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Excitation kinetics during induction of chlorophyll *a* fluorescence

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Abstract

We present a chlorophyll fluorometer module system which adapts the intensity to the individual leaf sample by adjusting the quantum flux density of the excitation light so that the fluorescence signal is kept constant. This is achieved by means of a feedback power adjustment of the fluorescence exciting laser diode. Thus, the intensity of the excitation light is adapted to the actual need of a particular sample for quantum conversion without applying exaggeratedly high quantum flux density. We demonstrate the influence of the initial laser power chosen at the onset of irradiation and kept constant during fluorescence rise transient within the first second. Examples are shown for measuring upper and lower leaf sides, a single leaf with different pre-darkening periods, as well as yellow, light green and dark green leaves. The novel excitation kinetics during the induction of chlorophyll fluorescence can be used to study the yield and regulation of photosynthesis and its related non-photochemical processes for an individual leaf. It allows not only to sense the present state of pre-darkening or pre-irradiation but also the light environment the leaf has experienced during its growth and development. Thus, the individual physiological capacity and plasticity of each leaf sample can be sensed being of high importance for basic and applied ecophysiological research which makes this new methodology both innovative and informative.

Additional key words: chlorophyll fluorescence; chlorophyll fluorometer module system; induction kinetics; irradiation; Kautsky effect; light adaptation.

Introduction

Induction kinetics (transients) of the chlorophyll (Chl) fluorescence are widely used in basic and applied photosynthesis research (Papageorgiou and Govindjee 2004). For inducing photosynthetic activity an actinic light is used which should be saturating when measured with continuous excitation irradiation (Strasser *et al.* 2004) or which should not be saturating when measured with pulsed excitation irradiation (PAM measurements) (Schreiber 2004). With continuous irradiation, the Chl fluorescence measured is excited by the same irradiance which also induces photosynthetic activity. With PAM measurements, only Chl fluorescence is measured which is excited by the extremely low intensity pulsed light and photosynthesis is induced by an additional constant ('actinic') light, which also excites Chl fluorescence but this constant Chl fluorescence is excluded from the measurement by a phase sensitive detection. The intensity of the light source is kept constant throughout the measurement and irrespectively of the sample used, *i.e.* one uses the same quantum fluence rate for leaf samples with high or low irradiance demand. There is a protocol for increasing or decreasing the intensity of the actinic light in several definite steps within a few minutes. With these 'rapid light curves' introduced by White and Critchley (1999) and later on applied for different samples (Ralph and Gademann 2005, Rascher *et al.* 2000,

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Abbreviations: Chl – chlorophyll; LP – laser power; LP_{max} – maximum laser power; F_0 – ground fluorescence in the dark adapted state (excitation with measuring light only); F_m – maximum fluorescence in the dark adapted state (measuring light plus saturation pulse); FMM – fluorometer module; F_P – maximum (peak) of the induction kinetics; F_{REF} – reference fluorescence to which the laser power is adjusted (either at 690 or 735 nm); F_s – steady state fluorescence at the end of the induction kinetics; PAM – Pulse Amplitude Modulation.
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Ritchie 2008), one is able to study the response of leaves to an equal set of sudden changes in irradiance conditions and judge the potential photosynthetic activity over a wide range of irradiances. Nedbal and Březina (2002) proposed the application of varying irradiance (harmonic irradiation) for judging the light adaptation of a photosynthetic organism, concluding later that a negative feedback regulation is responsible for the nonlinear modulation of photosynthetic activity in plants (Nedbal *et al.* 2003).

The Chl fluorescence transients of a leaf measured at the transfer from dark to light conditions are strongly influenced by the time periods of pre-darkening and/or pre-irradiation that determine the efficiency of energy transfer towards the two photosystems and the usage of ATP and $NADPH + H^+$ in the Calvin-Benson cycle (Nedbal and Koblížek 2006).

We present an instrument which uses a feedback power adjustment to adapt excitation light in order to maintain a constant fluorescence signal. Thus the intensity of the excitation light is adapted to the individual sample. In PAM measurements, 'saturating flashes' are often applied using the same quantum fluence rate several times higher than natural sunlight (= 2000 μmol $m^{-2}s^{-1}$), which – depending on the leaf type – may cause photoinhibition. On the other hand, it was demonstrated that quanta fluence rates of 13,000 μmol $m^{-2}s^{-1}$ are needed to achieve a real maximum fluorescence (Karageorgou *et al.* 2007).

The novel excitation kinetics allow studying the efficiency of photosynthesis and its physiological plasticity for an individual leaf sample taking into account the light environment the leaf has experienced during its growth and development.

Materials and methods

The leaves used in most of the experiments were taken from an indoor potted fig plant (*Ficus benjaminii* L.). Before starting the fluorescence measurements, the leaves were dark adapted for at least 20 min.

The fluorescence measurement was carried out with a chlorophyll fluorometer module system (FMM) (Fig. 1), which is based on its predecessor chlorophyll fluorometer (CFM) (Barócsi *et al.* 2000, 2003). The main instrument is equipped with a three-branch optical fiber and a sample holder. The sample holder consists of an eight-stage numbered revolvable disc with sample plates to which the fiber bundle is fixed. The main features of the new FMM system are its modularity (the device can be application optimized without system redesign) and watertight construction.

The keypad and display module (*FMM-1-LCD*) serves as the user interface of the instrument. The operation mode and data are shown on a 64 by 128 graphic dot-matrix liquid crystal display (LCD, *Lascar SP 5-GFX1*, Whiteparish, UK). The touch-button keypad consists of a wake-up and 12 function keys. In the present tests the optional module *FMM-2-GPS* (global positioning function) has not been used.

The central processing unit (module *FMM-3-CPU*) consists of a *CMD16686GX* single board computer (*Real Time Devices*, Pennsylvania, USA) with 128 MB of RAM and a 300 MHz *National Semiconductors Geode GX1 MMX* enhanced microprocessor. The instrument control program (firmware) and the recorded data are stored in a non-volatile Disk-On-Chip flash storage device (*M-Systems MD2202-D32*, Port Coquitlam, Canada). The data transfer and firmware update are performed through one of the serial ports.

The ISA-bus is decoded by a complex programmable logic device (*CPLD, Lattice M4A5-96/48*, Hillsboro, USA) containing, in addition, an 8-bit parallel-to-serial converter for the display, keypad readout, communication to the internal microcontroller, and the select lines together with a 16-bit parallel-to-serial interface for programming the digital potentiometers, necessary for setting the detector amplifier gain and offset as well as the light source intensities.

In module *FMM-4-ADC*, there are two 12-bit 4-channel simultaneous sampling analog-to-digital converters (*Analog Devices AD7864-2*, Norwood, USA) with low noise of $\pm$1 LSB (least significant bit: 1 LSB corresponds to 1 count of the 4095 maximum counts). An internal peripheral interface controller (*PIC, Microchip PIC18F876*, Wels, Austria) is used for hardware timing and triggering as well as for power-management functions.

In module *FMM-5-LDD*, a combination of interference filters (*NT43-089* for 690 nm and *NT43-091* for 730 nm, *Edmund Optics*; full width at half maximum: 10 nm each) and cut-off filters (665 nm *RG665*, *Edmund Optics*, Karlsruhe,Germany) are used to separate the detection wavelengths and to mask the scattered irradiation light. Low noise PIN photodetectors (*SD-200-14-21-241*, *Laser Components*, Olching, Germany) are applied with electrometer preamplifiers (*OPA129*, *Texas Instruments–Burr Brown*, Freising, Germany) and precision low noise resistors (*Vishay CMF55* series, Selb. Germany) for photocurrent-to-voltage conversion.

The internal light source is a 635 nm laser diode (*DL-4038-021*, *Sanyo*, München, Germany) with 10 mW maximum optical power. Fast, monolithic laser diode driver circuit is applied to minimize the power-on transients (*AD9661A*, *Analog Devices*) and realize digital optical power adjustment with linear response. A safety interlock circuitry monitors the laser diode temperature with calibrated temperature readout presented on the display.

A unique three-branch mixed optical fiber bundle is prepared to guide the laser beam onto the plant sample

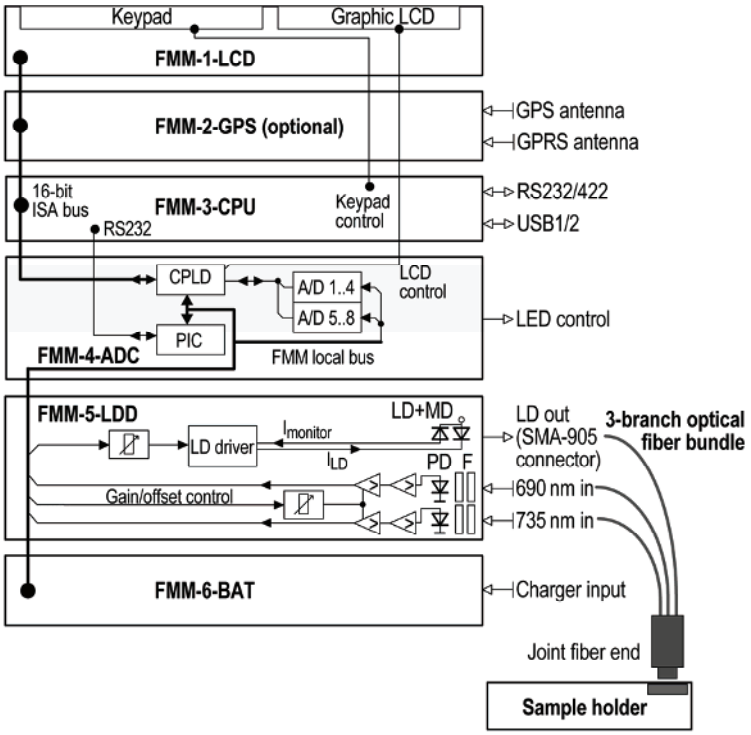

Fig. 1. Block scheme of the fluorometer module (FMM) system. The modules are from top to bottom: *FMM-1-LCD* (keypad and display), *FMM-2-GPS* (GPS/GPRS, global positioning and general packet radio service systems, respectively, optional), *FMM-3-CPU* (PC/104 single board computer), *FMM-4-ADC* (data acquisition and control), *FMM-5-LDD* (laser and detector unit) and *FMM-6-BAT* (battery). Further abbreviations not mentioned in the text: LD+MD – laser diode with monitor diode, PD – photodiodes, F – optical filters.

and the fluorescence signals back to the detectors. The ends of all three branches are joined together to form a fiber endface which is positioned into the sample holder (as close as 1 mm to the sample surface). The other ends of the three fiber arms are mounted to the instrument body. The laser guiding arm is a single unjacketed plastic fiber with an outer diameter of 2000 µm (*NT02-549*, *Edmund Optics*) and numerical aperture of NA=0.51 delivering 5.6 mW of the 9 mW input laser power. Around this central fiber, 2×39 unjacketed plastic fibers with an outer diameter of 500 µm (*NT02-532*, *Edmund Optics*, NA=0.51) are positioned in a mixed fashion at the fiber endface for the 2 detection wavelengths.

Module *FMM-6-BAT* powers the FMM system by an 11.1 V / 4000 mAh Li-ion mounted battery pack (*CH-UNLI72C04*, *Powerizer*, Richmond, USA).

The FMM allows the measuring of the traditional Kautsky induction kinetic curves detected simultaneously at the two maxima of the Chl *a* fluorescence of leaves (at the 690 nm red and 735 nm far-red bands) as described earlier for the CFM (Barócsi *et al.* 2000). In order to minimize the number of collected data, the hardware timing samples the entire kinetics in quasi-logarithmic time increments (i.e. 0.1 ms during the first 4 ms, 1 ms up to 1 s, 20 ms up to 15 s and later on 200 ms).

The novel excitation kinetics technique is achieved by adjusting a laser power (LP). The initial laser output is user selectable in percent of the maximal laser output power available (~5.6 mW corresponding to 775 µmol m^{-2} s^{-1} at the leaf surface). Recording starts with a preset (initial) LP kept constant within one second and the fluorescence reaches its maximum just as in the conventional induction kinetics measurement. After 1 s, however, the FMM system starts changing (most probably increasing) the LP to keep the fluorescence at the constant level reached after 1 s of irradiation. The time for this is selected so that the fast rise of the fluorescence has surely been elapsed. The LP can then be adjusted in 256 steps so that the fluorescence is kept constant with an error of $\varepsilon = \pm 5$ LSB referenced to the fluorescence value at t = 1 s. This fluorescence (F) reference is calculated as an average of 3 successive F values in order to minimize noise: $F_{REF} = {}^{1}/_{3}\,[F(1\text{ s}) + F(1\text{ s} - 1\text{ ms}) + F(1\text{ s} - 2\text{ ms})]$. The control is carried out by comparing the actual F(t) value with the reference value after the hardware timer tick is handled and the actual F(t) value is measured. If $\text{Abs}\,[F_{REF} - F(t)] > \varepsilon$ then the laser output power is incremented or decremented by 1 count. Then F(t) is measured again before the next hardware tick comes. If the difference is still large (>ε), the LP incrementing or decrementing is repeated (once, at present) and the F(t) is re-measured and stored. Note, that each time the LP is changed in a single count increment or decrement ($\Delta P = 2$ counts): no scaling is calculated to change the power with higher slope to avoid instability of the feedback control loop. If the LP reaches maximum, no feedback is possible any longer: the power is kept at maximum and, consequently, the fluorescence will decay.

During the kinetics measurement, the values are stored for the fluorescence at 690 nm and 735 nm as well as for the LP. For adjusting the LP, one of the two fluorescence bands can be selected as the reference wavelength which is kept constant after 1 s of irradiation.

Results

An example for a measurement of an excitation kinetics is given in Fig. 2 for a fully developed green leaf. During the first second of irradiation, the laser power (LP) was set to a constant value of 20% of the maximum (20% LP_{max}). One can follow the fast rise of the fluorescence intensity to a maximum reached at around 1 second. Later on, LP was adjusted to keep the fluorescence at a constant level. One can observe a slow increase of the LP to a maximum at around 300 seconds and the concomitantly unchanged Chl *a* fluorescence.

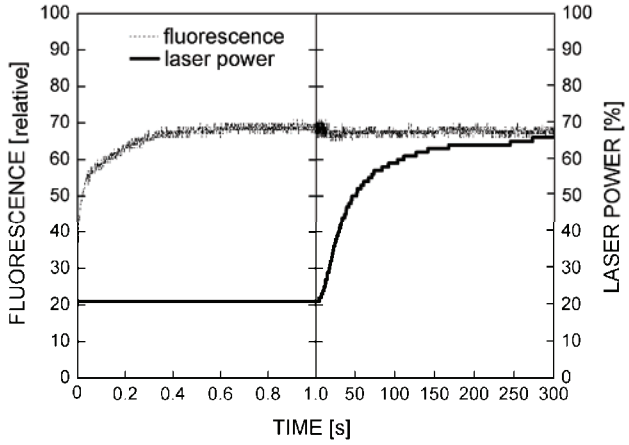

Fig. 2. Example for a measurement of excitation kinetics with a green leaf (*upper side*, *Ficus benjaminii* L.) showing the laser power (LP) and the Chl *a* fluorescence at 690 nm during 300 s of irradiation. During the first second of irradiation (*left*), LP was kept constant at 20% of the LP_{max}. After the first second of irradiation (*right*), LP was automatically adjusted to keep the fluorescence at a constant level.

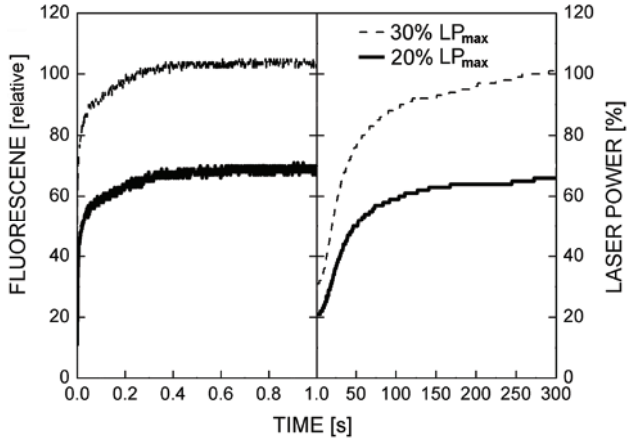

Fig. 3. Comparison of the effects of different initial intensities of excitation light: Excitation kinetics for the Chl *a* fluorescence at 690 nm starting with 20% and 30% maximum laser power (LP_{max}), respectively (upper side of a light-green leaf of *Ficus benjaminii* L.). *Left*: fluorescence during the first second with constant irradiation, *right*: laser power after the first second of irradiation (automatically adjusted to keep the fluorescence constant).

When starting the irradiation with 30% LP_{max} the fluorescence during the first second of irradiation showed a similar rise kinetics but leading to a higher fluorescence maximum than that with 20% LP_{max} (Fig. 3). The increase of LP within the subsequent 300 seconds was clearly slower in the beginning when starting with 20% than with 30% LP_{max}. After 150 s of irradiation, there was a stronger rise for the kinetics with 30% than with 20% of LP_{max}. After 300 s of irradiation a maximum steady state was not even fully reached for both treatments.

When comparing the Chl *a* fluorescence measured simultaneously at the two emission maxima, *i.e*. at 690 and 735 nm, the fluorescence signal was lower at 690 nm and rose faster during the first second of irradiation than the signal at 735 nm (Fig. 4). Subsequently, the fluorescence at 690 nm remained constant due to the adjustment of the LP. However, keeping the 690 nm fluorescence constant resulted in the rise of the fluorescence at 735 nm, at least within the first 100 s.

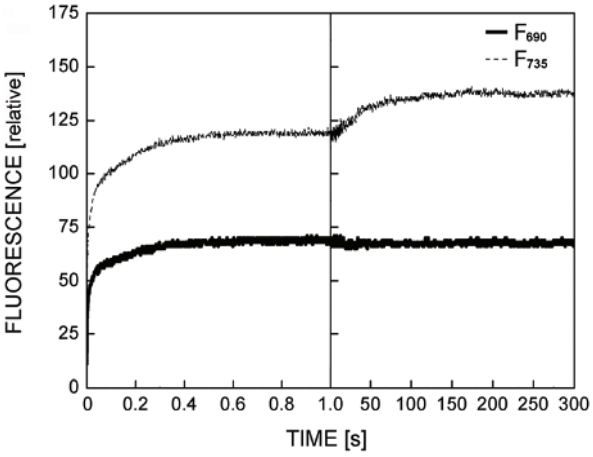

Fig. 4. Comparison of the fluorescence at 690 and 735 nm: Chl *a* fluorescence measured simultaneously at 690 nm (F_{690}) (*solid line*) and 735 nm (F_{735}) (*dashed line*) starting with 20% maximum laser power (LP_{max}) (upper side of a light-green leaf of *Ficus benjaminii* L.). *Left*: fluorescence during the first second with constant irradiation, *right*: fluorescence after the first second of irradiation.

The dark adaptation time before the onset of the fluorescence measurement strongly changed the fluorescence rise detected with constant LP (20% LP_{max}) and the subsequent excitation kinetics (Fig. 5). The first kinetics with the freshly collected leaf sample dark adapted for 30 min showed a fast rise to a high fluorescence maximum within the first second of irradiation and a rather slow increase of LP in the subsequent 5 minutes. The next kinetics, started after a dark period of one minute, exhibited a much lower fluorescence maximum and a much lower increase of LP in the excitation kinetics. After subsequent dark periods of 5, 12 and 30 min, the corresponding fluorescence maxima reached about the

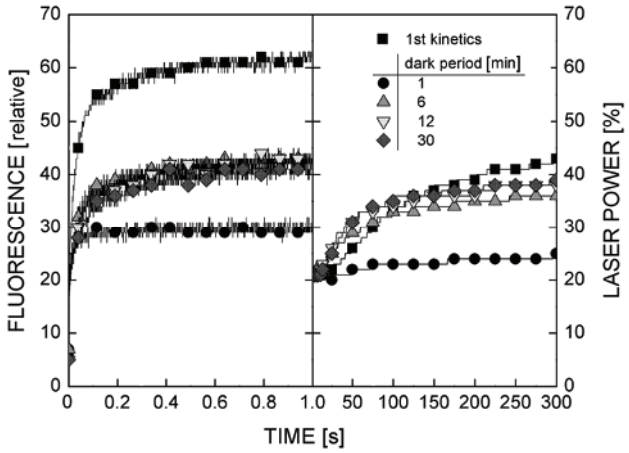

Fig. 5. Variation with different dark adaptation periods: Excitation kinetics for the Chl *a* fluorescence at 690 nm of the upper side of a dark green leaf (*Ficus benjaminii* L.) starting with 20% maximum laser power (LP_{max}). Recording of the '1st kinetics' was carried out after collecting the leaf sample and with a dark adaptation of 30 min, then followed by measurements with 1, 5, 12 and 30 min of dark periods. *Left*: fluorescence during the first second with constant irradiation, *right*: laser power after the first second of irradiation (automatically adjusted to keep the fluorescence constant).

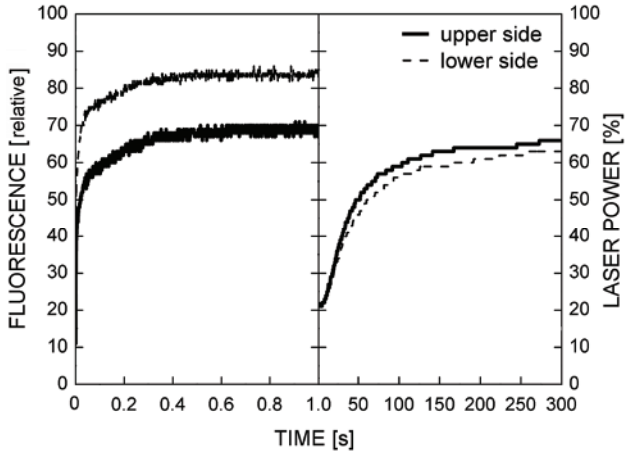

Fig. 6. Comparison of upper and lower leaf side: Excitation kinetics for the Chl *a* fluorescence at 690 nm of the upper and lower leaf side starting with 20% maximum laser power (LP_{max}) (light-green leaf of *Ficus benjaminii* L.). *Left*: fluorescence during the first second with constant irradiation, *right*: laser power after the first second of irradiation (automatically adjusted to keep the fluorescence constant).

Discussion

At the onset of irradiation, a low value of constant fluorescence is reached within nanoseconds (which can not be resolved in the figures presented with a scale expansion of 1 s). This fluorescence level termed O-level (O = origin) corresponds to the F_0-level of PAM measurements which is achieved without induction of photosynthetic activity. The interpretation of fluorescence transients is usually based on the principle that the fluorescence yield depends both on the redox state of Q_A, the primary electron acceptor of photosystem 2 (PS2) as introduced by Duysens and Sweer (1963) and on other processes only indirectly related to the photosynthetic

same level, after 1 second of irradiation, with fluorescence rise curves becoming more slowly with increasing dark period. Each corresponding maximum reached after 1 second of irradiation was clearly higher than that after 1 minute of dark adaptation. Each of the excitation curves after 5, 12 and 30 min of dark showed a rise kinetics close to that of the first one, but with a slightly faster rise and a lower LP_{max} after 5 min of irradiation.

The lower side of a leaf showed a higher Chl *a* fluorescence than the upper side (Fig. 6). However, LP required to keep the fluorescence constant showed similar excitation kinetics for both leaf sides with a slightly lower LP for the lower side.

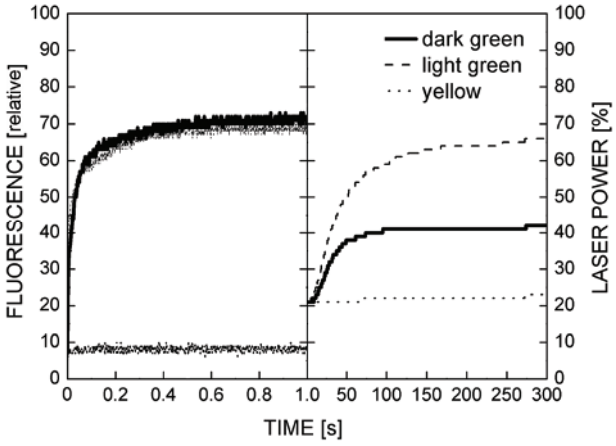

Fig. 7. Comparison of leaves with different Chl *a* content: Excitation kinetics for the Chl *a* fluorescence at 690 nm of a dark green, light green and yellow leaf starting with 20% maximum laser power (LP_{max}) (upper side of a leaf of *Ficus benjaminii* L.). *Left*: fluorescence during the first second with constant irradiation, *right*: laser power after the first second of irradiation (automatically adjusted to keep the fluorescence constant level).

The excitation kinetics were significantly different depending on the intactness of the photosynthetic function of leaves (Fig. 7). During the first second of irradiation, a dark green and a light green leaf showed a similar fluorescence rise whereas a yellow leaf exhibited only a low Chl *a* fluorescence with nearly no variation. During the subsequent 300 s, the LP remained unchanged for the yellow leaf at around 20% LP_{max}, but increased to 40% LP_{max} for the dark green leaf and to 65% LP_{max} for the light green leaf. The kinetics for the light green leaf was somewhat more slowly than that for the dark green leaf.

electron transport which are usually summarized under the term 'non-photochemical quenching' (Govindjee 2004). During the first second of irradiation the fluorescence rises from the F_0-level to a maximum (P-level or F_P, P = peak) due to the reduction of the redox substances downstream of the reaction center of PS2. In a dark period of at least 20 min prior to the onset of irradiation, these redox substances are oxidized (Strasser *et al.* 2004) and the several key enzymes of the Calvin-Benson cycle are de-activated (Buchanan 1980). Upon the onset of irradiation, the oxidized redox substances are reduced within 1 s (or less) and remain in the reduced state (the reaction centre is said to be 'closed') since they can not give off their electrons into the photosynthetic electron transport chain towards photosystem 1 (PS1) (Papageorgiou 1975, Bolhàr-Nordenkampf and Öquist 1993). Recently, the quantitative characterization of the induction of photosynthetic electron transport via a multi-exponential analysis of the fluorescence rise has been presented (Antal and Rubin 2008). The complete electron transport chain with the two photosystems working in series (state 2) becomes active only in the subsequent seconds and minutes of irradiation (Fork and Satoh 1986). The rise kinetics and the area over the fluorescence curve has been termed 'complementary area' have been used to determine the quantity of redox substances and the functioning of electron transport rate around PS2 (Lavorel *et al.* 1986).

A faster rise kinetics of LP (Fig. 6,7) can be explained by a more efficient light usage. The missing rise kinetics in the yellow leaf (Fig. 7) is, however, an indication of disturbance of energy transfer in the antenna system or electron transport which can also be seen in the missing fluorescence rise within the first second of irradiation (Fig. 7, *left*).

When the pre-darkening time is shortened, F_p reached after 1 s of irradiation becomes smaller (Fig. 5, *left*). With a pre-darkening time of 30 min, the pre-irradiated sample exhibits a lower F_P than the non-irradiated sample (1^{st} kinetics). This shows that 30 min of pre-darkening is not enough to oxidize all redox substances between PS2 and PS1 in pre-irradiated samples and to achieve a dark adaptation as for the first kinetics in which the sample was not pre-irradiated but kept in the dim laboratory light before the 30 min pre-darkening. The strong influence of quanta fluence rate applied during a pre-irradiation phase has been demonstrated recently (Schansker *et al.* 2006).

When applying a higher intensity of the incident light, the redox substances are faster reduced and the fluorescence rise is faster accomplished than at lower irradiance (Fig. 3). Under saturating irradiance, the ratio F_v/F_m of the 'variable' fluorescence (F_v) influenced by the photosynthetic activity ($F_v = F_m - F_0$) and the maximum fluorescence (F_m) is a measure of the maximum photosynthetic quantum yield (Kitajima and Butler 1975). With F_P detected when exciting with the non-saturating irradiance applied by the FMM, a similar ratio $(F_P - F_0)/F_P$ can be used for determining a relative photosynthetic quantum yield. This parameter can be used for comparison of different samples measured with the same initial LP.

When keeping the intensity of the excitation light constant after the first second of irradiation, the fluorescence intensity decreases slowly from the maximum to a steady state (F_s) reached after about 300 seconds. This is the time during which the linear photosynthetic electron transport from PS2 to PS1 and further on to NADPH is fully established. This electron transport depends on the connection of the light harvesting systems to the appropriate reaction centers of PS2 and PS1, respectively (state transitions) and the onset of the enzymatic Calvin-Benson cycle which consumes ATP and NADPH formed by the electron transport chain. The slow fluorescence decrease, reflecting the gradual oxidation of the redox substances fully reduced at the maximum of the fluorescence kinetics, is generally termed 'photochemical quenching'. In addition to this, the Chl *a* fluorescence decrease within the first 300 s of irradiation is also interpreted in terms of 'non-photochemical quenching' which summarizes the effects of the proton gradient, state transition and photoinhibition during the induction kinetics (Krause and Weis 1991, Maxwell and Johnson 2000).

Following the first second of irradiation, we adjusted the intensity of the exciting laser to the conditions of the leaf samples established by their antenna systems and electron transport components formed during earlier times of growth and development. Thus, we sense the uptake and consumption of energy for each individual sample instead of the usual procedure of confronting all samples with the same constant high (often more than saturating) irradiance.

In contrast to the existing rapid light curves that change irradiance for several seconds without waiting for a steady state reaction, in our excitation kinetics the incident light is continuously adjusted to the reaction of the leaf sample. Rapid light curves show the potential reaction to a wide range of different irradiances under non-steady state conditions, whereas with excitation kinetics the real individual response is sensed. The shape of the excitation kinetics characterizes the adaptation of the leaf to the light. As with 'forced harmonic irradiance' (Nedbal and Březina 2002), internal dynamics of the processes regulating photosynthetic activity can be monitored by excitation kinetics.

Fluorescence of leaves associated with photosynthetic activity is emitted only from Chl *a*. There are emission maxima at 690 nm (F_{690}) and 735 nm (F_{735}) which do not change their position during the induction kinetics (Buschmann and Schrey 1981). The ratio between the two maxima depends on the Chl *a* concentration of the leaf tissue since the short wavelength fluorescence at 690 nm overlaps with the absorption maximum of Chl *a* at 680 nm. The fluorescence at 690 nm is strongly re-

absorbed on its way to the leaf surface where it can be detected and thus the ratio F_{690}/F_{735} decreases with increasing Chl *a* content of the leaf (Buschmann 2007). During the induction kinetics, the ratio F_{690}/F_{735} decreases (Buschmann and Schrey 1981), in part, due to the faster kinetics of F_{690} emitted mainly from the vicinity of the sample surface where it receives higher irradiance and, in part, due to the gradual appearance of (otherwise non-variable) PS1 fluorescence during state 1/state 2 transition which is sensed mainly in F_{735} (with a maximum at 715 nm: Lombard and Strasser 1984). Therefore, when adjusting LP to keep the F_{690} fluorescence constant, the F_{735} signal increases slightly (Fig. 4).

The required increase of LP for adjusting the fluorescence to a constant level during the 300 s of the excitation kinetics monitors the rise of the energy input to achieve the steady state of the photosynthetic electron transport and its related processes which are usually summed up as non-photochemical processes (pH gradient at the thylakoid, state transitions and photoinhibition). With short times of pre-darkening (Fig. 5, *right*) or with reduced photosynthetic activity of yellow leaf (Fig. 7), only a small rise of energy input is required. With longer pre-darkening periods of 5, 12 and 30 min (Fig. 5) and fully intact leaves (Figures 2, 3, 6, and 7: green leaves), LP has to be increased by a factor of 2-3 indicating a stronger demand for light energy to achieve the maximum steady state of PS2 activity. The difference in the rise of LP in the excitation kinetics can be interpreted in terms of adaptation ability to the light environment and is taken to characterize samples. Thus, the excitation kinetics rises more slowly when starting with 30% LP_{max} (Fig. 3), without pre-irradiation (Fig. 5: 1st kinetics), on the upper leaf side (Fig. 6), and in light green leaves (Fig. 7), as compared to the respective counterpart samples.

In conclusion, the novel excitation kinetics affords a new look from a different angle which will need further studies to understand the regulation mechanisms of photochemical and non-photochemical processes. Measurements with the FMM can be used to study the efficiency of photosynthesis for an individual leaf. It allows not only to sense the present state of pre-darkening or pre-irradiation but also the light environment which the leaf has experienced during its growth and development. Thus, the individual physiological capacity and plasticity of each leaf sample can be sensed, which is of high importance for basic and applied ecophysiological research and makes this new methodology both innovative and informative.

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