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Growth tracking of basil by proximal remote sensing of chlorophyll fluorescence in growth chamber and greenhouse environments

Daniel Bånkestad[a,*], Torsten Wik[b]

[a] Heliospectra AB, Göteborg, Sweden
[b] Department of Signals and Systems, Chalmers University of Technology, Göteborg, Sweden

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ABSTRACT

Remote sensing is a promising tool for plant phenotyping and precision farming, as it allows for non-invasive, fast and automated measurements of relevant plant traits with spatial and temporal resolution. The simplest and most used remote sensing application in the field is to use reflectance vegetation indices, based on the optical properties of chlorophyll, as indicators of variables of interest. However, the applicability is limited by their sensitivity to environmental conditions and canopy structure. Another remotely sensed signal related to chlorophyll is chlorophyll fluorescence. Compared to reflectance it is plant specific and directly linked to plant physiological processes; but it is also weak, which complicates its use for in-field applications. This study evaluates the performance of an active proximal remote sensing system utilizing the chlorophyll fluorescence ratio method, measuring the ratio of red fluorescence to far-red fluorescence (termed SFR), for the assessment of growth and biomass as an alternative or complement to reflectance vegetation indices.

Basil plants were subject to chlorophyll fluorescence and weight measurements periodically throughout commercial growth cycles, both in a laboratory and commercial greenhouse environment. In the laboratory, SFR showed a strong linear relationship with dry weight on logarithmic scales. Further characterization of the method indicated that it is independent of background light and the same growth dynamics is obtained irrespective of point in time during chlorophyll fluorescence induction. The same trend that was observed in the laboratory was also observed in the greenhouse, but varying background light from the sun and from supplemental lighting added complexity that needs to be addressed in further studies. To our knowledge, the strong link between SFR and biomass, both in a closed environment and greenhouse setting, has not so clearly been demonstrated on canopy level before. Owing to the simplicity of the method, being relatively cheap and fast, it has potential for commercial applications.

1. Introduction

More efficient methods for plant phenotyping and precision agriculture are needed to meet future requirements in crop production and environmental sustainability. See Kumar et al. (2015), Fiorani and Schurr (2013), Deery et al. (2014), and Li et al. (2014) for recent introductory reviews on plant phenotyping and the concept of precision agriculture is explained by e.g. Diacono et al. (2013). Methods using optical remote sensing technology are particularly promising as they allow for non-invasive, fast and automated measurements with both spatial and temporal resolution in the field. They are based on transmittance, reflectance or fluorescence signals from the plants, which contain information about agronomic and physiological traits.

Canopy reflectance is the most accessible and widely studied signal for field applications. It is a function of leaf morphological properties, absorption of light by pigments, and plant architecture, such as varying arrangements of leaves, branches and stems (Winterhalter et al., 2013). The optical properties of chlorophyll, with strong absorption in the blue and red wavebands, are clearly observed in the reflectance spectrum. Since chlorophyll is involved in photosynthesis and other physiological processes, the pigment provides a link between remote sensing observations and plant physiology (Schlemmera et al., 2013). For example, chlorophyll is closely related to rubisco, which is the main sink for nitrogen, and thereby chlorophyll content is a good indicator of nitrogen status (Chappelle et al., 1984). The most common approach to estimate crop parameters from reflectance spectra is by the use of empirically derived reflectance vegetation indices (rVIs). These are simple formulas that consist of a combination of two or more

* Corresponding author.
E-mail address: daniel.bankestad@heliospectra.com (D. Bånkestad).

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reflectance wavebands. Typically two wavebands, one that is correlated and one that is uncorrelated to the parameter of interest, are expressed as a ratio or normalized difference in order to minimize the variability induced by external factors.

rVIs based on chlorophyll have been derived to assess nitrogen (N) status, green biomass, photosynthetic capacity, leaf-area index (LAI), plant stress and other important crop parameters. Many studies have demonstrated the utility of these indices and, as a result, commercial sensor systems for routine monitoring in the field have been developed, such as the Yara N Sensor™ ALS (Yara International ASA, Dülmen, Germany), the GreenSeeker™ RT200 (Trimble, Sunnyvale, USA), and the Crop-Circle ACS-470 Active Crop Canopy Sensor (Holland Scientific, Lincoln, USA). However, the full potential is yet to be explored. On the canopy scale, reflectance indices are affected by both sampling conditions and chlorophyll independent canopy features. Soil interference, illumination conditions (e.g. solar illumination angle and shadowing), sensor position (e.g. sensor view angle) and plant structure (e.g. leaf inclination angle, species-specific canopy architecture and leaf surface features) are some of the factors that influence the reflectance spectrum (Royo and Villegas, 2011)). They have mostly been neglected, leading to substantial imprecisions in the estimates and to the need of site-specific calibration (Jones, 2014). This has clearly limited the use of rVIs in plant phenotyping and the technical challenges need to be addressed. Active sensors, that are not dependent on the sun but possess their own light-emitting units, are less sensitive to varying illumination conditions and thereby improve robustness and timeliness of operation. The commercial instruments listed above are all based on active sensing technology. Major improvements can also be made by correcting for canopy structure and illumination conditions using canopy reflectance models based on radiative transfer theory (Feret et al., 2011; Knyazikhin et al., 2012), but more research is required.

An important limitation of rVIs based on chlorophyll is reflectance saturation. At high crop densities the reflectance reaches an asymptote making the rVI insensitive to changes in chlorophyll content. By employing spectral bands outside the major absorption bands, it is possible to retain sensitivity longer. For example, indices based on the red-edge- instead of the red region have shown better performance in this aspect (Nguy-Robertson et al., 2012). The performance can also be improved by using more than just a few wavebands. Various techniques have been tested for the selection of critical wavebands, including 2D correlation plots (Darvishzadeh et al., 2008), partial least square regression (Serbin et al., 2012) and principal component analysis (Dreccer et al., 2014).

The chlorophyll content of plant canopies can also be assessed remotely by measuring chlorophyll fluorescence (ChlF). ChlF is the red- and far-red emission by Chl *a* upon absorption of light. It is a de-excitation pathway, competing with photochemical conversion and heat dissipation, in the photosynthetic apparatus. The ratio between red and far-red fluorescence (RF/FRF), termed SFR for Simple Fluorescence Ratio in this study and elsewhere (Tremblay et al., 2012), is sensitive to chlorophyll content because red- but not far-red fluorescence is readily reabsorbed. As such, the ratio decreases with increasing chlorophyll content (Buschmann, 2007; Gitelson et al., 1998; Lichtenthaler and Rinderle, 1988).

The SFR parameter is offered by fluorometers using the so-called laser-induced two-wavelength ChlF technique (Tremblay et al., 2012). Most of them are hand-held devices, measuring single leaves at close distances, which cannot be used on-the-go. One exception is the tractor-mounted Planto N-Sensor (Planto GmbH, Leipzig, Germany), which can handle a measurement distance of 3–4 m between the sensor and the canopy. It measures ChlF, induced by red (630 nm) laser light, in the red (690 nm) and far-red (730 nm) wavebands. The instrument has been evaluated in a number of field studies: the ratio of the two wavebands showed strong correlation with aboveground N content of oilseed rape, with an accuracy comparable to reflectance-based methods (Thoren and Schmidhalter, 2009); and a strong relationship was also observed between SFR and both total aerial N and aerial dry mass in wheat, but the coefficient of determination was slightly lower when compared to some reflectance indices (Mistele and Schmidhalter, 2010). Other than that, there are only a few studies looking on SFR on canopy level using active sensing (Ač et al., 2015). Most of them investigate how it correlates with stress factors such as drought (Dahn et al., 1992; Valentini et al., 1994), nitrogen deficiency (Kuckenberg et al., 2009), pathogen infections (Kuckenberg et al., 2009), and temperature (Thoren et al., 2010).

A rather new commercially available fluorometer that potentially can be used for screening large number of plants in the field is MULTIPLEX RESEARCH™ (Force-A, Orsay, France; Tremblay et al., 2012). In addition to chlorophyll indices, it measures indices related to polyphenol content using the ChlF screening method. The method compares ChlF induced by two different excitation lights, one reference light that is not screened and one sampling light that is screened by the polyphenol of interest. For example, a smaller fraction of UV light compared to red light reaches the chlorophyll in the mesophyll because it is absorbed by flavonols in the epidermis. LEDs of four different colors are used as excitation lights: UV (375 nm), blue (450 nm), green (530 nm) and red (630 nm). Flavonol and chlorophyll have opposite N-dependence, suggesting that a combined index is a better indicator of N status than chlorophyll alone. The combined index (NBI, nitrogen balance index; Cartelat et al., 2005) proved to be better than the chlorophyll index in discriminating N levels applied to turfgrasses, and it was superior to reflectance-based indices (Agati et al., 2015; Agati et al., 2013). Padilla et al. (2016) demonstrated the applicability on vegetable crops by showing that the fluorescence indices for chlorphyll (SFR), flavonols (FLAV) and nitrogen (NBI) are able to distinguish deficient from optimal crop N status in cucumber. The Multiplex sensor was developed as a hand-held device measuring a small surface (8 cm in diameter) at 10 cm distance from the light source. A tractor-mounted version, which is able to work at 20–30 cm distance, has more recently been developed for on-the-go applications. It has been shown to be capable of assessing chlorophyll, nitrogen and anthocyanin levels on grapevines and grape berries in the field (Diago et al., 2016; Bramley et al., 2011).

ChlF has advantages over reflectance for remote sensing. The signal is plant specific and therefore less sensitive to soil interference. It is also more directly linked to plant physiological processes. However, the signal is weak and noisy, owing to the small quantum yield of ChlF (typically 0.5–3% and not exceeding 10%; Brody and Rabinowitch, 1957; Latimer et al., 1956; Krause and Weis, 1991), making remote measurements from mobile platforms technically challenging. Similar to reflectance-based methods the signal is also sensitive to saturation at high crop densities (Gitelson et al., 1998).

There are alternative approaches to the ChlF ratio method and the ChlF screening method. Measuring variable ChlF by using PAM (pulse amplitude modulation) technology is widely used in plant science for the assessment of photosynthetic functioning and crop health status (Tremblay et al., 2012). It involves measurements under light saturating conditions, it is sensitive to measurement distance and dark-adaption is required for full analysis. These properties are difficult to accommodate for in the field. A related technique called laser-induced fluorescence transients (LIFT) have been developed to remotely measure photosynthetic properties at a distance of up to 50 m (Kolber et al., 2005), and it was recently tested for the first time in an agriculture setting (Raesch et al., 2014). Passive remote sensing of chlorophyll fluorescence is possible through the infilling of radiation in the Fraunhofer lines in the