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TECHNICAL COMMUNICATION

A new monitoring PAM fluorometer (*MONI-PAM*) to study the short- and long-term acclimation of photosystem II in field conditions

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Abstract We present and evaluate the performance of a new field monitoring PAM fluorometer (*MONI-PAM*) which is intended for short- and long-term monitoring of the acclimation of photosystem II (PSII). The instrument measures chlorophyll fluorescence, photosynthetic photon flux density (PPFD), and temperature in the field, and monitors exactly the same leaf area over prolonged periods of time, facilitating the estimation of both rapidly reversible and sustained non-photochemical quenching (NPQ). The *MONI-PAM* performance is evaluated in the lab and under natural conditions in a Scots pine canopy during spring recovery of photosynthesis. The instrument provides a new tool to study in detail the acclimation of PSII to the environment under natural field conditions.

Keywords Chlorophyll fluorescence · Diurnal acclimation · Scots pine · Seasonal acclimation

Abbreviations

ETR	Electron transport rate
F_0 and F_m	Minimal and maximal chlorophyll fluorescence yield from dark-acclimated leaves
F_t and F'_m	Actual and maximal chlorophyll fluorescence yield from light-exposed leaves
Φ_P	PSII operating efficiency
NPQ	Non-photochemical quenching
$PPFD_{SL}$	Photosynthetic photon flux density at sample level
PSII	Photosystem II

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Introduction

Acclimation of photosystem II (PSII) to the light environment is of fundamental importance to adjust the photosynthetic electron flow to the requirements for ATP and NADPH by dark photosynthetic reactions. PSII acclimation involves modulation of the fractions of absorbed light energy that is utilized in photochemistry and that dissipated as heat. Acclimation of PSII takes place on different time scales: short-term adjustments are mostly reversible and occur in the range from minutes to hours in response to, for example, rapidly varying light environments due to temporary shading by clouds or sunflecks (Porcar-Castell et al. 2006); long-term acclimation of PSII, however, occurs in response to seasonal light and temperature changes, or to episodes of water, nutrient, or biotic stress (Öquist and Huner 2003), leading to slowly reversible acclimation in the PSII energy partitioning. Because variations in the energy partitioning in PSII affect the yield of chlorophyll fluorescence, measurements of chlorophyll fluorescence are widely used to study PSII acclimation status, both under laboratory and field conditions (Bolhàr-Nordenkampf et al. 1989; Maxwell and Johnson 2000; Demmig-Adams and Adams 1992).

To estimate PSII photochemistry fluorometrically, PSII maximum efficiency in dark-acclimated leaves ($\Phi_{Pmax} = (F_m - F_0)/F_m$; Kitajima and Butler 1975) and PSII operating efficiency in light-exposed leaves ($\Phi_P = (F'_m - F_t)/F'_m$; Genty et al. 1989) are commonly determined. These parameters can be established by fluorescence

measurements lasting a few seconds during which the sample is fixed relative to the fluorometer. Because, the particularities in the optical properties of individual samples cancel out when Φ_{Pmax} or Φ_P are calculated, long-term fluorometric monitoring of PSII photochemical efficiency of various leaves is possible, albeit laborious, by repeatedly examining individual leaves.

To estimate heat dissipation by PSII, the Stern-Volmer non-photochemical quenching parameter NPQ is often used (NPQ = $(F_m/F'_m) - 1$; Bilger and Björkman 1990). In practice, calculation of NPQ requires that the maximum chlorophyll fluorescence yield (F_m) is measured in a dark-acclimated leaf, when non-photochemical quenching is minimal, and the F'_m is determined with the same leaf in a light-exposed state, when non-photochemical quenching has increased to certain level. Both Φ_{Pmax} and Φ_P measurements, and F_m and F'_m measurements need to be carried out with the same leaf position so that the optical properties of the sample cancel out when NPQ is determined. Time intervals between measurements of F_m and F'_m, however, are much longer than the time needed to establish Φ_{Pmax} and Φ_P. Especially in long-term experiments, the requirement to keep probing areas constant creates logistical and technical problems which critically restrict NPQ determinations (Logan et al. 2007).

Therefore, to monitor both photochemical and non-photochemical fluorescence quenching during extended time intervals in the field, a multi-channel fluorometer system has been developed (*MONI-PAM*, Heinz Walz GmbH, Effeltrich, Germany). The system facilitates continuous measurements of the chlorophyll fluorescence from constant areas of several leaf samples simultaneously. The present study evaluates the performance of the *MONI-PAM* under realistic field conditions which include temperatures below 0°C. Specifically, we studied the diurnal and seasonal acclimation of PSII in Scots pine needles during spring recovery of photosynthesis.

Methods

The MONI-PAM fluorometer

Chlorophyll fluorescence was recorded with a "*MONITORING-PAM* Multi-Channel Chlorophyll Fluorometer" or *MONI-PAM* (Walz, Effeltrich, Germany). A measuring system can comprise up to 7 emitter-detector units (*MONI*-head/485). Each *MONI*-head/485 represents an independent fluorometer. The *MONI*-head/485 fluorometers are connected using RS-485 serial data communication *via* a storage-capable (1 GByte memory on microSD flash card) data acquisition system (*MONI-DA*) to a *MONI*-IB4/LAN central interface box. From the latter box to the computer, data are transferred using USB, RS232 or Ethernet communication. The data line between *MONI-DA* and *MONI*-IB4/LAN can be as long as 100 m but the lines between *MONI*-heads and data acquisition system, and between USB interface box and computer, normally should not exceed 10 m and 2 m, respectively. In the present study, we used an arrangement consisting of four *MONI*-heads installed within a tree canopy. Each *MONI*-head was connected with 2.5 m lines to a *MONI-DA* prototype without memory card.

The chassis of a *MONI*-head emitter-detector unit is a water-tight aluminum or stainless steel cylinder with a diameter of 30 mm and a length of 280 mm (Fig. 1). The *MONI*-head delivers measuring and actinic light to the sample through a window that transmits radiation in the range of 400–750 nm, situated at one end of the cylinder. The same blue LED emits actinic light and saturating flashes as well as measuring light: the LED emission maximum and full width at half maximum is 455 nm and 18 nm, respectively. Measuring pulses to excite modulated fluorescence are given at frequencies of 5 and 100 Hz for measurements of fluorescence under dark (F_0 fluorescence) and light conditions, respectively. The intensity of the measuring pulses can be adjusted to include between 1 and 5 probing flashes of 8 µs length and variable intensity, resulting in integrated photon flux densities at sample level ($PPFD_{SL}$) between 0.1 and 1 µmol m^{-2} s^{-1} at 5 Hz, or between 1 and 15 µmol m^{-2} s^{-1} at 100 Hz. Also, the *MONI*-head provides actinic light up to a $PPFD_{SL}$ of 1,500 µmol m^{-2} s^{-1} and saturating light pulses with maximal duration of 2 s and $PPFD_{SL}$ up to 4,000 µmol m^{-2} s^{-1}. The *MONI*-head employs PIN photodiodes to measure pulse-amplitude modulated (PAM) chlorophyll fluorescence at wavelengths longer than 625 nm, as well as $PPFD_{SL}$ in the range from 400 to 700 nm. Temperature is recorded by an integrated-circuit temperature sensor. In the present study, the intensity of the measuring light was 0.9 (at F_0 and F_t) and 9 µmol m^{-2} s^{-1} (at F_m), and the intensity of the saturating pulses was 4,000 µmol m^{-2} s^{-1}.

Saturating pulses were supplied every 5–10 min, and F_t, F'_m, $PPFD_{SL}$, and temperature were recorded for each measuring point. To keep actinic effects of the *MONI-PAM* minimal, the measuring light was switched off between measurements but automatically switched on a few seconds before each saturating-pulse analysis using the batch file feature of the WinControl-3 software.

Pine needles were fixed in the *MONI*-head's leaf clip consisting of two aluminum frames (35 × 25 mm) Fig. 1). The leaf clip is mounted at a distance of 25 mm from the *MONI*-head's optical window so that leaf clip area and longitudinal axis of the *MONI*-head form an angle of 120°. The sample holder includes a laterally mounted 13 × 7 mm area covered by white-reflecting material which directs

Fig. 1 *MONI*-head emitter-detector unit in a semi-permanent installation at the top of a crown of Scots pine (left), and details of the sample holder with Scots pine needles (right)

ambient light toward the *MONI*-head optical window in which the $PPFD_{SL}$ is measured. The most recent version of the *MONI*-head employs a 1-mm-thick Teflon layer (Spectralon®) as reflecting material. The *MONI*-heads employed in the present study saturated at a PPFD of 1,730 μmol m^{-2} s^{-1}, but the currently built devices are able to record PPFD up to 2,500 μmol m^{-2} s^{-1}. Since our pine needles covered only a part of the sample holder, we placed black foam backed by a black plate behind the samples to exclude possible fluorescence from the background (Fig. 1).

Field measurements

We measured chlorophyll fluorescence with the *MONI-PAM* in the needles of two 45-year-old Scots pine trees [*Pinus sylvestris* (L.)] growing at SMEAR-II station (Station for Measuring Forest-Ecosystem-Atmosphere Relations) in southern Finland (61°51′ N, 24°17′ E, and 181 m of elevation). Two branches were selected in each tree, one at the top of the canopy and the other in the lower part of the canopy, and both were accessed using permanently installed scaffolds. A *MONI*-head emitter-detector unit was installed in each of the four branches using ropes, nylon belts, and aluminum bars (see Fig. 1) so that both the *MONI*-head and the branch would move together in the wind.

Results and discussion

PPFD and temperature measurement, operations at extreme temperatures

Tests were performed in laboratory and field conditions to evaluate the precision and accuracy of the *MONI*-head unit for light and temperature measurements, as well as the performance of the instrument under severe field conditions. The response of the intensity of the saturating pulse to low temperatures was tested in laboratory conditions. At −15°C the *MONI*-head showed a 10% reduction of maximum saturating-pulse intensity, but the instrument operated normally at +50°C. In needles of Scots pine, the decrease in the intensity of the saturating pulse under below-zero temperatures did not impair the full reduction of the primary quinone acceptors, as indicated by the saturation of the chlorophyll fluorescence increase during the saturating pulse (data not shown).

Further, one-point calibration of the *MONI*-head PPFD sensor was achieved in sunlight under clear skies. Calibration was against a cosine-corrected quantum sensor (type MQS-B, Walz) which was mounted next to the light-reflecting area of the sample holder and in the same plane. After calibration, the relationship between the *MONI*-head and MQS-B sensor was linear throughout the range of light intensities during the day ($y = 0.9812x - 0.3335$, $R^2 = 0.9959$, PPFD range 0–1,250 μmol m^{-2} s^{-1}). The relationship between the *MONI*-head and MQS-B sensor did not depend on the angle of incidence of light: plotting *MONI*-head versus MQS-B response at an angle of incidence of 55° and 80° yielded similar slopes of first-order regressions (the ratio of slopes, slope 80°/slope 55°, was 1.01 with standard deviation of 0.06; $n = 3$). Overall, the PPDF estimates of the *MONI*-head gave satisfactory estimates of the incident light reaching the leaf, which facilitates the accurate estimation of the electron transport rate (ETR).

Finally, the built-in *MONI*-head temperature sensor was compared in the field against a thermocouple (TC) placed next to the needles in the sample holder. The relationship between *MONI*-head temperature sensor and TC was linear throughout the range of temperatures ($y = 0.9989x + 0.2695$, $R^2 = 0.89$, Temperature range: 0–20°C). A closer evaluation, however, revealed that differences between the sensors increased with increasing temperatures and reached values of ±5°C at 15°C.Correspondingly, linear regressions analysis yielded a value of R^2 of 0.9227 when the TC ranged from 0 to 10°C but an R^2 of 0.2485 was observed in the range of 10–20°C. The sign of the difference between TC temperature and $PPFD_{SL}$ did not reveal any relationship with environmental conditions like, for example, direct exposure to sunlight. Overall, the temperature estimate of the *MONI*-head should be used only as an approximation.

Seasonal acclimation of PSII

The *MONI-PAM* was employed to follow the seasonal acclimation of PSII to the natural environment in Scots pine needles. Left and right panels of Fig. 2, respectively, depict the results obtained with needles from a top branch of Scots pine before and after the spring recovery of photosynthesis. By definition, the daytime F_t and F'_m becomes F_0 and F_m chlorophyll fluorescence levels, respectively, during the night. Accordingly, fluorescence provides information on PSII operating efficiency, Φ_P, during the day and maximum photochemical yield of PSII, Φ_{Pmax}, during the night (Fig. 2c, h).

The Φ_{Pmax} increased from March 25 to 29 (Fig. 2c). The recovery of Φ_{Pmax} was characterized by maximum daytime temperatures above 15°C (Fig. 2b). Therefore, it is reasonable to assume that high-daytime temperatures triggered the rise of Φ_{Pmax}. Both, F_0 and F_m level fluorescence recovered concomitantly with Φ_{Pmax} (Fig. 2d, e). In particular, the increase in F_m is consistent with relaxation of sustained non-photochemical dissipation of excitation energy. After March 29, temperatures decreased again and night frosts appeared (Fig. 2b) and the F_0, F_m and Φ_{Pmax} decreased (Fig. 2d, e). Again, the long-term data for F_m indicated that sustained non-photochemical energy dissipation, causing decreasing Φ_{Pmax} values, builds up with falling temperatures.

The Φ_{Pmax}, together with F_m, decreased during some cold nights in March and early April (Fig. 2c, e). Similar decreases during cold nights have been observed when measuring greenhouse-grown grapevine leaves and these observations were attributed to photoinhibitory effects of saturating light pulses (Flexas et al. 2000). To test this idea, we disconnected two of the four *MONI*-heads for 4 h at night, and compared the change in F_m before and after the 4-h period. The results showed that F_m had decreased by 3.7% and 3.5% in the needles receiving saturating pulse, whereas F_m decreased by 0.4% and increased by 1.3% in the absence of saturating pulses. Therefore, photoinhibitory action of saturating pulses during cold March nights very probably caused the decreases in F_m and in Φ_{Pmax} values. Similarly, saturating light intensities could also explain why some needles presented visible signs of damage after being monitored for several days during cold winter conditions (not shown).

To alleviate the side effects of saturating pulses, we increased the interval between pulses from 5 to 10 min: nighttime depression of Φ_{Pmax} was still occasionally observed in cold nights during April. It is advisable, therefore, to decrease the frequency of saturating pulses during cold nights even further (e.g. every hour) and, during daytime, carry out measurements at higher frequencies (e.g. 5–10 min). The latter regime of data collection would still permit long- and short-term adjustments in PSII to be followed.

That the levels of F_t and F_m during June were lower than those during March (compare Fig. 2d, e with 2h, i) was predominantly caused by a sample change; i.e. a smaller leaf area was probed in June than in March. This re-emphasizes the importance of maintaining a constant area under examination to draw accurate conclusions on the true fluorescence levels. The latter intensity variations, however, do not influence the Φ_{Pmax} which was close to 0.8 in June but always below 0.65 in March measurements (Fig. 2c, h). We conclude from the low Φ_{Pmax} that sustained non-photochemical dissipation of energy did not completely disappear during the warm temperature phase in March.

Changes in leaf absorptance due to chlorophyll synthesis or changes of other optical properties (cf. Pfündel et al. 2006) might affect the determination of absolute fluorescence levels in long-term experiments. Similarly, changes in connectivity between PSII units will affect F_t, and changes in the relative absorption in cross sections of PSII:PSI will affect the intensity of measured fluorescence. Therefore, parallel investigation of these factors is needed to critically evaluate and correct long-term records of absolute fluorescence signals.

Diurnal acclimation of PSII

Over the diurnal scale, F_t and F'_m provided information on the functioning of the rapid acclimation processes of energy partitioning in PSII. For example, during the cold days in winter, F_t began increasing immediately after sunrise but F'_m began decreasing about 45 min later (Fig. 3a). This phenomenon is consistent with the assumption that the F_t increase corresponds to a reduction of the electron transport chain upon sunrise when low temperatures retard the use of ATP and NADPH by the Calvin cycle, and also the operation of the xanthophyll cycle and formation of ΔpH which both are required for non-photochemical dissipation of the excess excitation energy. Apparently, after 45 min, the build-up of ΔpH and de-epoxidation of violaxanthin into zeaxanthin was sufficient to result in noticeable non-photochemical energy dissipation which is reflected in decreasing F'_m. This phenomenon was observed daily during the first monitoring period in late March and early April. In contrast to cold weather conditions, F'_m decreased immediately after sunrise during warm days (Fig. 3b).

Apart from the seasonal variations observed immediately after sunrise, three general stages could be distinguished in the diurnal variations of F'_m and F_t (Fig. 3a, b): firstly, after sunrise, F'_m decreased and F_t increased denoting increasing

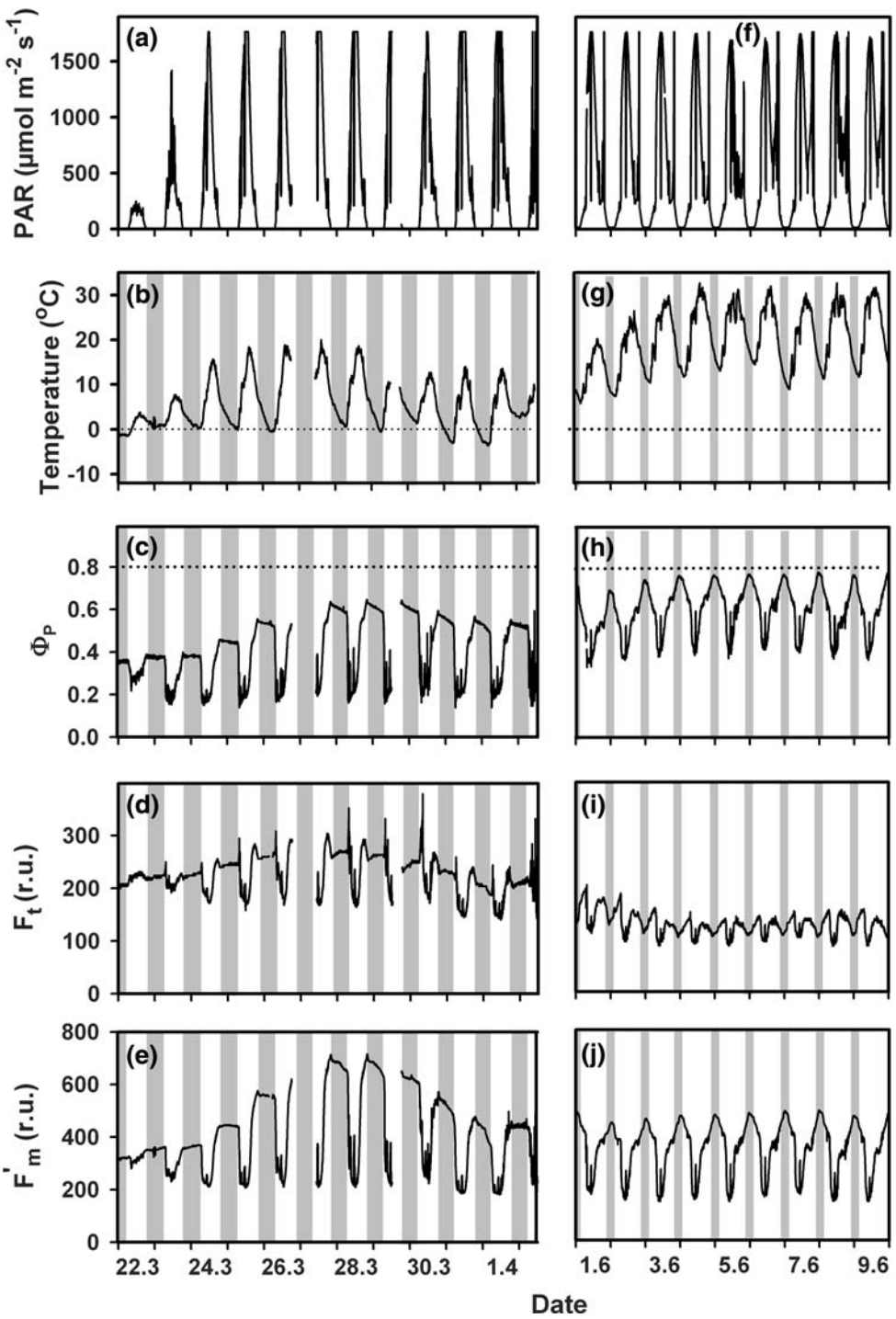

Fig. 2 Seasonal and diurnal changes in photosynthetic photon flux density (PPFD) (**a**, **f**); in air temperature (**b**, **g**); in photochemical yield (**c**, **h**); in current chlorophyll fluorescence intensity (F_t) (**d**, **i**); and in maximum chlorophyll fluorescence intensity (F'_m) (**e**, **j**). Data correspond to two different measuring periods from the end of March to early April (**a**–**e**), and during early June (**f**–**j**). Gray areas indicate nighttime (ambient PPFD = 0). Measurements were carried out every 5 min in the first period (**a**–**e**) and every 10 min in the second period (**f**–**j**)

heat dissipation together with saturation of the electron transport chain; secondly, during the day, both F'_m and F_t changed in a similar manner indicating the rapid adjustment in NPQ to fluctuations in the light environment; and, thirdly, before sunset, F'_m increased and F_t decreased, corresponding to a relaxation of non-photochemical energy dissipation and re-oxidation of the electron transport chain.

MONI-PAM versus other monitoring systems

During the last years several fluorometers have been developed for remote monitoring of the long-term changes in leaf photosynthetic properties. The "Frequency-Induced Pulse Amplitude Modulated" fluorometer (*FIPAM*) (Flexas et al. 2000) or the *Laser-PAM* (Ounis et al. 2001)

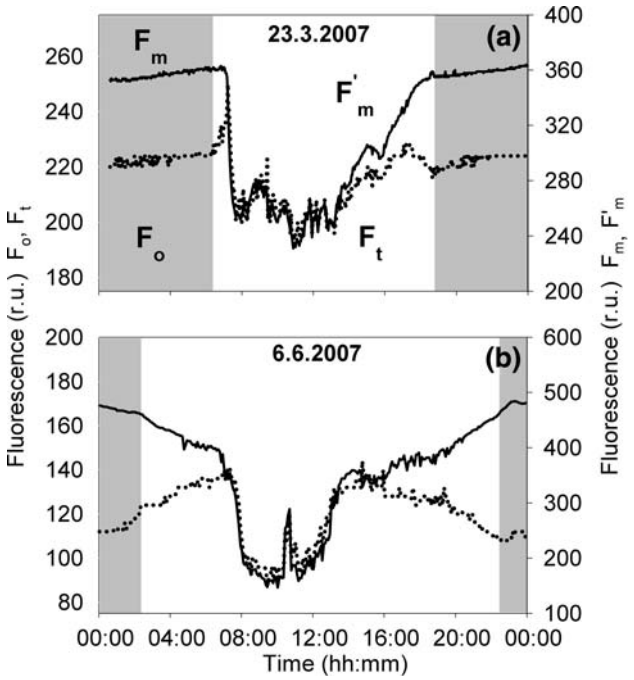

Fig. 3 Diurnal changes in the maximum chlorophyll fluorescence intensity (F'_m, or F_m during the night), and current chlorophyll fluorescence intensity (F_t or F_0 during the night), measured on two different days: (**a**) before the spring recovery of photosynthesis and (**b**) after the recovery. Gray areas represent nighttime (ambient PPFD = 0)

are both based on the saturating-pulse technique combined with fluorescence detection, which permit measurements to be made up to a few meters from the leaf. Other types of monitoring fluorometers utilize sunlight and measure the current fluorescence level (F_t); for example, the "Passive Multi-wavelenght Fluorescence Detector" (*PMFD*; see Louis et al. 2005) which measures F_t based on the Fraunhofer-lines principle (Moya et al. 2004). Such fluorometers are capable of measuring at greater distances (>10 m), but they do not supply a saturating light pulse to the leaf and hence do not measure F_m. Some intermediate technologies use sub-saturating light pulses and operate at intermediate distances of 5–30 m; for example, the "Laser-Induced Fluorescence Transient" (*LIFT*; see Ananyev et al. 2005). In this context, the *MONI-PAM* overlaps its application niche with the *FIPAM* or *Laser-PAM*. However, the main difference between these instruments is that the *MONI-PAM* is physically attached to the leaf and moves together with it, hence it has proved to be particularly suitable for long-term measurements under field conditions (i.e. rain, wind, and variable temperatures) by maintaining a constant leaf area under examination and, therefore, facilitating the interpretation of changes in F_0, F_t, F'_m, and F_m, levels throughout the monitoring period.

Conclusion

The new *MONI-PAM* is able to record continuously, and with adequate resolution, short- and long-term acclimation of photochemical and non-photochemical utilization of absorbed light energy by PSII under realistic field conditions. In particular, the fluorometer system provides the data required to assess seasonal adjustments of sustained NPQ which are difficult to obtain using conventional fluorometers.

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