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Seasonal stability of chlorophyll fluorescence quantified from airborne hyperspectral imagery as an indicator of net photosynthesis in the context of precision agriculture

P.J. Zarco-Tejada [a,*], M.V. González-Dugo [a], E. Fereres [a,b]

[a] Instituto de Agricultura Sostenible (IAS), Consejo Superior de Investigaciones Científicas (CSIC), Alameda del Obispo s/n, 14004 Córdoba, Spain
[b] Departamento de Agronomía, Universidad de Córdoba, Campus Universitario de Rabanales, 14014 Córdoba, Spain

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ABSTRACT

The seasonal stability of solar-induced chlorophyll fluorescence (SIF) vs field-measured leaf CO_2 assimilation (A) was assessed over a period of 2 years by means of airborne flights performed at midday and diurnally over a citrus (evergreen) crop canopy. The orchard was cultivated under a control treatment (ET) that received 100% of its water requirements and two regulated deficit irrigation (RDI) treatments with water supply reduced to 37% and 50% of the control level during the summer. Field measurements consisted of assimilation rate, stomatal conductance, stem water potential, leaf fluorescence and leaf reflectance. The airborne campaigns took place in 2012 and 2013, and were flown on the solar plane in order to acquire hyperspectral imagery at 40 cm resolution, 260 spectral bands and 1.85 nm/pixels in the 400–885 nm spectral region. A thermal camera was installed in tandem in all flights, acquiring imagery in the 7.5–13 μm spectral region at 640 × 480 pixel resolution, yielding a 50 cm pixel size. The robustness of the SIF quantification through the Fraunhofer Line Depth (FLD) principle based on three spectral bands (FLD3), as well as the performance of physiological and structural hyperspectral indices, was evaluated in order to understand their ability to track photosynthesis at different phenological and stress stages throughout the season. Solar induced fluorescence quantified as FLD3 was the most robust indicator of photosynthesis in all the airborne campaigns performed in the course of the two-year experiment, which comprised seven midday flights and two diurnals. The relationships between fluorescence (FLD3) and assimilation rates yielded correlation coefficients (R) between 0.64 and 0.82 across all dates, these being statistically significant with *p*-values between $p < 0.05$ and $p < 0.0001$. Fluorescence retrievals performed better than structural and physiological indices, with the structural MTVI1 index being the only other statistically significant indicator throughout the season, although it yielded lower levels of significance than FLD3. A normalization strategy proposed for SIF FLD3 for all dates using control (ET) trees on each flight date as a reference permitted the generation of a single relationship between FLD3 normalized (FLDn) and assimilation rates for the entire year, both at tree ($r^2 = 0.5$; $p < 0.0001$) and treatment level ($r^2 = 0.72$; $p < 0.0001$), a strategy that confirmed the ability of seasonal SIF retrievals to track photosynthesis from broader resolution hyperspectral imagery (i.e. spectral resolution 1–5 nm full-width at half maximum (FWHM)) for applications in the context of precision agriculture and crop-monitoring studies.

1. Introduction

Rising attention is being paid to solar-induced chlorophyll fluorescence (SIF) for global assessment of vegetation physiology, particularly as a means of monitoring crop photosynthesis on global scales (Guanter et al., 2014). Such worldwide interest in chlorophyll fluorescence is partly due to the need to improve the inputs and methods used in current carbon-cycle models for global estimates of gross primary production (GPP), which in some cases have produced large errors with results that differ by a factor of two compared to tower-flux data networks (Schaefer et al., 2012). The best estimates of the GPP of crop systems are widely accepted to be derived from direct measurement of carbon dioxide exchange by flux towers (Baldocchi et al., 2001) but these tend to sample small areas and are spatially scattered. Image-based remote quantification of SIF, emitted in the 650–850 nm region of the spectrum as a by-product of photosynthesis (Porcar-Castell et al., 2014; Meroni et al., 2009; Rascher et al., 2009), is therefore regarded as critical, as it has been proposed as a direct proxy for photosynthetic rate and related to vegetation stress conditions. Although the rise in interest is very recent, studies carried out around the end of the last century had already demonstrated the links between chlorophyll fluorescence

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* Corresponding author.
E-mail address: pablo.zarco@csic.es (P.J. Zarco-Tejada).
URL: http://quantalab.ias.csic.es (P.J. Zarco-Tejada).

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emission and photosynthesis (Papageorgiou, 1975; Krause & Weis, 1984; Schreiber & Bilger, 1987; Lichtenthaler & Rinderle, 1988; Lichtenthaler, 1992; Larcher, 1994; Schreiber, Bilger, & Neubauer, 1994), with most of the fluorescence quantification efforts being performed later at leaf and near-canopy levels (Pérez-Priego, Zarco-Tejada, Sepulcre-Cantó, Miller, & Fereres, 2005; Meroni, Colombo, & Cogliati, 2004, 2008a, 2008b, 2009, Moya et al., 2004; Cogliati et al., 2015). Specifically, some studies have focused on the technical side of the fluorescence quantification for monitoring global photosynthesis (Malenovsky, Mishra, Zemek, Rascher, & Nebal, 2009) or on leaf- to regional-level scaling issues using the O_2 bands (Rascher et al., 2009), in addition to modelling GPP from fluorescence/assimilation rates in diurnal patterns (Damm et al., 2010).

Besides the methodological advances in chlorophyll fluorescence emission retrieval, efforts to assess crop physiology from remote sensing have focused on the development of new indicators calculated from hyperspectral reflectance imagery based on narrow spectral bands related to canopy structure and photosynthetic pigments, including chlorophyll, xanthophylls and carotenoids (Quemada, Gabriel, & Zarco-Tejada, 2014; Hernandez-Clemente et al., 2014; Zarco-Tejada et al., 2013a). The interest in the development of these reflectance-based physiological indices relies on the fact that traditional vegetation indices such as the Normalized Difference Vegetation Index (NDVI) are highly sensitive to the phenology that usually drives the main structural changes that occur throughout the season, being saturated at larger leaf-area index values (Haboudane et al., 2004) and masking smaller physiological variations due to water or nutrient availability (Suárez et al., 2008; Suárez, Zarco-Tejada, Berni, González-Dugo, & Fereres, 2009). Among the proposed physiological indices, the Photochemical Reflectance Index (PRI) (Gamon, Peñuelas, & Field, 1992) has been suggested as a proxy for photosynthesis, demonstrating its dynamic changes along with photosynthesis in different vegetation canopies, including grapevines (Evain, Flexas, & Moya, 2004), Scots pine (Louis et al., 2005), experimental mangrove canopies (Nichol, Rascher, Matsubara, & Osmond, 2006) and coastal shrubs (Naumann, Young, & Anderson, 2008). Before these reflectance-based hyperspectral indices and fluorescence emission quantities can be readily applied on larger scales, a better understanding of fluorescence emission and the interpretation of physiological changes at different stress levels and spatial resolutions is necessary (Zarco-Tejada et al., 2013b); this is particularly important in the context of precision agriculture and decision-making to improve fruit quality and yields, and to reduce water and fertilizer use. In this context of water-stress detection and precision irrigation, seasonal monitoring of fluorescence from airborne imagery has demonstrated a link with assimilation rates in crops with little structural changes over the growing season (Zarco-Tejada, González Dugo, & Berni, 2012, 2013c), and for disease detection at pre-visual stages, using airborne-retrieved fluorescence data derived from high-resolution hyperspectral imagery (Calderon et al., 2013, 2015).

On the modelling side, important progress has been made since the first attempts as part of FluorMOD (Miller et al., 2004) with recent developments and updates on the integrated leaf-canopy fluorescence-temperature-photosynthesis model (SCOPE) (Van der Tol et al., 2009a, 2009b), used to assess the key variables that drive (SIF) from vegetation canopies (Verrelst et al., 2015) in the context of the FLuorescence EXplorer FLEX mission supported by the European Space Agency (ESA) Earth Explorer program. However, the large number of inputs required makes its direct application difficult outside of experimental studies, which thus imposes important limitations on its use for operational purposes in commercial orchards and precision agriculture applications. Regarding the imaging sensors available for fluorescence quantification, current state-of-the-art methodologies for fluorescence retrieval are based on sub-nanometer spectral resolution of hyperspectral data at both leaf and canopy levels. Nevertheless, actual access to very high spectral resolution imagery is still quite limited (e.g. HyPlant, an airborne sensor designed to cover the 670 to 780 nm region with 0.25 nm FWHM bands and 0.11 nm sampling interval), as these sensors are only available for scientific purposes and have demonstrated successful retrievals of fluorescence linked to photosynthetic efficiency (Rossini et al., 2015). Despite such *standard* sensor specifications and narrow-band requirements, recent studies have demonstrated that hyperspectral imagery acquired at 5–6 nm FWHM and long sampling intervals is capable of retrieving fluorescence with relatively high success. This has been demonstrated through modelling (Damm et al., 2011) and in experimental studies which displayed relationships with field-measured assimilation and fluorescence levels (Zarco-Tejada, Catalina, González, & Martín, 2013d), carbon data measured from a flux tower (Zarco-Tejada et al., 2013c), and ecosystem-specific fluorescence retrievals linked to gross primary production using the Airborne Prism EXperiment (APEX) characterized by a spectral sampling interval of 4.5 nm and a FWHM of 5.7 nm (Damm et al., 2015). The theoretical and experimental results obtained using broader bandwidths (i.e. FWHM > 1 nm) are of paramount importance for the operational and widespread application of chlorophyll fluorescence retrievals and hyperspectral indices for monitoring physiological status using commercially available sensors in the context of precision agriculture, plant phenotyping, precision forestry and disease detection. For some of these purposes, the actual retrieval of the fluorescence quantification in absolute terms is not absolutely critical, while the spatial variability (i.e. relative differences among stress levels, treatments and disease affection) is the important factor for precision agriculture and stress-detection purposes in an operational context (see Zarco-Tejada et al., 2012 for a demonstration of fluorescence retrievals for stress-detection purposes using a micro-hyperspectral imager carried on board an unmanned aerial vehicle). Nevertheless, the impact of fluorescence retrieval errors when using broader spectral bands (i.e. FWHM > 1 nm) is unknown in the context of seasonal studies performed for agronomic and stress detection purposes, and for the assessment of the temporal relationships between photosynthesis rates and fluorescence quantification measured under different irradiance levels and atmospheric conditions.

In this study we assess the stability of the fluorescence quantification over a two-year period of hyperspectral flights as compared to field-measured leaf assimilation in an evergreen crop canopy (orange) grown under different water stress levels. We evaluate the robustness of the fluorescence quantification and physiological and structural indices in order to understand their ability to track photosynthesis at different phenological and stress stages throughout the season, and suggest taking a fluorescence normalization approach to its operational use in precision agriculture.

2. Materials and methods

2.1. Field experiments and airborne campaigns

2.1.1. Study site and field data collection

The experiment was carried out in a commercial orange (*Citrus sinensis* L. cv. Powell) grove near La Campana, Seville (Spain (37.8°N, 5.4°W) in 2012 and 2013. The orchard was planted in 1997 in a 7 × 4 m grid on a deep alluvial loamy to sandy loam soil. For further information regarding the experimental set-up and local climate characteristics, see Zarco-Tejada et al. (2012). Three irrigation treatments were applied: a control treatment (ET), that received 100% of the estimated crop evapotranspiration and was never short of water, and two regulated deficit irrigation (RDI) treatments, in which water application was reduced to 37% (RDI1) and 50% (RDI2) during a deficit irrigation period in the summer (dates are shown in Table 1). Concurrently with the airborne flights over the study site at different crop development stages, a series of leaf measurements were performed (Table 1). Assimilation rate (A; $\mu mol/m^2/s$) and stomatal conductance (Gs, $mmol/m^2/s$) were measured by means of a photosynthesis measurement system (LCDpro-SD: ADC Bioscientific Ltd., Herts, U.K.) on two leaves per tree