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Simple reflectance indices track heat and water stress-induced changes in steady-state chlorophyll fluorescence at the canopy scale

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Abstract

Non-invasive remote sensing techniques for monitoring plant stress and photosynthetic status have received much attention. The majority of published vegetation indices are not sensitive to rapid changes in plant photosynthetic status brought on by common environmental stressors such as diurnal fluxes in irradiance and heat. This is due to the fact that most vegetation indices have no direct link to photosynthetic functioning beyond their sensitivity to canopy structure and pigment concentration changes. In contrast, this study makes progress on a more direct link between passive reflectance measurements and plant physiological status through an understanding of photochemical quenching (qP) and non-photochemical quenching processes. This is accomplished through the characterization of steady-state fluorescence (Fs) and its influence on apparent reflectance in the red-edge spectral region. A series of experiments were conducted under controlled environmental conditions, linking passive reflectance measurements of a grapevine canopy (*Vitis vinifera* L. cv. Cabernet Sauvignon) to leaf level estimates of CO_2 assimilation (A), stomatal conductance (g), qP, and Fs. Plant stress was induced by imposing a diurnal heat stress and recovery event and by withholding water from the plant canopy over the course of the experiment. We outlined evidence for a link between Fs and photosynthetic status, identified the Fs signal in passive remote sensing reflectance data, and related reflectance-derived estimates of Fs to plant photosynthetic status. These results provide evidence that simple reflectance indices calculated in the red-edge spectral region can track temperature and water-induced changes in Fs and, consequently, provide a rapid assessment of plant stress that is directly linked to plant physiological processes.

Keywords: Plant stress monitoring; Chlorophyll fluorescence; Remote sensing; Photochemical quenching; Non-photochemical quenching; Grapevines

1. Introduction

Much effort has been devoted to developing non-invasive remote sensing techniques for monitoring plant stress and photosynthetic status. Most remotely sensed vegetation indices are used for characterizing the amount and spatial distribution of vegetation (Baret & Guyot, 1991; Price, 1992). Vegetation indices have also been used to estimate potential levels of canopy photosynthesis and net primary productivity with mixed success (Choudhury, 2001; Gamon et al., 1995; Verma et al., 1993). Nevertheless, the majority of published vegetation indices are not sensitive to rapid changes in plant photosynthetic status brought on by common environmental stressors such as diurnal fluxes in irradiance and heat. This is due to the fact that most vegetation indices have no direct link to photosynthetic functioning beyond their sensitivity to canopy structure (e.g., leaf angle) and pigment concentrations. Consequently, measurements of canopy reflectance have proven less useful for real-time monitoring of plant photosynthesis and/or water status at the whole plant level (Gamon et al., 1990; Peñuelas et al., 1995).

One exception to this involves remote estimates of the xanthophyll cycle captured by the photochemical reflec-

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tance index (PRI) (Gamon et al., 1990, 1992, 1995, 1997). The PRI has been linked to the status of the epoxidation state of xanthophyll pigments, one of the components of the non-photochemical de-excitation pathway (Demmig-Adams & Adams, 1992). The PRI is intended for estimating changes in xanthophyll cycle pigments as they vary due to changes in photosynthetic light use efficiency (Gamon et al., 1992, 1997; Peñuelas et al., 1995, 1997). The PRI is one of the few spectral indices which has been shown to be sensitive to rapid changes in plant photosynthetic status (Gamon et al., 1990, 1992, 1997; Peñuelas et al., 1997).

A similar avenue of research gaining both past and recent attention is the study of chlorophyll fluorescence (CF) as a means for the remote estimation of plant physiological status. CF is a protective process by which plants dissipate energy that is in excess of photosynthetic demands. CF is emitted primarily from chlorophyll *a* of the antennae system of photosystem 2 (PS II). Any physiological process that influences the function of photosystem II and other photosystem de-excitation pathways will have an effect on CF. There are two main controls on the relaxation pathways: (i) the redox state of plastoquinone, the primary stable electron acceptor of PSII, which determines the level of quenching by photochemistry (qP); and (ii) the changes in non-photochemical quenching processes, which are light-induced protective processes that result in the de-excitation of the chlorophyll singlet to the ground state with the production of heat (Johnson et al., 1994; Müller et al., 2001; Pospisil, 1997). CF, qP, and non-photochemical quenching all have rate constants associated with them and any process that increases the rate constants of the other de-excitation pathways will decrease CF intensity (Bjorkman & Demmig-Adams, 1994; Demmig-Adams & Adams, 1992).

CF intensity can vary over time as a function of the photosynthetic activity of the plant tissue being measured. This relationship was initially described by Kautzky (Kautzky & Hirsch, 1931) and has garnered much attention from plant physiologists who have used leaf level CF measurements as a non-invasive plant monitoring tool for many years. More specifically, leaf level CF measurements utilizing a class of instruments known as pulse amplitude modulating fluorometers have been used with varying degrees of success to estimate plant stress, quantum yield, PS II efficiency, and electron transport rates. CF measurement, interpretation, and relation to photosynthesis and plant physiological status have been the subject of several detailed reviews (Larcher, 1994; Lazar, 1999; Lichtenthaler, 1992).

In addition to leaf level measurements, CF can be measured for entire leaves and plants. Fluorescence imaging, as it is commonly referred to, is conducted using laser or flash lamps to induce CF. Concurrently, spectral or imaging sensors are used to measure the CF signal for entire leaves, groups of leaves, or entire plants depending on the sensor type and configuration. These types of measurement systems have been shown to be effective for non-destructive monitoring of plant stress and functioning at near distances and far distances (for reviews, see Buschmann et al., 2000; Lang et al., 1996). Unfortunately, the application of these techniques to larger spatial scales is limited primarily by the small spatial extent of the laser induction pulse as well as incomplete coverage in the spatial domain.

An area of active CF research is exploring the link between steady-state fluorescence (Fs) and plant photosynthetic status. Fs is the fluorescence emitted under constant illumination without saturating flashes. Flexas et al. (1999, 2000, 2002a) showed that Fs exhibits a strong positive correlation with diurnal variations in H_2O stomatal conductance (g), and to a lesser extent, CO_2 assimilation (A) influenced by variable irradiance conditions and water stress. Their findings are promising in that Fs can be monitored directly without the use of laser induction pulses or saturation flashes. This provides an avenue for long-term plant stress monitoring using passive remote sensing strategies. Concurrent to these findings, a small but growing body of research has found support for the potential of identifying the Fs signal in reflectance data from passive platforms at near and far distances. This is further expanded below.

1.1. CF emission spectra

Understanding the spectral characteristics of Fs is critical if remote estimates of its properties are attempted. The emission spectra of chlorophyll fluorescence is characterized by two bands spanning the range between 600 nm and 800 nm but with maxima at 690 nm (F_{690}) and 740 nm (F_{740}) (Buschmann et al., 2000). The intensity, shape, and position of these emission bands are affected by a number of factors. Gitelson et al. (1998) showed that a significant portion of the shape of the CF emission spectra of PS II can be explained by re-absorption processes due to chlorophyll pigments. The re-absorption of fluorescence emission is greatest at F_{690} because this emission peak overlaps the in vivo chlorophyll *a*, and to a lesser extent, chlorophyll *b* absorption maxima located in this region of the spectrum. This suggests that the F_{690} intensity is sensitive to chlorophyll concentration of the leaf tissue as was demonstrated by a number of investigators (Gitelson et al., 1998, 1999). In contrast, F_{740} is minimally affected by chlorophyll concentration, and thus, the ratio F_{690}/F_{740} has been shown to be inversely related to chlorophyll content (Gitelson et al., 1998; Lichtenthaler et al., 1998).

1.2. Fs and remote sensing

The remote detection of Fs in vegetation using passive remote sensing techniques is in a nascent state. Early on, investigators showed that Fs was related to water stress and photosynthetic function using a Fraunhofer line discrim-

inator (Carter et al., 1990, 1996; McFarlane et al., 1980). Buschmann and Lichtenhaler (1988) found evidence for the superposition of a Fs signal on leaf apparent reflectance signatures. Subsequently, Zarco-Tejada et al. (2000a,2000b) demonstrated that the Fs signal could be detected in apparent reflectance data at both the leaf and canopy level using time-decay experiments and induction with long-pass filters in the laboratory. Furthermore, Zarco-Tejada et al. (2003a) showed that the Fs signal could be identified at the canopy scale using spectral subtraction and derivative reflectance-based optical indices under controlled environment conditions. More recently, studies have demonstrated evidence for Fs in-filling of the 760-nm atmospheric oxygen absorption band detectable in the radiance spectra of field instruments (Evain et al., 2004; Moya et al., 2004).

Despite these initial results, little work has been conducted that demonstrates a strong link between reflectance based Fs measurements at the canopy level and plant physiological status. This study explores the link between canopy-level reflectance-derived Fs measurements, and plant physiological status. The goals are (1) outline evidence for a link between Fs and photosynthetic status; (2) identify the Fs signal in passive remote sensing reflectance data; and (3) relate reflectance-derived estimates of Fs to plant photosynthetic status. These steps are critical if Fs measurements are to be used strategically for plant monitoring and if these techniques are scalable to larger stand and landscape levels.

2. Materials and methods

2.1. Plant materials and controlled environment

The experiment was conducted on 3-year-old grapevines of *Vitis vinifera* L. cv. Cabernet Sauvignon potted in 1-L containers. Nine plants were used in the experiment to create a vegetation canopy approximately 1 m × 1 m in size (herein referred to as the 'canopy'). The plants were grown under favorable conditions in a potting mixture containing 40% peat, 30% sand, and 30% vermiculite, at an outdoor facility under 30% shade cloth prior to the experiment. On the morning the experiment began, the plants were moved into a controlled environment facility and placed in a growth chamber (Conviron PGV36,Controlled Environments Ltd., Winnipeg, MN, Canada) in which temperature and moisture stress could be induced in the canopy under constant light conditions. The growth chamber area was 3.3 m^2, with dimensions of 2.4 m × 2.2 m × 2.0 m height. Photosynthetically active radiation (PAR) was measured in the CEF with a quantum sensor (LI-190SA, Li-Cor, Inc., Lincoln, NE, USA), yielding 700 μmol photons m^{-2} s^{-1} at the canopy surface. A balanced spectrum was provided using three types of light sources in the chamber: metal halide, high-pressure sodium, and incandescent lamps.

2.2. Experiment

Several trials were conducted to test for instrument capabilities and spectral calibration and measurement protocol design. The actual experiment was conducted in October 2002 over a 4-day period. Just prior to being placed in the growth chamber, the canopy was fully watered to field capacity. Subsequently, seven fully expanded leaves from separate plants in the upper portion of the canopy were selected and labeled. A light response curve was conducted on each leaf in order to pick a representative leaf for gas-exchange and fluorescence measurements over the course of the experiment.

The canopy was placed in the fully illuminated growth chamber. The light treatment was maintained from 1030 to 1930 h each day over the course of the 4-day experiment. Photosynthetic response was varied in the growth chamber over an 8-h period by ramping the temperature from 23 °C at the start of each day (1100 h) to 43 °C in the middle of the day (1530 h), followed by a recovery to initial conditions of 23 °C at the end of the day (1830 h). This diurnal temperature profile was implemented over a 3-day course. On day 4, the temperature was ramped up to 30 °C at 1330 h and held constant for the remainder of the light period in order to prevent heat stress from being induced. Additionally, water stress was induced over the course of the experiment by withholding water from the canopy for the first 3 days. The canopy was fully watered to field capacity at 1530 h on day 3 and was watered at again the beginning of day 4 in order to ensure that the canopy did not experience a water deficit.

2.3. Gas exchange and fluorescence measurements

Simultaneous measurements of gas-exchange and Fs were made over the 4-day experiment using an infrared gas analyzer (LI-Cor 6400 IRGA with an integrated 6400-40 leaf chamber fluorometer, Li-Cor, Inc., Lincoln, NE, USA). Over the course of the experiment, gas-exchange measurements including CO_2 assimilation rate (A), stomatal conductance (g), and related parameters were made every 10 min. A suite of CF measurements including Fs, ΔF/Fm', and qP were made every 20 min on the leaf exhibiting an average assimilation response as determined from the light response curve results. The leaf chamber was configured to track the temperature, humidity, and illumination conditions of the growth chamber. CO_2 levels were held fixed at 430 ppm within the leaf chamber corresponding to the average concentration in the growth chamber. Fluorescence parameters were set following recommended values published in the LICOR 6400 manual (LICOR Biosciences, Inc., Lincoln, NE) for light-adapted leaves. Saturation pulses of approximately 8000 μmol photons m^{-2} s^{-1} with a 0.8-s duration were applied in order to saturate the PS II reaction centers for estimating Fm'. Additionally, the 'dark pulse' routine was performed in order to estimate Fo'. This is

accomplished by preferentially exciting PSI with far-red light, causing electrons to drain from PSII (see Licor manual for further details). Given Fo', Fm', and Fs, the fraction of absorbed PS II photons used in photochemistry (ΔF/Fm') as well as photochemical quenching (qP) were estimated using the built in LICOR fluorometer functions following published equations (van Kooten & Snel, 1990).

2.4. Leaf water potential and chlorophyll measurements

Pre-dawn leaf water potential measurements were conducted just prior to the beginning of the experiment, at the height of water stress (day 3 prior to watering), and at the end of the experiment using a pressure bomb. Five fully expanded leaves were collected from the canopy outside of the field of view of the spectrometer (see Spectral measurements below) at each sampling period in order to quantify the effect of the water stress. Additionally, chlorophyll samples were taken at the beginning and end of the experiment in order to track any potential degradation of chlorophyll over the course of the experiment. The seven leaves labeled for the light response curves were used to excise two, 2.3-cm-diameter circles per leaf, which were subsequently used for extraction of chlorophyll using 5 ml of *N*,*N*-dimethylformamide (Spectral-analysis grade, Fisher). 3 ml of supernatant were centrifuged at 3000×*g*, placed in a cuvette and the absorbance was measured at 663.8 nm, 646.8 nm, and 480 nm with a spectrophotometer diode array (Hewlett Packard 8452A). Chlorophyll *a* and chlorophyll *b* concentrations were calculated using the extinction coefficients derived by Wellburn (1994).

2.5. Spectral measurements

The growth chamber was equipped with a spectrometer (ASD FieldSpec Pro, Analytical Spectral Devices, Inc., Boulder, CO, USA) measuring the 350–2500-nm spectral region. The instrument has a spectral resolution of 3 nm at 700 nm and a sampling interval of 1.4 nm between 350 and 1050 nm. A 200-μm-diameter single-mode fiber optic was used for data collection with an angular field of view of 25°. The fiber optic was placed 0.72 m from the vegetation canopy, resulting in a circular field of view (FOV) of 0.32 m in diameter at the top of the vegetation canopy. The porometer leaf chamber was located outside the FOV of the fiber optic.

Spectral measurements were conducted every half hour over the course of the experiment without disturbing the vine canopy. A 0.7×0.7-m $BaSO_4$ calibration panel was used for calculation of reflectance with every spectral measurement. Each vegetation and panel radiance measurement was the average of 50 scans. Additionally, a dark current correction was collected with every spectral measurement in order to account for instrument noise. This protocol ensured accurate reflectance measurements, independent of the temperature change occurring inside the growth chamber. A Savitzky–Golay third-order polynomial least-square function of 20 nm was used to smooth the signal between 350 nm and 1050 nm (Savitzky & Golay, 1964). Further details about the spectrometer configuration can be found in (Zarco-Tejada et al., 2003a).

2.6. Determining the effect of Fs on canopy reflectance

Two methods were used to quantify the fluorescence emission signal superimposed on the canopy reflectance. The first method involves calculating the reflectance difference between times in which fluorescence emissions vary due to the heat and water stress induced in the experiment. This method identifies the reflectance change between two times due to fluorescence (Zarco-Tejada et al., 2000a,2000b). The second method, and the focus of this study, is based on optical indices calculated from wavelengths affected by fluorescence emission (bands in the spectral region between 650 and 770 nm with emission peaks at 690 nm and 740 nm) normalized by wavelengths not affected by CF emission (e.g., 600 nm, 800 nm). Several optical indices of this type have been reported in the literature, such as the curvature index $(R_{675}\cdot R_{690})/R_{683}^2$ and ratio indices such as R_{750}/R_{800}, R_{685}/R_{655}, R_{690}/R_{655} (Zarco-Tejada et al., 2000a,2000b, 2003a).

2.7. Statistical analysis

Reflectance measurements were made at different times than the fluorescence and gas-exchange measurements. Therefore, a smoothing spline was fit to the data to interpolate fluorescence and gas-exchange measurements taken every 20 min and 10 min, respectively, to the 30-min interval of the reflectance measurements. The smoothed values were used to subsequently determine the correlation between these metrics. The smoothing parameter (analogous

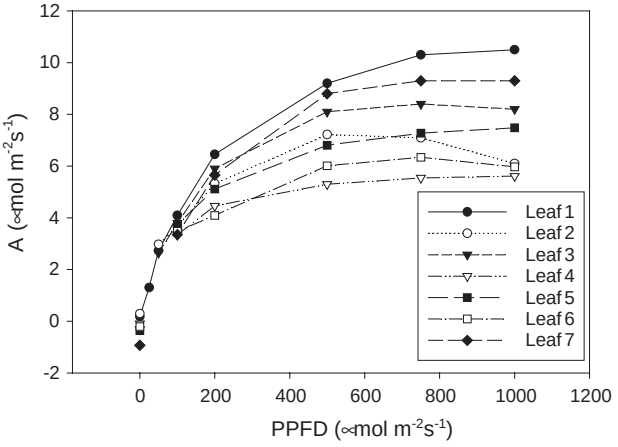

Fig. 1. Light response curves for seven fully expanded leaves from separate plants in the upper portion of the experimental canopy. Leaf 3 was chosen for use in all subsequent fluorescence and gas-exchange measurements.

to the degrees of freedom) was automatically selected using a cross-validation process (for further details, see Chambers & Hastie, 1992) to maximize fidelity to the original data. Consequently, the degrees of freedom for each smoothing operation varied depending on the characteristics of the data. All statistical analyses were done using Splus statistics software (Insightful.corp).

3. Results

3.1. Gas-exchange and fluorescence measurements

Light response curves for seven fully expanded leaves from separate plants in the experimental canopy are shown in Fig. 1. Assimilation rates approached their maxima at the ambient PPFD value of 700 μmol m^{-2} s^{-1} and varied between 5 and 10 μmol CO_2 m^{-2} s^{-1}. Leaf 3 was chosen for all subsequent gas-exchange and fluorescence measurements given that it showed an average assimilation response for the canopy.

3.2. Temperature and water stress effects on photosynthetic functioning are tracked by fluorescence (Fs)

CO_2 assimilation (A), stomatal conductance (g), and steady-state fluorescence (Fs) varied with growth chamber temperature over the course of the multi-day experiment (Fig. 2). Two major patterns in A and g values are apparent. The first is a diurnal pattern brought on by heat stress and recovery. Temperatures followed a diurnal cycle between 23 and 43 °C. A and g values increased with increasing temperature until approximately 30 °C. A and g subsequently decreased as temperatures increased beyond 30 °C resulting in mid-day depressions. This mid-day depression was followed by a general recovery in A as temperatures decreased, whereas g did not show a recovery. The lack of a pronounced mid-day depression in A and g on

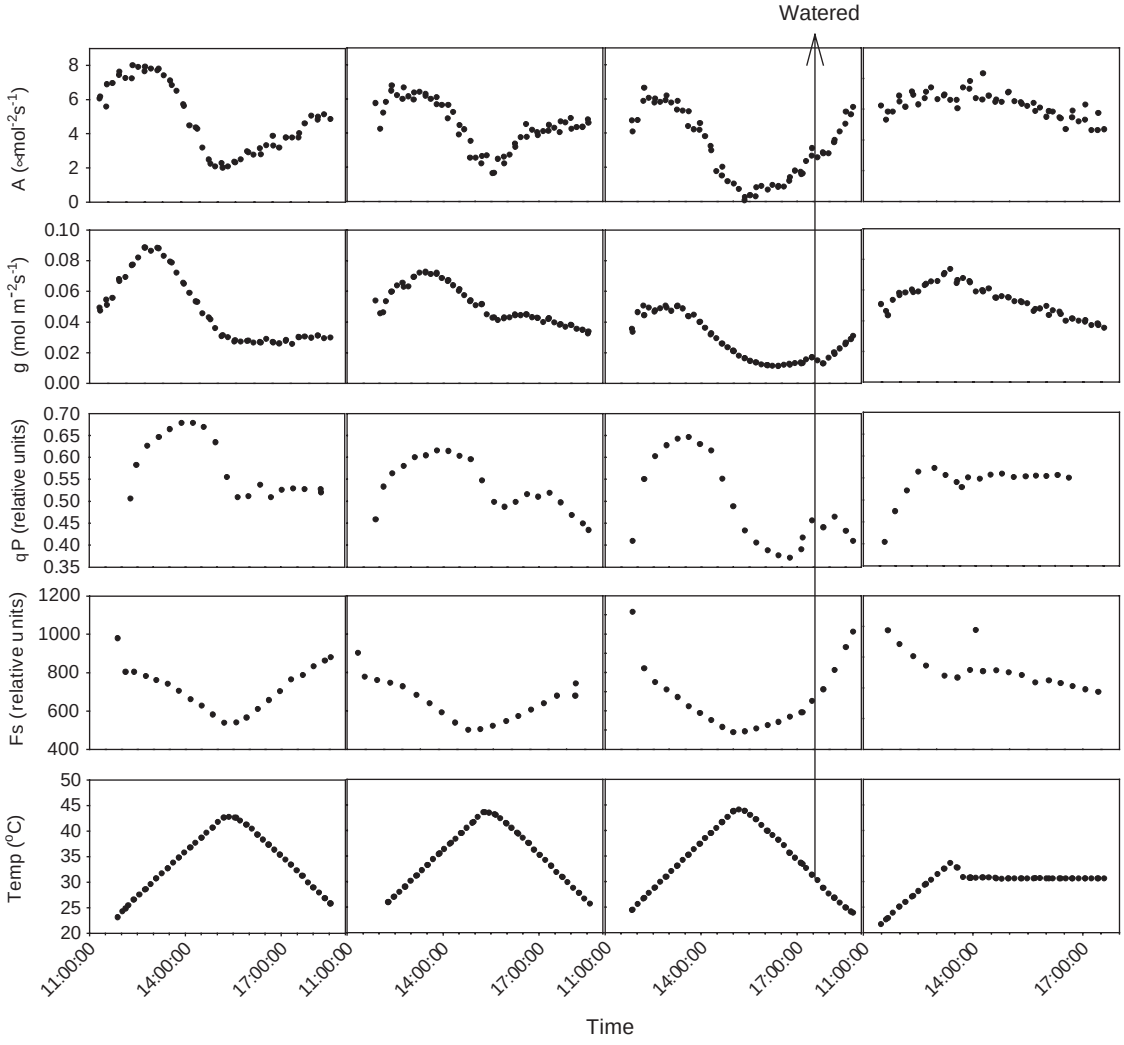

Fig. 2. Summary of gas-exchange and chlorophyll fluorescence measurements taken over the course of the 4-day experiment for leaf 3. All leaf measurements were taken at the ambient growth chamber photosynthetic photon flux density of 700 μmol $m^{-2}s^{-1}$. The canopy was watered prior to the beginning of the experiment as well as on day 3 (noted in plot).

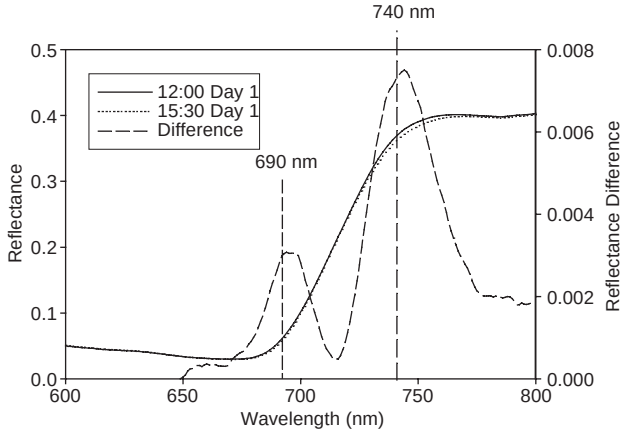

Fig. 3. Difference spectrum between unstressed (1200 h) and stressed (1530 h) canopy reflectance measurements showing the contribution of steady-state chlorophyll fluorescence to apparent reflectance. Note that the emission maxima for chlorophyll fluorescence are centered at 690 and 740 nm and coincide with the maximum reflectance difference values.

day 4 when the temperature was held constant at 30 °C supports the temperature-mediated basis of this diurnal pattern.

The second major pattern is the overall decline in maximum and minimum A and g values over the course of the first 3 days of the experiment followed by a recovery. This behavior was precipitated by withholding water from the canopy for the first 3 days and the subsequent removal of this water stress by day 4. Pressure-bomb measurements taken prior to the experiment averaged 0.21 MPa ($\sigma = 0.04$) increased to 1.73 MPa ($\sigma = 0.24$) during day 3 prior to watering and returned to 0.35 MPa ($\sigma = 0.07$) on day 4 after the water stress was removed by watering. As mentioned earlier, stomatal conductance did not show a pronounced diurnal recovery from the heat stress but instead remained at depressed values during the recovery periods. The recovery period of day 3 after watering was an exception to this. The concomitant sharp increase in A with g following watering suggests stomatal limitations on A brought on by water stress.

Fs showed both a direct and inverse relationship to A and g depending on the intensity of the stress and the status of alternate de-excitation pathways. Fs gradually decreased early in each day of the experiment consistent with an increase in qP, a competing electron pathway. As temperature values move beyond optimal ranges, Fs decreases more rapidly in concert with sharp declines in A, g, and qP, suggesting a shift towards non-photochemical pathways. Concurrent with this diurnal cycle, there is a gradual decrease in the minimum qP and Fs values over the experiment with the onset of extended heat and water stress. ΔF/Fm' values (not shown) followed a similar qualitative pattern to qP with a marked mid-day depression and a gradual decline in minimum values over the course of the experiment. After the canopy was watered on day 3, there was a sharp increase in Fs values that were consistent with an increase in A and g.

3.3. Canopy level reflectance measurements

The steady-state fluorescence signal was identifiable in the canopy level reflectance measurements. A difference spectra between the stressed canopy at 43 °C (1530 h) and the unstressed canopy at 25 °C (1200 h) shows the superposition of the dual band emission spectra of Fs at 690 and 740 nm (Fig. 3). Similar results were demonstrated at the canopy level by Zarco-Tejada et al. (2000a, 2003a) using spectral subtraction. Using the spectral subtraction technique, one can follow the effect of heat stress and recovery on the contribution of Fs to the apparent reflectance. Fig. 4 shows a steady decrease in Fs as the heat stress develops (1200–1500 h) followed by a recovery as temperatures return to more favorable conditions (1500–1800 h). This pattern was consistent with leaf level measurements of Fs (Fig. 2).

3.4. Reflectance indices track diurnal and multi-day patterns in Fs

Although the spectral subtraction technique is conceptually simple, it is limited due to the need for a baseline spectra (e.g., stressed or non-stressed canopy spectra) for its calculation. Consequently, two ratio-based indices were

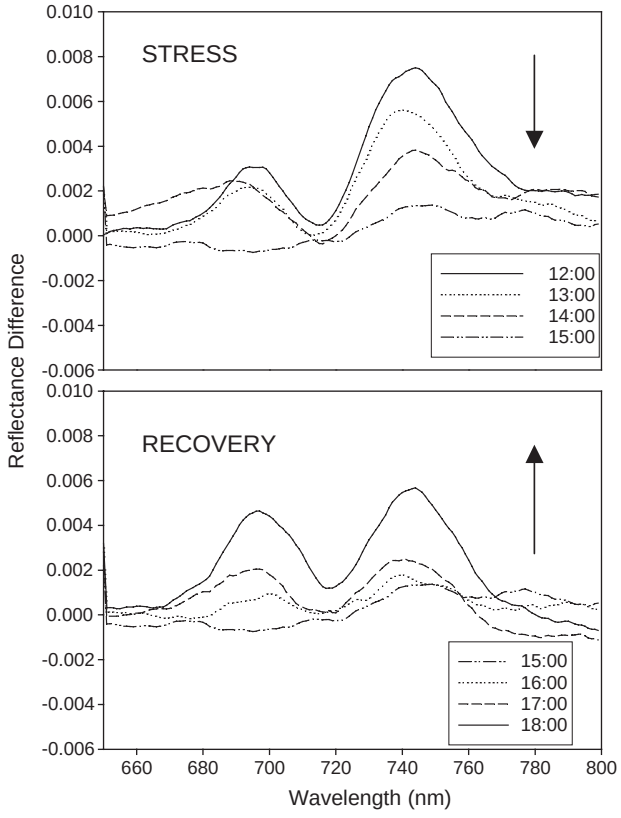

Fig. 4. Difference spectra calculated for day 1 showing a reduction in steady-state chlorophyll fluorescence as heat stress develops mid-day followed by and increase in steady-state chlorophyll fluorescence during the recovery period.

used to quantify the contribution of Fs to the canopy level apparent reflectance (Fig. 5). These include ratio indices that utilize bands located at the CF emission maxima (690 nm and 740 nm) normalized by bands not affected by CF (R_{690}/R_{600} and R_{740}/R_{800}). Both of these fluorescence ratio indices (FRI) qualitatively tracked the leaf level measurements of Fs including the diurnal depression and recovery. The relationship between the interpolated Fs values and the two fluorescence ratio indices is summarized in Fig. 6. Both indices show a strong positive curvilinear relationship with Fs ($r^2=0.75$ for R_{690}/R_{600}, and $r^2=0.8$ for R_{740}/R_{800}).

The photochemical reflectance index (PRI), formulated as $(R_{531}-R_{570})/(R_{531}+R_{570})$, is also included in Fig. 5 for comparative purposes. In our results, the PRI diurnal time course exhibits significant noise which qualitatively masks much of the apparent relation to the Fs measurements. However, the correlation structure is apparent in Fig. 6 with the PRI showing a positive curvilinear relation with Fs ($r^2=0.3$).

The mean chlorophyll *a* concentration taken from seven leaves in the upper canopy on day 1 of the experiment prior to stress induction was 0.2165 g/m^2. At the end of the experiment, the mean chlorophyll *a* concentration was 0.207 g/m^2, not statistically different at $p=0.05$ as determined through a *t*-test. Similarly, a comparison of the 1200 h spectra taken all 4 days of the experiment show a less than 1% difference in red reflectance (670 nm) and less than 3% difference in the NIR (800 nm). Consequently, traditional reflectance indices such as NDVI that track changes in pigment concentration and canopy structure are rather insensitive to the diurnal and multi-day stress event described in this experiment. The NDVI shows no diurnal pattern (Fig. 5) but instead shows a small downward trend over the course of the experiment. This is associated with the 3% drop in the NIR wavelengths over the course of the experiment likely brought on by small changes in leaf angles due to water stress. No relationship between NDVI and interpolated Fs (Fig. 6) was found.

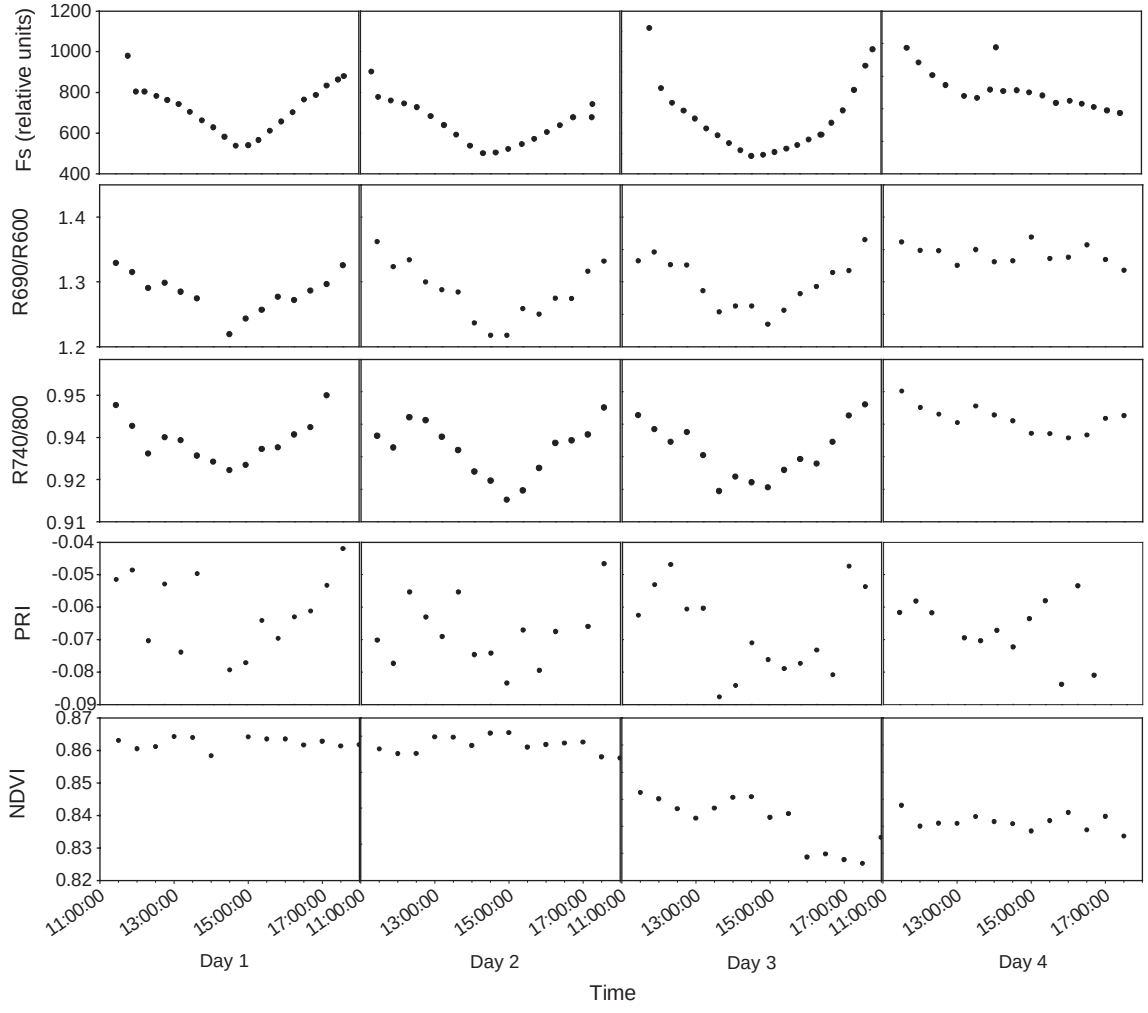

Fig. 5. Comparison of leaf level steady-state chlorophyll fluorescence measurements made with a fluorometer and canopy level reflectance indices. R690/R600 and R740/R800 are reflectance indices calculated using bands located at the chlorophyll fluorescence emission maxima normalized by bands not affected by chlorophyll fluorescence emission (600 and 800 nm). The photochemical reflectance index (PRI) (Gamon et al., 1992) and the normalized difference vegetation index (NDVI) are also included for comparative purposes.

The FRI indices showed a positive linear relation to interpolated A as summarized in Fig. 7. Given the direct and inverse relationship between Fs and A shown in Fig. 2, we expected the relationship between the FRI indices and interpolated A to be convoluted. Despite this, these simple reflectance indices accounted for a noteworthy amount of variance in the assimilation response (r^2 = 0.36, 0.42).

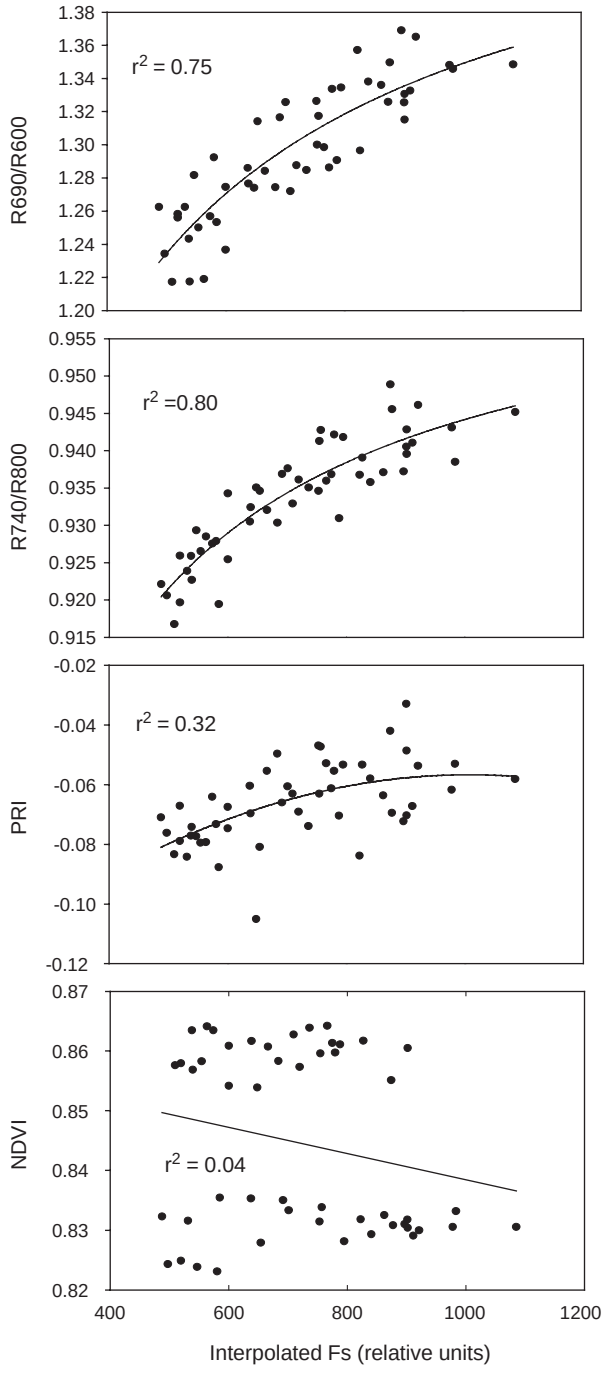

Fig. 6. Scatter plot showing the relationship between the canopy level fluorescence ratio indices (FRI), photochemical reflectance index (PRI), and normalized difference vegetation index (NDVI) with leaf level interpolated estimates of steady-state chlorophyll fluorescence (relative units). Plot includes measurements taken every half hour over the 4-day course of the experiment.

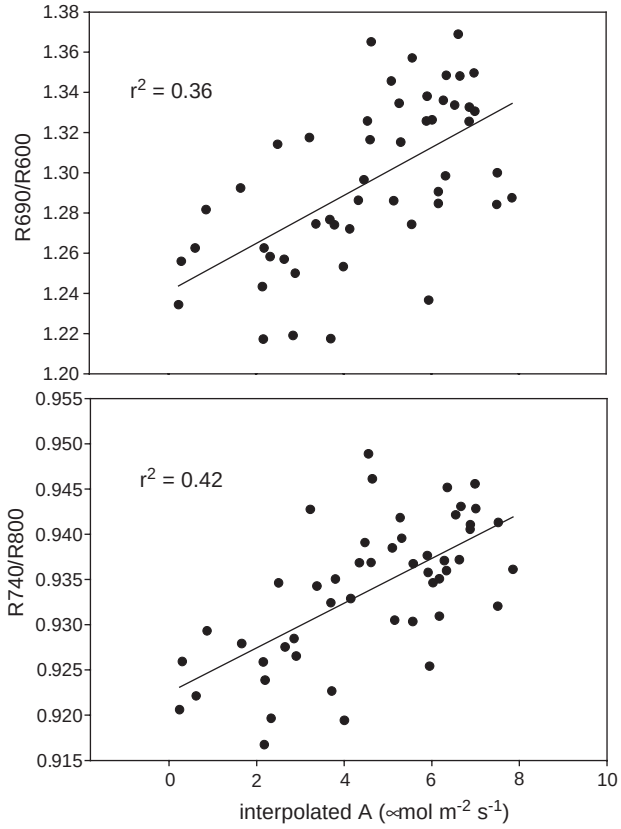

Fig. 7. Scatter plot of the relationship between the fluorescence ratio indices (FRI) and interpolated CO_2 assimilation rates. Plot includes measurements taken every half hour over the course of the 4-day experiment.

4. Discussion

4.1. Temperature and water stress effects on photosynthetic functioning are tracked by steady-state fluorescence (Fs) as mediated through photochemical (qP) and non-photochemical quenching

The relationship between Fs and A is complex as mediated through competing de-excitation pathways such as qP and non-photochemical quenching. This was demonstrated under varying temperature and drought conditions while under constant illumination. Early in the diurnal cycle, Fs exhibits an inverse relationship to A and g. This is potentially brought on by an increase in photochemical quenching (qP) under favorable temperature and drought conditions as shown in Fig. 2. Photochemical quenching is a competing de-excitation pathway with CF and an increase in its rate constant would result in a decrease in Fs. This mechanism is consistent with the concomitant increase in A and g values displayed early in the diurnal cycle. As temperature moves beyond optimal ranges and as the canopy dries down, qP, A, and Fs drops

sharply, suggesting a shift toward non-photochemical quenching.

Three primary components of non-photochemical quenching (qN) have been identified due to their relaxation kinetics. These are qE (rapidly inducible energy-dependent quenching), qT (state transition quenching brought on by the separation of the light harvesting complex from PSII), and qI (photoinhibitory quenching). qE is the most rapid and is brought on by a decrease in the thylakoid lumen ΔpH through the protonation of PSII proteins and the synthesis of xanthophylls via the xanthophyll cycle (Müller et al., 2001). With beans under favorable conditions, Pastenes and Horton (1996) showed that qN (particularly the qE component) decreased between 20 °C and 30 °C in conjunction with an increase in qP. Above 30 °C, qN increased, potentially brought on by a shift towards qT or qI quenching. Additionally, temperatures above 40 °C have been shown to increase Fo and suggest irreversible damage to the photosynthetic apparatus brought on by a break in the link between light harvesting complex II and the reaction center (Pastenes & Horton, 1999). Although qN was not directly measured in this experiment (due to the inability to estimate Fo under dark adapted conditions), a closely related measure, qP was shown to increase between temperatures of 35 °C and 40 °C and then sharply drop above this temperature threshold in a manner consistent with the results of Pastenes and Horton (1996, 1999). Moreover, our results show a decrease in CF (Fs and Fm'), a competing de-excitation pathway with qN, with increasing temperature above 40 °C consistent with previous research (Lu & Zhang, 1999; Pastenes & Horton, 1999).

PSII is recognized as the most sensitive component of the photosynthetic system (Berry & Björkman, 1980). As high temperatures are experienced, non-photochemical processes become strong electron sinks and reduce both qP and Fs (Rosema et al., 1998). High temperature affects the structure of the thylakoid membrane, as well as changes the rate constants of chemical reactions (Berry & Björkman, 1980; Sharkey et al., 2001). Fs tracks the depression in assimilation and stomatal conductance brought on by heat stress. As expected, under natural conditions, multiple environmental stressors co-occur frequently and can exhibit synergistic or antagonistic effects. In this experiment, the diurnal heat stress was accompanied by a longer term water stress. Water stress alone has been shown to have no effect on the primary photochemistry of PSII but has been shown to increase the thermo-stability of PSII in wheat (Lu & Zhang, 1999) and predisposes PSII to photoinhibitory damage (Giardi et al., 1996). Thus, it is likely that the onset of water stress somewhat ameliorated the effects of heat stress under the light-adapted conditions of this experiment.

To reiterate, Fs shows both a direct and inverse relationship to A and g depending upon the intensity of the stress and the status of the alternate de-excitation pathways. This suggests that it is not possible to estimate A directly from Fs without complimentary information. These results are consistent with the findings of Flexas et al. (1999, 2002a,2002b) which showed that Fs demonstrates a strong inverse relationship with non-photochemical quenching under varying illumination and drought conditions. They suggest that Fs is a rapid metric that distills the effects of declining stomatal conductance, CO_2 assimilation, and the onset of non-photochemical quenching. Our results also support the conclusion that the link between Fs and A is indirect as mediated through a suite of competing de-excitation pathways. Despite the lack of a consistent relationship between Fs and A, Fs still provides valuable information on plant stress response. Fs tracks the sharp decline in A, g, and qP likely brought on by the onset of non-photochemical quenching processes during heat stress and the subsequent recovery. This is demonstrated in Fig. 7, albeit indirectly, by the positive linear relationship between the remote estimates of Fs and interpolated A. Moreover, Fs provides an avenue for passive plant physiological monitoring under natural illumination without interfering with the plant growing environment. These characteristics make Fs an ideal candidate for passive remote sensing technologies.

4.2. Steady-state fluorescence signal is superimposed on passive reflectance measurements at the canopy scale

There is a body of conclusive evidence supporting a passive Fs signal superimposed on apparent reflectance signatures. Our results provide further support for this. A difference spectrum between the stressed canopy at 43 °C and the unstressed canopy at 25 °C shows the superposition of the dual-band emission spectra of CF at 690 and 740 nm. Additionally, this difference spectrum was shown to decrease with the onset of stress and subsequently increase during the recovery period in a manner consistent with leaf level measurements of Fs. Similar results were demonstrated at the canopy level by Zarco-Tejada et al. (2000b) using spectral subtraction and time-decay experiments in the laboratory. Additionally, a number of reflectance indices have been posited in the literature for tracking the contribution of Fs to apparent reflectance (see Zarco-Tejada et al., 2000a,2000b, 2003a for further details). The FRI reflectance indices outlined in this study were shown to track the multi-day pattern in leaf level Fs measurements.

These results also demonstrate a link between the PRI and leaf level estimates of Fs. We would expect the PRI to contain similar information as the ratio indices R_{690}/R_{600} and R_{740}/R_{800} given its inverse relationship to non-photochemical quenching processes. Sims and Gamon (2002) also showed that the PRI is sensitive to the carotenoid/chlorophyll ratio across a number of species. This is a consequence of its use of spectral bands located in the absorption regions of both chlorophyll and carotenoid pigments. Consequently, the use of the PRI may be convoluted given that spatial, and to a lesser extent, temporal variation in the index may be due to either

variability in radiation use efficiency or variability in carotenoid/chlorophyll ratios. In contrast, R_{690}/R_{600} and R_{740}/R_{800} utilize spectral bands located out of the absorption regions for carotenoid pigments. Additionally, R_{740}/R_{800} utilizes spectral bands outside of the absorption regions for chlorophyll pigments, thus potentially minimizing the influence of varying pigment concentrations on the Fs signal through re-absorption. However, this assertion is not experimentally verified in this study.

As expected, the NDVI time series showed no qualitative or quantitative link to the diurnal plant physiological measurements presented. This is expected given the lack of change in pigment concentrations and canopy structure during the experiment. Moreover, the sensitivity of the NDVI to changes in pigment concentration has been shown to saturate at medium to high chlorophyll concentrations (Buschmann & Nagel, 1993). The NDVI did show sensitivity to changes in leaf angle brought on by water stress late in the experiment. However, changes in leaf angle, much like changes in pigment concentrations, are non-rapid generalized responses to plant stress.

4.3. Remote sensing considerations

The magnitude of the contribution of Fs to apparent reflectance under a wide range of conditions is unknown. It is clear, however, that this will play a critical role in the efficacy of passive Fs remote sensing for monitoring plant physiological status under natural conditions. It has been shown that the contribution of Fs to apparent reflectance is small. The difference between stressed and unstressed canopy reflectance measurements attributed to Fs in this study is under 1%. This is likely due to the saturating light conditions in the growth chamber, which have been shown to depress Fs, due to the onset of competing non-photochemical quenching processes used in photoprotection of the PSII complexes (Flexas et al., 1999, 2000, 2002b; Rosema et al., 1998). Consequently, under natural solar illumination, CF will be lower for light-adapted leaves than under dark-adapted conditions using saturating pulses.

Despite this, recent studies suggest that the contribution of Fs to apparent reflectance may be greater than previously believed. Zarco-Tejada et al. (2003a) found Fs contributed up to 2% to apparent reflectance under saturating light conditions. Additionally, using long-pass filters to control the induction of CF, Campbell et al. (2002) showed that the contribution of Fs to apparent reflectance in *Zea mays* (corn) was over 2%. Moreover, Maier et al. (2002) and Zarco-Tejada et al. (2003b) found evidence for the detection of the Fs signal on apparent reflectance obtained in the field and from aerial sensors under solar illumination based on in-filling of fluorescence in the atmospheric oxygen absorption regions of the spectrum.

The variability of the contribution of Fs to apparent reflectance for different species is unknown. It is expected that varying physiognomy, canopy architecture, and leaf structures will affect the magnitude of the Fs signal in apparent reflectance data. Assessing this experimentally under a variety of illumination conditions and across a number of species will provide much needed information on the potential utility of these techniques at larger spatial scales. Information along these lines may help put bounds on the remote sensing problem such as the maximum distance we can expect to be able detect the Fs signal given atmospheric absorption, as well as minimum requirements of sensors in terms of their signal to noise characteristics. These issues are the focus of current research funded by the European Space Agency under the FluorMOD project which integrates a leaf fluorescence model and a canopy fluorescence model through a linking scheme in order to simulate the combined spectral reflected radiance and fluorescence emission signals at canopy level and top of the atmosphere (more details at http://www.ias.csic.es/fluormod/).

5. Conclusions

A strong link between plant pigment concentrations and spectral reflectance measurements are well developed in the remote sensing literature (Sims and Gamon, 2002). Barring a few exceptions (e.g., xanthophyll cycle), changes in pools of pigment concentrations are not rapid direct indicators of plant physiological status. Instead, they integrate the cumulative effects of the plant growing environment over longer periods of time (days to weeks) through generalized shifts in pigment concentrations or pigment degradation.

In contrast, results from this study posit a more direct link between passive reflectance measurements and plant physiological status through a mechanistic understanding of qP and non-photochemical quenching processes. This is accomplished through the characterization of Fs. The FRI indices, calculated in the red-edge spectral region, described in this study, showed superior results as compared with the PRI and NDVI indices for tracking plant stress and photosynthetic status. The use of the reflectance-derived fluorescence ratio indices described here should allow for a more direct link between passive reflectance measurements and plant photosynthetic functioning.

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