady-state fluorescence (Ft) and quantum yield $\Delta F/Fm'$ in water-stressed trees, yielding mean values of Ft $= 0.38$ (well-irrigated) and Ft $= 0.21$ (water-stressed trees). The agreement between Ft and water potential showed that steady-state fluorescence could be used to detect differences in water stress levels, with determination coefficients ranging between $r^2 = 0.48$ and $r^2 = 0.81$ for individual dates. Analysis in the 680–770-nm range showed that the chlorophyll fluorescence *in-filling* in the O_2-A band at 760 nm is sensitive to diurnal variations of fluorescence and water stress, yielding $r^2 = 0.76$ (well-watered treatment), $r^2 = 0.89$ (intermediate stress treatment), and $r^2 = 0.7$ (extreme stress treatment), demonstrating the close relationships between Ft and *in-filling* at the crown level.**

***Index Terms*—Chlorophyll fluorescence, fluorescence in-filling, hyperspectral, olive tree, oxygen O_2-A band, remote sensing, water stress.**

I. INTRODUCTION

THE importance of chlorophyll fluorescence (CF) is being investigated in several studies which focus on the theory, measurement methods, and interpretation, evaluating its relationship with photosynthesis and plant physiological status [1]–[7]. Light-induced oxidative damage is minimized through this protective process, with plant chloroplasts dissipating light energy that exceeds photosynthetic demands [8], [9]. Under increasing stress, plant tissues increase heat production to dissipate excess energy, reducing CF production, at least in the initial and intermediate stages of stress [10]. The relationship between steady-state fluorescence (Ft) and water stress was studied by Flexas and collaborators [11]–[13] showing a strong correlation between Ft and the diurnal variation of stomatal conductance, and to a lesser extent CO_2 assimilation, also affected by irradiance levels and water stress. These results suggested a potential for water stress monitoring in vegetation using solar-induced chlorophyll fluorescence measures, without requiring active fluorescence sensors.

Several studies attempted to measure the solar-induced chlorophyll fluorescence naturally emitted by plant tissues using passive remote sensing methods, which would enable the global monitoring of plant physiology and photosynthetic functioning at large spatial and temporal scales. As an example, measurements of solar-induced natural fluorescence in vegetation canopies were reported by McFarlane [14] who measured solar-induced fluorescence in a crop canopy using the H-α Fraunhofer line at 656 nm, and Carter [15], [16] using the H-α and O_2-B lines in leaf measurements. Later, Buschmann and Lichtenthaler [17] demonstrated that the solar-induced fluorescence signal is added on top of leaf reflectance signatures, with other qualitative studies suggesting the effect of fluorescence on apparent reflectance could be detected in the red edge spectral region [18]. Later, quantitative assessments in the laboratory of the Ft signal superimposed on the apparent reflectance at both the leaf and canopy levels using *time-decay* experiments, light blocking methods with long-pass filters in the 400–700-nm region, reflectance difference calculations, and optical indexes demonstrated that Ft contributed up to 2% on the apparent reflectance in the 750-nm spectral region [19]–[21]. Additionally, Campbell [22] using filters to control light induction showed that the contribution of Ft to apparent reflectance in *Zea mays* (corn) was over 2%.

Recent studies continue to provide additional evidence for fluorescence *in-filling* of the O_2-A atmospheric oxygen absorp-

Manuscript received June 20, 2005; revised August 2, 2005. This work was supported in part by the Consejo Superior de Investigaciones Científicas under the CSIC-PIF Program, in part by the European Space Agency (ESA), and in part by the Spanish Ministry of Science and Technology (MCyT) for the projects PIF-200440-F035, ESA-ESTEC Contract 16365/02/NL/FF (FluorMOD), and AGL2003-01468, respectively. The work of P. J. Zarco-Tejada was supported by the *Ramón y Cajal* (MCyT) and *Averroes* (JA) programs. The work of J. R. Miller was supported by the Natural Sciences and Engineering Research Council of Canada.

O. Pérez-Priego, P. J. Zarco-Tejada, and G. Sepulcre-Cantó are with the Instituto de Agricultura Sostenible (IAS), Consejo Superior de Investigaciones Científicas (CSIC), 14004 Córdoba, Spain (e-mail: pzarco@ias.csic.es).

J. R. Miller is with the Department of Earth and Space Science and Engineering, York University, Toronto, ON, M3J 1P3, Canada.

E. Fereres is with the Instituto de Agricultura Sostenible (IAS), Consejo Superior de Investigaciones Científicas (CSIC), 14004 Córdoba, Spain and also with the Departamento de Agronomía, Universidad de Córdoba, 14071 Córdoba, Spain.

Digital Object Identifier 10.1109/TGRS.2005.857906

tion band at 760 nm, as a detectable feature in the radiance spectra at the near-canopy levels [23]–[26]. At the airborne and far-field scales, Maier and Zarco-Tejada [27], [28] found evidence for the detection of the solar-induced fluorescence signal on apparent reflectance obtained from airborne sensors *Reflective Optics System Imaging Spectrometer* (ROSIS) and *Compact Airborne Spectrographic Imager* (CASI) based on the *in-filling* of fluorescence in the 760-nm atmospheric oxygen absorption band. Despite these initial results, little work has been conducted that quantitatively demonstrates the link between steady-state fluorescence and spectral reflectance under natural light conditions. Although the FRT and SLOPE models were developed to account for the effects of chlorophyll fluorescence on leaf apparent reflectance [19], [29], the need for an integrated leaf-canopy model to account for spectral fluorescence effects on leaf and canopy reflectance initiated the *Development of a Vegetation Fluorescence Canopy Model* (FluorMOD) funded by the European Space Agency (ESA) (http://www.ias.csic.es/fluormod). Current validation efforts for FluorMOD require the collection of vegetation radiance and reflectance spectra at leaf and canopy levels under different viewing geometries, stress levels, and spectral configurations. As a result of these efforts, the FluorMOD project [30] is making progress on the development of a leaf model that simulates leaf fluorescence, FluorMODleaf [31], and a linked leaf-canopy model that simulated canopy fluorescence, FluorSAIL [32]. The FluorMODgui integrated leaf-canopy model [33] enables the simulation of diurnal effects under different viewing geometries, atmospheric characteristics, and illumination dependency, modeling the effects of natural fluorescence on apparent canopy reflectance.

This manuscript presents progress made as part of FluorMOD validation efforts, assessing whether water stress levels caused by different irrigation treatments would affect the crown-level natural fluorescence emitted, assessing the potential for its detection through the fluorescence *in-filling* effects in the O_2-A band at 760 nm. The study focuses on the detection of steady-state fluorescence superimposed on the canopy reflectance, using water stress as a driver for obtaining different stress and fluorescence levels. Conclusions will facilitate the evaluation of the simulated fluorescence effects on canopy reflectance, assessing the potential for global monitoring of solar-induced chlorophyll fluorescence.

II. MATERIALS AND METHODS

A. Study Site Location and Experimental Design

The field experiment was conducted from June to November 2004 in a 4-ha irrigated olive orchard (*Olea europaea* L cv. "Arbequino") located in Córdoba, southern Spain (37.8°N, 4.8°W). The study site is located in an area with Mediterranean climate, with an average annual rainfall of 650 mm concentrated from autumn to spring, and potential evapotranspiration of 1390 mm. The soils in the study area are *Typic Xerofluvents*, with sandy slimy characteristics, sandy stratum at 1.5-m depth.

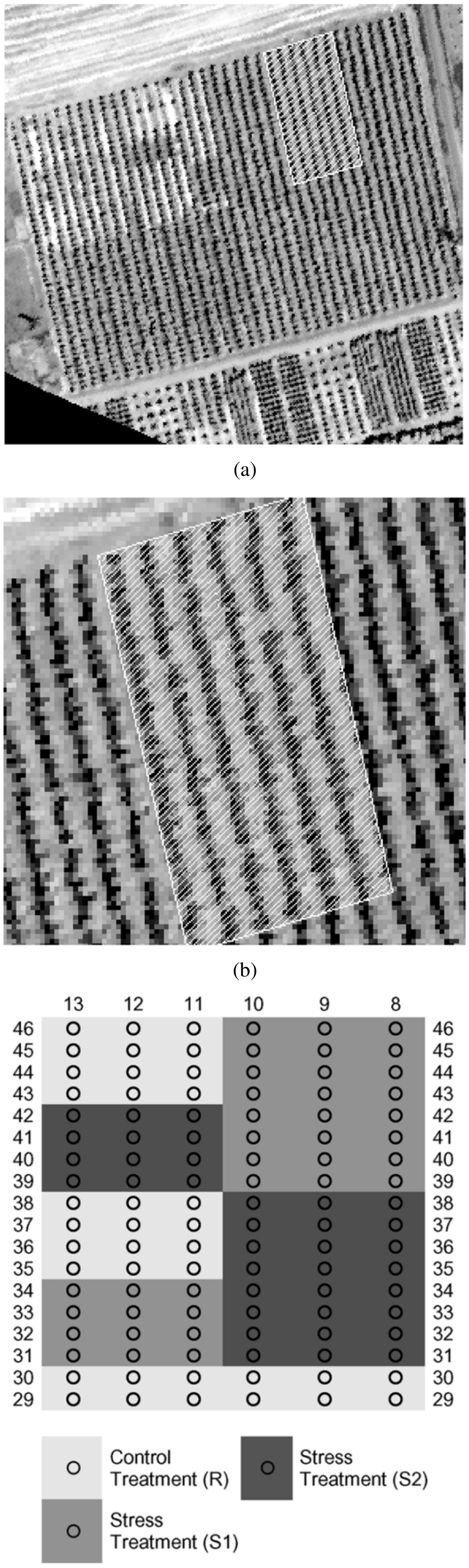
The orchard was planted in 1997 in a 3.5 m × 7.0 m grid (408 tree ha^{-1}) in a north–south direction. The trees were planted on ridges to avoid flood damage problems because of the drainage and the low percolation rate, using weed killer for soil maintenance. The drip irrigation method used in this study enabled different irrigation treatments over a single experimental area. Fig. 1(a) and (b) shows the study site area, the orchard field and the trees used for this experiment, as imaged by the CASI sensor at 1-m spatial resolution and eight spectral bands in the 400–950-nm spectral range. The CASI sensor flew the study site as part of the AgriSpectra airborne campaign conducted by Spanish and Canadian teams. A chlorophyll fluorescence gradient was sought through three irrigation treatments randomly applied in six rows of 18 olive trees covering an area of 2646 m^2, generating three water stress levels: 1) irrigation at 2.8 mm/day (well watered treatment, used as control, R); 2) 0.7 mm/day (deficit treatment, stress treatment S1); and 3) an intermittent treatment, with 1.2 mm/day from June 14, 2004 to July 5, 2004 and from September 6, 2004 to Otober 19, 2004, with no irrigation from July 5 to September 6 (deficit treatment, stress treatment S2) [Fig. 1(c)].

Trees located in the center of each treatment block were selected for weekly monitoring of tree physiological status, with measurements made of leaf water potential, stomatal conductance, photosynthesis and leaf chlorophyll fluorescence. In addition, crown measurements of reflectance in the 680–770-nm spectral range were conducted on trees under different stress levels and for different viewing geometries.

B. Field Data Collection

1) Leaf Measurements: Weekly measurements were conducted on 11 trees located in the center of the irrigation blocks to avoid treatment edge effects, to monitor water potential (Ψ_x) on four trees per treatment for S1 and S2 treatments, and three trees for the control treatment R. Among them, two trees per treatment were selected for weekly measurements of chlorophyll fluorescence, stomatal conductance, and photosynthesis. A Scholander pressure bomb (PWSC Model 3000, Soilmoisture Equipment Corp., California) was used to measure leaf water potential from 11 trees covering the three irrigation treatments, with measurements taken weekly at 10:00 solar time from two shaded leaves per tree located close to the trunk. Stomatal conductance was measured weekly with a leaf steady-state porometer (model PMR-4, PP Systems, Hitchin Herts, Great Britain), every hour from 7:30 to 11:30 solar time from three trees, on one tree per irrigation treatment, on five labeled sun-exposed leaves per tree. Leaf photosynthesis was measured weekly with a CIRAS-1 instrument (PP Systems, Hitchin Herts, Great Britain) at 7:00 and at 10:00 solar time from six trees on two labeled sun-exposed leaves per tree.

Leaf chlorophyll fluorescence measurements were conducted under field conditions using the *Pulse-Amplitude-Modulated Fluorometer* PAM-2100 (Heinz Walz GMBH, Effeltrich, Germany), an instrument that has been used widely in basic and applied fluorescence research [34]. The instrument enables the monitoring of steady-state and dark-adapted fluorescence features, providing flexibility for scientific research on chlorophyll fluorescence [11]. Therefore, the focus of this validation experiment was on measuring *steady-state* chlorophyll fluorescence as function of stress condition, enabling the assessment of natural fluorescence effects on canopy spectral reflectance for remote sensing detection under natural sun light conditions. Procedures



(a)

(b)

(c)

Fig. 1. Study area showing (a), (b) the orchard field used for the experiment as imaged by the CASI sensor at 1-m spatial resolution and eight spectral bands in the 400–950-nm spectral range. (c) Schematic view of the irrigation treatments randomly applied in six rows of 18 olive trees, obtaining three water stress levels.

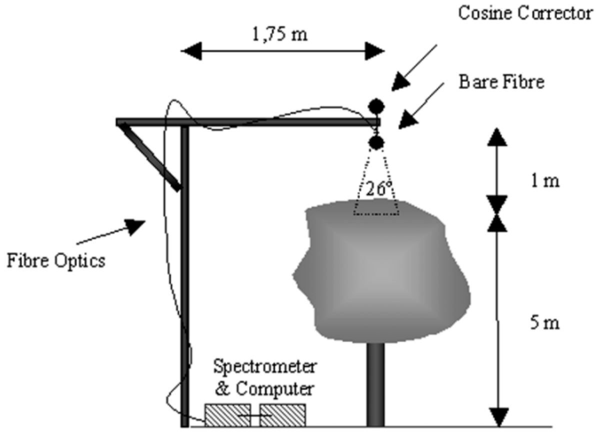


Fig. 2. Schematic view of the setup designed for downwelling irradiance and upwelling radiance acquisitions from the top of the tree crowns using a 0.065-nm FWHM spectrometer.

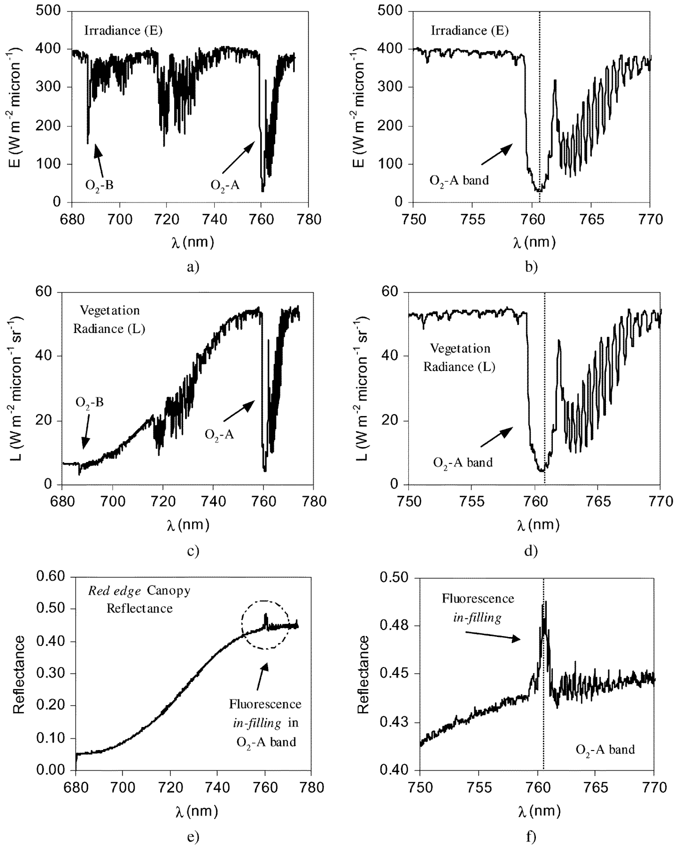
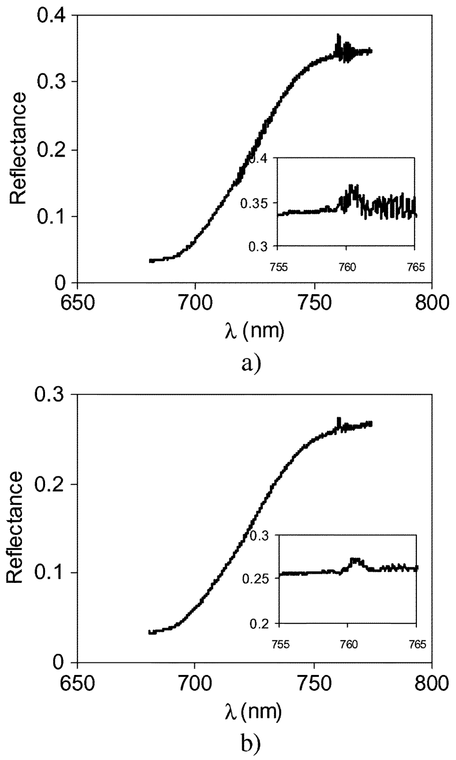


Fig. 3. (a), (b), Spectral irradiance and (c), (d) crown radiance measurements showing the O_2-A and O_2-B absorption bands collected with the narrow-band HR2000 spectrometer in the field. Calculation of reflectance in the (e), (f), red edge spectral region from (a), (b) irradiance and (c), (d) vegetation radiance spectra.

used for measuring steady-state (Ft) and fluorescence quantum yield ($\Delta\mathrm{F}/\mathrm{Fm}'$) were based on standard methodologies as documented in the PAM-2100 manual (Heinz-Walz-GmbH, 1993). The leaf was positioned in the PAM-2100 leaf clip holder, which exposes a sample area approximately 1-cm in diameter to the fiberoptic light emitter and detector array. Steady-state fluorescence Ft feature was measured along with the effective quantum yield $\Delta\mathrm{F}/\mathrm{Fm}'$, which denotes the actual efficiency of PSII photon capture in the light by closed Photosystem II (PSII) reaction centers, determined as $\Delta\mathrm{F}/\mathrm{Fm}' = (\mathrm{Fm}' - \mathrm{Ft})/\mathrm{Fm}'$, where Fm' is the maximal fluorescence of a preilluminated sample with PSII centers closed, and Ft is the fluorescence at

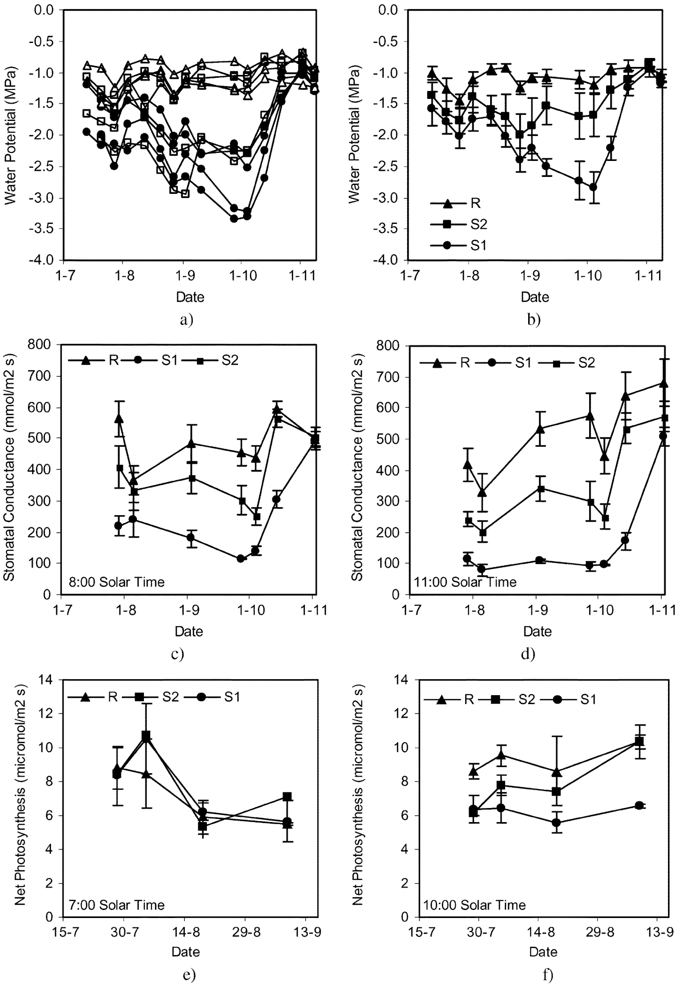


a)

b)

Fig. 4. Sample reflectance spectra collected with this high-resolution spectrometer from the crown of two trees, observing the red edge features between 700 and 750 nm, and the *in-filling* at 760 nm due to the vegetation fluorescence emission.

steady-state [35], [36]. An average of 50 exposed leaves per tree were used for Ft and $\Delta F/Fm'$ measurements five times per day between 6:30 h and 11:30 h solar time during June and November 2004.



a) b)

c) d)

e) f)

Fig. 5. Leaf water potential measurements conducted at 10:00 solar time from (a) 11 trees and (b) per treatment over the summer, showing large variations in water stress as function of the irrigation treatments. (c), (d) Stomatal conductance and (e), (f) photosynthesis rates were greater for the low-stressed well-watered treatment (R).

2) Canopy Spectral Measurements: Apparent reflectance data were obtained for the top of tree crowns using a 0.065-nm full-width half-maximum (FWHM) Ocean Optics HR2000 fiber-optics spectrometer (Ocean Optics, Dunedin, FL) installed on a 7.0-m height pole. The sensor head included a cosine collector for downwelling irradiance and a bare fiber for upwelling radiance (Fig. 2). The 0.065-nm FWHM HR2000 spectrometer provided spectral measurements in the 680–770-nm range with 2048 channels from top of the crowns in a diurnal data collection series coincident with leaf fluorescence measurements. The spectrometer was specifically designed for the experiment, and built with grating H11 of 1800 lines/mm, a 5-μm slit, an L2 detector collection lens, an OF1-OG590 longpass filter, and a set of high-reflectivity AgPlus mirrors model SAG-UPGD-HR to enhance sensitivity of the instrument for high-resolution measurements in low-light level experiments (all these model parts belong to the instrument manufacturer, Ocean Optics, Dunedin, FL). The instrument was connected to a 10-m long, 600-μm optical fiber using a CC-3 VIS-NIR cosine corrector-diffuser (Ocean Optics, Dunedin, FL) for irradiance measurements and the 26° field-of-view (FOV) bare fiber for vegetation radiance measurements giving an estimated viewing footprint of 1 m at the tree crown. The spectrometer was connected to a computer using an USB port, recording spectral data with the OOIBase32 software (Ocean Optics, Dunedin, FL). Due to dark current sensitivity of the instrument as function of ambient temperature, a Peltier thermally insulated box Model PT-100 (Magapor, Zaragoza, Spain) was used to house the spectrometer, keeping the internal temperature fixed at 24 °C ± 1 °C during field measurements.

Irradiance (E_g) calibration of the spectrometer attached to the fiber with the cosine corrector-diffuser was conducted in the laboratory using the LS-1-CAL calibrated tungsten halogen NIST-traceable light source (Ocean Optics, Dunedin, FL). Radiance (L) calibration was conducted with a Spectralon panel (Labsphere, North Sutton), calculating the apparent reflectance (R) of the tree crown with the equation $\mathrm{R} = \pi \cdot L/E_g$, which includes the reflected radiance and the fluorescence emission effects. Wavelength calibration of the instrument was conducted with the Hg-Ar HG-1 light source (Ocean Optics, Dunedin, FL). Aerosol optical measurements were acquired with a Microtops II sunphotometer (Solar Light, Philadelphia) measuring at 440-, 500-, 675-, 870-, and 936-nm spectral bands. The sunphotometer was connected to a GPS model GPS-12 (Garmin, Olathe, KS) for simultaneous readings of geographic location, altitude and solar geometry at the time of spectral acquisitions.

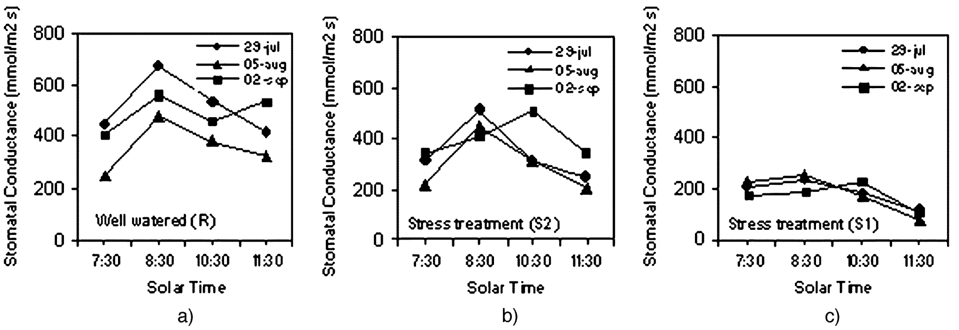


Fig. 6. Diurnal variations of stomatal conductance acquired throughout the season.

The spectral measurements were made over selected tree crowns under the three different water stress treatments, measuring irradiance and crown radiance at high spectral detail inside the O$_2$-A (760.5 nm) oxygen absorption band to study the detection of natural fluorescence *in-filling* [23]–[26] on spectral reflectance as function of stress status and diurnal variation of chlorophyll fluorescence. The prominent irradiance absorption lines due to atmospheric oxygen can be seen in Fig. 3(a) (O$_2$-A and O$_2$-B bands) measured with the HR2000 spectrometer at 0.065-nm FWHM in the 680–780-nm spectral range, observing the O$_2$-A absorption band in detail [Fig. 3(b)]. Internal structure and frequency features due to molecular oxygen can be observed in the very high spectral resolution of less than 1 Å FWHM used in this experiment. Field-measured vegetation radiance collected from pure tree crowns under sun conditions shows the oxygen absorption bands [Fig. 3(c)] and the spectral detail inside the O$_2$-A band in the radiance signal [Fig. 3(d)]. Calculation of the apparent reflectance spectra using the measured downwelling spectral irradiance [Fig. 3(a)] and vegetation radiance spectra [Fig. 3(c)] generates a *peak* or *in-filling* at the oxygen absorption maxima shown on the red edge vegetation spectra [Figs. 3(e) and (f)] which has been shown with potential to quantify natural chlorophyll fluorescence emission from vegetation. The high spectral resolution used in this experiment facilitates the observation of the structure of the *in-filling* of the O$_2$-A band, assessing the potential effects of the FWHM of the instrument used on the detection of the fluorescence signal superimposed on the canopy reflectance. The absolute *in-filling* amplitude was calculated using a band affected by fluorescence emission in O$_2$-A ($R_{760.59}$) and another band outside the fluorescence *in-filling* ($R_{759.5}$), obtaining a measure of the *in-filling* absolute amount by the subtraction $R_{760.59} - R_{759.5}$. The shape of the red edge reflectance spectrum collected with the HR2000 spectrometer, showing the *in-filling* at the O$_2$-A band [Fig. 3(e)] indicates the characteristic vegetation spectral shape in the red edge region normally acquired with other spectrometers of lower spectral resolution, suggesting that proper methods for spectral data collection were used. Crown spectral measurements and *in-filling* calculations were conducted in diurnal trials from trees under each water stress level, acquiring spectra and fluorescence measurements every 30 min between 8:30 and 11:30 solar time. Sample reflectance spectra obtained with this high-resolution spectrometer from the crown of two trees using the large pole are shown in Fig. 4 noting the red edge features between 700 and 750 nm, and at higher spectral detail between 755 and 765 nm with the figure insert showing the *in-filling* at 760 nm due to the vegetation natural fluorescence emission.

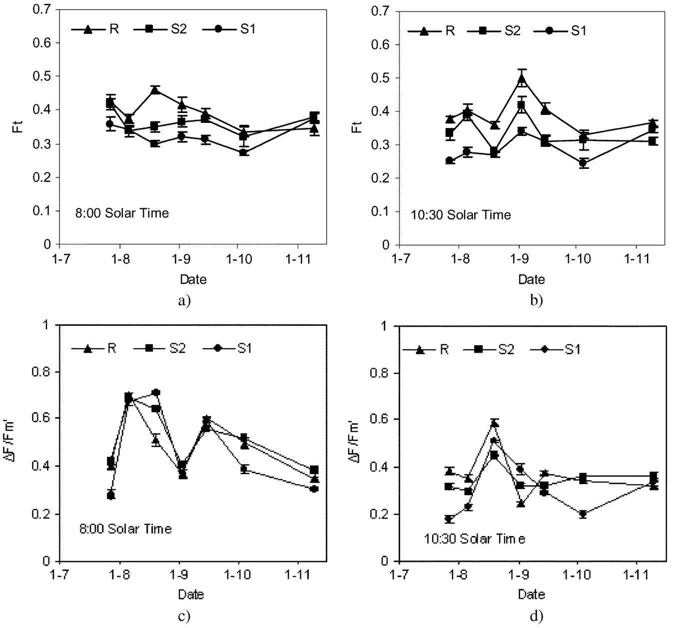
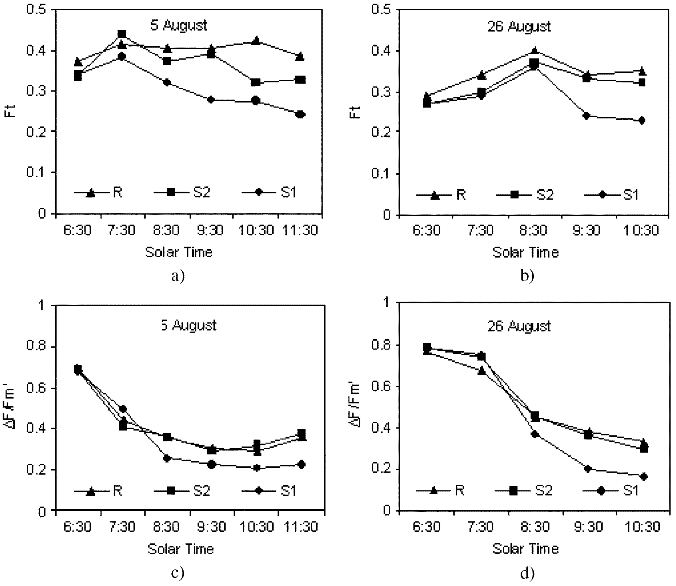


Fig. 7. Leaf chlorophyll fluorescence measurements for (a), (b) steady-state fluorescence Ft and (c), (d) fluorescence quantum yield $\Delta F/Fm'$.

III. RESULTS

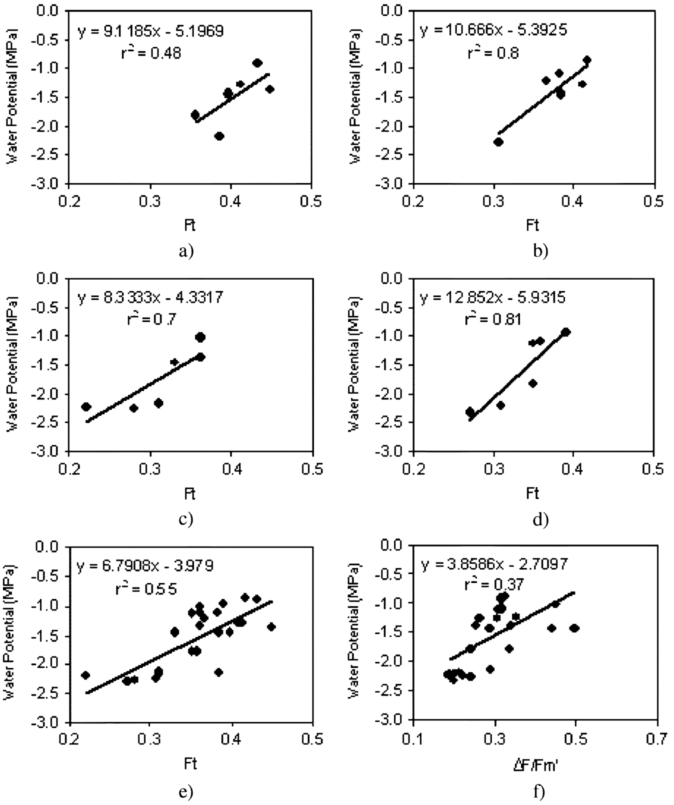
Leaf water potential measurements conducted at 10:00 solar time from the 11 trees monitored over the entire summer showed large variations in water stress as function of the irrigation treatment [Fig. 5(a)]. As expected, trees subjected to stress treatments S1 and S2 with lower irrigation doses were more stressed than control R trees with irrigation for full evapotranspiration (ET) [Fig. 5(b)]. Trees with maximum water stress (S1) reached -3.3 MPa on September 30, before the first rainfalls in early October. Control irrigation (R) trees showed stable water potential over the course of the experiment, reaching average values of -1 MPa with a minimum of -0.6 MPa, whereas the intermittent deficit irrigation treatment S2, yielded water potential values in between the R and S1 irrigation treatments [Fig. 5(b)]. A full recovery of the water potential at the end of the experiment was achieved as a consequence of the rainfalls after the summer. Consistently, both stomatal conductance [Fig. 5(c) and



a) b)

c) d)

Fig. 8. Diurnal monitoring of (a), (b) Ft and (c), (d) ΔF/Fm′ on two different dates with increasing water stress condition. (a), (c) For August 5, $\Psi_x = -1$ MPa (R), −1.7 MPa (S2), −1.8 MPa (S1). (b), (d) For August 26, $\Psi_x = -1.3$ MPa (R), −2 MPa (S2), −2.4 MPa (S1).



a) b)

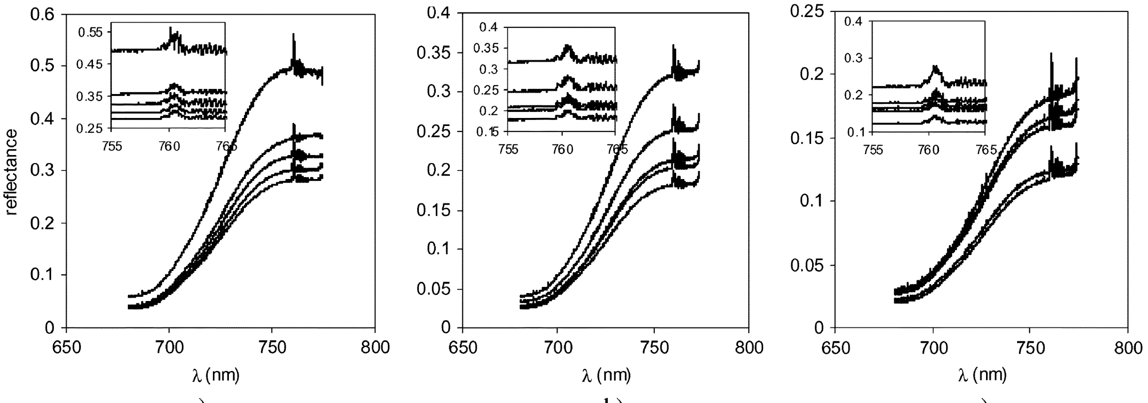
c) d)

e) f)

Fig. 9. Relationships obtained between leaf water potential Ψ_x and fluorescence measures Ft and ΔF/Fm′ for single days and for the entire experiment throughout the season.

(d)] and photosynthesis rates [Fig. 5(e) and (f)] were greater for the low-stressed well-watered treatment (R) trees at the two times of measurements in the morning (8.00 solar time) and midday (11.00 solar time). Stomatal conductance measured at 11:00 solar time was affected over the course of the experiment as function of stress status, increasing from 329 mmol/m^2 at the time of maximum stress to 680 mmol/m^2 at recovery for treatment R, from 203 to 573 mmol/m^2 for treatment S2, and from 80 to 507 mmol/m^2 for treatment S1. The photosynthesis rate measured at 10:00 solar time throughout the experiment consistently showed a reduction for treatment S1, as illustrated by 10.35 μmol/m^2 for well-irrigated trees and 6.5 μmol/m^2 for S1 stressed trees at the time of maximum stress (September 27). These measurements of water potential, conductance, and photosynthesis suggest that the experimental design to create a gradient in stress condition as function of water deficiency was successful, therefore enabling the study of the imprint of chlorophyll fluorescence on apparent crown spectral reflectance.

Diurnal variations of stomatal conductance acquired throughout the season between 7:30 and 11:30 solar time also showed a consistent behavior (Fig. 6) yielding maximum conductance rates for the control trees at 8:30 solar time, with a general decrease in the trend afterwards. Diurnal rates showed consistent trends for the control R [Fig. 6(a)] as compared with the deficit treatment S2 [Fig. 6(b)] and the highest stress S1 [Fig. 6(c)], yielding lower conductance rates over the course of the day. Both the seasonal and diurnal variation of conductance and photosynthesis measures, which showed a recovery at the end of the season, enabled the study of fluorescence effects as function of stress status. The leaf chlorophyll fluorescence measurements collected from selected tree crowns diurnally and throughout the season showed that steady-state fluorescence Ft [Fig. 7(a) and (b)] and fluorescence quantum yield ΔF/Fm′ [Fig. 7(c) and (d)] values for the low-stressed trees (R) were higher than for the high water-stressed treatments (S2, S1) at 8:00 [Fig. 7(a) and (c)] and 10:30 solar time [Fig. 7(b) and (d)] with measurements in three trees per irrigation treatment. Large differences were found between the extreme irrigation treatments R and S1 for both Ft and ΔF/Fm′, demonstrating that effects of deficit irrigation and water stress on steady-state fluorescence measures over the season were detected, tracking the water potential recovery at the end of the experiment. Results of the diurnal measurements for Ft [Fig. 8(a) and (b)] and ΔF/Fm′ [Fig. 8(c) and (d)] on two different dates with increasing water stress condition [Fig. 8(a) and (c) for August 5th, $\Psi_x = -1$ MPa (R), −1.7 MPa (S2), −1.8 MPa (S1); Fig. 8(b) and (d) for August 26th, $\Psi_x = -1.3$ MPa (R), −2 MPa (S2), −2.4 MPa (S1)] indicated that a maximum value for Ft was found between 7:30 and 8:30 solar time, decreasing afterwards. Moreover, the diurnal studies showed that the largest differences between treatments were found at 10:00 solar time for both Ft and ΔF/Fm′, with small differences in the emitted fluorescence signal as function of water stress at earlier times of the day. The diurnal measurements showed consistent lower Ft and ΔF/Fm′ values in water stressed trees, yielding mean values of Ft = 0.38 in well-irrigated trees with $\Psi_x = -1.3$ MPa (R) as compared to Ft = 0.21 in stressed trees with $\Psi_x = -2.4$ MPa (S1) on August 26th. Diurnal variations of steady-state fluorescence suggested that midday values acquired at 10:00 solar time for Ft and ΔF/Fm′ were therefore better for separating different irrigation treatments as function of water stress status.

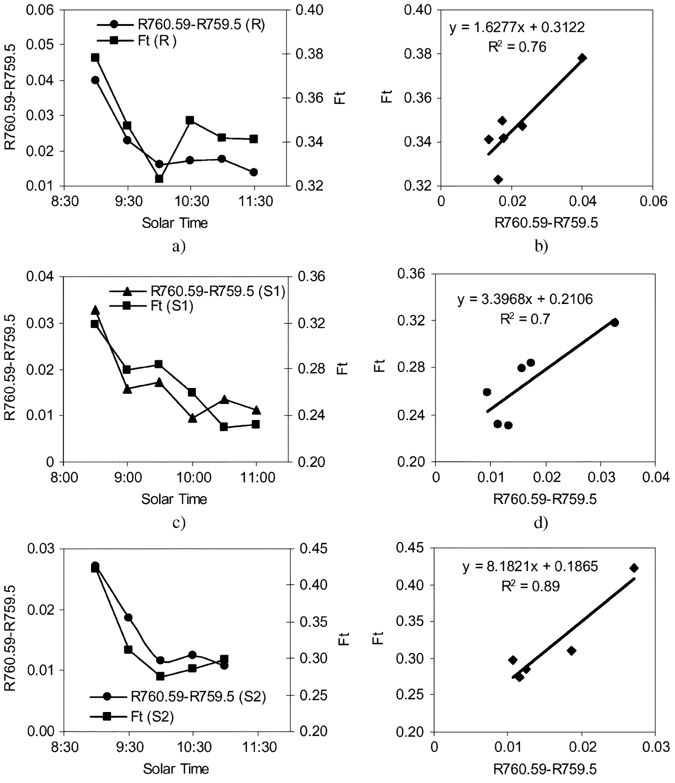


a) b) c)

Fig. 10. Diurnal tree-crown reflectance measurements acquired in the field with the HR2000 spectrometer at 0.065-nm FWHM in the 680–770-nm range.

The relationships obtained between leaf water potential Ψ_x and fluorescence measures Ft and $\Delta\mathrm{F/Fm'}$ for single days and for the entire experiment throughout the season indicated that steady-state fluorescence measures are good indicators of water stress status at the tree level (Fig. 9). The agreement between Ft and Ψ_x on different dates throughout the season for which coincident water potential and fluorescence measurements were made, showed that steady-state fluorescence Ft could detect differences in water potential, with $r^2 = 0.48$ [July 21; Fig. 9(a)], $r^2 = 0.8$ [August 4, Fig. 9(b)], $r^2 = 0.7$ [August 25, Fig. 9(c)], and $r^2 = 0.81$ [September 1, Fig. 9(d)]. For the entire experiment, the agreement between leaf water potential and Ft [$r^2 = 0.54$, Fig. 9(e)] and $\Delta\mathrm{F/Fm'}$ [$r^2 = 0.37$, Fig. 9(f)] suggested that steady-state fluorescence Ft can potentially be used as a consistent indicator for water stress in olive tree crops.

The diurnal measurements conducted with the Ocean Optics HR2000 spectrometer at 0.065-nm FWHM in the 680–770-nm range demonstrated that the fluorescence *in-filling* at the O_2-A band was detected and contributed to the tree crown reflectance signal, manifested in a sudden increase of the apparent reflectance at 760 nm (Figs. 3, 4, and 10). The amplitude of the 760-nm peak, calculated as $\mathrm{R}_{760.59} - \mathrm{R}_{759.5}$, potentially associated with the emission of natural fluorescence, was compared diurnally with steady-state fluorescence Ft measurements collected at the same time from the trees under the different stress levels (Fig. 10). The results obtained in diurnal trials between Ft and the O_2-A peak amplitude (Fig. 11) yielded correlations of $r^2 = 0.76$ (for a tree with $\Psi_x = -0.825$ MPa, treatment R well irrigated), $r^2 = 0.89$ ($\Psi_x = -1.05$ MPa, stress treatment S2) and $r^2 = 0.7(\Psi_x = -3.35$ MPa, stress treatment S1), indicating the close relationships between Ft and the fluorescence *in-filling* signal at the crown level on individual trees. These results suggest the potential monitoring of diurnal changes in fluorescence through apparent reflectance spectra at the canopy level. Moreover, results for the diurnal measurements on all trees under the three different stress conditions yielded $r^2 = 0.61$ (Fig. 12), showing that well-irrigated trees (R) exhibited higher Ft and *in-filling* values than stressed S1 and S2 trees. These results suggest that variations in natural fluorescence emission were successfully tracked through inferred reflectance spectra on all trees under different water stress conditions, enabling the identification of stress levels. Nevertheless, the dependency of the emission peak on reflectance bidirectional reflectance distribution function (Fig. 10) still requires critical attention due to the known changes as a function of the viewing geometry and solar angle that accompany diurnal changes [32].



a) b) c) d) e) f)

Fig. 11. Relationships obtained in diurnal trials between Ft and the O_2-A peak amplitude calculated from crown reflectance.

IV. CONCLUSION

Apparent reflectance measurements obtained from tree crowns with a high-resolution Ocean Optics HR2000 spectrometer at 0.065-nm FWHM in the 680–770-nm range demonstrated that the observed fluorescence *in-filling* in the O_2-A band at 760.5 nm varied with water stress status in orchard trees.

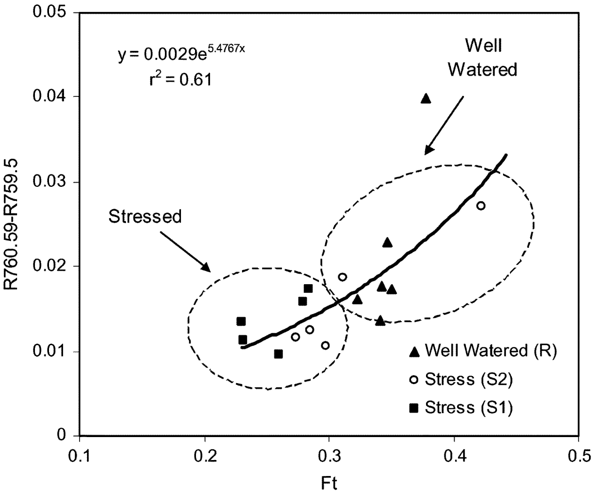


Fig. 12. Relationships obtained between field-measured Ft and fluorescence *in-filling* extracted from crown reflectance on all trees under the three stress levels.

Diurnal and seasonal measurements acquired over the course of the summer experiment showed consistently lower Ft and $\Delta\text{F}/\text{Fm}'$ values in water-stressed trees, yielding mean values of $\text{Ft} = 0.38$ in well-irrigated trees with $\Psi_x = -1.3$ MPa (R) and $\text{Ft} = 0.21$ in stressed trees with $\Psi_x = -2.4$ MPa (S1). Diurnal measurements of steady-state fluorescence demonstrated that midday values acquired at 10:00 solar time for Ft and $\Delta\text{F}/\text{Fm}'$ were better for separating different irrigation treatments as function of water stress status.

The relationships obtained for leaf water potential with Ft and $\Delta\text{F}/\text{Fm}'$ for single days and for the full dataset comprising the entire experiment throughout the season demonstrated that steady-state fluorescence Ft is a good indicator of water stress status at the tree level in olive canopies. The agreement between Ft and Ψ_x throughout the season showed that steady-state fluorescence could detect differences in water potential levels, with determination coefficients ranging between $r^2 = 0.48$ and $r^2 = 0.81$ for individual dates. For the entire experiment, the agreement between leaf water potential and Ft $(r^2 = 0.54)$ and $\Delta\text{F}/\text{Fm}'(r^2 = 0.37)$ demonstrated that steady-state fluorescence Ft shows potential as a consistent indicator of water stress in olive tree crops.

The amplitude of the 760-nm peak, calculated as $\text{R}_{760.59} - \text{R}_{759.5}$, associated with the emission of natural fluorescence, was compared diurnally with steady-state fluorescence Ft measurements collected at the same time from the trees under different stress levels. The results obtained in diurnal trials between Ft and the O_2-A peak amplitude yielded $r^2 = 0.76$ (for a tree with $\Psi_x = -0.825$ MPa, well-irrigated treatment R), $r^2 = 0.89$ ($\Psi_x = -1.05$ MPa, stress treatment S2) and $r^2 = 0.7$ ($\Psi_x = -3.35$ MPa, stress treatment S1), demonstrating the link between Ft and *in-filling* of the 760-nm apparent reflectance at the crown level on individual trees. Results for the diurnal measurements considering all trees under study yielded $r^2 = 0.61$, suggesting that natural fluorescence was successfully monitored through reflectance spectra on all trees under different water stress conditions.

These results obtained at canopy level for water stress monitoring through fluorescence detection are in agreement with studies conducted by Meroni [37] at the leaf level, and with canopy results by Moya [23], showing that fluorescence emission can be detected at the leaf level and on a corn canopy under diuron herbicide penetration using the O_2-A band. In this case the study was conducted on orchard trees, showing that water stress variations were responsible for corresponding fluorescence effects on canopy reflectance, enabling its detection with a narrow-band spectrometer under natural field conditions. Conclusions may lead toward important implications for water stress monitoring and water scheduling applications in agriculture, suggesting the potential application of high spectral resolution reflectance data for monitoring natural chlorophyll fluorescence emission at the canopy level as an indicator of water stress.

ACKNOWLEDGMENT

F. Villalobos, L. Testi, and I. Calatrava are acknowledged for scientific and technical support.

REFERENCES

[1] G. Papageorgiou, "Chlorophyll fluorescence: An intrinsic probe of photosynthesis," in *Bioenergetics of Photosynthesis*. New York: Academic, 1975, pp. 319–371.

[2] G. H. Krause and E. Weis, "Chlorophyll fluorescente as a tool in plant physiology. II. Interpretation of fluorescence signals," *Photosynthesis Res.*, vol. 5, pp. 139–157, 1984.

[3] U. Scheiber and W. Bilger, "Rapid assement of stress effects on plant leaves by chlorophyll fluorescence measurements," in *Plant Response to Stress*, J. D. Tenhunen and E. M. Catarino, Eds. Berlin, Germany: Springer-Verlag, 1987, pp. 27–53.

[4] H. K. Lichtenthaler and U. Rinderle, "The role of chlorophyll fluorescence in the detection of stress conditions in plant," *CRC Crit. Rev. Anal. Chen.*, vol. 19, pp. 529–585, 1988. (suppl).

[5] H. K. Lichtenthaler, "The kautzky effect: 60 years of chlorophyll fluorescence induction kinetics," *Photosynthetica*, vol. 27, pp. 45–55, 1992.

[6] W. Larcher, "Photosynthesis as a tool for indicating temperature stress events," in *Ecophysiology of Photosynthesis*, E. D. Schulze and M. M. Caldwell, Eds. Berlin: Springer-Verlag, 1994, pp. 261–277.

[7] U. Schreiber, W. Bilger, and C. Neubauer, "Chlorophyll fluorescence as a nondestructive indicator for rapid assessment of *in vivo* photosynthesis," *Ecol. Stud.*, vol. 100, pp. 49–70, 1994.

[8] A. Gilmore and A. Govindjee, "How higher plants respond to excess light: Energy dissipation in photosystem II," in *Concepts in Photobiology: Photosynthesis and Photomorphogenesis*, G. S. Singhal, G. Renger, S. K. Sopry, K.-D. Irrgang, and K.-D. Govindjee, Eds. Dordrecht, The Netherlands: Kluwer, 1999, pp. 513–548.

[9] H. A. Frank, A. J. Young, G. Britton, and R. J. Cogdell, Eds., *The Photochemistry of Carotenoids*. Dordrecht, The Netherlands: Kluwer, 1999.

[10] W. Yahyaoui, R. Harnois, and R. Carpentier, "Demonstration of thermal dissipation of absorbed quanta during energy-dependent quenching of chlorophyll fluorescence in photosynthetic membranes," *FEBS Lett.*, vol. 440, pp. 59–63, 1998.

[11] J. Flexas, J.-M. Briantais, Z. Cerovic, H. Medrano, and I. Moya, "Steady-State and maximum chlorophyll fluorescence responses to water stress in grapevine leaves: A new remote sensing system," *Remote Sens. Environ.*, vol. 73, pp. 282–297, 2000.

[12] J. Flexas, J. M. Escalona, S. Evain, J. Gulias, I. Moya, C. B. Osmond, and H. Medrano, "Steady-state chlorophyll fluorescence (Fs) measurements as a tool to follow variations of net CO2 assimilation and stomatal conductance during water-stress in C-3 plants," *Physiologia Plantarum.*, vol. 114, no. 2, pp. 231–240, 2002.

[13] J. Flexas, J. M. Escalona, and H. Medrano, "Water stress induces different levels of photosynthesis and electron transport rate regulation in grapevines," *Plant Cell Environ.*, vol. 22, pp. 39–48, 1999.

[14] J. C. McFarlane, R. D. Watson, A. F. Theisen, R. D. Jackson, W. L. Ehrler, P. J. Pinter, S. B. Idso, and R. J. Reginato, "Plant stress detection by remote measurement of fluorescence," *Appl. Opt.*, vol. 19, no. 19, pp. 3287–3289, 1980.

[15] G. A. Carter, A. F. Theisen, and R. J. Mitchell, "Chlorophyll fluorescence measured using the Fraunhofer line-depth principle and relationship to photosynthetic rate in the field," *Plant Cell Environ.*, vol. 13, pp. 79–83, 1990.

[16] G. A. Carter, J. H. Jones, R. J. Mitchell, and C. H. Brewer, "Detection of solar excited chlorophyll a fluorescence and leaf photosynthetic capacity using a Fraunhofer line radiometer," *Remote Sens. Environ.*, vol. 55, pp. 89–92, 1996.

[17] C. Buschmann and H. K. Lichtenthaler, "Reflectance and chlorophyll fluorescence signatures in leaves," in *Applications of Chlorophyll fluorescence*, H. K. Lichtenthaler, Ed. Dordrecht, The Netherlands: Kluwer, 1988, pp. 325–332.

[18] J. A. Gamon and J. S. Surfus, "Assesing leaf pigment content and activity with a reflectometer," *New. Phytol.*, vol. 143, pp. 105–117, 1999.

[19] P. J. Zarco-Tejada, J. R. Miller, G. H. Mohammed, and T. L. Noland, "Chlorophyll fluorescence effects on vegetation apparent relectance. II. Laboratory and airborne canopy-level measurements with hyperspectral data," *Remote Sens. Environ.*, vol. 74, pp. 582–595, 2000.

[20] P. J. Zarco-Tejada, J. R. Miller, G. H. Mohammed, T. L. Noland, and P. H. Sampson, "Chlorophyll fluorescence effects on vegetation apparent relectance. II. Laboratory and airborne canopy-level measurements with hyperspectral data," *Remote Sens. Environ.*, vol. 74, pp. 596–608, 2000.

[21] P. J. Zarco-Tejada, J. Pushnik, S. Dobrowski, and S. L. Ustin, "Steady-state chlorophyll a fluorescence detection from canopy derivative reflectance and *Double-Peak* Red-Edge effects," *Remote Sens. Environ.*, vol. 84, no. 2, pp. 283–294, 2003.

[22] P. K. E. Campbell, E. M. Middleton, L. A. Corp, J. E. McMutey, M. S. Kim, E. W. Chappelle, and L. M. Butcher, "Contribution of chlorophyll fluorescence to the reflectance of corn foliage," *Proc. IGARSS*, 2002.

[23] I. Moya, L. Camenen, S. Evain, Y. Goulas, Z. G. Cerovic, G. Latouche, J. Flexas, and A. Ounis, "A New instrument for passive remote sensing. 1. Measurements of sunlight-induced chlorophyll fluorescence," *Remote Sens. Environ.*, vol. 91, pp. 186–197, 2004.

[24] S. Evain, J. Flexas, and I. Moya, "A new instrument for passive remote sensing: 2. Measurement of leaf and canopy reflectance changes at 531 nm and their relationship with photosynthesis and chlorophyll fluorescence," *Remote Sens. Environ.*, vol. 91, no. 2, pp. 175–185, 2004.

[25] P. J. Zarco-Tejada, O. Pérez-Priego, G. Sepulcre-Cantó, J. R. Miller, and E. Fereres, "Chlorophyll fluorescence detection with a high-spectral resolution spectrometer through *in-filling* of the O_2-A band as function of water stress in olive trees," presented at the *2nd Int. Workshop on Remote Sensing of Vegetation Fluorescence*, Montreal, QC, Canada, Nov. 2004, pp. 17–19.

[26] L. Liu, Y. Zhang, J. Wang, and C. Zhao, "Detecting solar-induced chlorophyll fluorescence from field radiance spectra based on the Fraunhofer line principle," *IEEE Trans. Geosci. Remote Sens.*, vol. 43, no. Apr., pp. 827–832, 2005.

[27] S. W. Maier, K. P. Günther, and M. Stellmes, "Remote sensing and modeling of solar induced fluorescence," presented at the *1st Workshop on Remote Sensing of Solar Induced Vegetation Fluorescence*, Noordwijk, Netherlands, 2002.

[28] P. J. Zarco-Tejada, J. R. Miller, D. Haboudane, N. Tremblay, and S. Apostol, "Detection of chlorophyll fluorescence in vegetation from airborne hyperspectral CASI imagery in the red edge spectral region," in *Proc. IGARSS*, vol. 1, Toulouse, France, Jul. 21–25, 2004, pp. 598–600.

[29] S. W. Maier, "Modeling the radiative transfer in leaves in the 300 nm to 2.5 μm wavelength region taking into consideration chlorophyll fluorescence—The leaf model SLOPE," Ph.D. thesis, Tech. Univ. München, München, Germany, 2000.

[30] J. R. Miller, M. Berger, L. Alonso, Z. Cerovic, Y. Goulas, S. Jacquemoud, J. Louis, G. Mohammed, I. Moya, R. Pedros, J. F. Moreno, W. Verhoef, and P. J. Zarco-Tejada, "Progress on the development of an integrated canopy fluorescence model," in *Proc. IGARSS*, vol. 1, Toulouse, France, Jul. 21-25, 2004, pp. 601–603.

[31] R. Pedrós, S. Jacquemoud, Y. Goulas, J. Louis, and I. Moya, "A new leaf fluorescencemodel," presented at the *2nd Int. Workshop on Remote Sensing of Vegetation Fluorescence*, Montreal, QC, Canada, Nov. 17–19, 2004.

[32] W. Verhoef, "Extension of SAIL to model solar-induced canopy fluorescence spectra," presented at the *2nd Int. Workshop on Remote Sensing of Vegetation Fluorescence*, Montreal, QC, Canada, Nov. 17–19, 2004.

[33] P. J. Zarco-Tejada, J. R. Miller, R. Pedrós, W. Verhoef, and M. Berger, "FluorMODgui: A graphic user interface for the spectral simulation of leaf and canopy fluorescence effects," presented at the *2nd Int. Workshop on Remote Sensing of Vegetation Fluorescence*, Montreal, QC, Canada, Nov. 17–19, 2004.

[34] G. H. Mohammed, W. D. Binder, and S. L. Gillies, "Chlorophyll fluorescence: A review of its practical forestry applications and instrumentation," *Scand. J. Forest Res.*, vol. 10, pp. 383–410, 1995.

[35] B. Genty, J.-M. Briantais, and N. R. Baker, "The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence," *Biochim Biophys Acta*, vol. 990, pp. 87–92, 1989.

[36] O. Van Kooten and J. F. H. Snel, "The use of chlorophyll fluorescence nomenclature in plant stress physiology," *Photosynth. Res.*, vol. 25, pp. 147–150, 1990.

[37] M. Meroni, R. Colombo, and S. Cogliati, "High resolution leaf spectral signature for the detection of solar induced chlorophyll fluorescence," presented at the *2nd Int. Workshop on Remote Sensing of Vegetation Fluorescence*, Montreal, QC, Canada, Nov. 17–19, 2004.

Oscar Pérez-Priego received the degree in forest engineering from the University of Córdoba, Córdoba, Spain. He is currently pursuing the Ph.D. degree in carbon balance and water productivity at the Institute for Sustainable Agriculture, Spanish Council for Scientific Research, Córdoba.

His research interests are crop physiology, remote sensing detection of water stress through chlorophyll fluorescence, and methods based on high-resolution spectrometry at leaf and canopy levels for biochemistry and biophysical estimates using reflectance spectra.

Pablo J. Zarco-Tejada received the Agricultural Engineering degree from the University of Córdoba, Córdoba, Spain, the Masters degree in remote sensing from University of Dundee, Dundee, U.K., and the Ph.D. degree in earth and space science at York University, Toronto, ON, Canada.

He works with AVIRIS, CASI, HyMAP, ROSIS, and MERIS sensors to estimate leaf biochemical and canopy biophysical variables through leaf and canopy modeling, and the effects of chlorophyll fluorescence on leaf apparent reflectance through radiative transfer simulation. He was a Postdoctoral Researcher and Lecturer in remote sensing at the University of California, Davis. He is currently at the Institute for Sustainable Agriculture, Spanish Council for Scientific Research, Córdoba. He is a member of the Editorial Advisory Board for the *European Journal of Agronomy*.

John R. Miller received the B.Eng. degree in physics and the M.Sc. and Ph.D. degrees in space physics from the University of Saskatchewan, Saskatoon, SK, Canada, in 1963, 1966, and 1969, respectively. During his M.Sc, and Ph.D. work, he studied the aurora borealis using rocket-borne radiometers.

He then spent two years on a postdoctoral fellowship at the Herzberg Institute at the National Research Council, Ottawa, ON, Canada. He joined the faculty at York University, Toronto, ON, in 1972, where he is currently a Professor of physics and Chair of the Department of Earth and Space Science and Engineering. His remote sensing interests include extraction of biophysical surface parameters through models from forest canopy reflectance and water color reflectance. Over the past decade, his primary focus has been on the application of reflectance spectroscopic techniques in remote sensing using imaging spectrometer sensors.

Guadalupe Sepulcre-Cantó is currently pursuing the Ph.D. degree in remote sensing at the University of Valencia, Valencia, Spain, conducting her research work at the Institute for Sustainable Agriculture, Spanish Council for Scientific Research, Córdoba, Spain.

Her research interests are thermal remote sensing and water stress detection in agricultural canopies, investigating the effects of soil and shadow components on the retrieval of crown temperature. Her work is conducted with airborne hyperspectral and thermal sensors, such as the Airborne Hyperspectral Scanner, studying scaling up methods for operational application of methodologies for stress detection with the Advanced Spaceborne Thermal Emission and Reflection Radiometer.

Elias Fereres received the Dr.Ing.Agr. degree from the Polytechnic University of Madrid, Madrid, Spain, in 1969, and the Ph.D. degree in ecology from the University of California, Davis, in 1976.

He has broad interests in agricultural water management and conservation. He has directed 27 doctoral theses and has published over 110 papers and book chapters, including chapters in the irrigation monographs of the American Society of Agronomy and the American Society of Agricultural Engineers, Co-Editor of *Irrigation Science* (Springer), member of the Technical Advisory Committee of the CGIAR, and former President of the European Society for Agronomy.