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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

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Abstract

A previously described passive remote sensing fluorimeter (see companion paper) was modified to detect changes in the reflectance of vegetation. The utility of this remote sensing technique to measure the Physiological Reflectance Index (PRI) is shown at both leaf level under laboratory conditions and at the canopy level in the field. PRI, defined as the relative changes in reflectance at 531 nm with respect to those at 570 nm (PRI = R531 − R570/R531 + R570), is related to xanthophyll-related, dynamic changes of non-photochemical quenching of chlorophyll fluorescence. The robustness of this relationship by simultaneous remote sensing of PRI and chlorophyll fluorescence is strengthened. At the leaf level, the existence of two kinetically distinct components of PRI is shown. A fast (within seconds) component that is partly attributed to ΔpH induced chloroplast shrinkage, and a slow (within minutes), main component that is related to xanthophyll de-epoxidation, as demonstrated by its disappearance in the presence of DTT. Overall, PRI correlated better with non-photochemical quenching of chlorophyll fluorescence (NPQ) than with any other measured parameter, including the photochemical efficiency of PSII. Finally, at the canopy level and under field conditions, it is shown that PRI can be a useful tool for remote sensing of water stress in grapevines.

1. Introduction

In the field, plants are continuously subjected to environmental variations that affect their metabolic rates, such as photosynthesis. Among the most common variations that interact with plant photosynthesis are diurnal changes of incident irradiance, ambient temperature and humidity, wind direction and intensity, as well as seasonal changes in the availability of water and nutrients (Schulze & Caldwell, 1994).

Most remote sensing indices used in vegetation studies serve to detect the quantity and spatial distribution of green vegetation, and may be used to estimate canopy photosynthesis and net primary productivity (Peñuelas & Filella, 1998; Sellers, 1987). However, they fail to detect dynamic variations of photosynthesis rates, like those occurring during the day or under certain stress conditions (Running & Nemani, 1988). These short-term responses can be remotely sensed by active chlorophyll fluorescence techniques (Cecchi et al., 1994; Cerovic et al., 1996; Flexas et al., 2000; Rosema et al., 1998). Active techniques are limited in their measuring range due to the induced fluorescence signal decrease with distance, which can only be solved by increasing the power of the light source. In the companion paper we present a method for passive chlorophyll fluorescence detection, as an alternative to active methods. In the present paper we describe a second alternative using a modification of the same instrument, based on the reflectance variations in the green part of the spectrum. These changes, unlike other common reflectance indices, are short-term responses to variations in light intensity and leaf physiological status. For this reason, there is an increasing interest in the use of these reflectance indices for spatial missions as it was put

Abbreviations: BRDF, bidirectional reflectance distribution function; Chl, chlorophyll; DTT, dithiothreitol; FIPAM, frequency induced pulse amplitude modulation; FLIDAR, fluorescence LIDAR; F_s, stationary Chl fluorescence flux; LIDAR, light-induced detection and ranging; NPQ, non-photochemical quenching; PAM, pulse amplitude modulation; PPFD, photosynthetic photon flux density; PSI, photosytem I; PSII, photosytem II; Q_A, primary quinone acceptor of PSII; Q_P, photochemical quenching.

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forward in some recent conferences (Harris, 2002) and publications (Stylinski et al., 2002; Winkel et al., 2002).

Under conditions where light exceeds the amount that can be used for photosynthesis, ΔpH and the de-epoxidation state of xanthophylls both increase, and are involved in a photoprotective mechanism that dissipates excess light as heat (Demmig-Adams & Adams, 1992; Li et al., 2000; Niyogi, 1999). This photoprotective mechanism varies along the diurnal time course, as well as in response to temperature, water and nutrient stresses (Demmig-Adams & Adams, 1992). This mechanism is thought to involve zeaxanthin-induced changes in the conformation state of PSII light harvesting complexes (Moya et al., 2001; Ruban et al., 1993). Dynamic changes in the de-epoxidation state of the xanthophyll cycle are accompanied by absorbance changes at 505–515 nm (Bilger et al., 1989; Heber, 1969; Heber et al., 1986; Krause, 1973; Li et al., 2000; Yamamoto & Kamite, 1972). Also, the accumulation of zeaxanthin allows rapid changes in the aggregation state of antenna chlorophyll–protein complexes, which are reflected by absorbance changes centered near 531–535 nm (Bilger et al., 1989; Ruban et al., 1993).

Gamon et al. (1990) were the first to show that rapid reflectance changes from leaves and canopies around 531 nm, occurring upon sudden changes in incident light, could be sensed remotely and passively from leaves and canopies, using a portable radiometer. These changes were related to the above-described chloroplast conformational changes associated with increased ΔpH and de-epoxidation state of the xanthophyll cycle (Gamon et al., 1990). Therefore, passive remote sensing of reflectance changes at 531 nm has been proposed as a useful tool for nutrient and water stress detection, although the latter is not always possible, specially in water-stressed canopies undergoing severe wilting (Gamon et al., 1992, 1997; Peñuelas et al., 1994).

The objective of the present work was to examine the possibility of measuring canopy reflectance changes at 531 nm using a modification of the passive sensor described in the accompanying paper. Reflectance changes were first related to remotely sensed chlorophyll fluorescence parameters at the leaf level in laboratory experiments in order to gain insights about the physiological meaning of these reflectance changes. Afterwards, they were used to monitor daily changes and water stress-associated changes under field conditions in a grapevine canopy.

2. Material and methods

2.1. Plant material and treatments

Grapevine (*Vitis vinifera* L.) was used as a model plant to study PRI variations. An experiment under laboratory conditions was performed at Orsay (France). Plants were grown under greenhouse conditions as described by Flexas et al. (2000), irrigated daily with a commercial nutrient solution. At the onset of the experiment, they were about 0.5 m tall, and showed photosynthetic rates corresponding to fully developed, healthy leaves (between 10 and 15 μmol CO_2 m^{-2} s^{-1}). These irrigated plants were analyzed as control plants. Water stress was induced either rapidly, by cutting a leaf petiole in air, or gradually, by withholding water to plants for several days. To test the involvement of the xanthophyll cycle in PRI changes, some leaves were infiltrated with dithiothreitol (DTT), a well-known inhibitor of xanthophyll de-epoxidation. For the infiltration, a leaf petiole was cut under water and allowed to absorb DTT through the petiole for 3 h at 200 μmol photons m^{-2} s^{-1}, until it reached a tissue concentration of about 3 mM, calculated according to Bilger & Björkman (1994).

Outdoors experiments were performed in Mallorca (Spain), under the Mediterranean summer conditions described by Flexas et al. (1999). Plants were rooted in big (70 l) pots and maintained outdoors. They were 1.5 m tall at the onset of the experiments. Water stress was imposed gradually, by withholding water to the pots.

2.2. Principles and instrumentation for measuring green reflectance changes

The so-called «Physiological Reflectance Index» (PRI), introduced by Gamon et al. (1992), traduces reflectance changes in the green part of the spectrum that correlate with the de-epoxidation state of the xanthophyll cycle, as well as with changes in the trans-thylakoid ΔpH. The PRI is formulated as follows:

$$PRI = (R531 - R570)/(R531 + R570),$$

where R531 and R570 represent respectively the reflectance at 531 nm and the reflectance at a 570 nm reference waveband (Gamon et al., 1992).

Previous attempts of measuring PRI were performed with field spectroradiometers (Gamon et al., 1990, 1992, 1997; Peñuelas et al., 1994, 1995) or a two-channel hyperspectral radiometer specifically developed for this purpose (Méthy, 2000; Méthy et al., 1999). Here we introduce another instrument. With this, the reflectance signal is calculated by dividing the spectral radiance of the leaf or the canopy by the radiance of a reference panel. This instrument introduces a flip-flop mirror to alternate the field of view between the reference and the sample. The continuous measurement of a reference panel allows accounting for spectral variations of the incident light during the diurnal cycle or caused by intensity changes of artificial light. The reference signal is obtained from of a flat black or green panel, for laboratory or field conditions, respectively. The reflectance of both reference panels was previously calibrated by comparison with a standard white panel (Spectralon, Labsphere, North Dutton, NH).

PRI was measured continuously and at distance with a device initially developed to measure chlorophyll fluores-

cence at 760 nm by a passive technique (see companion paper). The field of view of the apparatus is about 4°. A beam splitter (aluminum coated microscope cover) with almost equal transmission and reflection is placed at 45° with respect to the beam axis. On each of the resulting beams an interference filter centered at 531 nm or at 570 nm (Omega, FWHM = 7 nm, 70% transmission, 25 mm diameter) defines the spectral bandwidth. Detection is ensured by amplified photodiodes (HUV 2000, EGG). A small chopped mirror, placed at the entrance of the device, automatically switches the field of view towards the vegetation or reference panel every second (see Fig. 1). The reference is a panel of Spectralon situated close and parallel to the target. Data acquisition for all the instruments used in the present experiments, except the gas exchange system, was performed using multimeters (HP34401A) connected to a PC by GPIB bus. A laboratory-made program developed with HP BASIC (HP E2060, Hewlett Packard, Les Ulis, France) allowed for on-line control and display of measured signals. Gas exchange measurements were made with a portable infrared gas analyzer, Li-6400 (Li-Cor, Nebraska, USA).

2.3. PRI, chlorophyll fluorescence and gas exchange measurements at the leaf level in laboratory conditions

In the laboratory experiments, PRI, chlorophyll fluorescence and gas exchange were simultaneously measured on the same leaf as described in Fig. 1. A leaf was placed vertically and enclosed in the gas-exchange analyzer chamber (Li-6400, Li-Cor). The leaf was dark-adapted, and maintained at ambient CO_2 concentration (360 ppm), 25 °C, and 60% relative humidity during the experiment. Measurements started in darkness and, then, light intensity, provided with a 650W white light projector (CCT, France) was manually increased at discrete steps (about 50, 200, 500 and 800 μmol photons m^{-2} s^{-1}) by voltage increase.

Gas exchange measurements were taken automatically every 2 s, and used to calculate net CO_2 assimilation and stomatal conductance, as already described (Flexas et al., 2000).

Chlorophyll fluorescence was measured through the transparent window of the Li-6400 using a Laser-PAM placed at 1 m distance as previously described (Ounis et al., 2001),. Steady-state chlorophyll fluorescence was continuously monitored every two seconds, both in dark- (F_o) and in light-adapted state (F_s). Saturating pulses of 5000 μmol m^{-2} s^{-1} were occasionally applied with a focused 60 W halogen lamp to obtain pulse-saturated variable fluorescence values (F_m and F'_m for dark and light-adapted leaves, respectively). The following chlorophyll fluorescence parameters were calculated:

- Quantum yield of linear electron transport, $\Delta F/F'_m = (F'_m - F_s)/F'_m$ (Genty et al., 1989)
- Stern-Volmer non-photochemical quenching, NPQ= $(F_m - F'_m)/F'_m$ (Bilger & Björkman, 1990)
- Normalized steady-state chlorophyll fluorescence, F_s/F_o (Flexas et al., 2002)

For PRI measurements in the laboratory, the black reference panel was placed vertically side by side with the sample leaf. The instrument was placed horizontally in front of them at 0.6 m distance. The measuring spot of PRI was adjusted to the window of the chamber of the gas exchange analyzer. A correction factor for the calculated PRI was introduced to account for the spectral changes in the reflectance pattern induced by the face of the chamber. This was made after comparing the reflectance changes of the reference panel outside and inside the Li-6400 chamber during a typical experiment.

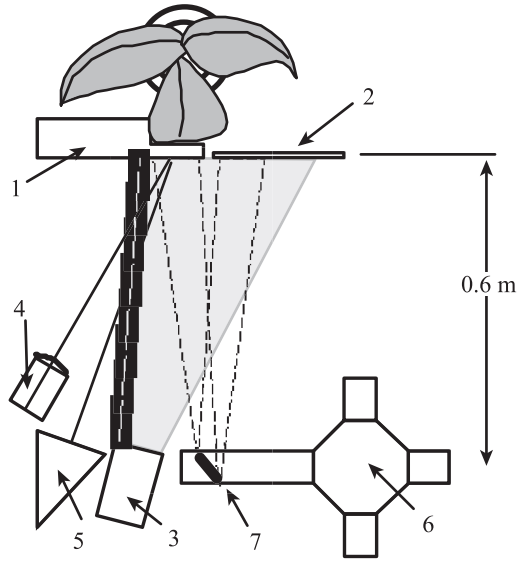

Fig. 1. Experimental set-up for laboratory experiments. A leaf was enclosed in the gas exchange chamber (1), and a reference panel (2) was placed vertically on its side. Actinic light was provided by a 650 W halogen projector (3). Light pulses of 5000 μmol m^{-2} s^{-1} were applied to saturate chlorophyll fluorescence using a focussed 60 W halogen lamp (4). Chorophyll fluorescence was sensed remotely through the transparent window of the gas exchange chamber using the Laser-PAM fluorimeter (5) described by Ounis et al. (2001). Reflectance variations were remotely sensed using the PRI device (6). To account for any spectral change in the actinic light, a flip-flop mirror (7) alternated the PRI field of view between the sample and the reference.

2.4. Chlorophyll fluorescence and PRI measurements in the field

For PRI measurements in the field, the green reference panel was placed beside the whole plant and inclined with an angle of about 20° from the vertical, in order to be near parallel to the average inclination of the canopy surface. PRI was measured over a canopy spot of 30 cm diameter at 4 m distance in the South–North direction, under natural conditions of light (Fig. 2). Data were collected each second during cloudless days. Major reflectance spectral changes

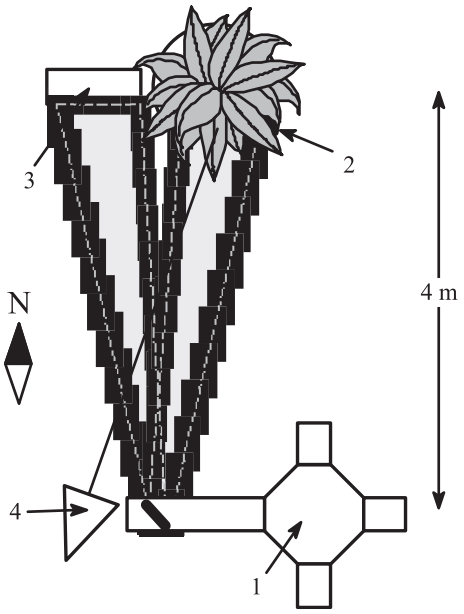

Fig. 2. Experimental set-up for field experiments. The PRI device (1) was placed at 4 m distance at the south side of the sample canopy (2), focused on a ca. 30 cm field of view. The reference panel (3) was placed near the plant, oriented towards the south. A single leaf from the PRI field of view, representative of the average orientation, was chosen for monitoring chlorophyll fluorescence using the FIPAM fluorimeter (4) described by Flexas et al. (2000).

during the day were taken into account by the measuring the reflectance of the reference panel every second. However, PRI measurements may be affected by changes on the Bidirectional Reflectance Distribution Function (BRDF) during the diurnal cycle (Gastellu-Etchegorry et al., 1999). Nevertheless, the results suggest that these effects are minor (see Results and discussion).

Chlorophyll fluorescence was measured on a single leaf of those included in the PRI field of view to compare with the variations of PRI. All fluorescence parameters described in the previous section were measured using the FIPAM (Flexas et al., 2000) placed at a 4 m distance. The FIPAM is a small FLIDAR based on a laser diode, able to measure variable chlorophyll fluorescence both under dark and light conditions.

3. Results and discussion

3.1. Processes involved in PRI changes at the leaf-level

Simultaneous measurements of PRI, chlorophyll fluorescence and gas exchange were conducted over a single leaf under varying light intensity. Fig. 3 shows a typical experiment performed on a control (irrigated) plant at 25 °C, in which light intensity was steeply changed from 0 to 50,

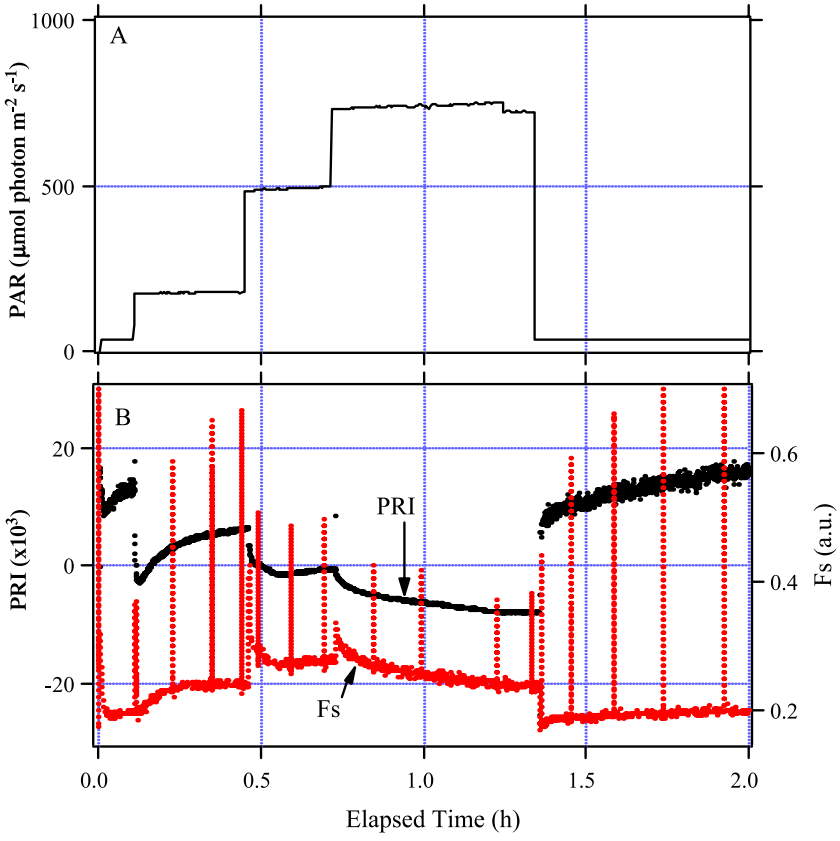

Fig. 3. (A) Time course variations of light incident on a leaf from a control (irrigated) plant. (B) Corresponding changes of PRI (solid dots) and F_s (dotted line). The spikes shown within the F_s pattern correspond to F_m values and their relaxation following the application of a light saturating pulse.

190, 450 and 750 μmol photons m^{-2} s^{-1} (Fig. 3A). Stomata opened progressively with increasing irradiance, which was accompanied by steeply increasing photosynthesis from -1 μmol CO_2 m^{-2} s^{-1} in darkness to 10 μmol CO_2 m^{-2} s^{-1} at the highest light intensity (data not shown). Steady-state chlorophyll fluorescence presented a typical pattern for a healthy irrigated plant (Fig. 3B), with F_s increasing in parallel to light intensity, except at the highest light intensity, in which F_s started to decrease (Cerovic et al., 1996; Flexas et al., 1999, 2000, 2002; Ounis et al., 2001). PRI presented two distinct phases of variation (Fig. 3B): a rapid phase, consisting in a sudden drop of PRI immediately after a step increase in light intensity (or a rise in PRI after a sudden light decrease, i.e. at the end of the experiment); and a slow phase of adjustment after the initial rapid phase that lasted several minutes. The presence of these two kinetically distinct phases confirms the earlier results by Gamon et al. (1990) on reflectance measurements and by Ruban et al. (1993) on absorbance measurements. The temporal resolution of the instrument described here permits to show that the rapid phase of absorbance changes takes place within seconds, as shown by Ruban et al. (1993). This fast kinetics imply that, at least in part, it may be related to a process other than xanthophyll de-epoxidation, which is described not to be completed until approximately a 5-min period (Gamon et al., 1990). The slow phase closely correlated to F_s variations, as already demonstrated for the absorbance changes at 505 and 535 nm (Heber, 1969; Heber et al., 1986; Krause, 1973; Ruban et al., 1993). The latter observation also supports the idea of a multi-component determinant of PRI, since F_s is governed by at least two opposite processes, namely photochemistry and energy-dependent heat dissipation (Cerovic et al., 1996; Flexas et al., 1999, 2000, 2002; Ounis et al., 2001).

Heber et al. (1986) suggested that absorbance (light scattering) changes at 535 nm were poor indicators of water stress, since the close correlation with F_s was abolished by cutting the leaf petiole in air. We therefore tested the relationship between the slow phase of PRI and F_s under water stress conditions. Fig. 4 shows an experiment similar to that in Fig. 3, except that changes in the water status of the leaf were induced by cutting the petiole on air. Following the cutting, a typical Iwanoff effect (Heber et al., 1986) was observed, consisting of a sudden increase of stomatal conductance and photosynthesis (not

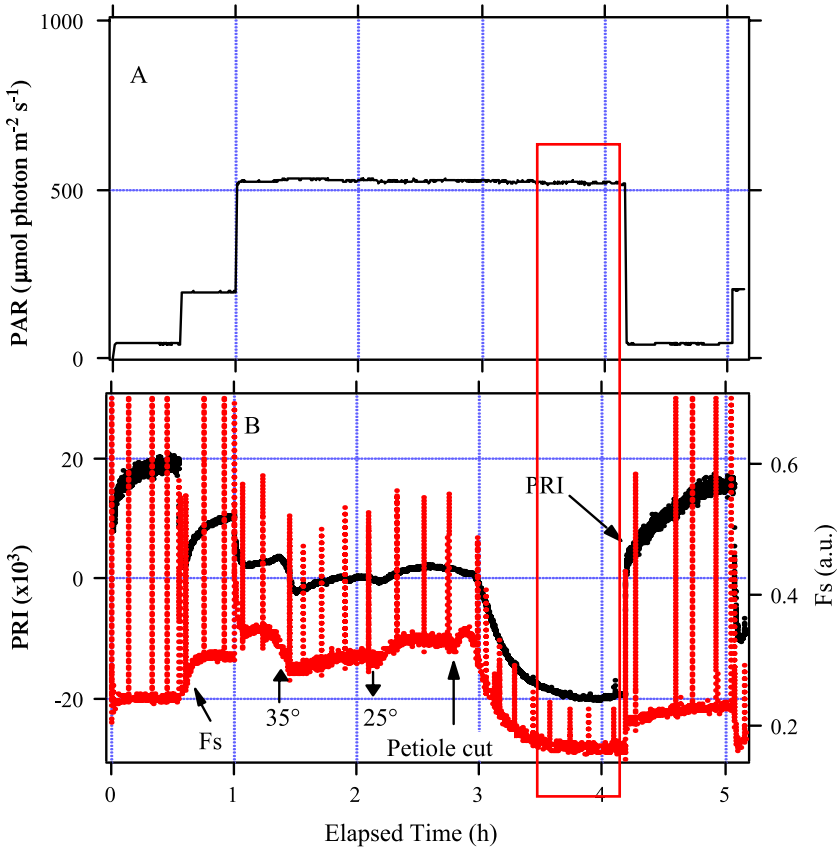

Fig. 4. (A) Time course variations of light incident on a leaf from a control (irrigated) plant, in which photosynthesis variations were induced by increasing the temperature from 25 to 35 °C (indicated in (B)) and by cutting the leaf petiole in air (indicated in (B), see text for details). (B) Corresponding changes of PRI (solid dots) and F_s (dotted line). The spikes shown within the F_s pattern correspond to F_m values and their relaxation following the application of a light saturating pulse.

shown), followed by a progressive decline of both parameters until reaching values close to zero about one hour after the cutting. The Iwanoff effect was closely followed by F_s, as already described (Flexas et al., 2002). F_s first increased as photosynthesis increased, and then dropped progressively until reaching values far below F_o. Interestingly, PRI changes closely followed F_s variations during the Iwanoff effect, contrarily to the observations by Heber et al. (1986). A very similar correspondence was observed when analyzing leaves from slowly dehydrated plants (not shown). Moreover, the close relationship between PRI and F_s was maintained when, prior to cutting the petiole, stomatal conductance and photosynthesis were depressed by increasing leaf temperature to 35 °C (indicated in Fig. 4B). It seems therefore that the relationship between the slow phase of PRI and F_s is very robust, which may indicate that the same processes that control F_s variations govern this phase of PRI.

To test the proposed implication of the xanthophyll cycle in PRI variations (Filella et al., 1996; Gamon et al., 1990, 1992, 1997; Peñuelas et al., 1994), some leaves were infiltrated with dithiothreitol (DTT), a well-known inhibitor of xanthophyll de-epoxidation. Fig. 5 shows an experiment performed with a control leaf, similar to that of Fig. 3, except that DTT was infiltrated. F_s rose strongly, due to the impairment of non-photochemical quenching (Flexas et al., 2002). Clearly, the treatment abolished almost completely the slow phase of PRI, and largely reduced the extent of the rapid phase. It can be therefore concluded that the slow phase of PRI was strongly related to a non-photochemical quenching type involving de-epoxidated xanthophylls. A fraction of the rapid phase is also related to this process, the rest being probably related to chloroplast shrinkage following increased ΔpH (Gamon et al., 1990). Similarly, it has been shown that DTT abolishes absorbance changes at both 505 and 535 nm (Bilger et al., 1990; Ruban et al., 1993; Yamamoto & Kamite, 1972).

If PRI really reflects changes associated to ΔpH and the xanthophyll cycle, a correlation might then expected between PRI and Stern-Volmer non-photochemical quenching of chlorophyll fluorescence (NPQ). Such a correlation is shown in Fig. 6A when combining data from experiments performed in plants under control conditions (Fig. 3), under different stresses (Fig. 4) and treated with DTT (Fig. 5), as well as in other similar experiments (not shown). Notice that all leaves, even those in which DTT was applied, hold a common relationship between PRI and NPQ, which strengths the tight relationship between xanthophyll de-epoxidation and PRI. Similarly to the present results, Ruban et al. (1993) showed a curvilinear relation-

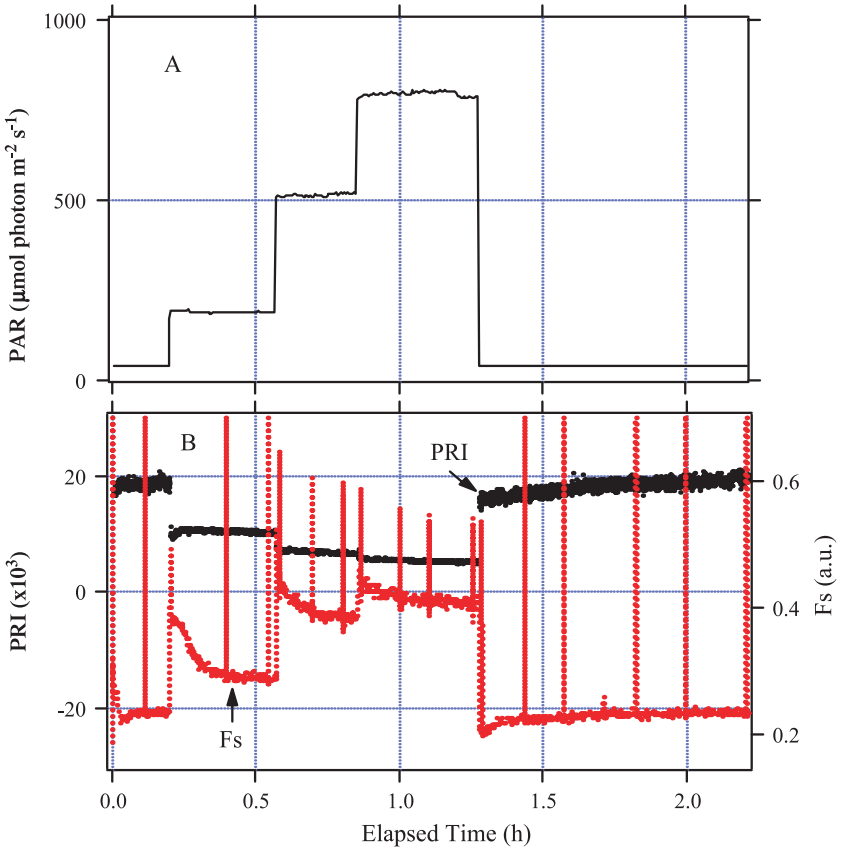

Fig. 5. (A) Time course variations of light incident on a leaf infiltrated with DTT. (B) Corresponding changes of PRI (solid dots) and F_s (dotted line). The spikes shown within the F_s pattern correspond to F_m values and their relaxation following the application of a light saturating pulse.

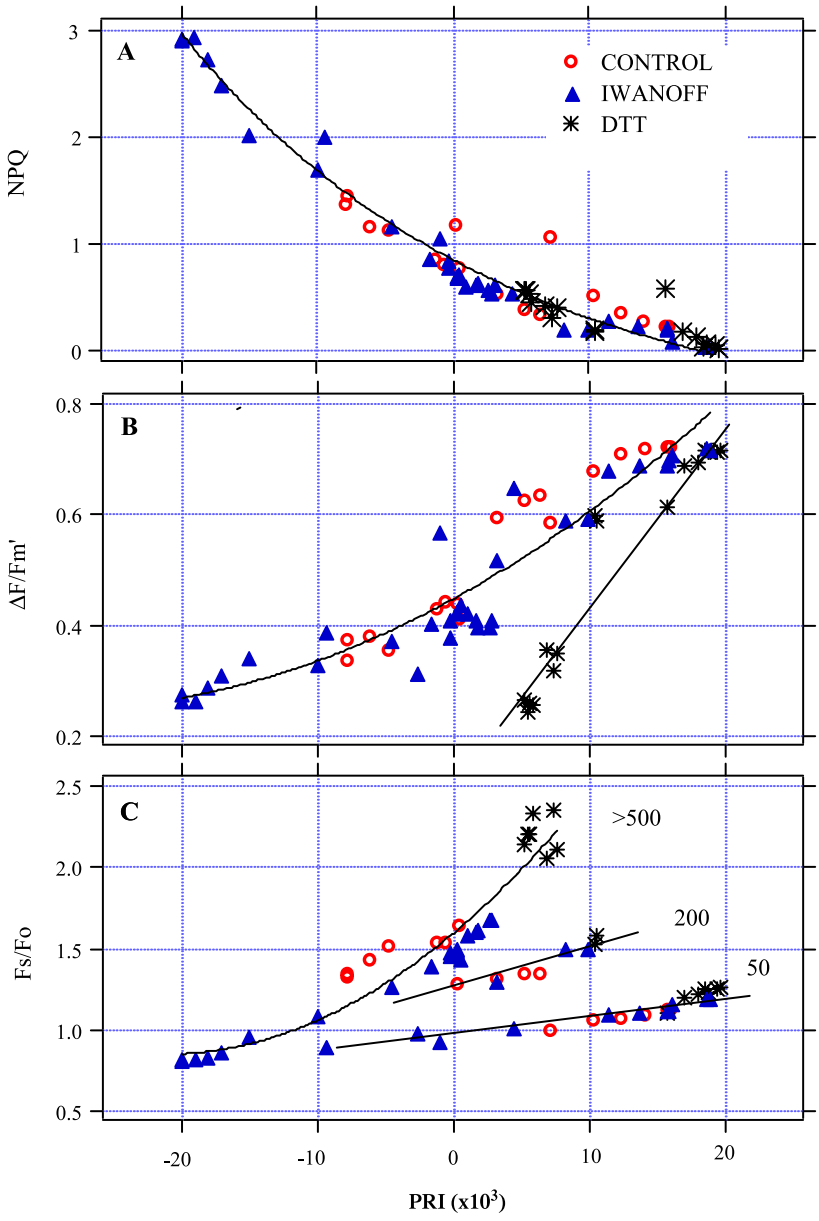

Fig. 6. Relationship between PRI and chlorophyll fluorescence parameters measured in the experiments in control plants (empty circles), stressed by heat or drought (solid triangles) and in the presence of DTT (stars). (A) Relationship between PRI and NPQ. (B) The relationship between PRI and $\Delta F/F'_m$. (C) The relationship between PRI and F_s/F_o.

ship between NPQ and absorbance changes at 505 nm, and a linear relationship between NPQ and absorbance changes at 535 nm.

Because of the tight inverse relationship between NPQ and $\Delta F/F'_m$ (Flexas et al., 2002), a correlation is also expected between $\Delta F/F'_m$ and PRI. Peñuelas et al. (1995) have shown a linear correlation between these two parameters, which was, however, species-dependent. Such a correlation was indeed observed in the present data, although it was curvilinear rather than linear. However, this correlation was clearly unpaired in plants treated with DTT (Fig. 6B). Because DTT inhibits xanthophyll de-epoxidation, but not electron transport nor ΔpH, the uncoupling of this correlation by DTT further supports a direct link between PRI and the xanthophyll cycle. The remaining variations of PRI (Figs. 5 and 6B) are probably reflecting other quenching mechanisms related to ΔpH.

Finally, the correlation between PRI and F_s/F_o was also high, but strongly dependent on the irradiance conditions (Fig. 6C). This was indeed expected, since the relative importance of photochemical and non-photochemical components of F_s varies with light intensity, the former being the most important at low light and the second at high light (Cerovic et al., 1996; Flexas et al., 2000). Also the relationship between F_s/F_o and NPQ has been shown to be strongly dependent on irradiance (Flexas et al., 2002).

In summary, the experiments conducted at the leaf level in laboratory conditions show that PRI involves two components, the most important of which is directly related to the xanthophyll cycle and NPQ. Since the xanthophyll cycle-dependent NPQ responds rapidly to both diurnal variations of light intensity and to different types of stress (Demmig-Adams & Adams, 1992), remote sensing of PRI seems a promising tool to detect dynamic changes of leaf photosynthesis and photoprotection under field conditions.

3.2. Remote sensing of PRI variations in the field

The usefulness of remote sensing of PRI under field conditions was tested with the new described instrument. PRI was measured over a growing canopy of grapevines in the field, and subjected to progressive water stress. Fig. 7 shows an example of these experiments. Fig. 7A shows the diurnal time course of PRI and F_s/F_o of a severely water-stressed grapevine plant, which had been without irrigation for 1 week. F_s/F_o shows a diurnal pattern of variation typical of a stressed plant, with an increase under low light in the early morning and late evening, and a strong midday depression dropping below dark-adapted values (Cerovic et al., 1996; Flexas et al., 1999, 2000, 2002; Ounis et al., 2001). PRI followed well the diurnal variations of F_s/F_o. The increase of both F_s/F_o and PRI at the early morning is concomitant with an increase of F_m' (not shown), which indicates a relaxation in dim light of a component of NPQ

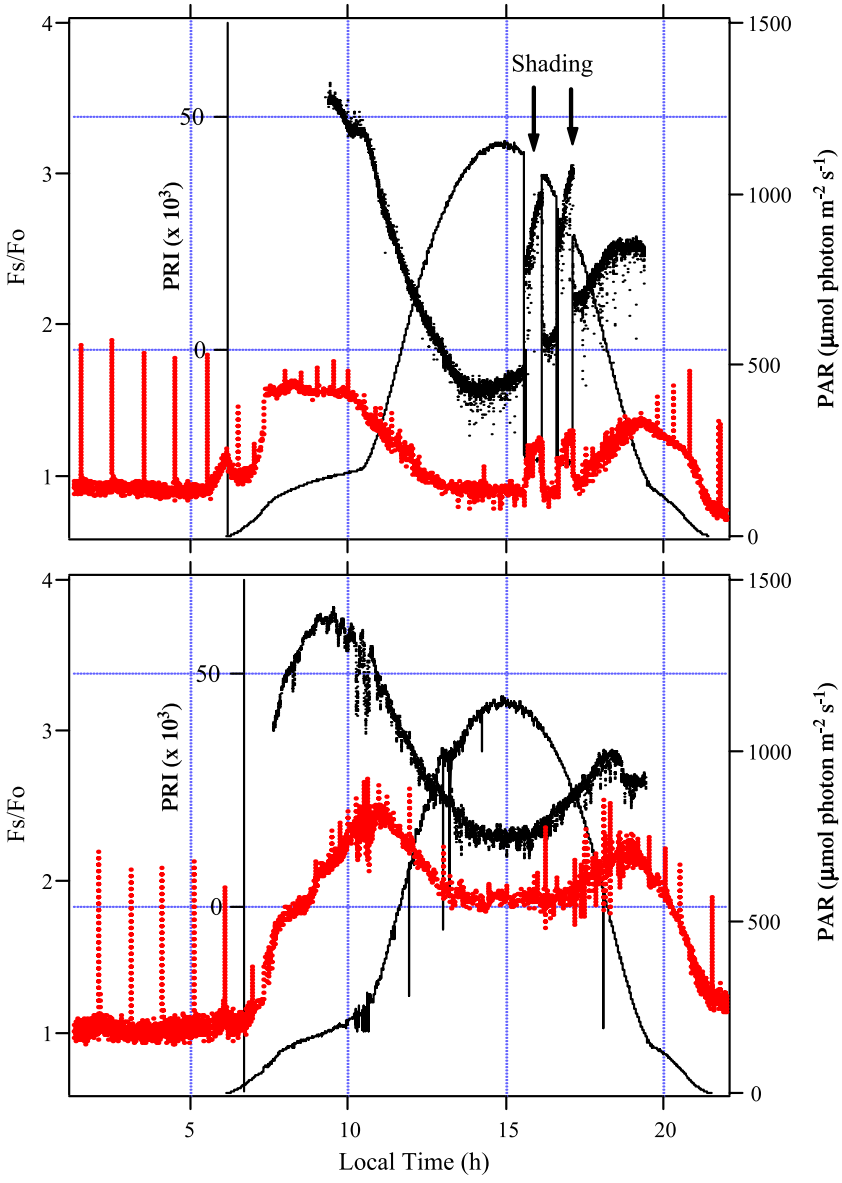

Fig. 7. Diurnal time course, under Mediterranean summer field conditions, of PAR (solid line), PRI (solid dots) and F_s (dotted line), for a water stressed grapevine canopy (A), and for the same plant after 2 days of irrigation (B). In the experiment shown (A), the canopy was twice (indicated by arrows) artificially shadowed to reveal the presence of the two PRI phases described. This was achieved by removing direct solar radiation with an opaque screen.

maintained overnight. The maintenance of an overnight quenching is consistent with the observed increased de-epoxidation state of the xanthophyll cycle as drought progresses (Medrano et al., 2002). Furthermore, a similar overnight quenching has been reported under winter stress (Adams & Demmig-Adams, 1995). Both F_s/F_o and PRI showed a strong depression at midday, corresponding to maximum development of NPQ. In the afternoon, both F_s/F_o and PRI incompletely reversed, corresponding to the well-known slow reversion of NPQ.

The two phases of PRI were not distinguishable outdoors due to the gradual changes on the light intensity existing in the field, as already pointed out by Gamon et al. (1990). However, the two phases could be distinguished by artificially shadowing the canopy, as shown in Fig. 7A. Fig. 7B shows the response of the same plant after two days of irrigation at field capacity. Clearly, F_s/F_o recovered, following a daily pattern typical of an irrigated plant, with only a small midday depression, with values still much higher than dark-adapted ones. Again, PRI closely followed the variations of F_s/F_o, and the range of the midday depression was largely reduced.

The absolute values of PRI obtained during field experiments were higher than those obtained during laboratory experiments. This was mainly due to scaling up from leaf to canopy level, although some effect could be also due to the different materials used as references in both experiments. Scaling up from the leaf to the canopy level is expected to result in higher levels of PRI for any given light intensity, since a large part of the field of view is occupied by shadowed areas that do reflect less at 531 nm because they present little de-epoxidation. Similarly, in sunflower, higher values of PRI were observed by Gamon et al. (1992) at the canopy level than by Peñuelas et al. (1994) at the leaf level. In spite of these differences in the absolute values, a similar correlation was observed between PRI (measured over a canopy area) and NPQ (measured over a single leaf) than found for laboratory experiments (Fig. 8). As mentioned in methods section, BRDF can affect PRI measurements. Nevertheless, the differences in PRI variations observed between the stressed and the control plant reveal a valid measurement of PRI despite these possible effects. Moreover, during short periods of artificial shading (Fig. 7A), the slow reversion kinetics of the recorded signal can only be attributed to actual PRI changes, since BRDF should be constant during this period. In addition, the strong parallelism observed with F_s variations along the day is in line with this interpretation.

Summarizing, it is shown that the described system allows remote sensing of PRI to follow water-stress associated changes at the canopy level, from a distance only limited by the homogeneity of the field of view. Gamon et al. (1992) and Peñuelas et al. (1994) suggested that PRI was not useful for water stress detection, especially in severely wilted canopies. Severe wilting will result in canopy structure rearrangements, which may in turn induce changes in the canopy areas that are under excess illumination, in the reflectance angles, etc. Here we show that water stress detection is possible by this method in grapevine canopies, possibly because this is a very drought-resistant species showing little wilting until drought is very severe. We have indeed observed very large changes in PRI level in wilting maize canopies (not shown) that are not explainable solely by increasing non-photochemical quenching.

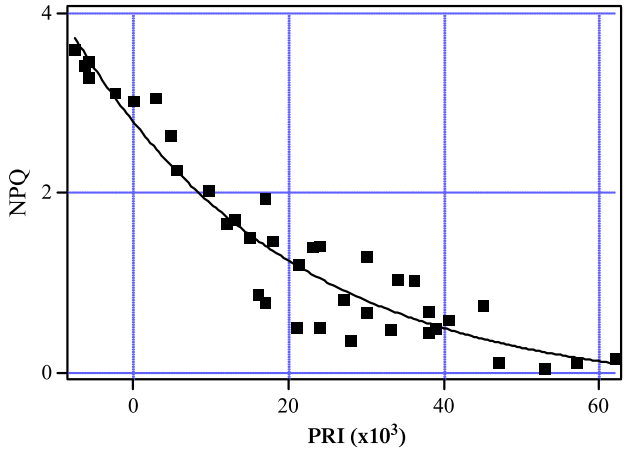

Fig. 8. The relationship between PRI measured at the canopy level and NPQ measured on a single leaf of those included in the PRI spot, from field experiments.

4. Conclusions

We describe an instrument capable of passive remote sensing of dynamic reflectance changes at around 531 nm in relation to a non-variable band at 570 nm, the so-called PRI, during the whole course of a day. This is achieved due to the use of a reference panel.

It is clearly shown that PRI better correlates with non-photochemical than with photochemical quenching. For this reason, we propose to maintain the original name of PRI, Physiological Reflectance Index (Gamon et al., 1992), instead of the revised name, Photochemical Reflectance Index (Peñuelas et al., 1995).

The interest of this signal lies in the feasibility of its measure, which can be extended to airborne remote sensing (Rahman et al., 2001). As exemplified by the water stress experiments presented, using the instrument described here allows two possibilities for the remote sensing of plant status, namely following the entire diurnal course of PRI to focus on its amplitude range variations, or determining differences in the absolute value of PRI under high light at noon.

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