Remote sensing of canopy photosynthetic performances: Two complementary ways for assessing the photochemical reflectance index

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Abstract

Two methods have been developed concurrently for hyperspectral measurements of plant canopy reflectance in two narrow wavelength bands centred around 531 and 570 nm. A laboratory-built two-channel radiometer provided an easy and quick estimation of the Photochemical Reflectance Index PRI = $(R_{531} - R_{570})/(R_{570} + R_{531})$ of a plot of alfalfa. A CCD digital camera provided multispectral imaging and the analysis of this index on the same target. The two devices are complementary. The results of measurements are complementary with those of chlorophyll fluorescence induction.

Additional key words: chlorophyll fluorescence; diurnal courses; *Ficus altissima*; *Medicago sativa.*

Introduction

Chlorophylls and carotenoids are the two major components of the pigment-protein complexes of thylakoid membranes of plant tissues. Using pulse-amplitude-modulation fluorometers, light-induced chlorophyll (Chl) fluorescence of green leaves provides information on the performance of photosynthesis and functioning of the photosynthetic apparatus.

Vegetation monitoring experiments were carried out with different active or passive approaches. Modified pulse-amplitude-modulation fluorometers were used for remote detection (0.5-1.0 m distance) by using a laser diode for excitation (Cerovic *et al.* 1996). In order to extend field fluorescence applications to greater distances, specific LIDARs have been developed for the remote sensing of Chl fluorescence of terrestrial vegetation, such as the FLIDAR (Cecchi *et al.* 1994) or the τ-LIDAR, which provides measurements of the fluorescence lifetime using

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picosecond laser pulses (Moya *et al.* 1995). Combined measurements of laser-induced fluorescence and CO_2 exchange have shown how the method behaves (Rosema *et al.* 1998).

The carotenoids of xanthophyll cycle, violaxanthin (diepoxy-zeaxanthin), antheraxanthin (monoepoxy-zeaxanthin), and zeaxanthin (dihydroxy-β-carotene), are of particular interest with respect to non-radiative energy dissipation; when there is an excess of excitation energy, a violaxanthin-antheraxanthin-zeaxanthin interconversion occurs. Conversely, when irradiance becomes limiting to photosynthesis, zeaxanthin is reepoxidized to violaxanthin (Björkman and Demmig-Adams 1995). These pigments are localized within the light-harvesting complexes of photosystem (PS) 2 as well as in PS1 (Thayer and Björkman 1992). Zeaxanthin-antheraxanthin-violaxanthin interconversion and chloroplast conformational changes are associated with optical changes (Bilger and Björkman 1990). In a survey of twenty angiosperm species, Gamon *et al.* (1993) showed that reflectance changes upon increased irradiance occur near 531 nm. In order to reduce the effects of chloroplast movement or sun angle, Peñuelas *et al.* (1995) combined this waveband with a reference waveband (570 nm) and calculated the Photochemical Reflectance Index as:

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

Moreover, they showed that leaves exposed to changing irradiance exhibit a functional relationship between PRI and PS2 radiation-use efficiency $\Delta F/F'_m$. Thus the PRI may be used for remote determination of the photosynthetic radiation-use efficiency of plant canopies.

The systems involved in this paper relate to two approaches for plant canopy monitoring: (*a*) two-channel radiometry, which provides a remote estimation of the PRI index of canopies, (*b*) multispectral imaging which provides a numerical image of the PRI of the same targets. Preliminary tests and comparisons with information obtained by the Chl fluorescence induction method are presented.

Materials and methods

The radiometer: It is necessary to detect simultaneously the photon flux reflected by the plants in the 531 and 570 nm regions of the spectrum. The present radiometer, the so-called PRI-meter, originates from a previously designed (Méthy 1977) two-channel radiometer used for a quantitative estimation of the plant above-ground biomass. The acceptance angle (15°) has been defined with a plano-convex lens (focal length: 10 cm) by the lens aperture method (Fig. 1). A mirror-type beam splitter with neutral colour characteristics (optical window with a semi-transparent mirrored coating) splits the beam into two separate beams. Due to their usual spectral response characteristics in the visible range, silicone photodiodes were used. Two encapsulated silicon solar cells (*Centralab Semiconductor*, El Monte, CA, USA, type *2AL*) with an active area of about 4.75 cm^2, each mounted with optical multi-layer dielectric interference filters (bandpass filters *Corion*, Holliston, MA, USA, type

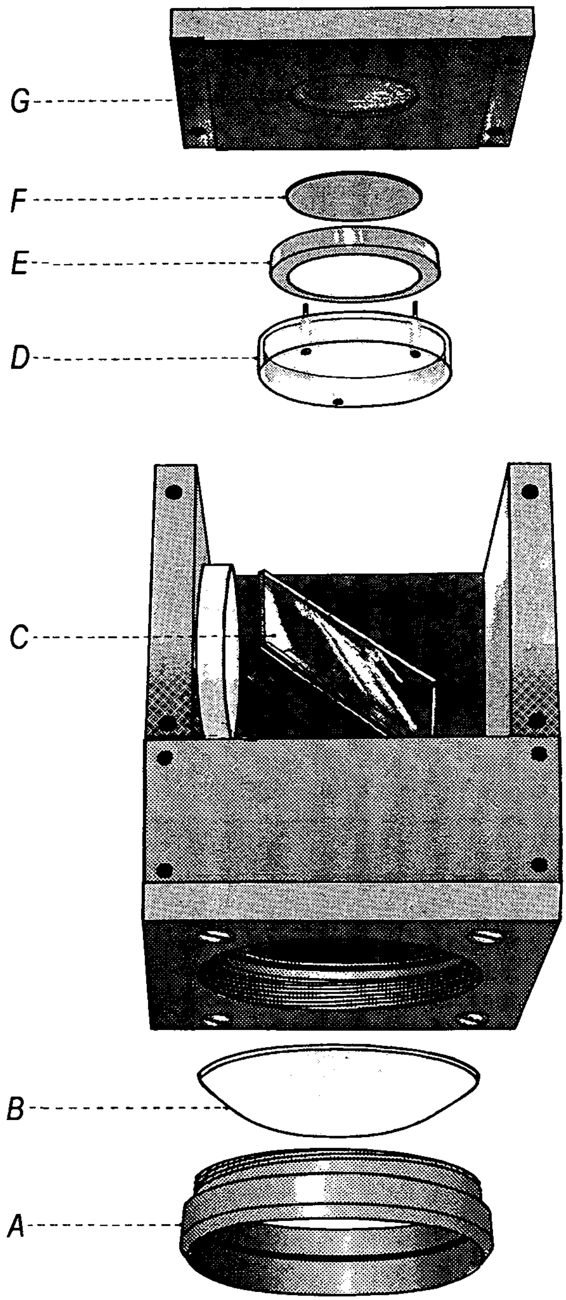

Fig. 1. View in section of the PRI-meter: *A*, lens ring; *B*, plano-convex lens, f = 10 cm; *C*, mirror type beam splitter; *D*, filter holder; *E*, interference filter; *F*, silicon photocell; *G*, photocell-housing (redrawn from Méthy 1977).

P10-530-F and *P10-570-F*, bandwidth 10±2 nm, minimum peak transmittance 50 %) deliver two photocurrents simultaneously. The short circuit currents are directly proportional to the radiance of the target in the two wavelengths 530 and 570 nm. The whole system is housed in a black delrin box. Two linear spirit-levels and a ballhead allow the PRI-meter to be mounted conveniently on any support.

Two load resistances (about 100 Ω) produce a voltage which is nearly proportional to the radiance of the target. A rugged digital multimeter (41/2-digit, 20 000 count LCD, DC voltage range 200 mV, 10 µV, accuracy ±0.05% of reading + 3 digits) was used satisfactorily in the first experiments.

The CCD camera *CCD800* from *Integrated Scientific Imaging Systems* (Santa Barbara, CA, USA) was used. The main specifications of this system are as follows: 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 gray levels; *Nikon F*-mount, lens: *Nikkor* 50 mm f/1.8. Taking into account the focal length of the lens and the size of the imaging area, the diagonal acceptance angle of the CCD camera is close to 10°. The detectable range of wavelengths resulting from the sensor, the window, and the optical properties of the lens is 400-1100 nm.

A built in eight-position filter wheel provides multispectral or colour imaging. It is controlled by the camera electronics, servo electronics, and an optical stop to sense position. The camera and filter positions can be operated by a PC (or a PC laptop) through a flexible parallel port (or PCMCIA interface) and the image acquisition software *Compuscope Procontrol 14HSD* (*Integrated Scientific Imaging Systems*, Santa Barbara, CA, USA). 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 IDRISI software (Clark University, Graduate School of Geography, MA, USA) was selected for image processing.

Tests of the two methods: First of all, the intercalibration of the two sensors was checked using wide leaves of a specially selected species, *Ficus altissima*. The tests themselves were carried out during summer 1998, on an experimental crop (4×4 m) of alfalfa (*Medicago sativa* L. cv. Du Puits) two days after the last irrigation. The crop height was 0.65 m and the leaf area index was 3.6. On sunny days, the PRI-meter and the CCD-camera were vertically positioned, facing down, 3 m above the top of the canopy using a traveler moving on rails. PRI was calculated from the radiances by normalizing canopy radiance with the radiance of test cards (*Kodak* neutral test cards). Five readings from equidistant points (0.5 m) were made six times a day on the same plots each time, with the PRI-meter.

Two separate images—one each in the 530 and 570 nm parts of the spectrum—were taken, from the same place, three times a day, with the CCD-camera. Time delay between these two images was around 60 s with exposures of about one second. In order to minimize the effect of wind-induced plant shift between two exposures, the effective part of each image (160 000 pixels) was split into 16 square sections of 100×100 pixels (16.5×16.5 cm); PRI was averaged in these sections.

Chl fluorescence measurements were also conducted for analysing the relation between the PRI and information obtained by fluorescence. Fluorescence parameters (for nomenclature, see van Kooten and Snel 1990) were measured using a pulse-amplitude-modulation fluorometer (*PAM 2000, H. Walz*, Effeltrich, Germany). The initial fluorescence level was determined with samples in position in sliding shutter dark leaf clips (*PEA/LC, Hansatech*, Norfolk, UK) by applying a weak modulated measuring beam (0.1 μmol m^{-2} s^{-1}, 655 nm) at a frequency of 600 Hz to dark-adapted (30 min) samples. This F_0 level is the fluorescence emission when all reaction centres are open and photochemical quenching is maximal. F_0 increases may occur if 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 "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) for the dark-adapted state. A leaf-clip holder (*2030-B, H. Walz*, Effeltrich, Germany) was used for light-adapted parameters. The tip of the fiberoptics was located at 1.0 cm and 60° from the surface of the sample. A Ni-Cr-Ni thermocouple and a quantum sensor integrated into the leaf-clip holder monitored the leaf temperature and photosynthetic photon flux density (PPFD). The steady-state value of fluorescence (F_s) was measured, and a second pulse of high irradiance "white light" was used to determine maximum Chl fluorescence in the light-adapted state (F_m'). The photochemical efficiencies of PS2 in the dark (maximum PS2 photochemical efficiency) and in the light (effective quantum yield of PS2) were calculated according to Genty *et al.* (1989):

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

$$\Delta F/F_m' = (F_m' - F_s)/F_m' \quad [2]$$

F_v/F_m is a good indicator of photoinhibitory damages. The value of F_v/F_m for intact tissues is the quantum yield of photochemistry (Butler and Kitajima 1975). These parameters suit laboratory and field measurements for single leaves at close (1 to 3 cm) sampling distance. They were measured on the adaxial surface of sun-exposed leaves randomly selected in the upper layer of the alfalfa crop. Five replicates were analyzed for each measurement time. According to the results of Peñuelas *et al.* (1995), on a range of species and photosynthetic types, PRI is linearly related to $\Delta F/F_m'$. The published relationships were averaged in order to calculate the PRI of sampled sun-exposed leaves.

Results

The two fluorescence parameters F_v/F_m and $\Delta F/F_m'$ showed a diurnal decline in relation to the solar irradiance. The lowest values were observed at noon. The beginning of a recovery at the end of the afternoon, near 18 h was also found (Fig. 2*A*).

Similar daily time-courses were observed for the estimated PRI of sampled sun-exposed leaves and for the PRI as measured with the PRI-meter (Fig. 2*B*). However, the values resulting from the estimate were lower throughout the day, the ratio being three times around 14 h.

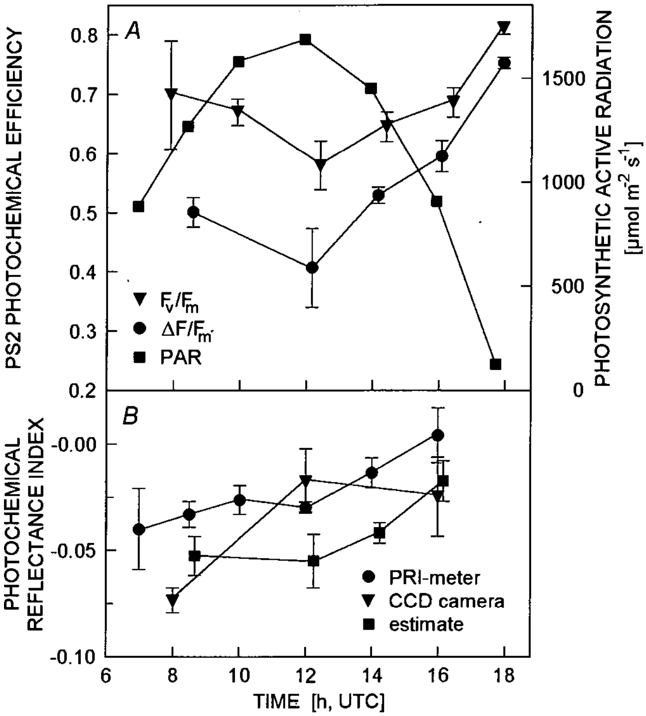

Fig. 2. Diurnal courses, for a canopy of alfalfa, of: (*A*) the photochemical efficiencies, at leaf level, of photosystem 2 (PS2) in the dark (F_v/F_m) and in the light ($\Delta F/F_m'$), the photosynthetically active radiation (PAR), and of (*B*) the Photochemical Reflectance Index (PRI) as estimated from fluorescence of sun-exposed leaves or as measured with the PRI-meter and the CCD camera. Vertical lines indicate ±1 SEM, n = 5 except for the CCD camera (n = 16).

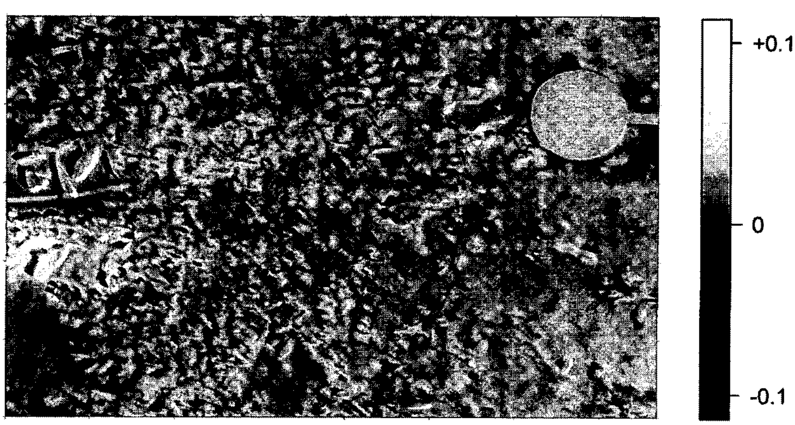

Fig. 3. Image of the Photochemical Reflectance Index (PRI) of the canopy of alfalfa, deduced from the CCD camera data (real size of the target: 33×20 cm). The reference surface is visible in the upper right corner.

Using the CCD camera, the high spatial variability of the values of PRI at the pixel level can be deduced from multispectral imaging (Fig. 3). In the absence of steady weather, and taking into account the effect of wind-induced blurring, a distribution analysis is nevertheless possible on the basis of the 16 square sections mentioned above (Fig. 4). The relationship between this distribution and the time of day is also presented. Changes in the distribution of the PRI are evident. The reflectance indices of about -0.14 and -0.16 were previously observed from radiometric measurements on bare soil and dead plant material, respectively. Due to fading of plants, the strength of the background and non-photosynthetic parts of plants in the signal increased during the day.

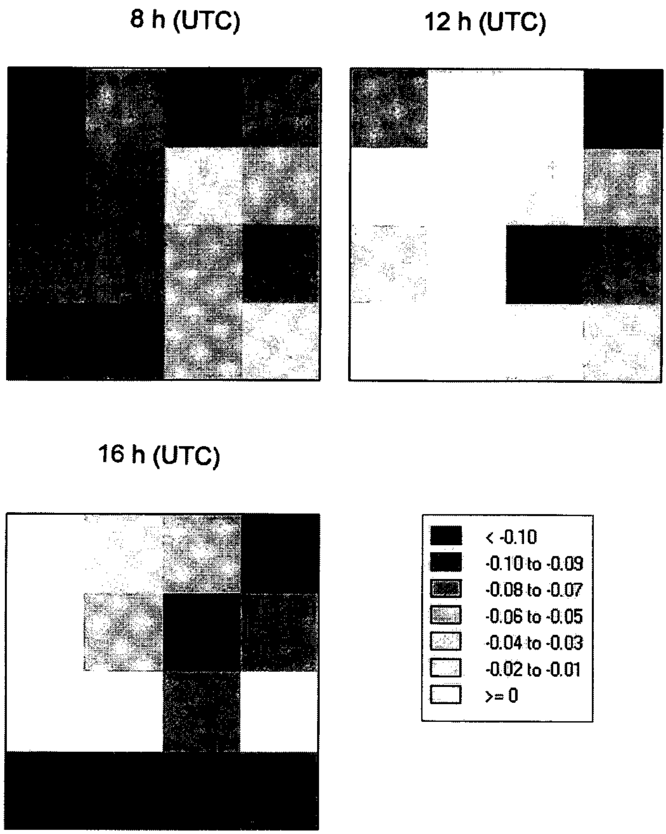

Fig. 4. Diurnal course of the distribution of the Photochemical Reflectance Index (PRI) of the canopy of alfalfa. Each square section includes 10^4 pixels. PRI was averaged in these sections.

Moreover, at the beginning and end of the day, the multispectral imagery processing gave values of the same order of magnitude as those obtained using the estimate, but they were up to five times larger at about midday. The values obtained using the PRI-meter were then two-times lower (Fig. 2*B*).

Discussion

The technical aspects of the above-mentioned measurements must be considered. The limited stock-list of bandpass filters provided by the manufacturers made it impossible to select exactly the centre wavelength of 531 nm. But, because of the bandwith (10 nm) of the filters, selecting the wavelength of 530 nm did not affect significantly the result. Our experiments were carried out with cheap neutral test cards as reference (*Eastman Kodak Co.*, Rochester, NY, USA). More expensive *Spectralon* diffuse reflectance standards, with highly Lambertian properties (*Labsphere*, North Sutton, NH, USA) offer a better stability and are more suitable for prolonged exposure to high solar irradiance. The radiance measurements can be done with one (or two) low-cost multimeters. Data acquisition using a data-logger or a laptop could facilitate the acquisition of radiance measurements and subsequent computing of reflectance and PRI. A suitable acquisition circuit board, or a microcontroller-based device, could also be considered.

However, even before such improvements, the good agreement between PRI, as measured with the PRI-meter, and the fluorescence parameters F_v/F_m and $\Delta F/F_m'$ suggests that 2-channel radiometry could be used for studying vegetation at spatial scales larger than individual leaves. Concerning the CCD camera, wind sensitive plant structures can be considered as unsuitable for conducting pixel-based image processing. However, by splitting up the images it was possible to use multispectral imaging at such scales. Because of their different acceptance angles, the PRI-meter and the camera did not analyze exactly the same area of plant canopy. Moreover, the sampling methods were not identical for the two types of apparatus. The homogeneity of plant arrangement and of the background reduced the effect of this discrepancy. However, the values produced by the two systems suggest a residual effect which needs to be further investigated.

Sampling at these scales introduces additional complexity to the reflectance signatures. Parameters such as the sun angle and canopy structure must be taken into account (Gamon *et al.* 1997). Moreover, soil or other non-green material and the varying pigment composition in the field of view of the sensor may interact with the physiological signal. Thus, the lowest values of PRI were obtained for sun-exposed leaves. The presence of shade leaves in the underlying layer, facing the PRI-meter and the CCD-camera explain the higher values of this index at the canopy scale. With radiometric sensors, these factors are to be taken into account in analysing the functioning of heterogeneous plant canopies such as Mediterranean forests. Two-channel radiometry does not provide details about the contribution of each level of PRI to the measured overall value, but additional information can be obtained with multispectral imagery. This technique is suitable for this type of research. The two methods are complementary.

Thus, previous results from Peñuelas *et al.* (1995) gave a detailed evaluation of the PRI as an indicator of efficiency of photosynthetic radiation use, at the individual leaf scale. The hyperspectral reflectance can also provide remote and non-destructive estimates of photosynthetic radiation-use efficiency. Two-channel radiometry and multispectral imagery provide a useful tool for non-destructive, non-contact optical study of photosynthetic function.

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