PHOTOPROTECTION DYNAMICS OBSERVED AT LEAF LEVEL FROM FAST TEMPORAL REFLECTANCE CHANGES

Shari Van Wittenberghe[1], Luis Alonso[1], Zbyněk Malenovský[2,3,4], José Moreno[1]

1 Laboratory of Earth Observation, University of Valencia, Valencia, Spain
2 School of Technology Engineering and Design, University of Tasmania, Hobart, Australia
3 Global Change Research Institute CAS, Brno, Czech Republic
4 School of Biological Sciences, University of Wollongong, Wollongong, Australia

ABSTRACT

Vegetation dynamically reacts to the available photosynthetically active radiation (PAR) by adjusting the photosynthetic apparatus to either a light harvesting or a photoprotective modus. When activating the photoprotection mechanism, either minor or major pigment-protein interactions may occur at the leaf level, resulting in different light absorption and consequently reflectance intensities. The reflectance changes were measured during sudden illumination transients designed to provoke fast adaptation to high irradiance. Different spectral reflectance change features were observed during different stages of photoprotection activation, extending over part of the visible spectral range (i.e. 490-650 nm). Due to this multiple wavelength reflectance modification, which affects also the reference band at 570 nm, the commonly used Photochemical Reflectance Index (PRI) is unable to trace and quantify such strong photoprotection mechanism. To quantify the entire photoprotection with a required accuracy, the spectral changes in the full visible range must be characterized.

Index Terms— Non-photochemical quenching (NPQ), conformational changes, photoprotective pigments, photosynthesis, FLEX, hyperspectral mission.

1. INTRODUCTION

To keep the balance between harvesting the sunlight for photosynthesis and protection against an irradiation excess, vegetation can switch between a light harvesting and a heat dissipating mode. For this, the electron excitation energy of Chlorophyll (Chl) *a*, which cannot be used for photosynthesis reactions, needs to be dissipated by either spontaneous or unprovoked de-excitations (fluorescence, internal conversions), or by de-excitations triggered and regulated by physiological and biochemical signals. The latter is known as the non-photochemical quenching (NPQ) of excitation energy of Chl *a* [1]. Under natural conditions, NPQ is mostly dominated by the rapidly reversible energy-dependent mechanism qE, or the controlled deactivation of singlet excited Chl (1Chl*) into heat [2]. qE involves complex dynamics of excitation-energy transfers between the excited states of Chl *a* and carotenoids [3], accompanied by structural or conformational changes of the light harvesting complexes of Photosystem II (LHCII) [4]. Carotenoids, the important accessory pigments of the leaf LHCII, play a crucial role in this process. They efficiently quench excitation energy, due to an ultrashort lifetime of the singlet excited state (S_1), which rapidly falls back to its ground state, releasing energy as heat [5]. The carotenoid S_1 state plays an essential role in the NPQ process, and its transition to higher excited states (S_n) has been seen as an electrochromic absorption shift between 505-600 nm at the LHCII level [3], [6]. In remote sensing this green absorption shift triggered by the photoprotective xanthophyll pigments' transformation is typically exploited in the Photochemical Reflectance Index (PRI), i.e. (R531-R570)/(R531+R570), which uses the reflectance at 531 and 570 nm, respectively, as sensitive and reference band for the photochemical changes due to photoprotection [7]. Although several studies have reported good correlations between PRI and energy-dependent NPQ during different short-term light phases [8]–[10], some discrepancies were acknowledged, assuming other interacting mechanisms being involved in NPQ at the short-term. Other absorption shifts than those purely caused by carotenoid excitation have been described for the qE-related conformational changes of the pigment bed [11]. However, these changes were observed for isolated chloroplasts, and not for leaves under natural environmental conditions. In this study we observed the contiguous reflectance dynamics during light adaptation of intact leaves under natural-like illumination. Our goal is to study the dynamic leaf reflectance changes during built-up of the photoprotection mechanisms under sudden high intensity light exposure. Additionally, the impact of those NPQ mechanisms on PRI is presented.

2. MATERIALS AND METHODS

Dark-to-high-light transients were set up in the laboratory to provoke the activation of leaf photoprotection mechanisms, while leaf level spectral responses were measured with a

FieldSpec spectroradiometer (ASD Inc., Boulder, CO, USA). The two similar set-ups were applied: (1) a leaf clip set-up with a strong LED illumination (High Cri LED 10W 17V 3050-5900 K, Yuji International Co., Ltd, Beijing, China) pointing to a leaf clip opening, and (2) a canopy set-up, illuminated with a LED panel (Photon System Instruments, s.r.o., Drásov, Czech Republic), in which a single leaf was measured with the bare fiber pointing to the adaxial leaf surface. In the set-up (1), healthy sunlit white mulberry (*Morus alba* L.) leaves collected from a mature tree were measured, and in the set-up (2) sunlit beech (*Fagus sylvatica* L.) leaves of potted saplings were sampled. Measurements were taken continuously on 2 h dark-adapted leaves with an integration time of 136 ms, resulting in approximately 1 recording per second during a 20-min light adaptation time to high intensity light (±1300 µmol m^{-2} s^{-1}). After each light transient a white reference (Spectralon, Labsphere Inc., North Sutton, US) was placed inside the leaf clip to measure the irradiance. Next, reflectance changes (dR) were calculated for the 490-650 nm spectral range, by subtracting the first reflectance signal from the dynamical reflectance during 20 min. PRI was calculated (i) based on the dynamic 570-nm reference band during the light adaptation/photoprotection, and, (ii) based on the fixed 570-nm reference band at t=0 min, i.e. the first reflectance right after illumination representing the un-photoprotected state.

3. RESULTS

Upon sudden high intensity illumination, absorbance changes associated with photo-induced redox changes in the electron transport and electrochromic shifts of the antenna pigments induced reflectance changes seen at the leaf level. Three cases of different photoprotection degree were observed through reflectance decrease and corresponding spectral behaviour during the light adaptation phase.

3.1. Reflectance change due to quick carotenoid excitation energy transfer upon high intensity light

Within a few minutes upon illumination, a common spectral change in the green region (500-570 nm) occurred in all cases, with a maximal decrease around 530-535 nm (Figure 1, top). This known electrochromic shift of absorption by carotenoids (xantophylls' transformation) indicates the excitation of photoprotective pigments, which dissipate energy as heat when falling back to their ground state. This spectral behaviour with a maximal decrease of approximately 1% at 531 nm was commonly observed. A second smaller absorption feature around 615 nm was also often observed, reflecting the simultaneous excitation of Chl *a* pigments during photoprotection. As a response to the quick carotenoid excitation transient absorption, PRI decreased quickly during the first 2-3 minutes, before stabilizing for the remaining adaptation period (Figure 1, middle, bottom). Nevertheless, both reflectance at the sensitive (R531) and reference (R570) PRI bands showed a decline during the first minutes (Figure 1, middle). The reference band remains constant after this, but declines again softly around t=10 min. Comparison of the dynamic PRI with the PRI calculated with fixed reference band (R570 at t=0 min) revealed the underestimating capacity of PRI in predicting photoprotection (Figure 1, bottom). The dynamic PRI (R570 variable) was sensitive to 86% of the total 531-nm decrease, indicated by PRI with fixed reference band (see the light grey versus total grey area in Figure 1, bottom).

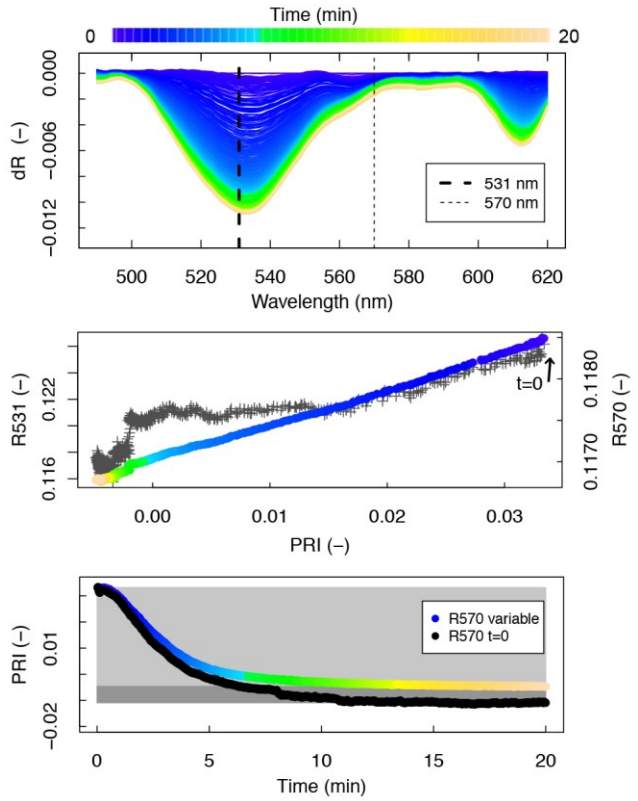

Figure 1 Reflectance change (dR) during a 20-min minimal photoprotection adaptation to high intensity irradiance of a sun adapted white mulberry leaf (top); R531 and R570 during PRI evolution (middle); and PRI calculated with variable and fixed reference band (bottom).

3.2. Reflectance changes during quick minor pigment-protein bed changes

A stronger degree of photoprotection was observed as a smooth reflectance decrease over the whole spectral region 490-650 nm, with a maximal change around 530-535 nm of approximately 2.5% (Figure 2). The reflectance changes, as shown in Figure 1, are smoother and the dR at 615 nm is not clearly visible anymore. Interestingly, reflectance of the reference band (R570) decreased continously during the first

5 min of intensive illumination, in parallel with R531 decrease (Figure 2, middle). PRI results were consequently affected by the variability in the reference band, causing a sensitivity of only 59% of the total 531-nm decrease, indicated by PRI with fixed reference band (see the light grey versus total grey area in Figure 2, bottom).

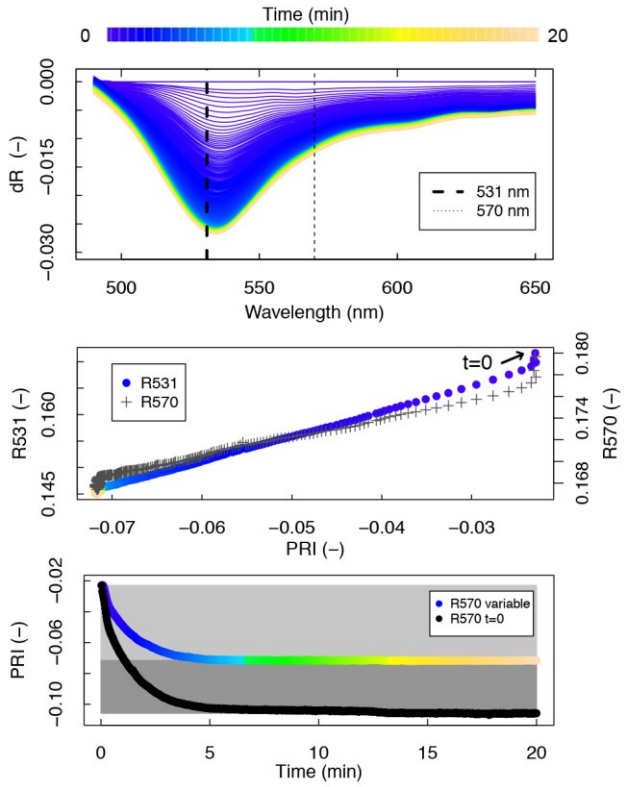

Figure 2 Reflectance change (dR) during a 20-min photoprotection adaptation to high irradiance of a sun adapted beech leaf (top); R531 and R570 during PRI evolution (middle); and PRI calculated with variable and fixed reference band (bottom).

3.3. Reflectance changes during slow major pigment-protein bed changes

An additional absorbance feature, which took place after an approximately 5 min long light adaptation, was observed for another sun adapted beech leaf (Figure 3). During the first minutes, rapid reflectance decline corresponding to the previously observed rapid reflectance changes (Figure 2) reappeared. Nonetheless, this phase was followed by a further reflectance change of a strong decrease over the whole 490-650 nm range, resulting in a decrease of about 3.5% around 531 nm. This additional reflectance decrease was not centred around the 530-535 nm region, but showed a strong absorbance shift over the whole 490-650 nm range. Consequently, a soft decrease of the reference band at 570 nm during first minutes upon light adaptation was followed by a stronger decrease in following minutes (Figure 3, middle). This strong R570 decline caused an PRI increase, despite a further R531 decrease. Hence, from the onset of this second mechanism, the PRI appeared to demonstrate the reverse response to the effect, for which indication it was designed.

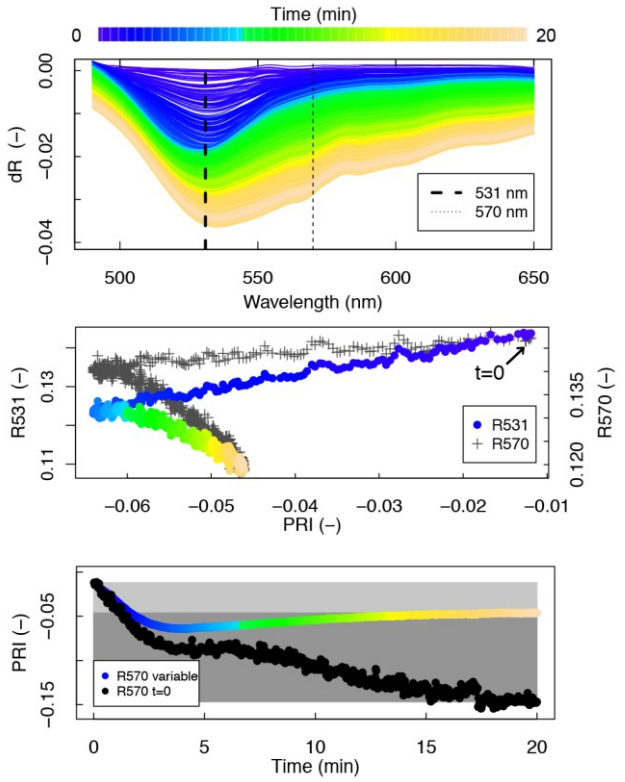

Figure 3 Reflectance change (dR) during a 20-min photoprotection adaptation to high irradiance of a sun adapted beech leaf (top); R531 and R570 during PRI evolution (middle); and PRI calculated with variable and fixed reference band (bottom).

In contrast, PRI based on the fixed reference band further declined (Figure 3, bottom). PRI with variable reference band finally represented only 25.5% of the total 531-nm decrease, indicated by PRI with fixed reference band.

4. DISCUSSION

Studies investigating isolated LHCII and related antenna complexes have provided important insights in the mechanism behind the plant photoprotection [12]. It has been shown that the LHCII can exist in different conformational states that have varying capacities for energy dissipation [4], [11]. This study illustrates the effect of different photoprotection mechanisms under a high irradiance observed as reflectance changes in the corresponding leaf spectral signature. Although it is hard to

identify when and under which conditions the different conformational states are activated, it shows that photoprotection affected the whole 490-650 nm spectral range in all investigated cases. These observations have important implications for spectral estimation of photoprotection and NPQ. PRI, designed for photoprotection and stress estimation, is based on the 570-nm reference band. However, also this band, and the full 490-650 nm range in general, is affected by photoprotection absorption mechanisms. Moreover, different overlapping absorbance changes in the 490-650 nm range during photoprotection causes difficulties to designate a single appropriate reference band. Some previous experiments have demonstrated the confounding effect of the 570-nm band variability on the PRI effectivity [13] and proposed a continuum removal index over the 511-557 nm region instead, but an overall absorbance shift under major pigment bed changes is expected also in those wavelengths. Consequently, the current efforts to estimate NPQ are unable to quantify the different absorbance changes occurring in the 490-650 nm region, linked to different quick and slow NPQ mechanisms. More complex signal processing methods characterizing vegetation reflectance signature shape by its different dynamical absorption features under photoprotection will be required to estimate photoprotection with a higher accuracy.

5. CONCLUSIONS

Photoprotection adaptation mechanisms activated under high intensity light can be observed as dynamic reflectance changes. During photoprotection, strong reflectance decrease in the 490-650 nm region are seen. Both fast (few min) and slower (5-20 min) changes take place upon sudden illumination, characterized by different absorbance features. Simple 2-band indices, such as PRI, cannot quantify the full complexity of energy-dependent NPQ. Especially when major pigment-protein changes take place, PRI fails to act as an appropriate indicator. Further studies, including photosynthetically dynamic radiative transfer modelling, should bring more insight on the dynamic reflectance/transmittance features linked to NPQ and their upscaling to the canopy level, as its quantification is of high importance to the optical retrieval of photosynthesis-related products. Accordingly, a high spectral resolution in the visible wavelengths of the imaging spectrometers, as also foreseen for ESA's 8th Earth Explorer mission FLEX, will be crucial to characterize the photoprotection state of vegetation.

6. ACKNOWLEDGEMENTS

The work presented is funded by the European Union's H2020 Marie Skłodowska-Curie actions under the grant agreement FLUOPHOT nº [701815].

7. REFERENCES

[1] B. Demmig-Adams and W. W. Adams III, "Photoprotection and other responses of plants to high light stress," *Annu. Rev. Plant Biol.*, vol. 43, no. 1, pp. 599–626, 1992.

[2] K. K. Niyogi, "PHOTOPROTECTION REVISITED : Genetic and Molecular Approaches," *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, vol. 50, pp. 333–359, 1999.

[3] A. V. Ruban *et al.*, "Identification of a mechanism of photoprotective energy dissipation in higher plants," *Nature*, vol. 450, no. November, pp. 575–579, 2007.

[4] A. R. Holzwarth, Y. Miloslavina, M. Nilkens, and P. Jahns, "Identification of two quenching sites active in the regulation of photosynthetic light-harvesting studied by time-resolved fluorescence," *Chem. Phys. Lett.*, vol. 483, pp. 262–267, 2009.

[5] R. Christensen, "The electronic states of carotenoids," in *The Photochemistry of Carotenoids. Advances in Photosynthesis and Respiration, Volume 8*, H. Frank, A. Young, Britton, G, and R. Cogdell, Eds. Springer, 2004, pp. 137–159.

[6] T. Polívka and H. A. Frank, "Spectroscopic investigation of carotenoids involved in non-photochemical fluorescence quenching," in *Non-Photochemical Quenching and Energy Dissipation in Plants, Algae and Cyanobacteria*, B. Demmig-Adams, G. Garab, W. W. Adams III, and Govindjee, Eds. 2016, pp. 203–228.

[7] J. A. Gamon, J. Peñuelas, and C. B. Field, "A Narrow-Waveband Spectral Index That Tracks Diurnal Changes in Photosynthetic Efficiency," vol. 44, pp. 35–44, 1992.

[8] J. Atherton, C. J. Nichol, and A. Porcar-Castell, "Using spectral chlorophyll fluorescence and the photochemical reflectance index to predict physiological dynamics," *Remote Sens. Environ.*, vol. 176, pp. 17–30, 2016.

[9] 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.

[10] P. Rahimzadeh-Bajgiran, M. Munehiro, and K. Omasa, "Relationships between the photochemical reflectance index (PRI) and chlorophyll fluorescence parameters and plant pigment indices at different leaf growth stages," *Photosynth. Res.*, vol. 113, no. 1–3, pp. 261–271, 2012.

[11] M. P. Johnson and A. V Ruban, "Rethinking the existence of a steady-state delta pfi component of the proton motive force across plant thylakoid membranes," *Photosynth. Res.*, vol. 119, pp. 233–242, 2014.

[12] I. Moya, M. Silvestri, O. Vallon, G. Cinque, R. Bassi, and A. Holzwarth, "Time-resolved fl uorescence analysis of the Photosystem II antenna proteins in detergent micelles and liposomes," *Biochemistry*, vol. 40, pp. 12552–12561, 2001.

[13] D. Kovác *et al.*, "Response of green reflectance continuum removal index to the xanthophyll de-epoxidation cycle in Norway spruce needles," *J. Exp. Bot.*, vol. 64, no. 7, pp. 1817–1827, 2013.