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Leaf level detection of solar induced chlorophyll fluorescence by means of a subnanometer resolution spectroradiometer

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Abstract

A leaf level investigation on the spectral signature of *Phaseolus vulgaris* was undertaken by using a very high spectral resolution spectroradiometer featuring full width at half maximum of 0.06 nm and spectral range of 635.5–802.5 nm. High spectral resolution allows detection of leaf reflected and emitted radiance fields in two narrow absorption bands at 687 and 760 nm, respectively, where solar irradiance is strongly reduced owing to molecular oxygen absorption of the terrestrial atmosphere. The flux emitted due to chlorophyll fluorescence was measured using the Fraunhofer line depth principle by spectrally modelling the signal, capitalizing on the high resolution of the spectroradiometer devices. An experiment was conducted on two potted bean plants. One was maintained in good health for use as a reference while the other was treated with a photosystem II inhibitor. Collected spectra show that the fluorescence emission produces a pair of characteristic peaks superimposed on the typical leaf-specific reflectance curve. The magnitude of the fluorescence signal of the herbicide-treated leaf was four times greater than that of the control plant, thus indicating damage to the photosynthetic apparatus of the plant.

Keywords: Subnanometer spectral signature; Fluorescence emission; Apparent reflectance; Fraunhofer line depth principle; Plant physiology; High spectral resolution spectroradiometer

1. Introduction

Investigation of leaf optical properties for plant status detection has intensified greatly since the 1960s, along with the development of spectrometry and remote sensing instrumentation. Leaf reflectance and transmittance have been studied more extensively than absorptance largely as a result of possible applications in remote sensing. Mainly due to technological problems, the investigation of solar-induced fluorescence emission in the visible and near infrared domain has received much less attention and only recently has it become an attractive measurement.

The leaf bidirectional reflectance factor (BRF) spectrum represents the plot of the fraction of radiation scattered in the direction of observation. For each incident wavelength, leaf scattering is produced at the leaf surface and interior levels, thus producing a "signature" that results from the contribution of many components: structure, chlorophyll and other pigments, water, proteins, starches, waxes, and structural biochemical molecules, such as lignin and cellulose (e.g., Walter-Shea & Norman, 1991).

During field campaigns, the hemispherical-directional reflectance factor (HDRF) is typically estimated by measuring total solar radiance incident on the target (L^i) and dividing it by the reflected radiance upwelling from the target (L^s) in a given direction of observation.

Indeed, the measured upwelling radiance L^s is the sum of two different fluxes originated by the reflection of incident light and the surface emission (F), respectively. The latter flux is due to fluorescence, a process by which the light energy absorbed at one wavelength is re-emitted at a different wavelength because excited electrons return to the ground state. Reflection and fluorescence act

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with different intensities in the visible and the near-infrared portions of the electromagnetic spectrum. Both processes occur simultaneously and are wavelength dependent, thus making the separation of the upwelling radiance into reflection and fluorescence problematic.

Among the other fluorophors contained in leaf tissues, chlorophyll *a* of the pigment–protein complexes exhibits a fluorescence emission spectrum in the red and near-infrared regions, characterized by two peaks at 690 nm and 740 nm (for an exhaustive review on fluorescence, see Papageorgiou & Govindjee, 2004). Chlorophyll *a* fluorescence induction kinetic studies, based on active measurements with artificial light sources, have shown that absorbed light energy is spent in three competing processes: fluorescence, light reactions of photosynthesis (photochemical quenching of the fluorescence signal, PQ), and heat dissipation (non-photochemical quenching, NPQ). Since these processes occur in competition, variation in the efficiency of one process affects the efficiencies of the others. This link justifies the use of the fluorescence signal to infer the vitality of the photosynthetic apparatus and hence to monitor plant stress. In fact, it has been demonstrated that the fluorescence signal is highly specific to vegetation functioning, stress, and vitality, and thus its retrieval in space and time potentially provides additional information useful in assessing the terrestrial carbon cycle at different scales (e.g., Field et al., 1994; Lichtentaler et al., 1998; Schreiber & Bilger, 1987).

Solar-induced chlorophyll fluorescence, or steady state fluorescence under natural illumination, is measured passively when no artificial source of excitation is used. At leaf and canopy level, this is indeed a very complex and poorly understood signal. At leaf level, the passive measurement of fluorescence flux F can be described as a functional relationship among a number of parameters and processes, as expressed by the simplified scheme of the following equation:

$$F = f(\alpha{*}\mathrm{PPFD}, \lambda, T, \mathrm{PQ}, \mathrm{NPQ}) \quad (1)$$

where $\alpha{*}\mathrm{PPFD}$ stands for spectral convolution of the leaf absorption coefficient and incident photon flux density in the photosynthetically active domain (both are wavelength dependent), λ for wavelength (which characterises emission and reabsorption), and T for leaf temperature.

Recent works have clarified the relationship between fluorescence and photosynthesis and the role played by the different terms in Eq. (1). For example, Rosema et al. (1998) proposed a framework model to interpret the F signal (laser-induced) considering photosystem deactivation. Flexas et al. (2002) recognized the role of NPQ by reporting that the diurnal time course of F tracks that of irradiance in well-watered plants, whereas water stress induces a diurnal pattern that mirrors the course of irradiance. Finally, Evain et al. (2004) demonstrated the feasibility of the remote sensing of NPQ by showing that it was highly correlated with the Physiological Reflectance Index (PRI) originally proposed by Gamon et al. (1992).

The F signal can be detected passively in very narrow dark lines of the spectrum in which solar incident irradiance is strongly reduced (Fraunhofer lines). For example, at 760 nm terrestrial atmospheric molecular oxygen strongly absorbs incoming solar light and the resulting incident radiation at ground level is significantly reduced with respect to the surrounding *continuum* (up to more than 90% of attenuation). In such dark lines, fluorescence can be detected by measuring to what extent the "wells" are filled by fluorescence relative to the *continuum* (Elachi, 1987). This technique forms the basis of the Fraunhofer line depth (FLD) principle. Interest in its application has been increasing due to the effort of several international agencies, research groups, and networks (e.g., Carter et al., 1996; McFarlane et al., 1980; Meroni et al., 2004; Moya et al., 2004; Plascyk, 1975; Sioris et al., 2003; Smorenburg et al., 2002; Stoll et al., 1999).

Ground level and aircraft level passive fluorescence measurements and their potential use in plant photochemistry have been investigated by using advanced instruments that collect the signal in few selected wavelengths, using interference filters and a photodiode detector (e.g., Moya et al., 2004; Plascyk, 1975), or dedicated systems based on an induced fluorescence approach (Kebabian et al., 1999). In this way, fluorescence has been detected successfully but without capturing a continuous signature and generally providing its quantification only in relative units.

Traditional portable spectroradiometers are used to estimate HDRF in the visible-near infrared range, and they do not provide a suitable spectral resolution for the detection of the F signal since the exploitable Fraunhofer lines are typically 0.5 to 2 nm in width. In fact, with conventional spectral resolution (2 to 5 nm for most commercial spectrometers), the aforementioned "in-filling effects" are averaged out (smoothed), so that the resulting features are largely attenuated and thus difficult to detect. For such reasons, fluorescence under natural illumination and its effect on reflectance have not been fully investigated in the past. Nevertheless, some studies have been successful: for example Buschmann et al. (1994) studied the fluorescence induction kinetic and recognised its effect on leaf reflectance using a prototype spectrometer and, only recently, some studies have shown a decoupling of spectral reflected radiance and the fluorescence signal (Miller et al., 2003; Zarco-Tejada et al., 2000).

In this context, the aim of this study was to determine a continuous spectral signature of leaves at subnanometer resolution to identify new spectral vegetation characteristics. Specifically, our objectives were twofold: (i) to detect and quantify chlorophyll fluorescence emission by exploiting two Fraunhofer lines centred at 687 and 760 nm, and (ii) to investigate the effect of this emission on the apparent reflectance spectrum in the framework of a stress-induction experiment.

2. System description

A typical fluorescence emission spectrum of a green leaf excited by UV radiation shows several emission *maxima* in the visible/near-infrared region (Fig. 1). Two Fraunhofer lines (dashed vertical lines in Fig. 1) are positioned in proximity to the 690 and 740 nm peaks. These lines, due to terrestrial molecular oxygen absorption, can be exploited in order to determine