

Single-turnover flashes to saturate the Q_A reduction in a leaf were generated by the light-emitting diodes from a double modulation kinetic chlorophyll fluorometer

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Abstract

The earlier developed double-modulation chlorophyll (Chl) fluorometer was modified for measurements with intact leaves of higher plants. The Chl fluorometer is based on a non-periodic modulation of both actinic and measuring flashes. In addition, continuous orange actinic and far-red radiation were produced by separate arrays of light-emitting diodes (LEDs). Programmable timing of the flashes allows to cover a wide dynamic range from microseconds to minutes. We have demonstrated that the LEDs can produce single-turnover flashes that saturate Q_A reduction of intact leaves of *Glyceria maxima* and shoots of *Picea abies*.

Additional key words: chlorophyll fluorescence; *Glyceria maxima*; *Picea abies*; Q_A reduction.

Introduction

A leaf is a complex optical system that imposes specific requirements on the measurement of Chl *a* fluorescence emission. The Chl concentration in a leaf is typically 0.2 to 0.7 g(Chl *a*+*b*) m⁻² (Šesták 1971). The high pigment concentration requires a high power of the single-turnover flashes to saturate the turnover of the reaction centres. Also, the excitation radiation and the emitted Chl fluorescence are

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Abbreviations: Chl – chlorophyll; DCMU – 3-(3',4'-dichlorophenyl)-1,1-dimethylurea; LED – light emitting diode; PS – photosystem; Q_A – primary quinone acceptor of photosystem 2.

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absorbed and re-absorbed much stronger by the highly concentrated pigments of a leaf (Bolhàr-Nordenkampf and Öquist 1993) compared to the usually used dilute suspensions of algae or of isolated photosynthetic structures. The Chl fluorescence emission of a leaf originates, in general, from cells at various depths in the tissue. The upper layer cells are exposed to the exciting irradiance that is not attenuated by the absorption; they exhibit the fastest turnover rates reflected by the fastest fluorescence induction. The cells deep in the tissue are exposed to significantly reduced irradiance that is reflected by a slower induction kinetics. The measured integral fluorescence kinetics is then a convolution of contributions originating from all depths of the leaf that reflect variable excitation rates.

Problems caused by the radiation gradient in a leaf may be reduced by using a measuring radiation that is incident on the leaf surface at a sharp angle so that most of the photons are absorbed in the upper layer of the leaf (Evans *et al.* 1993). This approach, however, limits the capacity of the method to yield information on the cells deep in the tissue. Alternatively, an application of very strong flashes that would turnover all reaction centres in the leaf in synchrony would ensure a uniform kinetic behaviour of the cells regardless of how deep in the tissue they are. Such flashes, called single-turnover saturating, can be generated by LEDs when applied to dilute suspensions of cyanobacteria (Nedbal and Trtílek 1995, Trtílek *et al.* 1997) or green algae (Nedbal *et al.* 1999).

Here, we describe a system that allows to generate single-turnover flashes from LEDs that saturate also the Q_A reduction in higher plants. The capacity of the instrument is demonstrated using one of the most challenging examples — a shoot of Norway spruce (*Picea abies* [L.] Karst.) that is distinct by a complex topology and a high Chl concentration in the needles (Marek *et al.* 1997). Measurements were also done with *Glyceria maxima* that has typical flat thick leaves.

Materials and methods

The Chl fluorometer was constructed as a modification of the earlier developed double modulation fluorometer (Nedbal and Trtílek 1995, Nedbal *et al.* 1999; *P.S. Instruments*, Brno, Czech Republic). The LEDs were distributed on two hemispheres of 3 cm diameter so that the exposed part of the leaf (*ca.* 3 cm²) was in the focus of radiation (7 in Fig. 1). The two hemispheres are parts of a mechanical clip that allows to fix the position of the leaf surface relative to the diodes without detaching it from the plant.

The measuring flashes were generated in 11 orange LEDs ($\lambda_{\max} \approx 620$ nm, *HLMP-DH08*, *Hewlett-Packard*, USA) that were positioned in the upper hemisphere at a small angle of *ca.* 20° to the leaf area (2 in Fig. 1) so that the upper layer of the leaf was excited preferentially. The actinic flashes were generated in 56 red LEDs ($\lambda_{\max} \approx 660$ nm, *HLMP-8103*, *Hewlett-Packard*, USA) distributed evenly at the upper hemisphere and 91 more LEDs at the lower hemisphere (1 in Fig. 1). Continuous irradiance was produced either in 12 orange LEDs (*HLMP-DH08*) at the upper hemisphere with *ca.* 65° angle of incidence (3 in Fig. 1) or in 7 far-red LEDs

($\lambda_{\text{max}} \approx 735$ nm, *QD-735*, *Quantum Devices*, Barneveld, WI, USA) with *ca.* 90° angle of incidence (4 in Fig. 1). The far-red LEDs were used to excite preferentially photosystem (PS) 1 and to oxidize the plastoquinone pool (Feild *et al.* 1998). The far-red radiation was spectrally limited by passing through a broad-band interference filter (*S10-730-A*, *Corion*, Franklin, MA, USA) that transmits photons at *ca.* 730 nm and eliminates photons of wavelengths shorter than 710 nm that would otherwise excite PS2.

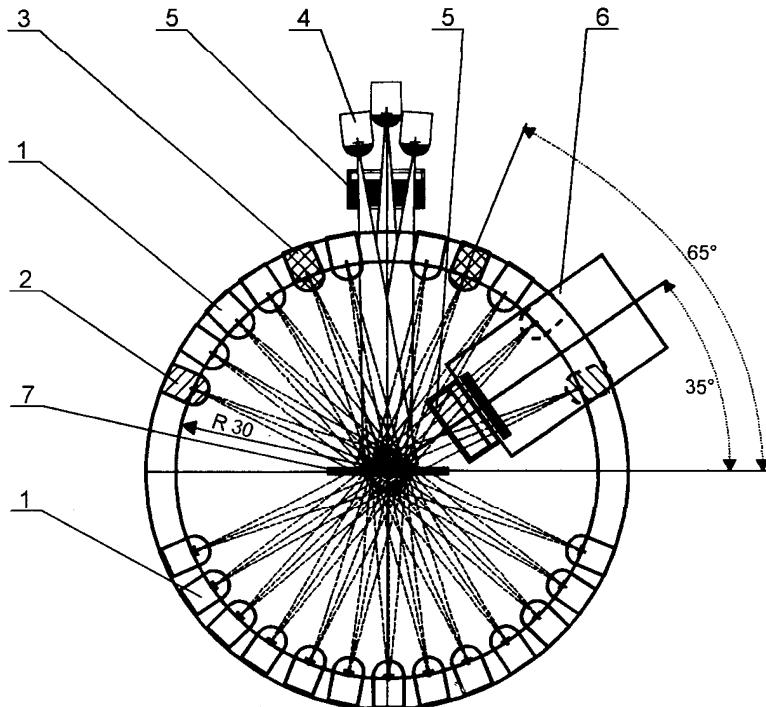


Fig. 1. Scheme of the sample compartment. 1 - light-emitting diodes (LEDs) generating red actinic single-turnover flashes. 2 - LEDs generating orange measuring flashes. 3 - LEDs generating continuous or pulsed orange actinic radiation. 4 - LEDs generating continuous or pulsed far-red actinic radiation. 5 - interference filter limiting far-red emission to dominant 730 nm. 6 - PIN photodiode with a 730 nm interference filter. 7 - leaf sample. The detector container includes a pre-amplifier and an analog-to-digital converter.

The PIN detector was positioned in the upper hemisphere at *ca.* 35° angle to the leaf area (6 in Fig. 1). The detector sensitivity was limited to the wavelengths of fluorescence by a broad-band interference filter (*S10-730-A*, *Corion*, Franklin, MA, USA). The signal amplifier and the analog-digital converter (16 bit, 100 kHz) were placed directly in the detector housing so that only digital signals were transduced by the cable to the control unit of the fluorometer. The digital part of the control unit and the *FluorWin* software were preserved identical to the earlier versions (Nedbal and Trtilek 1995, Trtilek *et al.* 1997).

For testing the instrument, second leaves of *Glyceria maxima* (Hartman) Holmberg were collected at the shore of a fishpond near our laboratory. One-year-old shoots of Norway spruce (*Picea abies* [L.] Karst.) were collected in the laboratory garden. The plant samples were collected in mid-April.

Results and discussion

An ideal modulated measuring radiation would produce no photochemistry and, yet, excite enough Chl molecules to produce measurable fluorescence. These two requirements are incompatible and therefore every modulated fluorescence experiment must be a compromise. The best way to test if the actinic effects of the selected measuring radiation are negligible is to change the number of measurement flashes in the experiment and check for the difference in the measured fluorescence kinetics. If there is no difference in the kinetics upon an increase of the measuring flashes, one may conclude that the observed kinetics are not affected by the measuring radiation. Fig. 2 shows the fluorescence transients elicited in a leaf of *G. maxima* by an actinic flash (red LEDs, 30 μ s duration, 100 % power). The circles show the kinetics obtained with 4 measuring flashes per decade and diamonds show

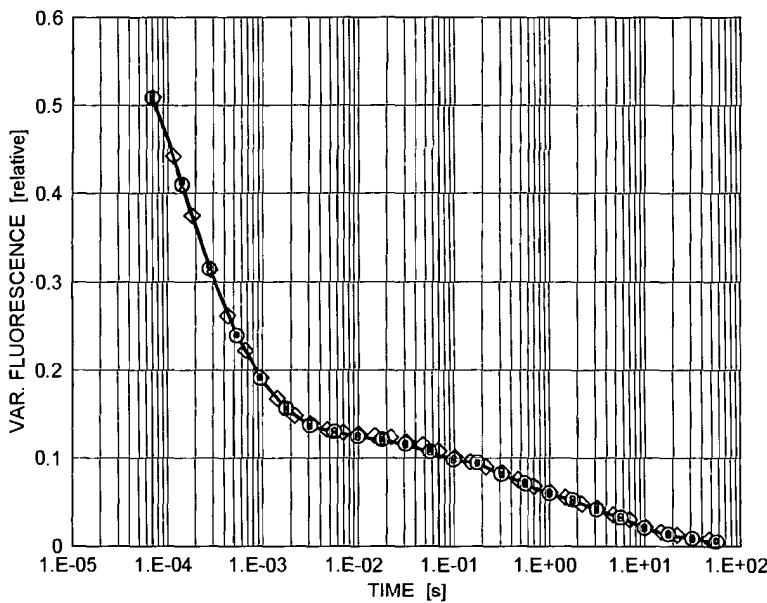


Fig. 2. Relative variable fluorescence $(F - F_0)/(F_M - F_0)$ following an actinic flash (red LEDs, 30 μ s duration) that reduced the primary quinone acceptor Q_A in the leaf of *Glyceria maxima*. The large circles show an average of three measurements done on the same leaf. Results of the individual measurements are shown by the small dots within the large circles. The individual measurements were separated by 5 min of dark adaptation. The values were obtained using 4 measuring flashes (orange LEDs, 80 % power, 2.5 μ s duration) per decade with the first data point taken 70 μ s after the actinic flash. The diamonds show results of a similar experiment in which 6 data points per decade were used instead of 4 data points per decade.

the kinetics with 6 measuring flashes per decade. The two kinetics overlap completely within the range of measuring error. This suggests that the measuring beam does not affect the resulting kinetics and the protocols may be used in further experiments. This conclusion was confirmed in an identical experiment done with a Norway spruce shoot and spinach (results not shown).

The capacity of the fluorometer to produce single-turnover flashes that saturate the reduction of the primary PS2 quinone acceptor Q_A was tested in two experiments. In the first one, we measured transients that are elicited by several actinic flashes spaced 200 ms apart. Each of these flashes generated in PS2 a charge separation that resulted on the acceptor side in one electron being sent through pheophytin to Q_A and further to the plastoquinone pool. On the donor side, the S-states were advanced by one turn in a flash. Because of the advancing S-states, the transients following individual actinic flashes exhibit a periodicity of 4 pattern (Lavergne and Leci 1993). Any difference in the transients elicited by flashes of different energy would indicate that the flashes are not saturating the charge separation in PS2.

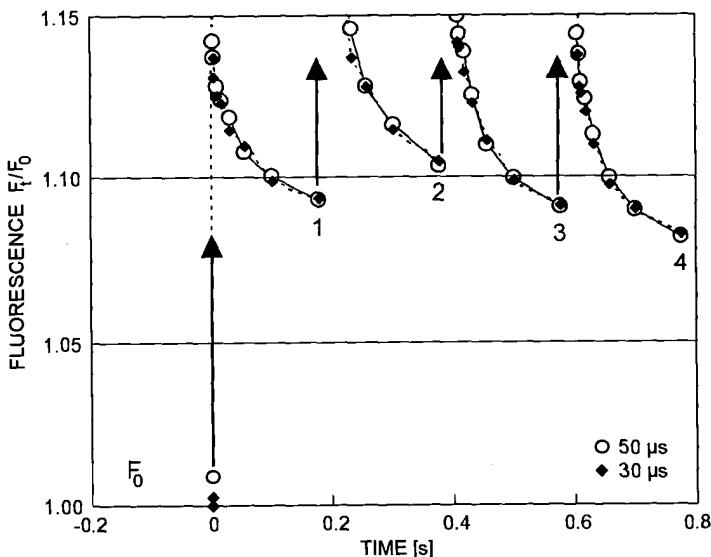


Fig. 3. Fluorescence transients elicited in a shoot of Norway spruce by 4 actinic flashes of 30 μ s (open circles, full line) and of 50 μ s (closed diamonds, dashed line) duration. The positions of the flashes are marked by arrows. The residual fluorescence difference ($F_n(200 \text{ ms}) - F_0$)/ F_0 after the n -th flash is marked by numbers and exhibits periodicity of four (Lavergne and Leci 1993). The transients are independent of the actinic flash energy.

In Fig. 3, two representative transients are shown. The transient with circles (*full line*) was elicited by actinic flashes of 30 μ s duration (100 % power), and the transient marked with full diamonds (*dashed line*) was elicited by actinic flashes of 50 μ s duration (100 % power). There was no difference in the two transients exceeding the experimental error. The lack of difference indicates that with both durations (30 and 50 μ s) we had a good approximation of the single-turnover

saturating flashes. In a similar experiment (not shown), we used 40 μ s flashes of two different relative powers (100 and 75 %) and again no difference in ensuing transients was observed.

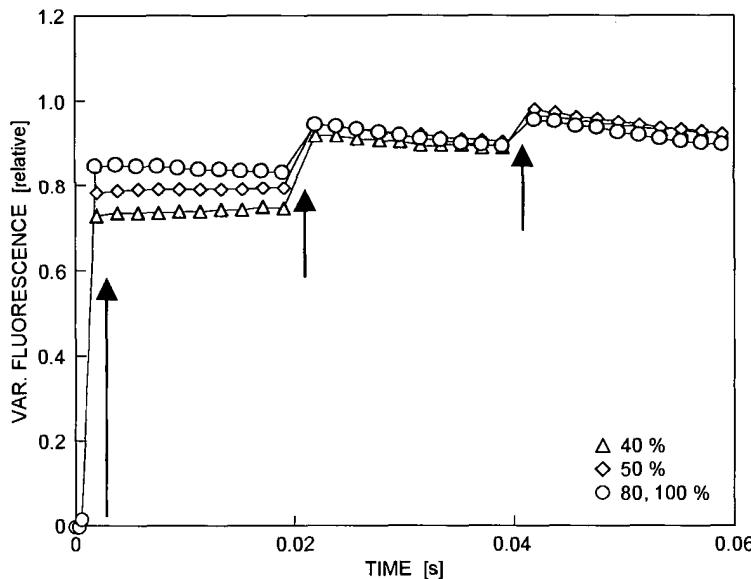


Fig. 4. Modulated fluorescence transients observed with a *Glyceria maxima* leaf poisoned by surface application with the herbicide DCMU (100 μ M, 15 min). The transients were elicited by 3 actinic flashes each lasting 30 μ s (arrows). The relative power of the flashes was 40 % (triangles), 50 % (diamonds), and 80 and 100 % giving identical transients (circles).

The capacity of actinic flashes to complete reduction of Q_A was further tested in an experiment shown in Fig. 4. The herbicide DCMU was used to block the re-oxidation of the Q_A^- acceptor that was reduced in an actinic flash. The differences between the transients elicited by actinic flashes of 40, 50, and 80 % relative powers show that a reduction to *ca.* one half of the flash relative power resulted in a small number of PS2 reaction centres that did not turn over during the first flash. The overlap of transients observed with 80 and 100 % relative power flashes, on the other hand, confirmed the earlier conclusion that a 30 μ s-long flash of 100 % relative power is a good approximation of a single-turnover, saturating flash.

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