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Analysis of photosynthetic activity at the leaf and canopy levels from reflectance measurements: a case study

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Abstract

The photochemical reflectance index (PRI), based on reflectance signatures at 531 and 570 nm, and associated with xanthophyll pigment inter-conversion and related thylakoid energisation, was evaluated as an indicator of photosynthetic function in a Mediterranean holm oak (*Quercus ilex* L.) coppice. The chlorophyll fluorescence pulse-amplitude-modulation and the eddy correlation techniques were used to estimate the photosystem 2 photochemical efficiency of leaves and the CO_2 flux over the canopy, respectively. The reflectance and fluorescence techniques yielded identical estimates of the photosynthetic activity in leaves exposed to dark-light-dark cycles or to a variable irradiance in laboratory. However, there was no such correlation between photosynthetic performance and PRI when applied to a sun-exposed canopy in field conditions. Fluorescence profiles inside the canopy and especially a helpful use of multispectral reflectance imaging highlight the limitations of such method.

Additional key words: fluorescence; imaging; photochemical reflectance index; photosynthetic activity; *Quercus ilex*.

Introduction

Properties of light-induced fluorescence and reflected radiation have been used in the last ten years by agronomists and ecologists for the non-destructive analysis of the structural or physiological status of plants. Chlorophyll (Chl) fluorescence, using the pulse-amplitude modulation technique, is a powerful tool suitable for studying photosynthetic processes and stress physiology. However, up until now, notwithstanding promising results (McFarlane *et al.* 1980, Cecchi *et al.* 1994, Günther *et al.* 1994, Cerovic *et al.* 1996), fluorescence based methods are still not sufficiently perfected for remote sensing applications.

The optical behaviour of leaves depends on their anatomical (leaf surface properties and internal structure) and biochemical (concentration and distribution of biochemical components) properties (Ourcival *et al.* 1999). Thus, analysis of reflectance gave rise to the development of passive optical remote sensing of aboveground biomass and leaf area index, LAI (Jackson 1983) and of the photosynthetic pigment contents (Gitelson and Merzlyak 1996, Blackburn 1998, Datt 1999). A number of works have dealt with identifying reflectance indices that could be used as indicators of environmental stresses (Carter 1994, Peñuelas *et al.* 1997a,b), nutritional deficits (Demetriades-Shah and Steven 1988), pollutants (Peñuelas *et al.* 1995a), or pest attacks (Malthus and Madeira 1993).

Among these parameters, the photochemical reflectance index (PRI) has been proposed and can be formulated as follows (Peñuelas *et al.* 1995b):

$$PRI = (R_{531} - R_{570})/(R_{531} + R_{570})$$

where R_{531} and R_{570} are the reflectances at 531 and 570 nm, respectively. Reflectance changes at 531 nm upon changing irradiance are associated with the thylakoid energisation and zeaxanthin-antheraxantin-violaxanthin inter-conversion. The relative concentration of xanthophyll cycle pigments is closely related to PS2 photochemical efficiency. Moreover, heat dissipation and optical changes adjust rapidly to excess photosynthetically photon flux density (PPFD). Using the reference wavelength 570 nm reduces the effects of changes in reflectance produced by chloroplast movements. Linear relationships were observed at leaf level

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between PRI and photochemical efficiency (Peñuelas *et al.* 1995b). Gamon *et al.* (1995) analysed grasslands, chaparral, and deciduous and evergreen woodlands using at the canopy level calibrated imaging spectrometer and NASA's airborne visible/infrared imaging spectrometer (AVIRIS) values. These experiments showed the difficulty in using this type of index at a scale larger than the leaf.

The present work aims to test, by a case study, the use of PRI as a tool for near or remote detection of photosynthetic performance. Is it possible to quantify plant responses to environmental stress at either the leaf or canopy level and what are the main difficulties inherent in this type of measurement?

Materials and methods

Plants: An evergreen tree, holm oak (*Quercus ilex* L.), dominant in the Mediterranean Basin, was selected. Measurements were carried out from 1 June to 31 July 1999 in a 57-year old *Q. ilex* coppice stand, the Puéchabon State forest, 35 km NW from Montpellier (France), 3°35'50"E and 43°44'30"N, and 250 m above sea level. The annual mean temperature and precipitation (1984-1996) were 13.4 °C and 812 mm, respectively. The estimated annual evapotranspiration was 950 mm. Annual short wave irradiation was 4867 MJ m^{-2}.

The average tree height was about 5 m. The stem density was 1.0 per m^2 (diameter at breast height >1 cm) and 0.1 (DBH >7.5 cm) (Floret *et al.* 1992). The main other characteristics of this site, located in a karstic zone with a Mediterranean sub-humid climate, were described by Floret *et al.* (1989).

The leaf area index (LAI), deduced from optical estimates with the *LAI-2000* plant canopy analyser (*LI-Cor*, Lincoln, NE, USA), was about 2.96 ± 0.30 (Joffre *et al.* 1996). Remote estimates by *Landsat TM* normalized difference vegetation index (NDVI) gave 3.8 (Teixeira Filho *et al.* 1996).

Reflectance measurements:

Radiometric technique: Reflectance was measured with a two-channel radiometer mounted with a 35 cm long fibre optic probe extension and a two-channel amplifier (*SKR 116* and *SKP 120*, *Skye Instruments*, Powys, UK). The "opamp" virtual ground current to voltage converter of the amplifier produced an output of 0.1 V per µmol m^{-2} s^{-1}. A rugged liquid crystal display multimeter (41/2-digit, 20 000 count, DC voltage range from 200 mV to 10 µV, and accuracy ±0.05 % of reading + 3 digits) was used for reading the output voltages. The two spectral bands of the radiometer were 530 and 570 nm (maximum transmission wavelengths). The transmission half width of the filters was 10 nm. Each reading was calibrated using an *Eastman Kodak* standard 18 % reflectance grey card. Measurements made with a laboratory spectrometer (*NIRSystems*, model *6500*) indicated that the reflectivity of the grey card was 0.180 ± 0.005 in the visible range.

Imaging technique: A CCD camera (*CCD800*, *Integrated Scientific Imaging Systems*, Santa Barbara, CA, USA) was selected (imaging device *CCD 800 KAF 0400* front irradiated sensor, pixel size 9 µm^2, array format 768×512 pixels, imaging area 6.91×4.60 mm, dynamic range 16 384 grey levels). This device was used with a 50 mm f/1.8 lens (*Nikkor*, *Nikon*, Japan). The sub-sequent diagonal acceptance angle was close to 10°. The detectable range of wavelengths resulting from the sensor, the window, and the optical properties of the lens was 400-1100 nm.

A built-in eight-position filter wheel provided multi-spectral imaging. It was controlled by camera electronics, servo electronics, and optical stop to sense position. Two optical multi-layer dielectric interference filters (bandpass filters *Corion*, Holliston, MA, USA, types *P10-530-F* and *P10-570-F*, bandwidth 10±2 nm, minimum peak transmittance 50 %) were used. The camera and filter positions were operated by a PC laptop through a *PCMCIA* interface and the image acquisition software *Compuscope Procontrol 14HSD* (*Integrated Scientific Imaging Systems*, Santa Barbara, CA, USA). Readings were calibrated using the same standard reflectance card. The *IDRISI* software (Clark University, Graduate School of Geography, MA, USA) was used for image processing.

Reflectance measurements at the leaf level were made in the laboratory with the two-channel radiometer. All samples were taken from the same aspect (south-east) and at the same time of day (near solar noon). Branches were immediately put in water and allowed a minimum of 30 min dark adaptation in a dark room. Measurements were conducted in a leaf-clip holder (*2030-B*, *H. Walz*, Effeltrich, Germany) on the adaxial surface of one-year-old leaves of *Q. ilex* under steady-state conditions. The samples were irradiated with actinic "white light" delivered by a 20 W dichroic halogen lamp fitted with a short pass interference filter (cut-off wavelength, 5 % of transmittance, 712 nm). Two electrical fans (*V245L*, *Micronel*, Zurich, Switzerland) were used to prevent heating by the lamp. Light was collected by the fibre optic of the radiometer as a cone extending from its polished surface at 40 degrees. The tip of the fibre optics was located at 1.0 cm and 60° from the surface of the leaf.

Reflectance measurements at the canopy level: Reflectance factors of the *Q. ilex* stand were measured on sunny days at about 4 m from the top of the plant canopy (8.5 m from the soil), from a scaffolding tower just above the canopy. Radiometric and imaging techniques were used by turns. An acceptance angle of about 15° was selected for the two-channel radiometer by mean of a suitable diaphragm down of the tip of the fibre optics. Thus, the diameter of the analysed area was about 1 m. Reflection measurements were made on a 2.80 m long transect at points spaced 5 cm apart. The 56 points had N, E, and W facing aspects; due to permanent measuring devices in the field view of the radiometer, the S aspect was not available. The measuring sequence for one aspect lasted about 3 min. Reflectance factors were calculated for each of the two spectral bands using the calibration values obtained with the radiance measurements from the nadir direction of the reference panels just before and after the series of measurements for the given aspect. Measurements were made six times a day. The CCD camera was set facing down from the same platform of the tower. Pictures were taken from the same points within the same times. A reference card was set in the field of view of the camera.

Measurement of photosynthesis and climate values: Continuous measurement of gaseous (H_2O and CO_2) and energy exchanges were deduced from the eddy co-variance (turbulent fluctuations) method, a reliable tool for directly monitoring net gaseous exchanges of Mediterranean plants, at the canopy scale (Valentini *et al.* 1991). Eddy covariance devices and a weather station were located at 10.5 m on the same tower. The system consisted mainly of a fast-responding, closed-path infra-red gas analyser to measure CO_2 and H_2O (*LI6262*, *Li-Cor*, Lincoln, NE, USA), a three-dimensional sonic anemometer to measure wind velocity and air temperature (*R3*, *Gill Instrument Solent*, Lymington, UK), a pumping unit, and a *Pentium 133MHz MMX* laptop computer (*Toshiba Power Satellite 230 CDX*) with the *EDISOL* software to enable the flux calculations to be made as half-hour averages. Automated weather values were also available on the top of the tower and in a clearing at a distance of 200 m. Short-wave radiation was measured with a silicon cell pyranometer (*SKS1110 Skye Instruments*, Powys, UK). Air temperature and humidity were monitored with platinum resistance and polymer humidity sensors (*MP100 Rotronic Instrument Corp.*, New York, USA) both in a radiation shield (*Gill Instrument Solent*, Lymington, UK).

Chl fluorescence measurements:

Fluorometer and calculated parameters: Chl fluorescence was measured at the leaf level. Fluorescence parameters (for nomenclature see van Kooten and Snel 1990) were determined on the adaxial surface of one-year-old leaves of *Q. ilex*, using a pulse-amplitude-modulation fluorometer (*PAM 2000*, *H. Walz*, Effeltrich, Germany).

The initial fluorescence F_0 was first determined. The F_0 level is the fluorescence emission when all reaction centres are open and photochemical quenching is maximal. F_0 level increases may occur if photosystem 2 (PS2) reaction centres are damaged, or if the transfer of excitation energy from the antenna to the reaction centres is impeded. F_0 depends on the size of PS2 Chl antenna and on the functional integrity of reaction centres (Krause and Weis 1991). A 1 s pulse of high irradiance by "white light" (max. 12 000 μmol m^{-2} s^{-1}) from the halogen lamp of the fluorometer (<710 nm) was used to determine maximum fluorescence (F_m). The steady-state value of fluorescence (F_s) was measured, and a second pulse of high intensity "white light" was used to determine maximum fluorescence in the light-adapted state (F_m'). The photochemical efficiency of PS2 in the dark (maximum PS2 quantum yield) was calculated according to Butler and Kitajima 1975):

$$F_v/F_m = (F_m - F_0)/F_m \quad [1]$$

The photochemical efficiency of PS2 in the light (actual PS2 quantum yield) was calculated according to Genty *et al.* (1989):

$$\Delta F/F_m' = (F_m' - F_s)/F_m'$$

Fluorescence measurements in the laboratory: Nearly simultaneous measurements of the fluorescence parameter $\Delta F/F_m'$ and spectral reflectance of *Q. ilex* leaves were done in a dark room with the above-mentioned leaf-clip holder. Leaf temperature and photosynthetic photon flux density (PPFD) were monitored by a Ni-Cr-Ni thermocouple and a quantum sensor integrated into this system.

Fluorescence measurements in the field: $\Delta F/F_m'$ was measured on 40 leaves with 10 leaves randomly selected in each of four levels above soil: 1, 3, 4, and 5 m. Moreover, five leaf samples were randomly selected in each of the same levels as above. Dark leaf-clips (*PEA/LC*, *Hansatech*, UK) were used for the dark adaptation (30 min) of samples in the daytime. Then, F_v/F_m was measured on these 20 leaves five times during the sun's daily course.

Results

Radiometric measurement of PRI: PRI at leaf level and fluorescence parameters of *Q. ilex* leaves were closely related with changing irradiance. Thus, "dark" (10 μmol m^{-2} s^{-1}) – light (1 500 μmol m^{-2} s^{-1}) – "dark" (10 μmol m^{-2} s^{-1}) sequences produced nearly identical kinetics with changing irradiance in both the $\Delta F/F_m'$ and PRI of leaves, under steady-state conditions, in the leaf clip holder of the fluorometer (Fig. 1). PRI decreased from 0.03 to -0.05 while at the same time $\Delta F/F_m'$ decreased from 0.7 to 0.2 with irradiances increasing from 10 to 1500 μmol m^{-2} s^{-1}. Moreover, using a leaf under steady-state conditions, a linear relationship between PRI and $\Delta F/F_m'$ (Fig. 2) was observed over a wide range of irradiances (100 to 2 000 μmol m^{-2} s^{-1}).

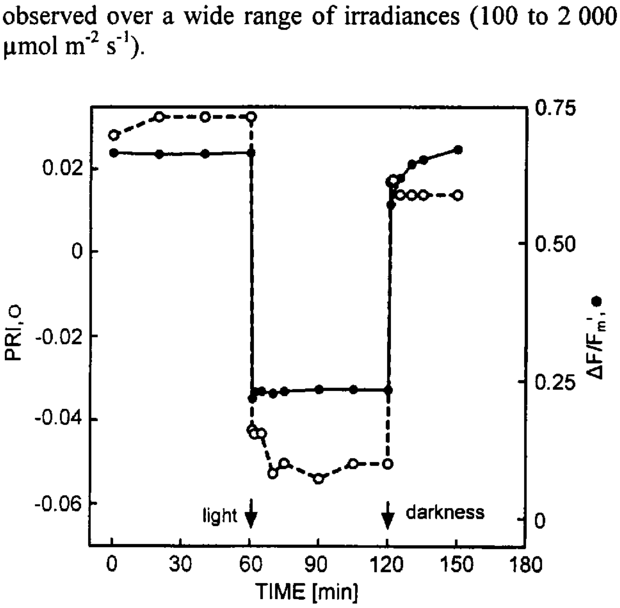

Fig. 1. Changes in the Photochemical Reflectance Index (PRI) and the actual photosystem 2 (PS2) photochemical efficiency ($\Delta F/F_m'$) in a *Q. ilex* leaf exposed to a dark-light-dark sequence.

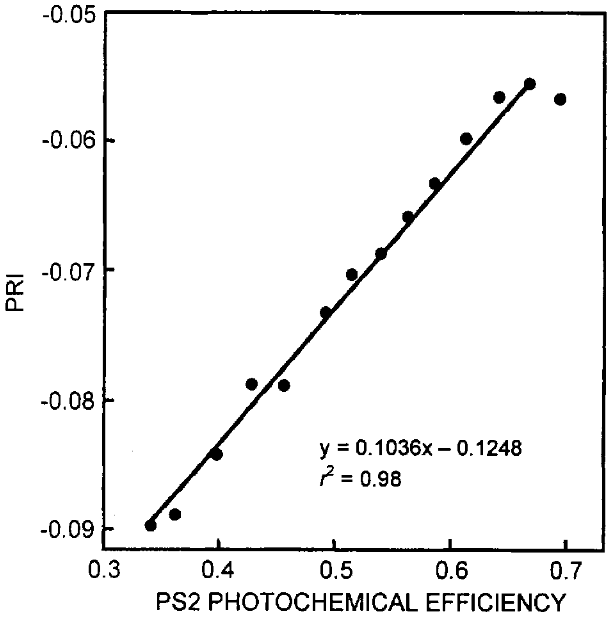

Fig. 2. Relationship between the Photochemical Reflectance Index (PRI) and the actual photosystem 2 (PS2) photochemical efficiency ($\Delta F/F_m'$) in *Q. ilex* leaves.

PRI at canopy level: Spectral reflectances were analysed under typical sunny and cloud-free conditions. As anexample, the air temperature and the relative air humidity, both measured during a daily course from the upper part of the canopy, reached values typical of summer conditions, with 25 °C and 16 % for air temperature and relative air humidity, respectively, in the afternoon. Solar PPFD increased to 1 892 μmol m^{-2} s^{-1} at solar noon (Fig. 3*A*). The related CO_2 flux into the canopy was closely correlated with radiation only in the morning. It reached its maximum (-8.8 μmol m^{-2} s^{-1}) at about 09:00 local solar time (LST). Then, the usual photosynthetic down-regulation was observable with a plateau level until 12:00 at about -7.03 μmol m^{-2} s^{-1}. A decrease was observed at least until 18:00 without recovery (Fig. 3*B*).

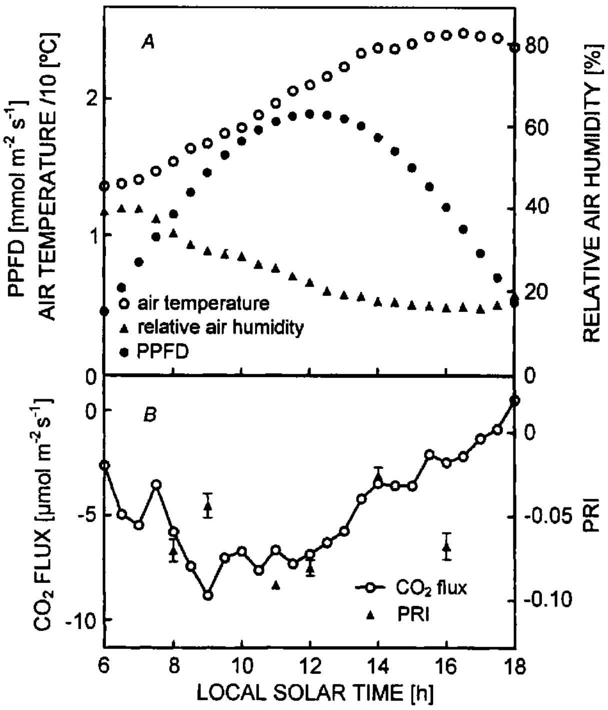

Fig. 3. Daily courses of (*A*) climatic parameters recorded on the top of the canopy on 22 June 1999: ● - photosynthetic photon flux density; O - air temperature; ▲ - relative air humidity, and (*B*) CO_2 flux measured by the eddy covariance method (O) and photochemical reflectance index (PRI) measured from the top of the tower (▲). Vertical lines indicate ± SE (n = 56).

Fig. 3*B* also demonstrates the lack of correlation between the photosynthetic activity measured by CO_2 flux and PRI. A deep divergence was even observed in the late afternoon and no relationship was evident when PRI was plotted *versus* CO_2 flux (graph not presented). Additional measurements during the same week and two weeks later confirmed this discrepancy.

Imaging the canopy in the field of view of the radiometer made clear the weight of trees structure: photosynthetic and non-photosynthetic materials (branches, soil), lower and upper layers of the canopy took place together in the monitored spectral reflectances in the 530 and 570 nm wavebands (Fig. 4*A*). The subsequent image of the photochemical index deduced from these values (Fig. 4*B*) shows the actual space distribution and the wide range—between –0.78 and+0.91—of PRI

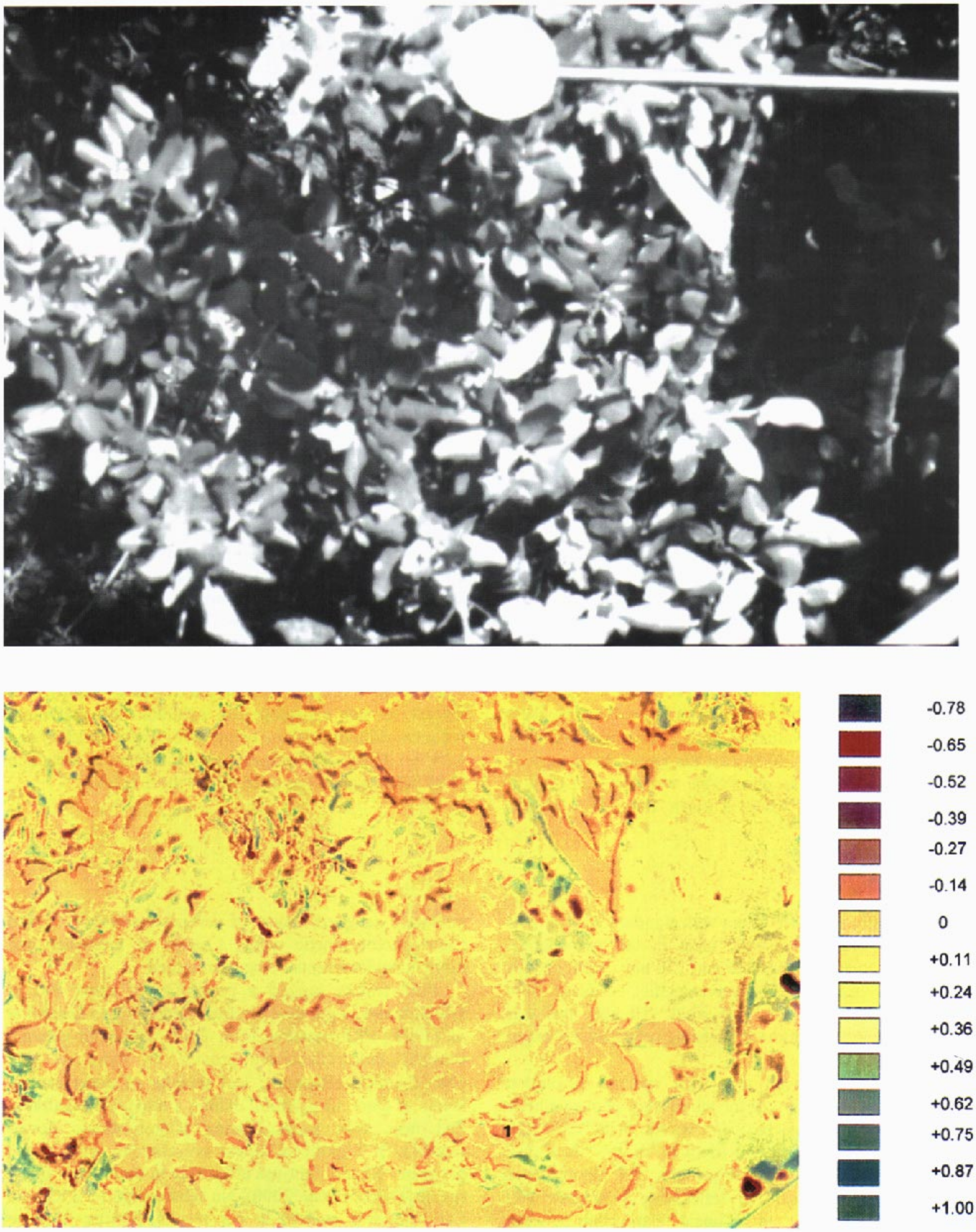

Fig. 4. (*A, top*) Picture of the canopy of *Q. ilex*. The reference card is visible in the upper part. (*B, bottom*) Image of the Photochemical Reflectance Index (PRI) of the same canopy, deduced from the CCD camera data (real size of the target: 37×55 cm).

in the sighted area. Fourty five percent of pixels were in the –0.028 to +0.065 class (Fig. 5). Only 25 % take values lower than –0.028.

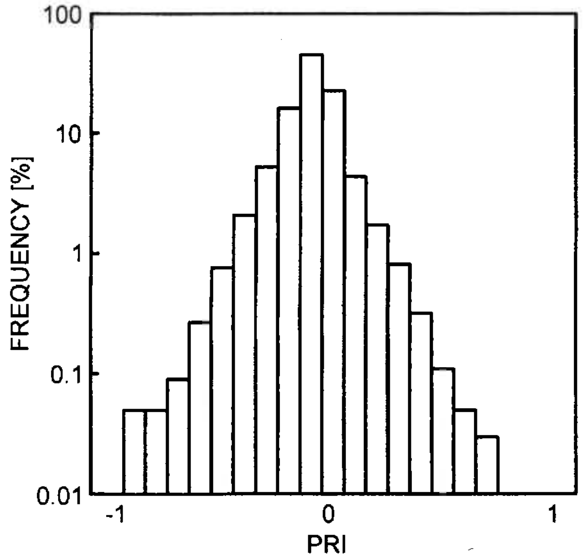

Fig. 5. Statistical distribution of the Photochemical Reflectance Index (PRI) deduced from the image of Fig. 4*B*.

Fluorescence parameter profiles: The subsequent actual PS2 photochemical efficiency of leaves was not homogeneous in the field of view of the radiometer. Near solar noon (Fig. 6) high values (0.70) of $\Delta F/F_m$' were recorded from 1 m up to nearly 4 m above the soil. From this level, measured values of $\Delta F/F_m$' decreased and were reduced by more than 40 % near the top of the canopy. A less sharp profile was observable with the F_v/F_m parameter at solar noon where a mean value of 0.79 was measured from the soil to the top of the canopy.

A divergence in these profiles was observable during the daily solar course (Fig. 7). Thus, the maximal PS2

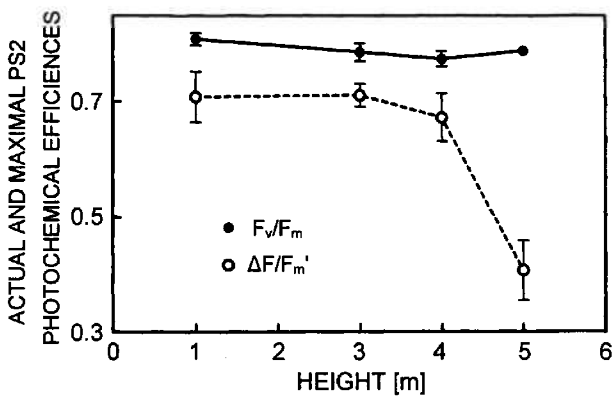

Fig. 6. Vertical profiles of the actual ($\Delta F/F_m$') and maximal (F_v/F_m) photosystem 2 (PS2) photochemical efficiencies measured near solar noon inside the *Q. ilex* forest. Vertical lines indicate ± SE (n = 10 for $\Delta F/F_m$' and n = 5 for F_v/F_m).

photochemical efficiency F_v/F_m of leaves near the soil level (1 m) was stable and remained close to 0.813 throughout the day. In contrast, the F_v/F_m parameter of leaves located near the top of the canopy (5 m) was more affected by sun position and by solar irradiance: the lowest mean values (0.75) were observed at about 14:00 (LST). The F_v/F_m of leaves at intermediate levels (3 and 4 m above the soil level) showed intermediate stability with respect to sun elevation and azimuth: the maximum reduction in mean values being 8.76 % (08:00 LST) and 4.28 % (10:00 LST), respectively. A minimum reduction (0.83 %) was observed at 16:00 for the 3 m level.

Discussion

Commercially available filters with standard wavelengths in 10 nm increments were used in the two-channel radiometer to analyse the 530 and 570 nm spectral bands (maximum transmission wavelengths). Taking into account the transmission half width (10 nm), this was without appreciable effect for sensing reflectance changes in the 531 nm region.

The results showed that high spectral resolution reflectance is a useful tool for monitoring photosynthetic radiation-use efficiency in *Q. ilex* leaves. With "dark" (10 µmol m^{-2} s^{-1}) – light (1 500 µmol m^{-2} s^{-1}) – "dark" (10 µmol m^{-2} s^{-1}) irradiance sequences, the fluorescence parameter $\Delta F/F_m$' and the reflectance parameter PRI exhibited similar changes. A linear relationship between these indices was found over a range of irradiances as wide as 100-2 000 µmol m^{-2} s^{-1}. Similar results were found by Peñuelas *et al.* (1995b) on a bean leaf and from randomly sampled leaves of several species of C_3 and CAM photosynthetic types, a range of irradiance and nutrient environments being represented.

Such results suggest a possibility of using PRI at spatial scales larger than the leaf. According to Gamon *et al.* (1995), PRI measured from above with nadir-viewing sensors could effectively provide an index of PS2 photochemical efficiency; leaf samples of upper canopy layers of *Quercus agrifolia* exhibit a midday reduction in PRI near solar noon (during photosynthetic down-regulation). Significant linear correlations were found between PRI and light-use efficiency in four boreal forest species (Nichol *et al.* 2000). No relationship for PRI with CO_2 flux was evident in our results. Similar negative results have been reported previously by Carter (1998) for photosynthetic capacity in pine canopies and by Gamon *et al.* (1992) in sunflower canopies with photosynthetic efficiency. Because of the relatively small reflectance signal, confounding effects are to be identified.

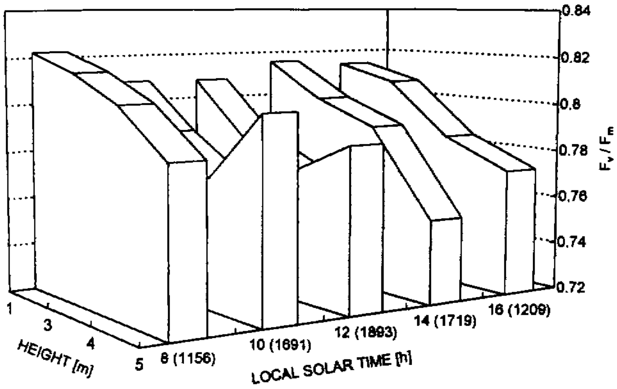

Fig. 7. Daily course of the vertical profile of the maximal photosystem 2 photochemical efficiency (F_v/F_m) measured at four levels inside the *Q. ilex* forest. Photosynthetic photon flux densities in μmol m^{-2} s^{-1} (the same as in Fig. 3) are indicated in brackets.

Temporal and spatial mismatch between eddy covariance (which measures whole-ecosystem fluxes for half-hour periods) and instantaneous point sampling of canopy reflectance can explain a part of this discrepancy. Furthermore, as a consequence of the long-term radiation regime, leaves can be classified into a range of leaf mass per area (LMA) categories ranging from 84 (shaded leaves) to 240 g m^{-2} (sun-exposed leaves). This pattern is consistent with the observations of Rambal *et al.* (1996) in ten canopies of Mediterranean evergreen oaks: five canopies of *Quercus coccifera* and five canopies of *Q. ilex*. A wide range of pigment contents and leaf optical and physiological properties can be associated with such range of LMAs. This is accompanied by a subsequent range of photosynthetic performances and hence photosynthetic rates per unit of leaf area, with saturation for leaves from the top of the canopy at higher PPFD than those from the bottom of the canopy (Hollinger 1989). Thus, at all times a wide variety of structural and functional properties can be observed. The distribution of solar irradiances inside the canopy increases at the time of measuring this variety in the field of view of the radiometer. The temporal and spatial patterns of PS2 regulation within the canopy, as deduced from our fluorescence measurements, are dynamic and complex and highlight the difficulty in interpreting the PRI as sensed from above.

In terms of the structural properties of the canopy, the top canopy layer (some tens of cm) showed an erectophyle tendency of leaves. The same tendency was observed by Eckardt *et al.* (1975). Furthermore, a phototropism phenomenon with plagiophototropic movements was observed, the abaxial leaf surfaces being sun-exposed in the afternoon. Viewing the highly reflective lower surface of the leaves may explain the observed divergence at about 16:00 (local solar time). According to Gamon *et al.* (1992), PRI does not correlate with photosynthetic efficiency in water-stressed canopies undergoing midday wilting; moreover, increased use of reductant by photorespiration and other processes besides carboxylation cannot be neglected.

Finally, taking into account the small size of the *Q. ilex* leaves and the apparent diameter of the sun, penumbra effects must be taken into consideration in such canopy (Eckardt *et al.* 1975). The related radiation dispersion can also partly explain some of the observed divergence.

This physiological and structural heterogeneity of leaves in the field of view of the radiometer and the possibly changing structure of the canopy show that spectral reflectance and PRI are complex parameters. To explore this complexity, it might be valuable to consider absolute canopy irradiance as an indicator of irradiation of the canopy portion in the field of view of the sensor. According to Méthy *et al.* (1999) and the above results, imaging spectrometry can fully and rapidly explore weighting of sunlit and shaded canopy components. However, using imaging spectrometry across AVIRIS data demonstrated the possible interference by the relative content of leaf pigments and LAI (Gamon *et al.* 1995). A more detailed information is given by the fluorescence techniques at leaf level.

Remote sensing is still carried out often with half bands much higher than 10 nm. However, with the complementary help of fluorescence and reflectance imaging techniques the use of PRI signatures is a promising tool for detecting stress-induced injuries (and to determine stress tolerance). Further investigation is needed before this tool can be applied by managers as a diagnostic technique in stress physiology and remote sensing of vegetation

References

Blackburn, G.A.: Spectral indices for estimating photosynthetic pigment concentrations: a test using senescent tree leaves. - Int. J. remote Sens. **19**: 657-675, 1998.

Butler, W.L., Kitajima, M.: Fluorescence quenching in Photosystem II of chloroplasts. - Biochim. biophys. Acta **376**: 116-125, 1975.

Carter, G.A.: Ratios of leaf reflectances in narrow wavebands as indicators of plant stress. - Int. J. remote Sens. **15**: 697-703, 1994.

Carter, G.A.: Reflectance wavebands and indices for remote estimation of photosynthesis and stomatal conductance in pine canopies. - Remote Sens. Environ. **63**: 61-72, 1998.

Cecchi, G., Mazzinghi, P., Pantani, L., Valentini, R., Tirelli, D., De Angelis, P.: Remote sensing of chlorophyll-*a* fluorescence of vegetation canopies: I. Near and far field measurements techniques. - Remote Sens. Environ. **47**: 18-28, 1994.

Cerovic, Z.G., Goulas, Y., Gorbunov, M., Briantais, J.M., Camenen, L., Moya, I.: Fluorosensing of water stress in plants: diurnal changes of the mean lifetime and yield of chlorophyll fluorescence, measured simultaneously and at distace with a τ-LIDAR and a modified PAM-fluorimeter, in maize, sugar beet, and kalanchoë. - Remote Sens. Environ. **58**: 311-321, 1996.

Datt, B.: A new reflectance index for remote sensing of chlorophyll content in higher plants: Tests using *Eucalyptus* leaves. – J. Plant Physiol. **154**: 30-36, 1999.

Demetriades-Shah, T.H., Steven, M.D.: High spectral resolution indices for monitoring crop growth and chlorosis. – In: Proceedings of the 4th International Colloquium on Spectral Signatures of Objects in Remote Sensing. Pp. 299-302. European Space Agency, Paris 1988.

Eckardt, F.E., Heim, G., Méthy, M., Sauvezon, R.: Interception de l'énergie rayonnante, échanges gazeux et croissance dans une fôret méditerranéenne à feuillage persistant (*Quercetum ilicis*). - Photosynthetica **9**: 145-156, 1975.

Floret, C., Galan, M.J., Le Floch, E., Rapp, M., Romane, F., Serrano, L., Blanchard, A., Grandjanny, M., Maistre, M., Perret, P.: Organisation de la structure, de la biomasse et de la minéralomasse d'un taillis ouvert de chêne vert (*Quercus ilex* L.). - Acta oecol. Oecol. Plant. **10**: 245-262, 1989.

Floret, C., Galan, M.-J., Le Floc'h, E., Romane, F.: Dynamics of holm oak (*Quercus ilex* L.) coppices after clearcutting in southern France. Flora and life cycles changes. - Vegetatio **99-100**: 97-105, 1992.

Gamon, J.A., Green, R.O., Roberts, D.A.: Deriving photosynthetic function from calibrated imaging spectrometry. – In: Proceedings of the International Colloquium Photosynthesis and Remote Sensing. Pp. 55-60. EARSeL, ISPRS, Montpellier 1995.

Gamon, J.A., Peñuelas, J., Field, C.B.: A narrow-waveband spectral index that tracks diurnal changes in photosynthetic efficiency. - Remote Sens. Environ. **41**: 35-44, 1992.

Genty, B., Briantais, J.-M., Baker, N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. – Biochim. biophys. Acta **990**: 87-92, 1989.

Gitelson, A.A., Merzlyak, M.N.: Signature analysis of leaf reflectance spectra: Algorithm development for remote sensing of chlorophyll. – J. Plant Physiol. **148**: 494-500, 1996.

Günther, K.P., Dahn, H.-G., Lüdeker, W.: Remote sensing of vegetation status by laser-induced fluorescence. - Remote Sens. Environ. **47**: 10-17, 1994.

Hollinger, D.Y.: Canopy organisation and foliage photosynthetic capacity in a broad-leaved evergreen montane forest. – Funct. Ecol. **3**: 53-62, 1989.

Jackson, R.D.: Spectral indices in *n*-space. - Remote Sens. Environ. **13**: 409-421, 1983.

Joffre, R., Rambal, S., Romane, F.: Local variations of ecosystems functions in Mediterranean evergreen oak woodland. – Ann. Sci. forest. **53**: 561-570, 1996.

Krause, G.H., Weis, E.: Chlorophyll fluorescence and photosynthesis: The basics. – Annu. Rev. Plant Physiol. Plant mol. Biol. **42**: 313-349, 1991.

Malthus, T.J., Madeira, A.C.: High resolution spectroradiometry: spectral reflectance of field bean leaves infected by *Botrytis fabae*. - Remote Sens. Environ. **45**: 107-116, 1993.

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

Méthy, M., Joffre, R., Rambal, S.: Remote sensing of canopy photosynthetic performances: Two complementary ways for assessing the photochemical reflectance index. – Photosynthetica **37**: 239-247, 1999.

Nichol, C.J., Huemmrich, K.F., Black, T.A., Jarvis, P.G., Walthall, C.L., Grace, J., Hall, F.G.: Remote sensing of photosynthetic-light-use efficiency of boreal forest. – Agr. Forest Meteorol. **101**: 131-142, 2000.

Ourcival, J.M., Joffre, R., Rambal, S.: Exploring the relationships between reflectance and anatomical and biochemical properties in *Quercus ilex* leaves. - New Phytol. **143**: 351-364, 1999.

Peñuelas, J., Filella, I., Elvira, S., Inclán, R.: Reflectance assessment of summer ozone fumigated Mediterranean white pine seedlings. – Environ. exp. Bot. **35**: 299-307, 1995a.

Peñuelas, J., Filella, I., Gamon, J.A.: Assessment of photosynthetic radiation-use efficiency with spectral reflectance. - New Phytol. **131**: 291-296, 1995b.

Peñuelas, J., Llusia, J., Piñol, J., Filella, I.: Photochemical reflectance index and leaf photosynthetic radiation-use-efficiency assessment in Mediterranean trees. - Int. J. remote Sens. **18**: 2863-2868, 1997a.

Peñuelas, J., Piñol, J., Ogaya, R., Filella, I.: Estimation of plant water concentration by the reflectance water index WI (R900/R970). - Int. J. remote Sens. **18**: 2869-2875, 1997b.

Rambal, S., Damesin, C., Joffre, R., Méthy, M., Lo Seen, D.: Optimatization of carbon gain in canopies of mediterranean evergreen oaks. – Ann. Sci. forest. **53**: 547-560, 1996.

Teixeira Filho, J., Rambal, S., Lacaze, B., Lointier, M.: Mapping maximal canopy transpiration over a Mediterranean watershed. - In: Parlow, E. (ed.): Progress in Environmental Remote Sensing Research and Applications. Pp. 379-384. A. Balkema, Rotterdam 1996.

Valentini, R., Scarascia Mugnozza, G.E., De Angelis, P., Bimbi, R.: An experimental test of the eddy correlation technique over a Mediterranean macchia canopy. - Plant Cell Environ. **14**: 987-994, 1991.

van Kooten, O., Snel, J.F.H.: The use of chlorophyll fluorescence nomenclature in plant stress physiology. – Photosynth. Res. **25**: 147-150, 1990.