Chapter 35

Chlorophyll Fluorescence as a Reporter on in vivo Electron Transport and Regulation in Plants

Ladislav Nedbal[1,2,*] and Michal Koblížek[2,3]
[1]Laboratory of Systems Biology, Institute of Landscape Ecology and [2]Institute of Physical Biology, Zámek 136, 37333 Nové Hrady, Czech Republic; [3]Laboratory of Photosynthesis, Institute of Microbiology, 379 81 Třeboň, Czech Republic

Summary 507
I. Introduction 508
II. Time Scales 508
A. Before Photochemistry: In Hundreds of Picoseconds and Earlier 508
B. Photochemistry and Soon Thereafter: From Nanoseconds to Seconds 510
C. Regulation and Development: From Seconds to Lifetime? 510
III. Analysis of Chlorophyll Fluorescence Transients 511
A. Flash of Light Removes Photochemical Quenching 511
B. Single-turnover Induction Reveals Antenna Functional Cross Section and Connectivity 512
C. Multi-turnover Induction Reflects Complexity 513
D. Non-photochemical Quenching Reflects Photoprotection 513
IV. Beyond the Conventional Analysis 515
A. Modulation by Redox Components 515
B. PS II Heterogeneity 516
V. Prospects of the Technique and Instrumentation 516
Acknowledgments 516
References 516

Summary

Transients of chlorophyll (Chl) fluorescence emission are widely used to estimate kinetics, yield and regulation of photosynthetic processes in intact plants. In this chapter, we introduce concepts and terms required for a qualified application of the technique. An overview of relevant processes that occur on different timescales, from picoseconds to organism lifetime, is provided as a reference framework for description of approximations and models connecting physiologically relevant photosynthetic parameters and the fluorescence data. Reaching beyond the conventional analysis, we also describe models including Photosystem II heterogeneity and short-living radicals that can affect plant-Chl fluorescence emission. Current state-of-the-art and future prospects for Chl-fluorescence instrumentation are described at the end of the chapter.

*Author for correspondence, email: nedbal@greentech.cz

Bernhard Grimm, Robert J. Porra, Wolfhart Rüdiger and Hugo Scheer (eds): Chlorophylls and Bacteriochlorophylls: Biochemistry, Biophysics, Functions and Applications, pp. 507–519.

I. Introduction

A rapid transition from dark to light elicits in plants a complex transient of fluorescence emission that was first reported in late 19th century by Müller (1874) and was then introduced to modern science more than 70 years ago by Kautsky and Hirsch (1931). At present, the dynamic changes in the chlorophyll (Chl) fluorescence emission are widely used as a reporter on photosynthetic activity and regulation in plants (Dau, 1994; Govindjee, 1995; Falkowski and Kolber, 1995; Kramer and Crofts, 1996; Strasser et al., 1998; Lazár, 1999; Krause and Jahns, 2002). The technique is well established in photosynthesis research with emerging applications in ecology, biotechnology and in precision farming (Bolhár-Nordenkampf et al., 1989; Mohammed et al., 1995; Lichtenthaler and Miehé, 1997; Jalink et al., 1998; DeEll et al., 1999; Nedbal et al., 2000).

Abbreviations: a – optical cross section; F_K, F_I, F_J, F_P – intermediate fluorescence levels of the fluorescence induction curve measured during a transition from dark adapted to light adapted state of a plant; F_0 – fluorescence emission of a dark adapted plant with the primary acceptor Q_A oxidized and non-photochemical quenching inactive; F_0' – fluorescence emission of a light adapted plant measured with the primary acceptor Q_A oxidized and non-photochemical quenching active ($F_0' < F_0$); F_M – fluorescence emission of a dark adapted plant exposed to a brief pulse of a strong light leading to a transient reduction of Q_A without induction of non-photochemical quenching; F_M' – fluorescence emission of a light adapted plant measured during a strong pulse of light with Q_A and the plastoquinone pool reduced and non-photochemical quenching active ($F_M' < F_M$); F_V, F_V' – variable fluorescence measured in dark-adapted ($F_V = F_M - F_0$) and in light-adapted plants ($F_V' = F_M' - F_0'$); Chl – chlorophyll; Cyt b_6f– cytochrome b_6f complex; *DCMU* – 3-(3,4-dichlorophenyl)-1,1′-dimethylurea; Fd – ferredoxin; FNR – ferredoxin-NADP$^+$ oxidoreductase; I – irradiance; J – parameter quantifying the inter-unit exciton transfer and the resulting sigmoidicity of the fluorescence induction curve ($J \equiv p/(1-p)$); k_P – rate constant of primary photochemistry in the reaction center of PS II; k_N – rate constant of non-radiative energy dissipation; k_F – rate constant of fluorescence emission; N – number of PS II units; *NPQ* – Stern-Volmer non-photochemical quenching parameter; p – probability that an exciton captured by a PS II antenna will migrate to antenna of a neighboring PS II reaction center; P680 – primary electron donor of Photosystem II; P700 – primary electron donor of Photosystem I; PC – plastocyanin; Phe – pheophytin; PS I – Photosystem I; PS II – Photosystem II; PS II^{open} – fraction of open reaction centers; PS II^{closed} – fraction of closed reaction centers; Q_A, Q_B – primary and secondary quinone acceptors of PS II; q_P – photochemical quenching; q_N – non-photochemical quenching parameter; S_n – oxidation states of manganese oxygen-evolving cluster; t – time; Y_z – tyrosine Z, secondary electron donor of PS II; Φ_p^{max}, $\Phi_p(t)$ – maximum and effective photochemical yield of PS II; $\sigma_{PS\,II}$ – functional cross section of PS II.

Plant Chl fluorescence originates predominantly from the lowest excited singlet state of Chl *a* adjacent to the Photosystem II (PS II) reaction center which, in turn, drives primary charge separation. Since the fluorescence emission competes with photochemistry for excitation energy, its measurement provides non-invasive and real-time insight into the dynamics of photosynthetic reactions.

The primary photosynthetic reactions are accomplished in a complex network of mutually coupled linear as well as cyclic processes that serve to transform light into chemical energy (Fig. l., reviewed in Ort and Yocum, 1996). The chemical energy, in the form of ATP and NADPH·H$^+$, is subsequently utilized in the dark phase of photosynthesis to assimilate inorganic carbon (Leegood et al., 2000). As in any complex metabolic system (Csete and Doyle, 2002), robustness of photosynthesis relies on a number of regulatory processes that sustain, coordinate and optimize its function in an uncertain dynamic environment. The complexity of forward redox reactions (Fig. l) combined with feedback regulations (Nedbal and Březina, 2002; Nedbal et al., 2003, 2005) is reflected in complex fluorescence transients occurring in response of plants to a light stimulus. The regulation makes the system-level response of photosynthesis non-linear with significant memory effects.

II. Time Scales

A. Before Photochemistry: In Hundreds of Picoseconds and Earlier

A photon of visible light has a wavelength comparable to the size of a thylakoid granum (Mehta et al., 1999) leading to increased scattering and optical path-length within the photosynthetic membranes. Photons cover the granum diameter in several femtoseconds (~10^{-15} s). If absorbed, its energy brings the pigment molecule to an initially de-localized excited singlet state, called an exciton. The exciton interacts with nuclear vibrations allowing thermal equilibration into the lowest excited singlet state of pigment molecule(s). The electronic dipole-dipole intermolecular interactions cause rapid movement of the molecular excited state towards Chl *a* in pigment-protein complexes of PS I or PS II (Fig. l). The equilibration within the Chl *a* pigment bed occurs effectively on a time scale comparable to the period of nuclear vibrations (~10^{-12} s), which makes the fate of

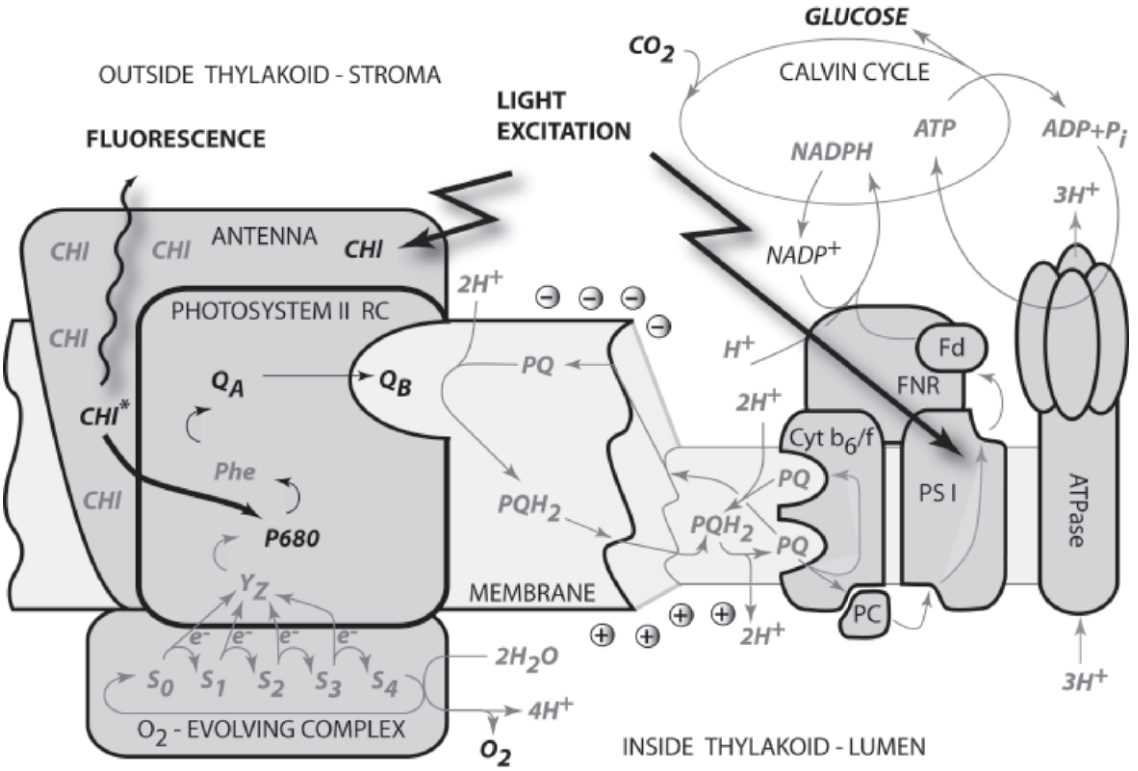

Fig. 1. Schematic presentation of a thylakoid membrane with key photosynthetic modules. Light is absorbed by antenna pigments of PS II (in front) and of PS I (at the back). The excitons generated in the antennae are rapidly transferred to the reaction centers where their energy serves to drive the primary charge separation. In PS II, the primary charge separation to $P680^{+\bullet}$-$Phe^{-\bullet}$ is followed by secondary charge transfer processes: the electrons are extracted by the oxidized primary donor $P680^{+\bullet}$ from water by the O_2-evolving complex and by the Y_Z donor. On the acceptor side, the electron is rapidly stabilized by a transfer from pheophytin ($Phe^{-\bullet}$) to the primary quinone acceptor Q_A. A mobile plastoquinone pool shuttles two electrons sequentially taken from $Q_A^{-\bullet}$ and two protons taken from the stromal side of the membrane to the lumenal side of cytochrome b_6f complex where the protons are released and electrons are sent to PS I. The PS I is using the excitonic energy to generate reductant $NADPH{\cdot}H^+$. The charge transfer reactions in the thylakoid membrane result in an accumulation of protons on the lumenal side and depletion on the stromal side of the thylakoid. The difference in electrochemical potentials is used by ATPase to generate ATP that is used together with $NADPH{\cdot}H^+$ in the Calvin-Benson cycle to assimilate inorganic CO_2 into sugars.

the excitation largely independent of the wavelength of the absorbed photons[1]. With the major fraction of the absorbed photons used for photochemistry in the reaction centers, only several percent are lost by fluorescence emission, thermal de-excitation or intersystem crossing (Latimer et al., 1956).

In higher plants at a physiological temperature, most of the total Chl fluorescence emission originates from PS II. At 683 nm, PS II contributes dominantly, with only a few percent of the total emission originating in PS I (Roelofs et al., 1992). The small fraction of Chl fluorescence that is emitted from PS I and from accessory pigment systems can be distinguished from the dominant PS II emission by its emission wavelength: PS II antenna emits fluorescence that is typically blue-shifted[2], and PS I emits fluorescence that is red-shifted relative to fluorescence from PS II core (Genty et al., 1990). The proportion of PS I fluorescence can increase several times in the long-wavelength range above 700 nm and at low temperatures.

[1] In contrast, fluorescence emission spectra depend on excitation wavelength in organisms such as cyanobacteria or red algae that have spectrally more widely spread peripheral antenna systems transferring excitonic energy less efficiently than the antennae of higher plants. Upon their excitation, the fluorescence emission occurs also from pigments with their singlet excited states higher than those of Chl.

[2] For an exception see Koehne et al., 1999.

B. Photochemistry and Soon Thereafter: From Nanoseconds to Seconds

Upon excitation of the primary donor P680 in the PS II reaction center (Fig. 1), a transient steady-state equilibrium is established between the excited state P680*-Phe and the radical pair state $P680^{+\bullet}$-$Phe^{-\bullet}$ (Schatz et al., 1988). The probability that the separated charges in $P680^{+\bullet}$-$Phe^{-\bullet}$ recombine is reduced in an open PS II reaction center by a rapid transfer of the electron from $Phe^{-\bullet}$ to the primary quinone acceptor Q_A (Fig. 1). The oxidized $P680^{+\bullet}$ accepts an electron from the tyrosine residue Y_Z that, subsequently, oxidizes the manganese cluster, advancing its redox state by one step from S_n to S_{n+1}. Photochemistry in the open PS II reaction centers shortens the excitation lifetime to hundreds of picoseconds, thereby lowering the fluorescence quantum yield to its minimum level F_0 — a phenomenon called photochemical quenching (Duysens and Sweers, 1963).

In contrast, the reduced primary quinone acceptor ($Q_A^{-\bullet}$) in closed PS II reaction centers hinders the primary charge separation by an electrostatic repulsive force (Schatz et al., 1988; Dau and Sauer, 1992), extending the excitation lifetime to several nanoseconds. The longer lifetime in the closed reaction centers leads to an increased fluorescence emission with maximal level F_M ($F_M \approx 5 \times F_0$). The variable part of fluorescence emission, $F_V = F_M - F_0$, reflects the limiting change in the photochemical quenching during transition from open to closed PS II reaction centers[3].

The closed reaction centers re-open with $Q_A^{-\bullet}$ oxidized by the plastoquinone molecule, Q_B, that is reversibly bound to the D1 protein of PS II, close to the stromal side of the thylakoid membrane (Fig. 1). The re-opened reaction center $Q_A/Q_B^{-\bullet}$ can undergo another stable charge separation resulting in $Q_A^{-\bullet}/Q_B^{-\bullet}$ and, eventually, in Q_A/Q_B^{2-}. By accepting two protons from the stroma, a neutral plastoquinol molecule Q_BH_2 is generated and released from PS II. This, in turn, is replaced by another molecule from the mobile plastoquinone pool Q_B. The plastoquinol migrates to the lumenal side of the Cyt b_6f complex where it is oxidized and protons are released. The electrons are transferred further through the chain of redox reactions, moved by another photochemical event in PS I towards the terminal acceptor $NADP^+$ reducing it into $NADPH{\cdot}H^+$.

On the donor side of PS II, the oxidative power of the manganese cluster increases with each turnover of the reaction center in $S_n \rightarrow S_{n+1}$ transitions until S_4 is reached. The S_4 state has oxidative potential capable of oxidizing water, which is split into protons and molecular oxygen, O_2. Four electrons return the manganese cluster to the S_0 state, and four protons add to the difference in the electrochemical potential between the stromal and lumenal sides of the thylakoid membrane. The electrochemical difference across the membrane drives phosphorylation of ADP into ATP. The ATP molecules combined with $NADPH{\cdot}H^+$ provide chemical energy and reducing power to the Calvin-Benson cycle assimilating inorganic CO_2 into sugars in C3 plants.

C. Regulation and Development: From Seconds to Lifetime?

The photochemical activities of both photosystems are coupled by the mobile plastoquinone pool, the Cyt b_6f complex and the soluble plastocyanine/cytochrome carrier. Multiple mobile redox elements make the entire system flexible and robust. PS II centers work in parallel so that damage to a small fraction of the centers has little effect on the overall performance (Behrenfeld et al., 1998). Also, temporal fluctuations and disharmony in the operation of the two photosystems are well buffered by the capacity of mobile carriers. Yet, the light environment of plants is highly dynamic and the long-term balance in the operation of both photosystems must be maintained by regulatory adjustments in optical cross section of their antenna systems or by redistribution of the excitation energy between the photosystems (Senger and Bauer, 1987; Wollman, 2001). Regulation also occurs when ATP and $NADPH{\cdot}H^+$ generated by the concerted operation of both photosystems cannot be effectively used by dark reactions of the Calvin-Benson cycle (Kanazawa and Kramer, 2002). This can be the case, for example, at limiting CO_2 concentrations or at supersaturating irradiance. Then, plants respond with multiple protective mechanisms that lower the excitonic flow to the reaction centers (Horton and Ruban, 1992). The reduction can be achieved by changes in leaf, cellular and thylakoid architectures, the decreased number of pigment-molecules or reaction centers or by quenching of excitonic states in the antenna systems. Either of these mechanisms is reflected in

[3] There is little or no variable fluorescence from PS I because its closed reaction center ($P700^+$) is quenching fluorescence as efficiently as an open reaction center (Nuijs et al., 1986, Dau, 1994).

a decrease of fluorescence emission conventionally called non-photochemical quenching.

The photosynthetic apparatus is also adapting to the biological changes during a cell cycle (Kaftan et al., 1999; Strasser et al., 1999) or during plant and leaf development in response to changing demand for assimilates (Maheswaran et al., 1987; Croxdale and Omasa, 1990; Huner et al., 1993) and the thylakoids are operating in different modes in source and sink leaves or leaf segments.

III. Analysis of Chlorophyll Fluorescence Transients

A. Flash of Light Removes Photochemical Quenching

The redox state of the PS II primary acceptor Q_A can be accurately manipulated by a short intense flash of light. Before the flash, reaction centers are open and have low fluorescence yield (i.e. minimal fluorescence F_0 in the interval 0–0.001s in Fig. 2) because the absorbed energy delivered by the dim measuring light is effectively used by photochemistry without perturbing the dark-adapted state of the plant. During the flash, which lasts typically no more than tens of microseconds, the acceptor Q_A is reduced in all reaction centers (single turnover). In the transiently closed centers, any absorbed energy from the dim measuring light cannot be used for photochemistry and contributes increasingly to fluorescence that is reaching its maximum, F_M (Fig. 2, 0.001 s). Neglecting PS I fluorescence, the limiting fluorescence levels, F_0 and F_M, can be expressed mathematically in relation to the number of PS II units N that are contributing to the measured fluorescence signal, local irradiance I, optical cross section a of PS II (Mauzerall and Greenbaum, 1989) and the rate-constants representing the three major pathways of de-excitation in PS II: k_P^{dark}, rate constant of PS II photochemistry; k_F, rate constant of PS II fluorescence emission; k_N^{dark}, rate constant of non-radiative dissipation which includes thermal dissipation, conversion to triplets and energy transfer outside the PS II reaction centers. The optical cross section of PS II is defined as the black-body area having identical frequency of photon capture with PS II antenna. The rate constant of PS II photochemistry k_P^{dark} is assumed to be negligible in closed reaction centers whereas the other rate constants and optical cross section are generally assumed to be invariant

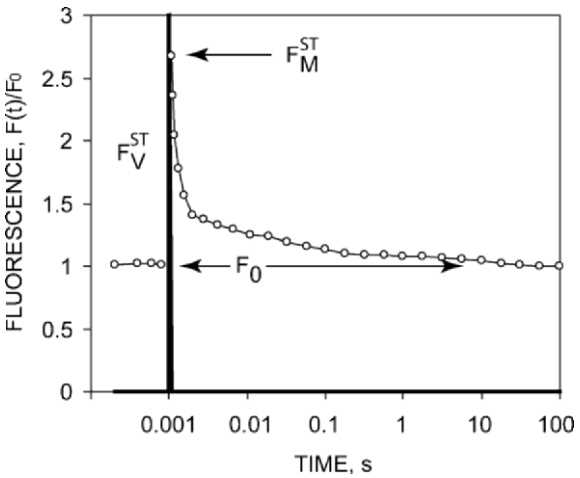

Fig. 2. Fluorescence transient induced in a suspension of the green alga, *Scenedesmus quadricauda,* by a single-turnover flash. The flash profile is schematically indicated by the heavy vertical line at 1 ms after starting the measurement. The irradiance during the flash reaches >0.1 mol (photons) $m^{-2}s^{-1}$ and the flash duration is typically less than 50 µs. Fluorescence was measured before and after the single-turnover flash using sufficiently weak excitation not altering the mean redox state of Q_A (Trtílek et al., 1997). First, 4 dim measuring flashes 250 µs apart were given to measure F_0. After the saturating flash at 1 ms, the dim measuring flashes were logarithmically spaced (4 measurements/decade) to increase the dynamic range of the data collection with very rapid sampling of the active centers in the initial phase and progressively slower sampling of inactive reaction centers in the final phase of the fluorescence transient.

in the measurements of F_0 and of F_M:

$$F_0 = (N \cdot I \cdot a)^{\text{dark}} \cdot k_F/(k_F + k_N^{\text{dark}} + k_P^{\text{dark}}) \quad (1)$$

$$F_M = (N \cdot I \cdot a)^{\text{dark}} \cdot k_F/(k_F + k_N^{\text{dark}}) \quad (2)$$

The maximum quantum yield of photochemistry, $\Phi_P^{\max}$, in open reaction centers is controlled by the same rate constants[4] and, thus, can be calculated from the measured fluorescence levels, F_0 and F_M (Malkin and Kok, 1966):

$$\Phi_P^{\max} = k_P^{\text{dark}}/(k_F + k_N^{\text{dark}} + k_P^{\text{dark}}) = (F_M - F_0)/F_M \equiv F_V/F_M \quad (3)$$

After the flash in Fig. 2, the Q_A acceptor is gradually oxidized by the plastoquinone Q_B, thereby restoring the charge separation capacity of the reaction center.

[4] This assumption may not be always true: k_N^{dark} constants may differ in open and closed reaction center (Koblížek et al., 1999)

Proportionately with the increasing number of re-opened reaction centers, the fluorescence drops as the photochemical quenching recovers (0.001–100 s in Fig. 2). The photochemical quenching, q_P, is frequently used to estimate the relative fraction of PS II reaction centers that are open at time t when the intermediate fluorescence levels, $F(t)$ are measured[5]:

$$q_P(t) \equiv (F_M - F(t))/(F_M - F_0) \approx [PS\,II^{open}/(PS\,II^{open} + PS\,II^{closed})](t) \quad (4)$$

The drop of fluorescence caused by the re-opening of the reaction centers after the flash proceeds with multiphase kinetics, that are usually resolved into three exponential phases, or, alternatively, into two exponentials and one hyperbolic phase (Vass et al., 1999). These are interpreted as reflecting heterogeneity of the acceptor side of PS II.

Product of the maximum quantum yield of photochemistry, Φ_P^{max} (see Eq. 3), multiplied by the fraction of open reaction centers measured by photochemical quenching, q_P (see Eq. 4) gives an estimate of the instantaneous quantum yield of photochemistry, $\Phi_P(t)$ (Paillotin, 1978):

$$\Phi_P(t) = \Phi_P^{max} \cdot q_P(t) = (F_M - F(t)) / F_M \quad (5)$$

Equations (4) and (5) link physiologically important parameters with the measured fluorescence signals.

B. Single-turnover Induction Reveals Antenna Functional Cross Section and Connectivity

The kinetics of the fluorescence transient from F_0 to F_M, induced by a single-turnover flash, reports on rates at which the light is captured and processed in PS II reaction centers (Kolber et al., 1998, Nedbal et al., 1999). The single-turnover transient is similar to that observed on a much longer time scale and in a weaker light when plants have the re-oxidation of $Q_A^{-\bullet}$ blocked by herbicides (e.g., DCMU). Simple models assuming uniform and isolated PS II (used in Eqs.4 and 5) predict a mono-exponential character of the transients. However, the measured induction transients in higher plants and green algae are typically sigmoidal (Joliot and Joliot, 1964; Koblížek et al., 2001). The sigmoidal kinetics can be explained assuming that an excitation generated in an antenna of a closed PS II reaction center can move to a neighboring PS II reaction center. The phenomenon of inter-unit excitation transfer is usually termed 'antenna connectivity.' With p being the probability of such a process, photochemical quenching can be related to the fraction of open reaction centers by Eq. (6a):

$$q_P \equiv (F_M - F_{(t)}) / (F_M - F_0) = \frac{PS\,II^{open}}{PS\,II^{open} + PS\,II^{closed} \cdot (1-p)} \quad (6a)$$

Equation (6a) is frequently re-formulated to show explicitly the hyperbolic relation between the photochemical quenching and the fraction of open reaction centers. To achieve that, a hyperbola constant C was introduced initially by Strasser (Strasser, 1978, 1981). Alternatively, the sigmoidicity parameter, $J \equiv p/(1-p)$ (Lavergne and Trissl, 1995), which is a numeric equivalent of the earlier Strasser's C is used dominantly in current literature to re-formulate Eq.(6a) into Eq.(6b):

$$q_P = \frac{PS\,II^{open}}{PS\,II^{open} + PS\,II^{closed}} \cdot (1+J) / (1 + J \cdot \frac{PS\,II^{open}}{PS\,II^{open} + PS\,II^{closed}}) \quad (6b)$$

The rate at which the PS II reaction centers are closed by irradiance I can be estimated from Eq. (7):

$$-\frac{d}{dt}\frac{PS\,II^{open}}{PS\,II^{open} + PS\,II^{closed}} = I \cdot \sigma_{PSII} \frac{PS\,II^{open}}{PS\,II^{open} + PS\,II^{closed}} (1+J) / (1 + J \cdot \frac{PS\,II^{open}}{PS\,II^{open} + PS\,II^{closed}}) \quad (7)$$

Numerical methods applied to Eqs. (6) and (7) can be used to find the sigmoidicity factor, J, and the PS II functional cross section, $\sigma_{PS\,II} = a \cdot \Phi_P^{max}$, that yield the best fit of experimental data by model kinetics. J values are typically found (Koblížek et al., 2001) in

[5] The estimate is based on an assumption of uniform and mutually isolated PS II. Corrections are necessary when other quenching mechanisms are present, PS II reaction centers are heterogeneous and the connectivity of antenna systems is considered.

the range from 0 (no connectivity, $p \approx 0$) to 1.5 (high connectivity, $p \approx 0.6$) with examples of fluorescence kinetics of extreme sigmoidicity shown in Fig. 3.

The introduction of connectivity does not perturb Eq. (5) which relates the instantaneous quantum yield of photochemistry $\Phi_P(t)$ with fluorescence data. Identical formula to Eq. (5) can be derived by combining Eqs. (6b) and Eq. (7).

C. Multi-turnover Induction Reflects Complexity

Plants, exposed to light of lower irradiance compared to that of a single-turnover flash, respond by a complex transient of fluorescence emission that is reflecting incoherent multiple-turnover transitions (Strasser et al., 1995; Lazár, 1999). The fluorescence transients consist of two major phases: the initial rise from the F_0 level to the fluorescence maximum labeled F_P (or F_M when Q_A is fully reduced) and the decline from F_P to the steady-state F_S (Fig. 4). The shape of the initial rise is polyphasic depending on the applied irradiance (Strasser et al., 1995; Kolber et al., 1998). In high irradiance, it consists of three (F_J, F_I, F_P in Strasser et al., 1995, alternatively, I_1-I_2-P in Neubauer and Schreiber, 1987) or, in heat stressed plants, four (F_K, F_J, F_I, F_P) phases, that are conveniently visualized on a logarithmic time-scale covering an interval from tens of microseconds to a second (Strasser et al., 1998).

The polyphasic fluorescence rise is characterized by the extreme emission levels F_0 and F_M (F_P), the initial rate of the rise, $(dF_V/dt)_{t\to 0+}$, the time integral,

$$\int_0^\infty (F_M - F(t))dt \,,$$

and by the independent amplitudes of the fluorescence rise phases, (F_{Vj}, F_{Vi}). A model was proposed (Strasser et al., 1998) to interpret the individual fluorescence characteristics on a molecular level. The parameters of the initial fluorescence rise are highly sensitive to the physiological state of the plant and may serve as an efficient screening tool (Strasser et al., 1998).

D. Non-photochemical Quenching Reflects Photoprotection

In a light-adapted plant, steady-state fluorescence, F_S, reflects a dynamic equilibrium between Q_A oxidation and reduction leading to a steady-state ratio of open and closed PS II reaction centers and, thus, to an intermediate level of photochemical quenching, q_P (see Eq. 6). Similar to the protocol described in Section III.A, the photochemical quenching can be transiently eliminated by a saturating flash to measure the maximum fluorescence, F_M', with all PS II centers closed (Fig. 4). F_M' measured in a light-adapted plant,

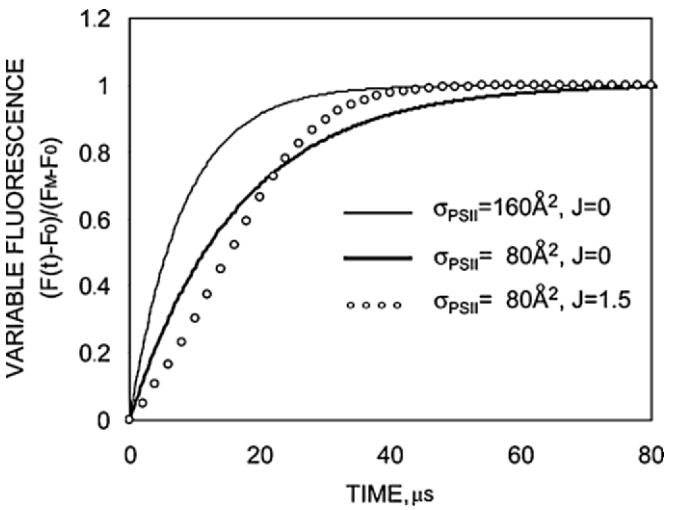

Fig. 3. Variable fluorescence calculated from Eqs. (6) and (7) for 3 types of PS II antenna systems. Isolated PS II antenna of a large functional cross section (thin line); isolated PS II antenna of a small functional cross section (heavy line); and, connected PS II antenna systems of a small functional cross section (open circles). The timescale corresponds to a light flux 0.1 mol (photons) $m^{-2}s^{-1}$ during a rectangular single-turnover flash of 650 nm.

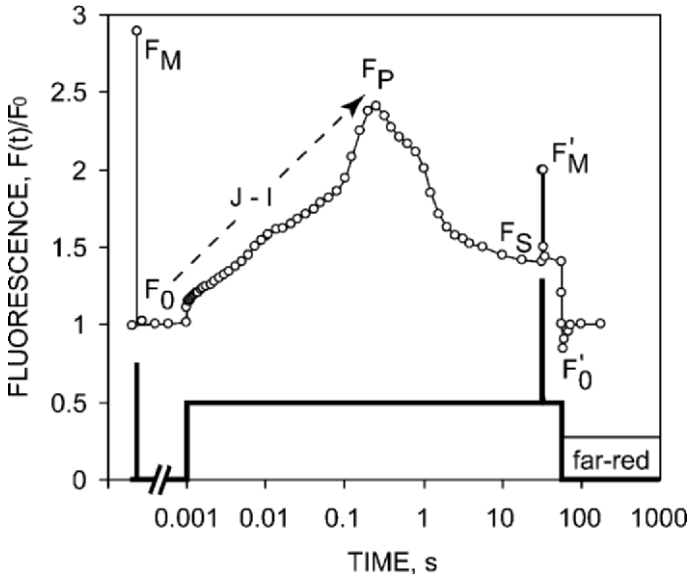

Fig. 4. Schematic presentation of a fluorescence transient based on a measurement with a suspension of the green alga, *Scenedesmus quadricauda*. The actinic light profile is schematically indicated by the heavy line at the bottom of the figure. The irradiance of the actinic light inducing the transient is typically in hundreds of μmol (photons) m^{-2} s^{-1}, whereas the irradiance of the saturating flashes (indicated by vertical lines) is typically stronger by one order of magnitude. After F_0 measurement, the saturating flash transiently reduced the plastoquinone pool to read F_M in dark-adapted cells. The F_0 emission was restored by a dark adaptation before actinic light was switched on (0.001s). The fluorescence rose to F_P level passing phases *J* and *I*. The decline to steady-state level reflected acclimation to the actinic irradiance. A second saturating flash was given to determine maximal fluorescence in the light-adapted plant F_M' that was diminished relative to F_M by non-photochemical quenching. The visible actinic light was replaced by far-red light at the end of the experiment to reveal the F_0' emission of the light-adapted plant.

is typically lower than the corresponding parameter F_M measured in the dark (Fig. 4)[6]. The difference between F_M and F_M' reflects non-photochemical quenching that is used by plants to cope with irradiance that is exceeding the photosynthetic capacity (Horton and Ruban, 1992). The non-photochemical quenching affects also the F_0' level. The F_0' fluorescence is measured in light-adapted plants after the visible light is switched off and replaced by a far-red irradiance that can rapidly oxidize the plastoquinone pool thus opening all PS II centers and maximizing the photochemical quenching (Fig. 4). In analogy to Eqs. (1) and (2), the following relations are derived for the limiting levels of fluorescence in light-adapted plants[7]:

$$F_0' = (N \cdot I \cdot a)^{light} \cdot k_F / (k_F + k_N^{\,\mathrm{light}} + k_P^{\,\mathrm{light}}) \qquad (8)$$

$$F_M' = (N \cdot I \cdot a)^{light} \cdot k_F / (k_F + k_N^{\,\mathrm{light}}) \qquad (9)$$

The non-photochemical quenching reducing F_M', F_0' relative to F_M, F_0 can be caused by increased non-photochemical dissipation $k_N^{light} > k_N^{dark}$ that is accompanied by corresponding shortening of the fluorescence lifetime (Dau and Hansen, 1990). Though terminologically incorrect, the term non-photochemical quenching is conventionally also used to describe the lowering of fluorescence due to lower light absorption ($I^{light}.a^{light} < I^{dark}.a^{dark}$) or a decrease in PS II numbers ($N^{light} < N^{dark}$). Lower light absorp-

[6] The prime symbol (e.g., in F_M', F_0' and F_V' and in Φ_P' and q_P') was introduced to indicate that the respective parameters were measured in a light-adapted plant. This 'prime' symbolism is, however, used inconsistently, e.g., *F(t)* is often used to represent fluorescence after *t* seconds of irradiance or F_S stands for steady-state equilibrium fluorescence reached after a long light exposure. Here, we combine the widely accepted 'prime' symbolism with explicit superscript labels 'light' and 'dark' wherever no historical precedent is known to us.

[7] Due to the homology between Eqs.8, 9 and Eqs.1, 2, the maximum quantum yield of PS II photochemistry ($\Phi_P^{\max}$), the instantaneous photochemical quenching $q_P(t)$ and the instantaneous quantum yield of PS II photochemistry $\Phi_P(t)$ can be quantified in light-adapted plants using Eqs.3-5 derived for dark-adapted plants. Only, F_M' and F_0' parameters must be used instead of F_M and F_0.

tion or a lower proportion of PS II centers preserves measured fluorescence lifetimes, and can thereby be distinguished from changes affecting the rate constant of non-photochemical energy dissipation, k_N.

In another classification, diverse types of non-photochemical quenching are distinguished by relaxation kinetics in the dark (Horton et al., 2000; Krause and Jahns, 2002). The fastest kinetics of relaxation (tens of seconds) is attributed to energy-dependent quenching mechanisms, $q_{N,E}$, that fade away with the difference in proton gradient across the thylakoid membrane (Briantais et al., 1979, 1980). The slower recovery kinetics (tens of minutes), $q_{N,T}$, is usually interpreted as reflecting detachment of an outer light harvesting antennae complex from PS II (Bennett et al., 1980; Wollman, 2001). Finally, the slowest phase is interpreted as recovery from photoinhibitory quenching, $q_{N,I}$, that is due to photodamage of PS II centers by high irradiance (Šetlík et al., 1990; Tyystjärvi and Aro, 1996).

The non-photochemical quenching is usually quantified using the difference between the maximum fluorescence measured in dark, F_M, and the one measured after a light exposure, F_M': $NPQ=(F_M - F_M')/F_M'$ (Bilger and Björkman, 1990)[8]. Assuming that the integral excitonic flow to reaction centers is constant, $(N.I.a)^{dark} \approx (N.I.a)^{light}$, and that the photoprotection is provided by an increase in the Stern-Volmer non-photochemical de-excitation rate, k_N^{light} relative to k_N^{dark}, NPQ can be used to measure light-induced changes in the concentration of the quencher. In the other limiting model case of reduced optical cross section or reduced number of contributing PS II units, NPQ quantifies the relative reduction in the integral flow of excitation to PS II centers that is caused by the light acclimation.

Assuming that the non-photochemical quenching is caused solely by change of k_N (i.e. no change in N, a or I), the relationship between the instantaneous yield of photochemistry, Φ_P', and the fluorescence parameters similar to Eq. (5) can be extended also to plants acclimated to light:

$$\Phi_P' = (F_M' - F_S)/F_M' \qquad (10)$$

Here, prime symbols indicate steady-state parameters measured in actinic light and F_S steady-state fluorescence. Eq. (10) is frequently used in plant physiology to estimate steady-state photosynthetic electron transport rates in conditions when primary photochemistry drives dominantly the linear photosynthetic electron flow. This relationship was experimentally verified in higher plants by Genty et al. (1989).

IV. Beyond the Conventional Analysis

A. Modulation by Redox Components

In a plant exposed to a strong nanosecond laser pulse, the fluorescence emission reaches its maximum only after a 35 μs delay once the Q_A acceptor is fully reduced (Mauzerall, 1972). The delay in reaching full F_M level is explained by a quenching of fluorescence due to transient appearance of oxidized PS II donor $P680^{+\bullet}$ (Sonneveld et al., 1979; Steffen et al., 2001) and of carotenoid triplets generated in the nanosecond flash (Breton et al., 1979).

Significant variability of F_M is also found with different techniques of Q_A reduction (Delosme, 1967; Kolber et al., 1998): F_M^{MT} measured in a multiple turnover pulse is typically higher than F_M^{ST} obtained using a single turnover flash. F_M^{DCMU} measured in presence of DCMU, the herbicide that inhibits Q_A reoxidation, is typically slightly lower than F_M^{MT} obtained in a multiple-turnover flash and higher than F_M^{ST} measured by the single turnover flash: that is, $F_M^{MT} > F_M^{DCMU} > F_M^{ST}$. Static quenching, by an oxidized plastoquinone pool directly interacting with the LHCII antenna chlorophylls during a single-turnover measurement, was proposed to account for the difference between F_M^{MT} and F_M^{ST} (Samson and Bruce, 1996; Vasil'ev and Bruce, 1998). Samson et al. (1999) tentatively explained the lowering of the single-turnover F_M^{ST} relative to F_M^{DCMU} by a quenching by the oxidized plastoquinone molecule bound in the Q_B pocket of herbicide-free PS II reaction centers.

Soon after the discovery of the sequential four-step mechanism of oxygen evolution by Joliot and Kok (Kok et al., 1970; Joliot et al., 1971), it was found that individual S-states of the water-splitting complex modulate both F_0 and F_M (Delosme, 1971; Joliot and Joliot, 1971; Zankel, 1973). Sonneveld et al. (1979) proposed that the life-time of $P680^{+\bullet}$ is S-state dependent, thereby affecting the F_M yield. However, this cannot explain the modulation of F_0 since it is

[8] Alternatively to the NPQ parameter, the photoprotective mechanisms are frequently quantified using light-induced reduction of variable fluorescence (van Kooten and Snel,, 1990: $q_N \equiv (F_V - F_V')/F_V$. The interpretation of q_N is more complex than with NPQ because it reflects not only non-photochemical quenching but also light-induced changes in photochemical rates.

measured on a time scale substantially exceeding the life-time of $P680^{+\bullet}$. Koblížek et al. (2001) showed that individual S-states differ in their photochemical yields, which suggests that the F_0 modulation by the S-states may be caused by the changes in the rate constant of the primary photochemistry, k_P.

B. PS II Heterogeneity

Uniform PS II units are expected to yield monophasic induction kinetics. However, the measured fluorescence transients consist frequently of multiple kinetically distinct components (Melis and Homann, 1975). Melis and Duysens (1979) interpreted such kinetics as reflecting the presence of two distinct sets of PS II units: PS II_α having large, mutually connected antenna systems and PS II_β with smaller isolated antenna systems. In higher plants, PS II_β typically account for about 30% of the total PS II having an optical cross section about half that of PS II_α (Lavergne and Briantais, 1996).

Another type of heterogeneity was found in the rates of $Q_A^{-\bullet}$ reoxidation. A substantial fraction of PS II reaction centers is unable to oxidize $Q_A^{-\bullet}$ at physiologically meaningful rates (Chylla et al., 1987). These inactive reaction centers are manifested in fluorescence induction in low light as the fastest kinetic component reflecting rapid accumulation of $Q_A^{-\bullet}$. In the fluorescence decline following the single-turnover flash, the inactive PS II reaction centers are responsible for the slowest component. The physiological role of the inactive reaction centers remains controversial (Lavergne and Briantais, 1996).

V. Prospects of the Technique and Instrumentation

Chl fluorescence measurements became widespread with the introduction of Pulse Amplitude Modulation fluorometers (PAM), separating fluorescence emission excited by measuring flashes from slowly changing background signals (Schreiber et al., 1986). Recently, the availability of high intensity light emitting diodes and the use of processor-controlled electronics made it possible to design a new generation of fluorometers which achieve time resolution in the microsecond range (Trtílek et al., 1997; Kolber et al., 1998; Strasser et al., 1998). The new light sources provide rectangular single-turnover pulses used for measurements of functional cross section and connectivity analysis (Kolber et al., 1998; Nedbal et al., 1999). Heterogeneity in Chl fluorescence emission over a plant surface can be mapped by kinetic fluorescence imaging (reviewed in Nedbal and Whitmarsh, 2004). The measured leaf or canopy area will be increased in the near future to facilitate applications of Chl fluorescence imaging in precision farming, a post-harvest quality control and in high-throughput mutant screening. Light Detection and Ranging (LIDAR) fluorometers resolving multiple kinetic parameters are being developed for applications in ecophysiology (Kolber et al., 2005). Substantial activity is emerging in the remote sensing of vegetation fluorescence detected in and near Fraunhofer lines and O_2 absorption lines in red and far-red bands (Theisen, 2002). At the other extreme, the resolution of the kinetic fluorescence imaging will be increased to optical diffraction limits to facilitate monitoring of photochemical activities within a single chloroplast (Küpper et al., 2000). Novel fluorescence techniques need to be designed to resolve regulatory processes that have, until now, escaped fluorescence detection because of their relatively slow kinetics (Nedbal and Březina, 2002, Nedbal et al., 2003, 2005).

Acknowledgments

This work was supported in part by the Czech Department of Education, Sports and Youth under Grant MSM6007665808, by the Institute of Landscape Ecology Grant AV0Z60870520 and by the Grant Agency of the Czech Republic GACR 206/05/0894 and 204/05/0307. Critical reading and helpful comments of Drs. Govindjee, Quigg, Strasser, Tsimilli–Michael and Trissl are gratefully acknowledged.

References

Behrenfeld M, Prášil O, Kolber Z, Babin M and Falkowski P (1998) Compensatory changes in Photosystem II electron turnover rates protect photosynthesis from photoinhibition. Photosynth Res 58: 259–268

Bennett J, Steinback K and Arntzen C (1980) Chloroplast phosphoproteins: Regulation of excitation energy transfer by phosphorylation of thylakoid membranes. Proc Natl Acad Sci USA 77: 5253–5257

Bilger W and Björkman O (1990) Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. Photosynth Res 25: 173–185

Bolhár-Nordenkampf H, Long S, Baker N, Öquist G, Schreiber U

and Lechner E (1989) Chlorophyll fluorescence as a probe of the photosynthetic competence of leaves in the field: A review of current instrumentation. Funct Ecol 3: 497–514

Breton J, Geacintov N and Swenberg C (1979) Quenching of fluorescence by triplet excited states in chloroplasts. Biochim Biophys Acta 548: 616–635

Briantais J, Vernotte C, Picaud M and Krause G (1979) A quantitative study of the slow decline of chlorophyll a fluorescence in isolated chloroplasts. Biochim Biophys Acta 548: 128–138

Briantais J, Vernotte C, Picaud M and Krause G (1980) Chlorophyll fluorescence as a probe for the determination of the photo-induced proton gradient in isolated chloroplasts. Biochim Biophys Acta 591: 198–202

Chylla RA, Garab G and Whitmarsh J (1987) Evidence for slow turnover in a fraction of PS II complexes in thylakoid membranes. Biochim Biophys Acta 894: 562–571

Croxdale JG and Omasa K (1990) Chlorophyll-*a* fluorescence and carbon assimilation in developing leaves of light-grown cucumber. Plant Physiol 93: 1078–1082

Csete M and Doyle J (2002) Reverse engineering of biological complexity. Science 295: 1664–1669

Dau H (1994) Molecular mechanisms and quantitative models of variable Photosystem II fluorescence. Photochem Photobiol 60: 1–23

Dau H and Hansen U (1990) A study on the energy-dependent quenching of chlorophyll fluorescence by means of photoacoustic measurements. Photosynth Res 25: 269–278

Dau H and Sauer K (1992) Electric field effect on the picosecond fluorescence of Photosystem II and its relation to the energetics and kinetics of primary charge separation. Biochim Biophys Acta 1102: 91–106

DeEll J, van Kooten O, Prange R and Murr D (1999) Applications of chlorophyll fluorescence techniques in post harvest physiology. Hort Rev 23: 69–107

Delosme R (1967) Etude de l'induction de fluorescence des algues vertes et des chloroplastes au debut d'une illumination intense. Biochim Biophys Acta 143: 108–128

Delosme R (1971) New results about chlorophyll fluorescence 'in vivo.' In: Forti G, Avron M and Melandri A (eds) 2nd International Congress on Photosynthesis Research, pp 187–195. Dr. W. Junk, The Hague

Duysens LNM and Sweers HE (1963) Mechanism of the two photochemical reactions in algae as studied by means of fluorescence. In*:* Studies on Microalgae and Photosynthetic Bacteria, Japanese Soc Plant Physiol, pp 353–372. University of Tokyo Press, Tokyo

Falkowski P and Kolber Z (1995) Variations in chlorophyll fluorescence yields in phytoplankton in the world oceans. Aust J Plant Physiol 22: 341–355

Genty B, Briantais J-M and Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim Biophys Acta 990: 87–92

Genty B, Wonders J and Baker N (1990) Non-photochemical quenching of Fo in leaves is emission wavelength dependent—Consequences for quenching analysis and its interpretation. Photosynth Res 26: 133–139

Govindjee (1995) Sixty-three years since Kautsky: Chlorophyll *a* fluorescence. Aust J Plant Physiol 22: 131–160

Horton P and Ruban A (1992) Regulation of Photosystem II. Photosynth Res 34: 375–385

Horton P, Ruban A and Wentworth M (2000) Allosteric regulation of the light-harvesting system of Photosystem II. Phil Trans R Soc Lond B 355: 1361–1370

Huner N, Öquist G, Hurry V, Krol M, Falk S and Griffith M (1993) Photosynthesis, photoinhibition and low temperature acclimation in cold tolerant plants. Photosynth Res 37: 19–39

Jalink H, van der Schoor R, Frandas A, van Pijlen J and Bino R (1998) Chlorophyll fluorescence of *Brassica oleracea* seeds as a non-destructive marker for seed maturity and seed performance. Seed Sci Res 8: 437–443

Joliot A and Joliot P (1964) Etudes cinétique de la réaction photochimique libérant l'oxygène au cours de la photosynthèse. CR Acad Sci Paris 258D: 4622–4625

Joliot P and Joliot A (1971) Studies on the quenching properties of Photosystem II electron acceptor. In*:* Forti G, Avron M and Melandri A (eds) Proceedings of the 2nd international Congress on Photosynth Research, pp 26–38. Dr. W Junk NV Publishers, The Hague

Joliot P, Joliot A, Bouges B and Barbieri G (1971) Studies of System II photocenters by comparative measurements of luminescence, fluorescence and oxygen emission. Photochem Photobiol 14: 287–305

Kaftan D, Meszaros T, Whitmarsh J and Nedbal L (1999) Characterization of Photosystem II activity and heterogeneity during the cell cycle of the green alga *Scenedesmus quadricauda*. Plant Physiol 120: 433–441

Kanazawa A and Kramer D (2002) In vivo modulation of nonphotochemical exciton quenching (NPQ) by regulation of the chloroplast ATP synthase. Proc Natl Acad Sci USA 99: 12789–12794

Kautsky H and Hirsch A (1931) Neue Versuche zur Kohlensäureassimilation. Naturwissenschaften 19: 964

Koblížek M, Ciscato M, Komenda J, Kopecký J, Šiffel P and Masojídek J (1999) Photoadaptation in the green alga *Spongiochloris* sp. A three-fluorometer study. Photosynthetica 37: 307–323

Koblížek M, Kaftan D and Nedbal L (2001) On the relationship between the non-photochemical quenching of the chlorophyll fluorescence and the Photosystem II light harvesting efficiency. A repetitive flash fluorescence induction study. Photosynth Res 68: 141–152.

Koehne B, Elli G, Jennings R, Wilhelm C and Trissl H (1999) Spectroscopic and molecular characterization of a long wavelength absorbing antenna of *Ostreobium* sp. Biochim Biophys Acta 1412: 94–107

Kok B, Forbush B and McGloin M (1970) Cooperation of charges in photosynthetic O_2 evolution — 1. A linear four step mechanism. Photochem Photobiol 11: 457–475

Kolber Z, Klimov D, Ananyev G, Rascher U, Berry JA and Osmond CB (2005) Measuring photosynthetic parameters at a distance: Laser induced fluorescence transient (LIFT) method for remote measurements of PSII in terrestrial vegetation. Photosynth Res 84: 121–129

Kolber Z, Prášil O and Falkowski P (1998) Measurements of variable chlorophyll fluorescence using fast repetition rate techniques. I. Defining methodology and experimental protocols. Biochim Biophys Acta 1367: 88–106

Kramer D and Crofts A (1996) Control and measurement of photosynthetic electron transport in vivo. In*:* Baker N (ed) Photosynthesis and the Environment, pp 25–66. Kluwer Academic Publishers, Dordrecht

Krause G and Jahns P (2002) Pulse amplitude modulated chlorophyll fluorometry and its application in plant science. In: Green B and Parson W (eds) Light Harvesting Antennas, pp 373–399. Kluwer Academic Publishers, Dordrecht

Küpper H, Šetlík I, Trtílek M and Nedbal L (2000) A microscope for two-dimensional measurements of in vivo chlorophyll fluorescence kinetics using pulsed measuring light, continuous actinic light and saturating flashes. Photosynthetica 38: 553–570

Latimer P, Bannister T and Rabinowitch E (1956) Quantum yields of fluorescence of plant pigments. Science 124: 585–586

Lavergne J and Briantais J-M (1996) Photosystem II heterogeneity. In: Ort D and Yocum C (eds) Oxygenic Photosynthesis: The Light Reactions, pp 265–287. Kluwer Academic Publishers, Dordrecht

Lavergne J and Trissl H (1995) Theory of fluorescence induction in Photosystem II: Derivation of analytical expressions in a model including exciton-radical-pair equilibrium and restricted energy transfer between photosynthetic units. Biophys J 68: 2474–2492

Lazár D (1999) Chlorophyll *a* fluorescence induction. Biochim Biophys Acta 1412: 1–28

Leegood R, Sharkey T and von Caemmerer S (eds) (2000) Photosynthesis: Physiology and Metabolism. Kluwer Academic Publishers, Dordrecht

Lichtenthaler H and Miehé J (1997) Fluorescence imaging as a diagnostic tool for plant stress. Trends Plant Sci 2: 316–320

Maheswaran S, Popovic R, Colbow K and Vidaver W (1987) Chlorophyll fluorescence in leaves and cells of light-grown maize (*Zea mays*) during development. J Plant Physiol 130: 173–179

Malkin S and Kok B (1966) Fluorescence induction studies in isolated chloroplasts. I. Number of components involved in the reaction and quantum yields. Biochim Biophys Acta 126: 413–432

Mauzerall D (1972) Light induced fluorescence changes in *Chlorella*, and the primary photoreactions for the production of oxygen. Proc Natl Acad Sci USA 69: 1358–1362

Mauzerall D and Greenbaum N (1989) The absolute size of a photosynthetic unit. Biochim Biophys Acta 974: 119–140

Mehta M, Sarafis V and Critchley C (1999) Thylakoid membrane architecture. Plant Physiol 26: 709–716

Melis A and Duysens L (1979) Biphasic energy conversion kinetics and absorbance difference spectra of Photosystem II of chloroplasts. Evidence for two different Photosystem II reaction centers. Photochem Photobiol 29: 373–382

Melis A and Homann P (1975) Kinetic analysis of the fluorescence induction in DCMU poisoned chloroplasts. Photochem Photobiol 21: 431–437

Mohammed G, Binder W and Gillies S (1995) Chlorophyll fluorescence: A review of its practical forestry applications and instrumentation. Scan J Forest Res 10: 383–410

Müller N (1874) Beziehungen zwischen Assimilation, Absorption und Fluoreszenz im Chlorophyll des lebenden Blattes. Jahrb Wiss Bot 9: 42–49

Nedbal L and Březina V (2002) Complex metabolic oscillations in plants forced by harmonic irradiance. Biophys J 83: 2180–2189

Nedbal L and Whitmarsh J (2005) Chlorophyll fluorescence imaging of leaves. In: Papageorgiou G and Govindjee (eds) Chlorophyll *a* fluorescence: A Signature of Photosynthesis, pp 389–407. Kluwer Academic Publishers, Dordrecht

Nedbal L, Trtílek M and Kaftan D (1999) Flash fluorescence induction: A novel method to study regulation of Photosystem II. J Photochem Photobiol 48: 154–157

Nedbal L, Soukupová J, Whitmarsh J and Trtílek M (2000) Postharvest imaging of chlorophyll fluorescence from lemons can be used to predict fruit quality. Photosynthetica 38: 571–579

Nedbal L, Březina V, Adamec F, Štys D, Oja V, Laisk A and Govindjee (2003) Negative feedback regulation is responsible for the non-linear modulation of photosynthetic activity in plants and cyanobacteria exposed to a dynamic light environment. Biochim Biophys Acta 1607: 5-17

Nedbal L, Březina V, Červený J and Trtílek M (2005) Photosynthesis in dynamic light: Systems biology of unconventional chlorophyll fluorescence transients in *Synechocystis* sp. PCC6803. Photosynth Res 84: 99–104

Neubauer C and Schreiber U (1987) The polyphasic rise of chlorophyll fluorescence upon onset of strong continuous illumination: I. Saturation characteristics and partial control by the Photosystem II acceptor side. Z Naturforsch 42C: 1246–1254

Nuijs A, Shuvalov V, van Gorkom H, Plijter J and Duysens L (1986) Picosecond absorbance-difference spectroscopy on the primary reactions and antenna-excited states in Photosystem I particles. Biochim Biophys Acta 850: 310–318

Ort D and Yocum C (eds) (1996) Oxygenic Photosynthesis: The Light Reactions. Kluwer Academic Publishers, Dordrecht

Paillotin G (1978) Organization of the photosynthetic pigments and transfer of excitation energy. In: Hall D, Coombs, J and Goodwin TW (ed) Photosynthesis '77, pp 33–44. The Biochemical Society, London

Roelofs T, Lee C-H and Holzwarth A (1992) Global target analysis of picosecond chlorophyll fluorescence kinetics from pea chloroplasts. Biophys J 61: 1147–1163

Samson G and Bruce D (1996) Origins of the low-yield of chlorophyll-*a* fluorescence induced by single turnover flash in spinach thylakoids. Biochim Biophys Acta 1276: 147–153

Samson G, Prášil O and Yaakoubd B (1999) Photochemical and thermal phases of chlorophyll *a* fluorescence. Photosynthetica 37: 163–182

Schatz G, Brock H and Holzwarth A (1988) Kinetic and energetic model for the primary processes in Photosystem II. Biophys J 54: 397–405

Schreiber U, Schliwa U and Bilger W (1986) Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. Photosynth Res 10: 51–62

Senger H and Bauer B (1987) The influence of light-quality on adaptation and function of the photosynthetic apparatus. Photochem Photobiol 45: 939–946

Šetlík I, Allakhverdiev S, Nedbal L, Šetlíková E and Klimov V (1990) Three types of Photosystem II photoinactivation. 1. Damaging processes on the acceptor side. Photosynth Res 23: 39–48

Sonneveld A, Rademaker H and Duysens L (1979) Chlorophyll *a* fluorescence as a monitor of nanosecond reduction of the photooxidized pripary donor P-680$^+$ of Photosystem II. Biochim Biophys Acta 548: 536–551

Steffen R, Christen G and Renger G (2001) Time-resolved monitoring of flash-induced changes of fluorescence quantum yield and decay of delayed light emission in oxygen-evolving photosynthetic organisms. Biochemistry 40: 173–180

Strasser R (1978) The grouping model of plant photosynthesis. In: Akoyunoglou G (ed) Chloroplast Development, pp 513–542. Elsevier, Amsterdam

Strasser R (1981) The grouping model of plant photosynthesis: Heterogeneity of photosynthetic units in thylakoids. In: Akoyunoglou G (ed) Photosynthesis III. Structure And Molecular Organisation of the Photosynthetic Apparatus, pp 727–737. Balaban International Science Service, Philadelphia

Strasser R, Srivastava A and Govindjee (1995) Polyphasic chlorophyll *a* fluorescence transient in plants and cyanobacteria. Photochem Photobiol 61: 32–42

Strasser RJ, Srivastava A and Tsimilli–Michael M (1998) The fluorescence transient as a tool to characterize and screen photosynthetic samples. In: Mohanty P, Yunus M and Pathre U (eds) Probing Photosynthesis: Mechanism, Regulation and Adaptation, pp 1–53. Taylor & Francis, London

Strasser B, Dau H, Heinze I and Senger H (1999) Comparison of light induced and cell cycle dependent changes in the photosynthetic apparatus: A fluorescence induction study on the green alga *Scenedesmus obliquus*. Photosynth Res 60: 217–227

Theisen A (2002) Detecting chlorophyll fluorescence from orbit: The Fraunhofer line depth model. In: Muttiah R (ed) From Laboratory Spectroscopy to Remotely Sensed Spectra of Terrestrial Ecosystems., pp 203-232. Kluwer Academic Publishers, Dordrecht

Trtílek M, Kramer D, Koblížek M and Nedbal L (1997) Dual-modulation LED kinetic fluorometer. J Luminesc 72-74: 597–599

Tyystjärvi E and Aro E (1996) The rate constant of photoinhibition, measured in lincomycin-treated leaves is directly proportional to light-intensity. Proc Natl Acad Sci USA 93: 2213–2218

van Kooten O and Snel J (1990) The use of chlorophyll fluorescence nomenclature in plant stress physiology. Photosynth Res 25: 147–150

Vasil'ev S and Bruce D (1998) Nonphotochemical quenching of excitation energy in Photosystem II. A picosecond time-resolved study of the low yield of chlorophyll *a* fluorescence induced by single-turnover flash in isolated spinach thylakoids. Biochemistry 37: 11046–11054

Vass I, Kirilovsky D and Etienne A-L (1999) UV-B radiation-induced donor- and acceptor-side modifications of Photosystem II in the cyanobacterium *Synechocystis* sp. PCC 6803. Biochemistry 38: 12786–12794

Wollman F-A (2001) State transitions reveal the dynamics and flexibility of the photosynthetic apparatus. EMBO J 20: 3623–3630

Zankel K (1973) Rapid fluorescence changes observed in chloroplasts: Their relationship to the O_2 evolving system. Biochim Biophys Acta 325: 138–148