Study of the diurnal cycle of stressed vegetation for the improvement of fluorescence remote sensing

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ABSTRACT

Chlorophyll fluorescence (Chf) emission allows estimating the photosynthetic activity of vegetation -a key parameter for the carbon cycle models- in a quite direct way. However, measuring Chf is difficult because it represents a small fraction of the radiance to be measured by the sensor.

This paper analyzes the relationship between the solar induced Chf emission and the photosynthetically active radiation (PAR) in plants under water stress condition. The solar induced fluorescence emission is measured at leaf level by means of three different methodologies. Firstly, an active modulated light fluorometer gives the relative fluorescence yield. Secondly, a quantitative measurement of the Chf signal is derived from the leaf radiance by using the Fraunhofer Line-Discriminator (FLD) principle, which allows the measurement of Chf in the atmospheric absorption bands. Finally, the actual radiance spectrum of the leaf fluorescence emission is measured by a field spectroradiometer using a device that filters out the incident light in the Chf emission spectral range.

The diurnal cycle of fluorescence emission has been measured for both healthy and stressed plants in natural and simulated conditions. The main achievements of this work have been: (1) successful radiometric spectral measurement of the solar induced fluorescence; (2) identification of fluorescence behavior under stress conditions; and (3) establishing a relationship between full spectral measurements with the signal provided by the FLD method. These results suggest the best time of the day to maximize signal levels while identifying vegetation stress status.

Keywords: Chlorophyll fluorescence, emission spectrum, Fraunhofer Line Depth Method, field spectroradiometer, remote sensing, stress, diurnal cycle.

1. INTRODUCTION

Chlorophyll fluorescence (Chf) implies red and far-red light is emitted from photosynthetic green plant tissues in response to photosynthetically active radiation.[1] Chf is a protection mechanism for the plant to dissipate the excess of energy in the photosynthesis process. Chf occurs in competition with heat dissipation, which is the other pathway of energy de-excitation, and the photosynthesis itself.[2] Therefore, any increase in the efficiency of one process will result in a decrease in the yield of the other two. By measuring the yield of chlorophyll fluorescence, information about changes in the efficiency of photochemistry and heat dissipation can be gained. Moreover, Chf is sensitive to rapid changes in plant photosynthetic status, as the one produced by the sudden illumination of a dark-adapted sample (Kautsky effect).[3]

Although the total amount of Chf is very small (only 1% or 2% of total absorbed light), measurement is feasible because the emission spectrum of fluorescence is different from that of absorbed light. Fluorescence spectrum is characterized by two broad bands that span from 600nm to 800nm and present a maxima at 690nm and 740nm.[4] The intensity, shape and position of these emission bands are affected by a number of factors. However, it is agreed that the first band is affected by the re-absorption of chlorophyll pigments while the second band is minimally affected by chlorophyll re-absorption effects.[5]

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Chf emission is an accurate estimator of the health status of plants and their photosynthetic activity.[6] Many studies relate the Chf emission to different plant stress conditions.[7, 8] Water stress induces marked effects on the daily pattern of steady-state Chf.[9] Other works analyze the effect of an herbicide (DCMU) on the Chf emission intensity,[10] or the accumulation of over-saturating light pulses.[11] The variation of the Chf with the plant temperature has been also studied under natural conditions[12, 13] or under stress.[14]

During recent years, the measurement of Chf has become irreplaceable in plant ecophysiology studies, in both laboratory and field conditions. This fact has been boosted by the introduction of reliable, portable measuring instruments based on the Pulse Amplitude Modulation technique (PAM).[15] PAM fluorometers measure the relative quantum yield of Chf by applying a modulated measuring light with constant amplitude.[16] The detector is tuned to detect only fluorescence excited by the measuring light and, therefore, the relative fluorescence yield can be measured in the presence of background illumination. However, the active measuring principle of these instruments limits their use to short distances (from several cm to some meters).[17]

Passive measurement of Chf, despite not very explored yet, looks promising for the far distance estimation of Chf. One possibility consists on extracting Chf emission from reflectance measurements using the FLD (Fraunhofer Line Discrimination) principle. FLD is based on reflectance measurements in the vicinity of absorption lines of the Sun/Earth atmosphere in which solar radiation is greatly reduced.[18, 19] Oxygen absorption bands have been used to monitor Chf in relation to photosynthesis. Some experiments have been conduit successfully from airborne instruments, and future satellite missions are foreseen.[20-22] Plant reflectance is generally insensitive to rapid changes in plant photosynthetic status. However, the photochemical reflectance index (PRI) can track diurnal and seasonal changes. The PRI is intended for estimating changes in xanthophylls cycle pigments as they vary due to changes in photosynthetic light use efficiency.[23-25]

Nowadays, the proliferation of modulated fluorometers has led to a great number of studies about Chf. However, the PAM measuring principle allows obtaining only the relative variation of the fluorescence yield (the ratio between the emitted fluorescence at a determined wavelength range and the incoming photosynthetic photon flux density, PPFD). As a result, the actual radiometric quantification of the Chf emission is still largely unknown, despite the fact that the relative values can be very useful for the remote sensing community, because it will eventually determine the possibility of a satellite-based estimation of Chf.

In this paper, we present a study of the actual Chf emission spectrum in radiometric units by using a spectroradiometer, during a complete diurnal cycle. From these measurements we can infer the absolute fluorescence yield of the plants, in contrast to the relative measures given by the PAM methodology. We have tracked the evolution of the Chf during both simulated and real diurnal cycles, for healthy and stressed plants, and we have obtained the diurnal evolution of the absolute fluorescence yield, which results to be perfectly correlated with the relative fluorescence yield obtained with a conventional PAM instrument. The study is completed with the analysis of the fluorescence emission in the oxygen absorption bands, in order to relate the values given by the FLD method with the actual ChF emission and, finally, the analysis of the PRI evolution during the diurnal cycle.

2. METHODOLOGY

This study on the actual Chf emission at leaf level of live plants has been divided into two parts. Firstly, the study was performed under controlled conditions, and for this reason, a simulated diurnal cycle was performed on the laboratory to simultaneously record the fluorescence yield given by the PAM method and the one measured from the actual spectral emission of the plant. The experiment was conducted on two plants species, and the cycle was repeated several times on each plant in order to produce an increasing water stress (the plants were only watered one day before the start of the experiment). Secondly, the solar induced Chf emission was studied during a complete diurnal cycle for four different plants (corresponding to the two different species in stressed and healthy conditions). In this part of the study, the fluorescence was measured from the spectral response of the leaf under the excitation of solar light. A cyan filter was used to block the incident light in the Chf emission region, in order to acquire the actual Chf emission spectrum, while the filter was removed afterwards in order to estimate the Chf from the leaf reflected light using the FLD method.

The two species analyzed in this experiment both in natural and laboratory conditions were sunflower (*Helianthus annuus*) and Chinese hibiscus (*Hibiscus rosa-sinensis*).

2.1 Chlorophyll fluorescence during a simulated diurnal cycle

In order to analyze the behavior of the fluorescence during a diurnal cycle, an experiment was previously conducted under repetitive conditions in the laboratory. The experiment simulated the PAR received by a plant during a normal sunny summer day in Valencia on a single leaf of the plant, where the fluorescence was recorded simultaneously by a PAM-2000 instrument (Waltz GmbH, Effeltrich, Germany) and a FieldSpec FR spectroradiometer (ASD Inc., Boulder CO, USA). Both measurements were taken over a small area of the leaf, which correspond to the area seen by the fiberoptics of the PAM-2000 positioned on the instrument leaf-clip holder. The fiberoptics of the ASD spectroradiometer was adjusted to integrate the same area with the same relative inclination (30 deg. from the leaf normal angle).

The PAM-2000 instrument acquired every 2min the PAR, the temperature of the leaf and the fluorescence yield; and every 20min generated a saturation pulse in order to measure the quantum yield. The ASD spectroradiometer acquired the Chf emission spectrum, with a spectral resolution of 1nm, every 2min synchronised with the PAM fluorescence measurements.

The leaf illumination was provided by two LED modules (Optospot OSP/LF6/L3, from VLM S.p.A., Milano, Italy) formed by three high power white LEDs with built-in lenses. Each module had a cyan filter mounted in front, which blocked the light on the spectral region of the Chf emission. The LED modules were driven by a current source controlled by an analog voltage. This illumination setup allows generating a PAR ranging from 0 to 3300 µmoles/m^2/s, measured over the analyzed leaf with the micro-quantum sensor of the PAM-200 leaf-chip holder. All the elements were controlled from a personal computer running an automated program in MATLAB. Fig. 1 shows a picture of the experimental setup.

Fig 1. Experimental set-up for the simulation of a diurnal cycle on the laboratory.

For each plant, three consecutive diurnal cycles were applied without watering the plant at all, producing an increasing stress condition. The radiance of the illuminant was also acquired in order to calculate the reflectance of the plants in the region of the spectrum filled with the lighting (from 350nm to 650nm), which will be used to compute PRI.

2.2 Chlorophyll fluorescence during a sunlight diurnal cycle

A complete diurnal cycle of Chf was tracked on the 23th of June of 2006 on the campus of the University of Valencia (39º30'N, 0º25'W), in Burjassot (Spain). Four different plants were studied, two from each of the species. One plant of each pair was watered (WS and WH, sunflower and hibiscus respectively), while the other was withdrawn watering two days before the experiment (SS and SH). From each plant a leaf was selected and properly labeled to assure that the same samples were used throughout the experiment. These leaves were analyzed in periodic intervals. The temperature range during the experiment was between 21.7º to 28ºC.

The Chf emission spectrum was acquired with the ASD spectroradiometer. A special device, designed as a portable dark chamber, was attached to the optical fiber of the spectroradiometer in order to eliminate the incoming solar irradiance usually being reflected at the same spectral range as the radiance being emitted as Chf from the selected leaf.

The device is a V-shaped piece consisting on two folded tubes, one for the illumination and the other for reading the reflected/emitted light, with a diameter of 4 cm and a folding angle of 40 degrees, which avoids specular reflectance.[26] The base, located at the intersection of the two tubes, rest on a slide where the leaf can be positioned. The other ends are attached to the ASD optical fiber and a filter holder, respectively. The filter holder allows placing and removing a cyan filter as needed, which removes around 99% of the incident light above 650nm. Figure 2 shows a diagram and a picture of the device.

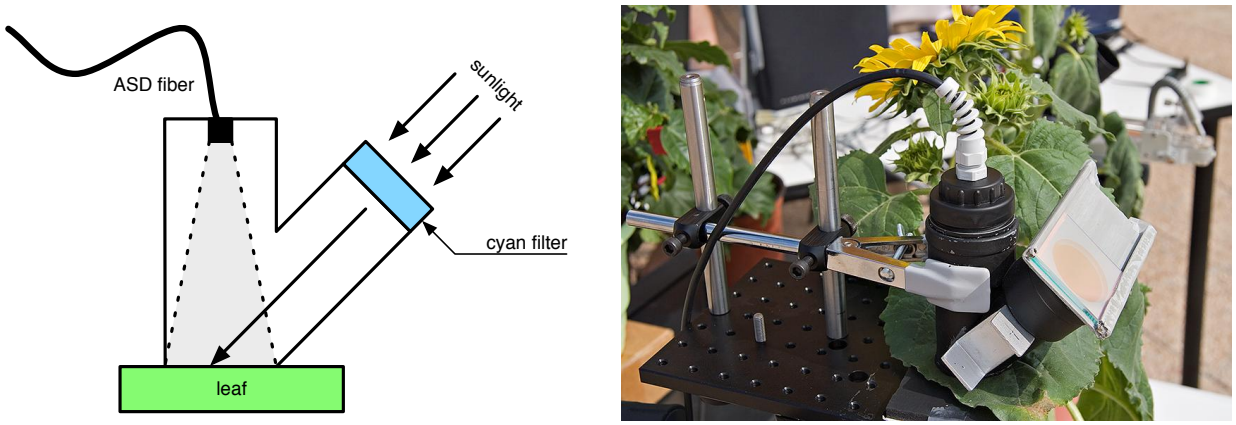

Fig 2. Diagram of the V-shaped device (left). Setup for Chf measurement of a sunflower plant under sunlight illumination (right).

The leaf under study is positioned on the slide and the device is oriented towards the sun (a metal attachment allows a precise alignment of the device). The sunlight hits the surface of the leaf through a cyan filter, exciting the plant on the UV and blue regions. The chlorophyll emits fluorescence on the red and NIR spectral range, and this emission is acquired by the ASD spectroradiometer. As no sunlight reaches the leaf in this region, all the measured radiance is due to the fluorescence emission rather than reflected light. When the cyan filter is removed, the device can be used to measure the reflected radiance of the leaves, and to derive from it the leaf fluorescence using the FLD method.

For each time period, seven consecutive measurements were taken. The first measurement acquires the white reference radiance without any filter, and the second one acquires the white reference radiance with the cyan filter put on the instrument. The third measurement acquires the spectrum of the leaf when using the filter and the fourth one acquires the leaf reflectance without filtering. Measurements five to seven are a repetition of the measurements one to three in reverse order, and are used only to validate the stability of the sunlight during the measure.[26]

2.3 Data analysis procedure

The Chf emission spectrum acquired with the ASD spectroradiometer has been used to calculate the fluorescence yield of the plants, the PRI and the estimation of the fluorescence at the oxygen absorption bands (FLD method). The PAM-2000 instrument has been used to read the relative fluorescence yield (internally calculated by the instrument) and to estimate the quantum yield of the plant.

The fluorescence yield is calculated as the ratio of the Fluorescence Photon Flux Density (FPFD) over the Photosynthetic Photon Flux Density (PPFD). The first value counts the number of photons emitted by the Chf in the range from 650 to 850 nm, while the second one counts the photons emitted by the illuminant in the photosynthetic active region (400 to 700 nm). The PRI is calculated from two bands of the leaf reflectance with the following equation:

$$\mathrm{PRI}=(\lambda_2-\lambda_1)/(\lambda_2+\lambda_1)$$

where λ_1=531nm and λ_2=570nm. The FLD estimation is calculated with the methodology described by Plascyk.[18, 19]

The PAM-2000 instrument allows the calculation of the quantum yield by applying a saturating light pulse to the leaf and measuring the maximum fluorescence (Fm'). Using this value and the fluorescence yield before the pulse (Ft), the quantum yield is computed as follows:

$$Q=(Fm'-Ft)/Fm'$$

3. RESULTS

3.1 Simulated diurnal cycle

Three consecutive diurnal cycles were simulated for each plant of the experiment. The desired PAR values were extracted from a model of the ideal illumination that a plant would receive a summer day on the coordinates of the campus of the University of Valencia. In this model, the sunrise took place at 5:00 (local solar time), the PAR reached a maximum of 1895 µmoles/m^2/s at noon, and the sunset was estimated at 19:00. The actual PAR applied to the plants in each cycle was tracked and agreed the desired PAR curve for all the cycles within a very close margin. The temperature was held quite high and constant around 33ºC in order to further increase the stressing process on the plants.

The simulation of the diurnal cycle and the data acquisition was controlled from a single function running under MATLAB. This function updated the PAR value applied to the plant every 15s; every 2 min recorded the fluorescence, actual PAR and temperature, and ordered the ASD control system to acquire one spectrum; and every 20min it generated a saturating pulse of the PAM-2000 instrument.

Figure 3 shows some of the acquired spectra of the Chf emission for the sunflower plant on the first cycle, expressed in radiance units. Studying the complete set of acquired spectra, one can track the evolution of the Chf emission at the wavelength on which the atmospheric O_2 absorption takes place, as shown on Fig 4. In this figure and the following ones, we have represented the received PAR in dashed grey line with a normalized scale. The PAM instrument recorded the evolution of the fluorescence yield during the cycle, expressed in arbitrary units (a.u.). The fluorescence yield was also calculated from the Chf emission spectra acquired with the ASD spectroradiometer, giving the actual yield on the plant expressed as the percentage of the incoming energy (PPFD) that is emitted as fluorescence (FPFD). Both yields are plotted on Fig. 5 for the sunflower and hibiscus plants.

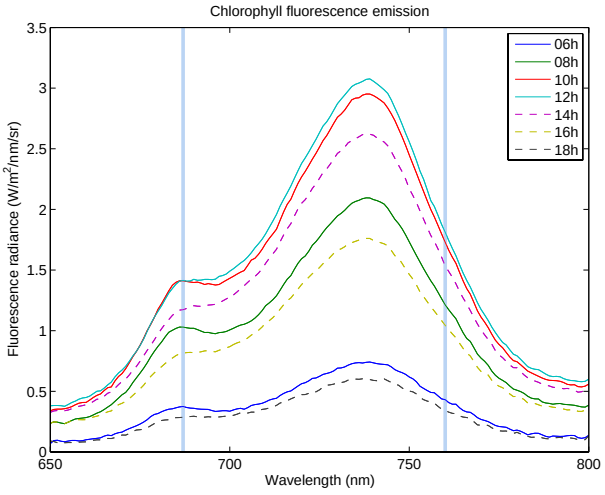

Fig 3. Chlorophyll fluorescence spectra in radiance units of a sunflower leaf during the simulated diurnal cycle. The vertical lines indicate the location of the oxygen absorption bands (at 687nm and 760nm).

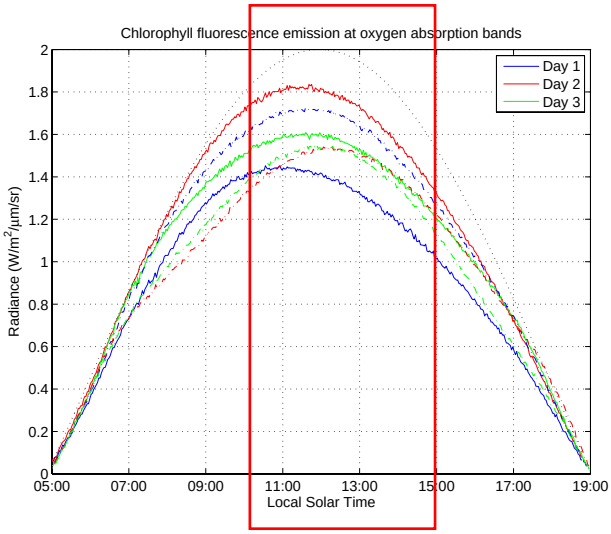

Fig 4. Chlorophyll fluorescence diurnal evolution measured at wavelengths corresponding to the oxygen absorption bands of a sunflower leaf (687nm in continuous line and 760nm in dashed line).

The saturation pulses gave information about the maximum fluorescence of the plant, from which the quantum yield can be extracted. Figure 6 represents the quantum yield measured over the three cycles of the plants.

The reflectance of the leaves (from 350nm to 600nm) was also calculated for each acquired spectrum, and the Photochemical Reflectance Index (PRI) was extracted from this information. The PRI value followed a characteristic curve during the diurnal cycle, which also showed a noticeable dependence with the increasing stress of the plant (Fig 7).

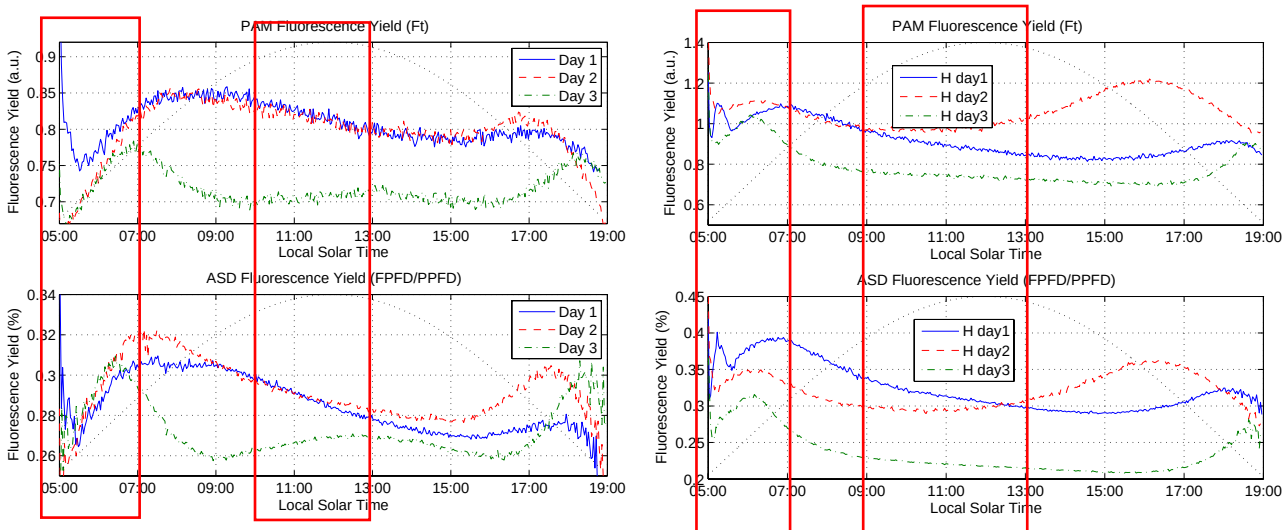

Fig 5. Fluorescence Yield measured of a sunflower (left) and a hibiscus (right) leaves with the PAM-2000 Flourometer (top); and Fluorescence Photon Flux Density measured with the ASD (bottom). Note that the PAM-2000 uses arbitrary units (a.u.).

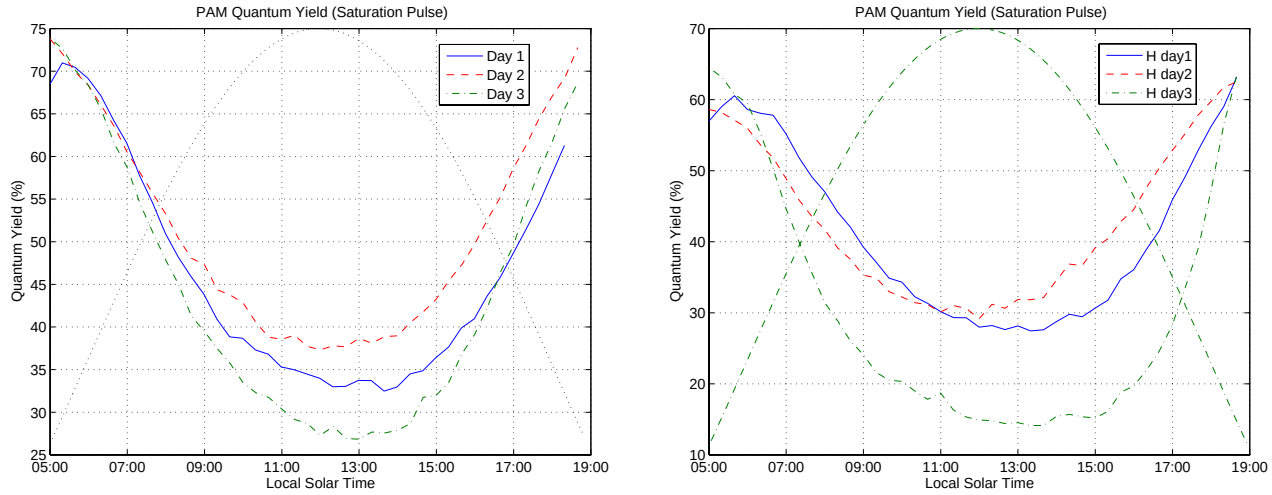

Fig 6. Quantum yield obtained with the PAM-2000 fluorometer applying a saturate pulse to a sunflower (left) and a hibiscus (right) leaves every 20min.

3.2 Sunlight diurnal cycle

The day chosen for the analysis of the sunlight diurnal cycle started with some haze and intermittent clouds that disappeared completely during the morning. The chlorophyll fluorescence was acquired with the ASD spectroradiometer using the aforementioned device. The four plants were analyzed consecutively in regular periods ranging between 10min and 20min, depending on the changing illumination conditions. At the same time that the plants were measured, the PAR was recorded. Due to the effect of the cyan filter, the PAR received by the plant with the filter inserted is about one half of the PAR received under natural sunlight. However, it is worth noting that in the range from 600 to 700nm there is almost no light arriving to the leaf, whereas from 400 to 600 the amount of received light is just around 75% of the incoming light.

The fluorescence yield of the plants was calculated from the Chf emission and the received PAR. The evolution of this value for the four plants is presented in Fig 8. This experiment showed the actual radiance of the Chf emission in its entire radiometric spectrum (from 650nm to 850nm). It is of special interest the radiance in the O_2 absorption bands, since these bands can be used for remote monitoring of the fluorescence using the FLD method. Fig 9 shows the evolution of the actual radiance at the 760nm for the four plants over the day, which corresponds to one of the oxygen absorption bands used for the FLD method. Fig. 10 shows the actual radiance of the Chf measured with the spectroradiometer, and also the FLD estimation for 760nm absorption band for the two species analyzed. The graphs show clearly the effect of the PAR reduction due to the cyan filter when measuring the actual radiance of the Chf, resulting in lower values with respect to the estimated Chf. The PRI evolution during the day was also calculated, and has been plotted in Fig. 11.

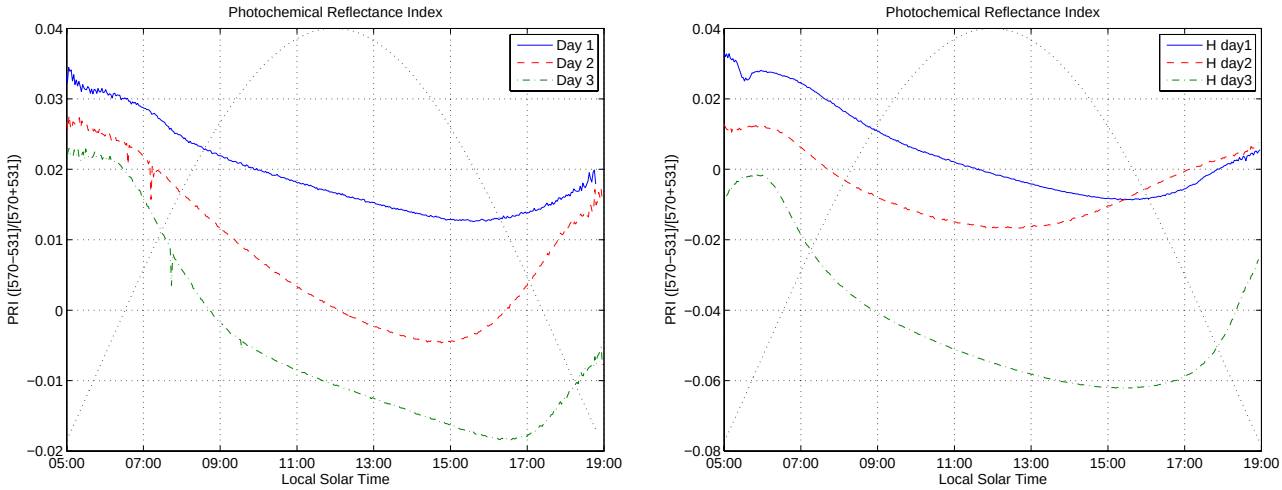

Fig 7. Evolution of the PRI for the three days on the sunflower (left) and the hibiscus (right) plants.

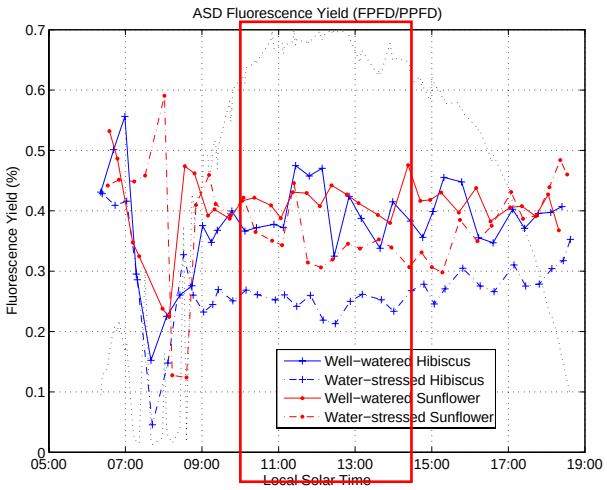

Fig 8. Fluorescence Yield of the well-watered and the water–stressed plants measured on 23th of July 2006 under natural illumination.

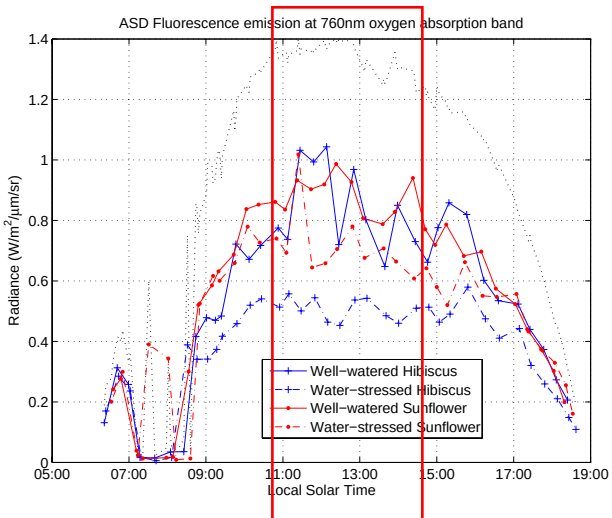

Fig 9. Fluorescence emission at the 760nm oxygen absorption band in radiance units of the well-watered and the water–stressed plants.

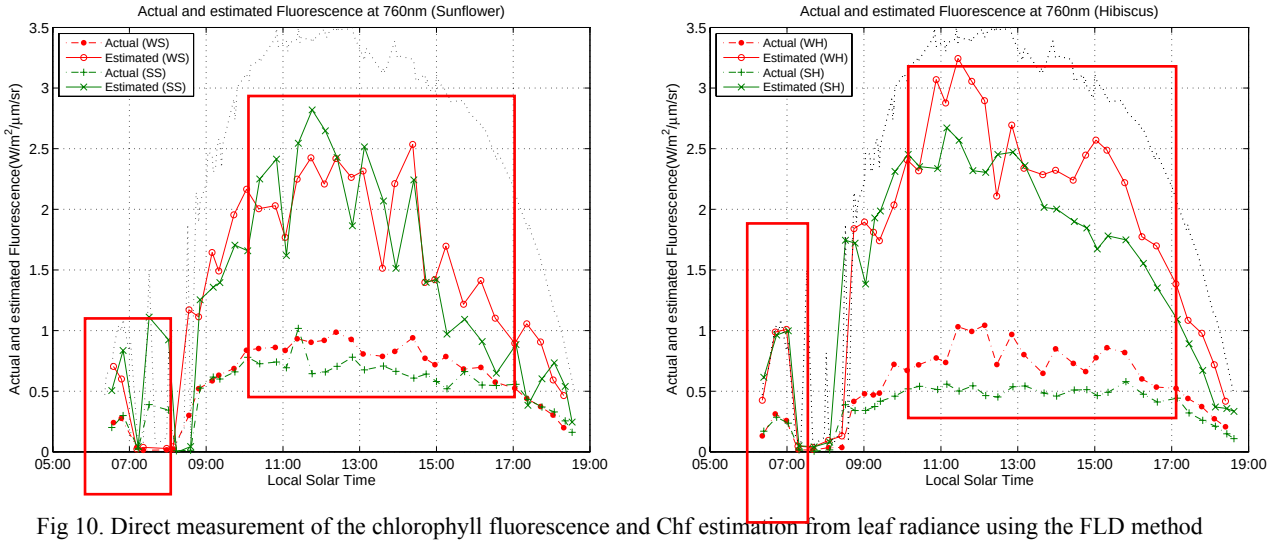

Fig 10. Direct measurement of the chlorophyll fluorescence and Chf estimation from leaf radiance using the FLD method (left) of a watered (WS) and a stressed (SS) sunflower plants; (right) of a watered (WH) and a stressed (SH) hibiscus plants. Note that the actual Chf has lower values than the estimated Chf using the FLD method due to reduction of the PAR caused by the cyan filter.

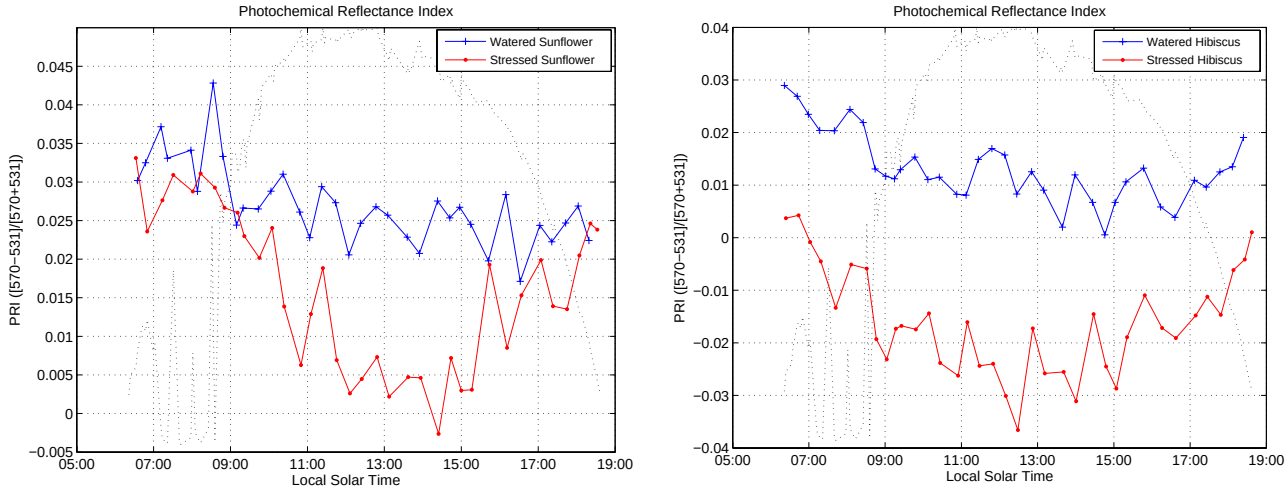

Fig 11. Evolution of the PRI for the sunflower (left) and the hibiscus (right) plants over the sunlight diurnal cycle.

4. DISCUSSION

The experiment on the simulated diurnal cycle shows that the fluorescence yield obtained from the radiance of the Chf emission presents a high correlation with the measurements (r=0.99) obtained with the PAM-2000, as shown in Fig. 12. Besides, the fluorescence yield obtained from this method gives an accurate estimation of the plant state, as seen in Fig. 5; while providing a quantitative value for the yield, thus making the results comparable between different measurement experiments.

The evolution of the Chf emission (Fig. 3) follows closely the PAR received by healthy plants. However, as the water stress increases, the plant is not able to keep the working rates as in healthy conditions, thus reducing fluorescence

emission. This is clearly appreciable on the plot of the fluorescence yield, especially for the third day, when the PAR decays in the evening, and the stressed plant is able to cope again with the amount of absorbed light, increasing the yield.

During the diurnal cycle, the quantum yield of the plants follows inversely the evolution of PAR. This behavior is repeated for both plants during the three simulated cycles, and it is also appreciable that the quantum yield is higher when the plant is not in perfect conditions but decays deeply when the plant suffers a very severe drought.

Similar trends are appreciated in the PRI evolution. During each cycle of the experiment in the laboratory, the PRI follows an opposite behavior to the PAR, as reflected in Fig. 7. However, as the plant state is getting worse, the PRI values decrease in a larger range. The PRI curve for each cycle does not show symmetry around noon, and the PRI value at the end of the diurnal cycle does not return to the level at the beginning of the cycle, so that the initial values for the PRI in each cycle seem to follow the end value of the previous cycle. This fact shows that the plant is not able to recover its condition during the night in an increasing stress condition. During the diurnal cycle under natural conditions, the PRI is also opposite to the PAR, and it is lower for the stressed plants.

In the experiment under actual sunlight conditions, we compared the estimated Chf emission given by the FLD method with the actual Chf emission measured with the ASD spectroradiometer, under different light intensities. Fig. 13 shows the correlation between both emission values. It is important to point here that each actual Chf emission value was measured with half the light received by the plant for its matching FLD estimation, which causes systematic deviation of the slope from the nominal 1:1 line. This effect can be compensated by applying a correction factor to account for the different illumination conditions in normal light (when measuring both the reflected radiance and the fluorescence emission) and with the filter used to insolate fluorescence emission. Another feature worth noting is the larger dispersion at larger levels of fluorescence, these happen at the maximum of illumination, but are proportionally to PAR smaller than Chf levels at lower illumination resulting in a smaller signal-to-noise ratio, thus the errors in the signal increase.

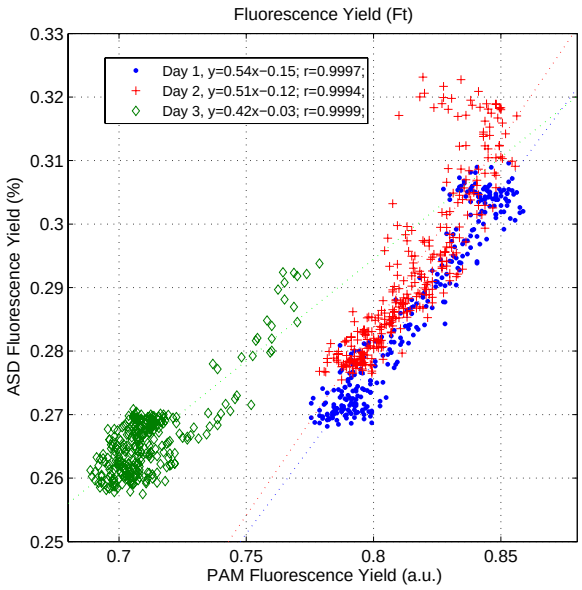

Fig 12. Correlation between ASD fluorescence yield and the fluorescence yield of a sunflower plant measured with the PAM-2000 in the laboratory.

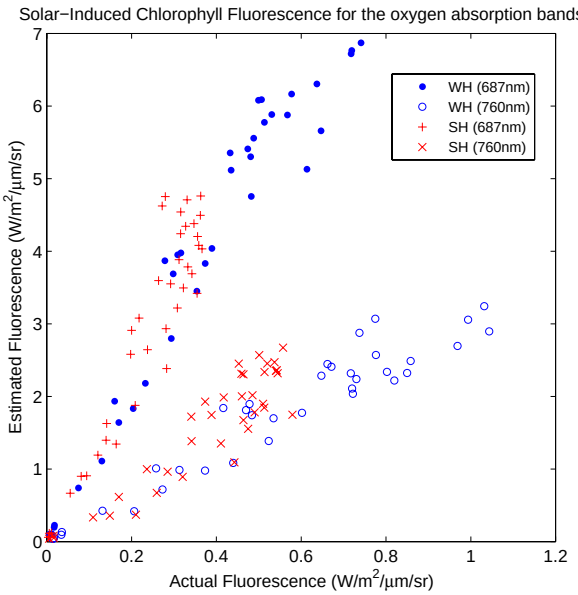

Fig 13.Correlation between the direct measurement of the Chf and the estimated Chf using the FLD method in a well-watered and water-stressed hibiscus plants under natural conditions.

The Chf emission is directly related to the PAR intensity. That means that the maximum Chf radiance can be measured around noon (in local solar time). However, in order to discriminate the healthy state of the plants, the morning period seems to contain more information, as the range of variation in the fluorescence yield suggests. The election of a best overpass time for a satellite remote observation of Chf must take into account this fluorescence yield variation rather than the absolute maximum value of the fluorescence emission.

5. CONCLUSIONS

This paper presents a study on the chlorophyll fluorescence evolution during a complete diurnal cycle, in both simulated and natural conditions at the leaf level for two species under different stress conditions. The authors have measured the absolute spectral radiance of the Chf emission, which allowed a quantitative derivation of the fluorescence yield of the Chf rather than measuring a relative value as the one given by modulated fluorometers. In addition, it provided a way of evaluating the performance of the FLD method as a technique to estimate the Chf by remote sensing.

The studied cases show that the Chf emission is mainly driven by the PAR during the whole cycle, but the fluorescence yield is severely reduced during the central hours of the day when the plant is under stress. Similar trends are observed on the quantum yield and the PRI measurements.

The order of magnitude of the measured Chf emission during the major part of the day is high enough not only to allow for a remote sensing estimation of Chf, but also to discriminate between different health conditions of the plants. The late morning period seems to be the most appropriate part of the day to acquire Chf in order to maximize the discrimination power of the measurement while maintaining a high level of signal. Moreover, the good agreement found between the estimated fluorescence emission with the actual measurements, despite the need for further validation, points to the FLD method as an accurate way of retrieving quantitative values of Chf.

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REFERENCES

1. P. J. Zarco-Tejada, J. Pushnik, S. Dobrowski, and S. L. Ustin, “Steadystate chlorophyll a fluorescence detection from canopy derivative reflectance and Double-Peak Red-Edge effects”, Remote Sens. Environ. 84(2), pp. 283–294, 2003.
2. K. Maxwell and G.N. Johnson, “Chlorophyll fluorescence - a practical guide”, J. Exper. Botany. 51, pp. 659-668, 2000.
3. H. Kautsky, W. Apel and H. Amann, “Chlorophyllfluorescenz und Kohlensaureassimilation”, Biochem Z. 322, pp. 277–292, 1960.
4. C. Buschmann and H. K. Lichtenthaler, “Reflectance and chlorophyll fluorescence signatures in leaves”, in Applications of Chlorophyll fluorescence, H. K. Lichtenthaler, Ed. Dordrecht, The Netherlands: Kluwer, pp. 325–332, 1988.
5. A.A. Gitelson, C. Buschmann and H.K. Lichtenthaler, “Leaf chlorophyll fluorescence corrected for re-absorption by means of absorption and reflectance measurements”, Journal of Plant Physiology 152, pp. 283-296, 1998.
6. G. Papageorgiou, *Chlorophyll fluorescence: an intrinsic probe of photosynthesis*. In: Bioenergetics of Photosynthesis (G. Govindjee, Ed) NY, Academic Press, pp. 313-371, 1975
7. H. Lichtenthaler and U. Rinderle, “The role of chlorophyll fluorescence in the detection of stress conditions in plants”, CRC Critical Reviews in Analytical Chemistry 19(1), pp. 529–585, 1988.
8. H.K. Lichtenthaler, “Vegetation stress: An introduction to the stress concept in plants”, J. Plant Physiol. 148, pp. 4–14, 1996.
9. J. Flexas, M. Briantais, Z. Cerovic, H. Medrano, and I. Moya, “Steady-State and maximum chlorophyll fluorescence responses to water stress in grapevine leaves: A new remote sensing system”, Remote Sens. Environ. 73, pp. 282-297, 2000.
10. H. K. Lichtenhaler, C. Buschmann, U. Rinderle, and G. Shmuck, “Application of chlorophyll fluorescence in ecophysiology”, Radiat. Environ. Biophys. 25, pp. 297–308, 1986.
11. S. Apostol, J.M. Briantais, N. Moise, Z.G. Cerovic and I. Moya, “Photoinactivation of the photosynthetic electron transport chain by accumulation of over-saturating light pulses given to dark adapted pea leaves”, Photosynthesis Research 67(3), pp. 215-227, 2001.

12. G. Agati, Z. Cerovic and I. Moya, “The Effect of Temperature on Chlorophyll Fluorescence: The role of PSI in 735 nm Fluorescence”, Photochemistry and Photobiology 72, pp. 75-84, 2000.
13. G. Agati, “Response of the in vivo chlorophyll fluorescence spectrum to environmental factors and laser excitation wavelength”, Pure Appl. Opt. 7, pp. 797–807, 1998.
14. S.Z. Dobrowski, J.C. Pushnik, P.J. Zarco-Tejada and S.L. Ustin, “Simple reflectance indices track heat and water stress induced changes in steady state chlorophyll fluorescence at the canopy scale”, Remote Sensing Environment 97, pp. 403-414, 2005.
15. U. Schreiber, “Pulse-Amplitude-Modulation (PAM) fluorometry and saturation pulse method: An overview”, in *Chlorophyll a Fluorescence: A Signature of Photosynthesis*. Series: Advances in Photosynthesis and Respiration 19, G.C. Papageorgiou and Govindjee (Eds.), pp. 279-319, 2004.
16. W.P. Quick and P. Horton, “Studies on the induction of chlorophyll fluorescence in barley protoplasts. I. Factors affecting the observation of oscillations in yield of chlorophyll fluorescence and the rate of oxygen evolution”, Proc R Soc Lond B 220, pp. 361-370, 1984.
17. J. Louis, A. Ounis, J.M. Ducruet, S. Evain, T. Laurila, T. Thum, M. Aurela, G. Wingsle, L. Alonso, R. Pedros and I. Moya, “Remote sensing of sunlight-induced chlorophyll fluorescence and reflectance of Scots pine in the boreal forest during spring recovery”, Remote sensing of environment 96(1), pp. 37-48, 2005.
18. J. Plascyk, “The MKII Fraunhofer Line Discriminator (FLD-II) for airborne and orbital remote sensing of solar stimulated luminescence”, Optical Engineering, vol. 14(4), pp. 339–346, 1975.
19. J. Plascyk and F. Gabriel, “The Fraunhofer Line Discriminator MKII - an airborne instrument for precise and standardized ecological luminescence measurements”, IEEE Trans. Instr. Measure 24, pp. 306–313, 1975.
20. I. Moya and Z.G. Cerovic, “Remote sensing of chlorophyll Fluorescence: Instrumentation and analysis”, in *Chlorophyll a Fluorescence: A Signature of Photosynthesis*, Series: Advances in Photosynthesis and Respiration, vol. 19, G.C. Papageorgiou and Govindjee (Eds.), pp. 429-445, 2004.
21. L. Liu, Y. Zhang, J.Wang, and C. Zhao, “Detecting solar-induced chlorophyll fluorescence from field radiance spectra based on the fraunhofer line principle”, IEEE Trans. Geosci. Remote Sensing 43(4), pp. 827–832, 2005.
22. K. Smorenburg, et al., “Remote Sensing of solar induced fluorescence of vegetation”. Remote Sensing for agriculture, ecosystems and hydrology III, Toulouse 17-19 Sept., SPIE 4542, pp. 178-190, 2001.
23. J.A. Gamon, J. Penuelas and C.B. Field, “A narrow-waveband spectral index that tracks diurnal changes in photosynthetic efficiency”, Remote Sensing of Environment 41(1), pp. 35–44, 1992.
24. J. Peñuelas, I. Fililla and J.A. Gamon, “Assessment of photosynthetic radiation-use efficiency with spectral reflectance”, New Phytologist 131(3), pp. 291– 296, 1995.
25. 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 Sensing of Environment 91, pp. 175–185, 2004.
26. L. Gomez-Chova, L. Alonso-Chorda, J. Amoros-Lopez, J. Vila-Frances, S. del Valle-Tascon, J. Calpe Maravilla, J. Moreno. "Solar induced fluorescence measurements using a field spectroradiometer". Proceedings on International Conference on Earth Observation for vegetation monitoring and water management. Napoli, November 2005. In press (pp. 63 abstract book)