Plant Cell Physiol. 36(5): 839–848 (1995)

The Steady State Chlorophyll *a* Fluorescence Exhibits in Vivo an Optimum as a Function of Light Intensity which Reflects the Physiological State of the Plant

Alaka Srivastava [1], H. Greppin [2] and Reto J. Strasser [1]

[1] *Bioenergetics Laboratory, University of Geneva, 1254, Jussy-Geneva, Switzerland*
[2] *Laboratory of Plant Physiology and Biochemistry, CH-1211, Geneva-4, Switzerland*

Modulated (690 and 730 nm), as well as direct chlorophyll (Chl) *a* fluorescence and changes in the concentration of the oxidized P700 were measured under steady state conditions in leaves of higher plants adapted to different light intensities. All the leaf samples exhibit an optimum curve of steady state fluorescence yield (F_s) versus the light intensity but its position with respect to light intensity varies considerably from one species to another or from one sample to other even in the same plant or within the same leaf sample. However, the optimum level of F_s was always at a moderate light intensity. By using the modulated fluorescence technique, the system with all closed (F'_m) or open reaction center (F'_o) were measured in steady state conditions. Each experimentally measured fluorescence yield was separated into a fluorescence emission of open ($F_{open}=F'_o\cdot(1-V_s)$) and closed ($F_{closed}=(F'_m\cdot V_s)$) reaction center (RC) of photosystem II where $V_s=(F_s-F'_o)/(F'_m-F'_o)$ is the function of fraction of closed reaction centers. With increasing light intensity, the fraction of open RC decreased while the fraction of closed RC increased. Maximum quantum efficiency (ΦP_o) and actual quantum efficiency (ΦP) decreased by increasing light intensity. An optimum level of F_s was observed, when the fraction of closed reaction centers V_s of each sample was about 0.2 showing a common quenching mechanism which determines the fluorescence properties under steady state condition. This explains the apparent phenomenological contradiction that the fluorescence yield under steady state conditions can increase or decrease upon an increase of actinic light.

Key words: Chlorophyll *a* fluorescence — Electron transfer — Open and closed reaction centers — Photosynthesis.

When dark adapted green leaves are illuminated with continuous light, Chl *a* fluorescence displays characteristic changes in intensity (Kautsky effect) accompanying the induction of photosynthetic activity (Kautsky and Hirsch 1931, Kautsky and Franck 1943). Under continuous illumination, photosynthetic organisms attain a steady state rate of photosynthesis and a relatively low Chl *a* fluorescence intensity. In spite of concerns raised by Trissl et al. (1993), measurements of the Chl *a* fluorescence induction curves have contributed significantly to the understanding of the photosynthetic mechanism (for reviews see, Papageorgiou 1975, Briantais et al. 1986, Govindjee and Satoh 1986, Krause and Weis 1991). However, not much information about the mechanisms which control the steady state fluorescence is available. At steady state, many factors are involved in optimizing the photosynthetic fluxes to maintain the optimum quantum yield of photosynthesis. However, the main considerations that control the Chl *a* fluorescence yield are (1) the redox state of the primary electron accep-

Abbreviations: Chl *a*, chlorophyll *a*; F_o^d and F_m^d, initial and maximal Chl *a* fluorescence in dark adapted leaf; F_s, steady state Chl *a* florescence; F'_m and F'_o, Chl *a* fluorescence yield under steady state conditions with saturating or without actinic light; F_s(dir), direct Chl *a* fluorescence; F_{op} and F_{cl}, fluorescence due to open and closed reaction centers; Mod L, modulated light; AL, actinic light; SL, saturating light; P700, reaction center of PSI; PS (I, II), photosystem (I, II); Q_A, primary quinone acceptor of PSII; ΦP_o, maximum quantum yield efficiency of PSII ($1-F'_o/F'_m$); ΦP, actual quantum yield efficiency ($\Phi P_o\cdot(1-V)$); V_s, relative variable Chl *a* fluorescence in steady state condition under any given light intensity ($(F_s-F'_o)/(F'_m-F'_o)$).

tor Q_A of photosystem II (PSII), with oxidized Q_A being a quencher and reduced Q_A a non quencher of Chl *a* fluorescence (Duysens and Sweers 1963) and (2) the state transition phenomenon, which provides a mechanism whereby a more balanced excitation of the two photosystems can be achieved (see e.g., Briantais et al. 1986, Strasser 1984). To achieve the optimum photosynthesis, the electron transport and photophosphorylation systems must be in balance (Heber et al. 1986). If this is not the case optimal efficiency is not possible, and often excess light is shown to cause damage to the system (Powles 1984, Demmig-Adams and Adams 1992). However, plants have developed many controlling mechanisms which quench excess excitation energy from the pigment system by many non-photochemical de-excitation mechanisms (Demmig-Adams 1990).

The diversity of mechanisms controlling Chl *a* fluorescence yield makes the fluorescence signals complex and difficult to interpret. The modulated fluorescence technique (Schreiber et al. 1986) has, however, widened the possibilities to analyze the fluorescence signals in the steady state conditions. Quenching analysis of steady-state Chl *a* fluorescence emission has been used to examine photosynthetic electron transport rates and their regulation in vivo (Peterson et al. 1988, Genty et al. 1989, Holmes et al. 1989, Havaux et al. 1991a, b). An almost linear relationship was observed by Genty et al. (1989) under their experimental conditions between $1-F_s/F_m$ and the quantum yield of photosynthesis as predicted by the energy flux theory of Strasser (1978). According to which $\Phi P=\Phi P_o\cdot(1-V_s)=1-(F_s/F_m)$, where $\Phi P_o=1-(F_o/F_m)$ and $V_s=(F_s-F_o^l)/(F_m^l-F_o^l)$.

Previous studies in our laboratory (Havaux et al. 1991b) suggested the existence of biological homeostasis under steady state conditions in leaves exposed to different light intensities. For instance, the level of modulated fluorescence emission from a dark adapted leaf with all PSII reaction centers in open configuration was similar to the steady-state modulated fluorescence emission measured in leaves illuminated with a strong photosynthetically saturating light with all photosystem II traps closed. However, in some cases, for instance, in young leaves and those leaves exposed to unfavorable environmental conditions, higher steady state fluorescence yields (F_s) were observed (Havaux et al. 1991b).

In the present work, a careful analysis of modulated fluorescence of steady state signals (F_s) shows that in all plant leaves tested, the deviation from homeostasis seems to be a general behavior of the plants. The changes in the F_s level of each leaf is the same if compared with respect to the fraction of closed and open reaction centers.

Materials and Methods

Plant material—Experiments were done with intact leaves of 3 week old pea (*Pisum sativum* L.), and 1 year old oleander (*Nerium oleander*) and *Ficus* sp. plants. Peas were grown as described earlier (Havaux et al. 1991a); oleander and *Ficus* sp. were grown under similar conditions but in larger pots than used for peas. Most of the data presented in this paper are those measured with the leaves of oleander because they are stiff, and they don't suffer any damage for several hours, often needed for our experiments. Data on pea leaves show that similar results can be obtained with softer leaves.

Chlorophyll a fluorescence measurements—Chl *a* fluorescence emission was measured at room temperature in open air with intact leaves using a twin channel modulated Chl *a* fluorescence system (Hansatech Inst., King's Lynn, Norfolk, England). The two sensor units of the system measured the fluorescence signals at 690 and at 730 nm. The instrument provided four (two modulated and two direct) fluorescence signals simultaneously. Those four signals were recorded by a 6 channel chart recorder (Goerz Metrawatt, SE 400). For a better resolution, the modulated fluorescence signals were recorded simultaneously at two amplifications (×1 and ×4). In this way, the recorded signals of F_o (the initial minimal fluorescence) and F_m (max-

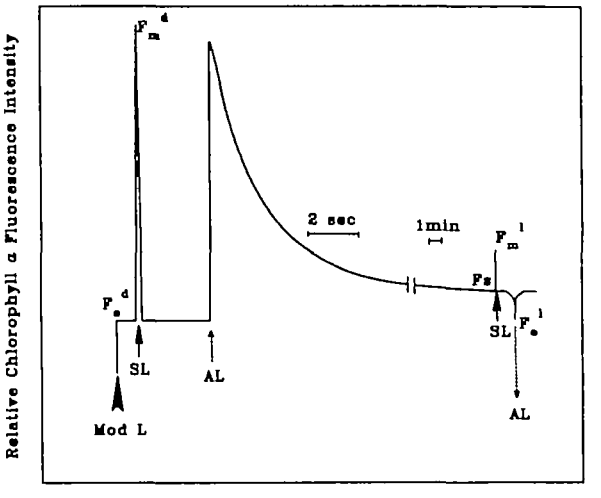

Fig. 1 A typical modulated Chl *a* fluorescence curve obtained from attached *Nerium oleander* leaf showing different fluorescence parameters used in the present work. After dark adaptation for 1 hour, the leaf was excited with the measuring beam of a weak modulated light, Mod L, to obtain dark level of fluorescence (F_o^d). During a 1 sec pulse of saturating light, SL, (300 W m^{-2}), the maximum fluorescence emission (F_m^d) was measured. The samples were then irradiated with actinic light (AL). In the measurement shown, the intensity of the actinic light was 100 W m^{-2}. After a prolonged illumination with AL, the Chl *a* fluorescence reached a low steady state level (F_s). The maximum (F_m^l) level in the steady state condition was obtained by applying a pulse of saturating light, SL. The dark (F_o^l) fluorescence level was obtained after switching off AL.

imal fluorescence) appear on the same paper with similar amplitudes.

Chl *a* fluorescence was excited with modulated yellow light (maximum transmittance at 585 nm; modulated frequency, 4.8 kHz). In this way it is possible to follow the fluorescence signals at any wavelength of the Chl *a* fluorescence emission spectrum. This is an advantage over several other commercial instruments where the modulated fluorescence can only be detected for wave lengths larger than 700 nm. The intensity of this modulated light beam was very low (<0.025 W m^{-2}) and was thus not able to induce any variable fluorescence. A typical example of different levels of modulated Chl *a* fluorescence signals at a measured wavelength is shown in Fig. 1. The yellow modulated beam (Mod L) was turned on and the low fluorescence level was measured when it reached a constant value and is referred to as F_o^d (d stand for dark adapted leaf). Then, a short pulse (1 s) of saturating blue light, SL (Schott 1500 lamp with a Corning filter, CS 4-96 and a built in shutter system) was given to measure the maximum level of modulated Chl *a* fluorescence (F_m^d) in the previously dark adapted sample. The variable Chl *a* fluorescence was induced by an actinic continuous blue light (AL) which was adjusted from 0–280 W m^{-2} using an electronically controlled light source (Schott, KL1500 Electronic lamp with a Corning filter, CS 4-96). After showing a transient (minutes), the Chl *a* fluorescence yield reached a stable and low steady state level (F_s). Later on, the AL was increased or decreased and a new F_s level for that light intensity was recorded when the fluorescence level again reached the stable steady state level. The maximum fluorescence level (F_m^l, where l refers to the fact that the samples had been under continuous illumination with actinic light) at any steady state condition was obtained by giving a short pulse of saturating light (SL) and immediately after that the F_o level (F_o^l) was measured by turning off the actinic light (AL). The intensity of the actinic light (AL) was measured and recorded continuously during the experiments as shown in the bottom half of the experiment (see e.g., Fig. 2B).

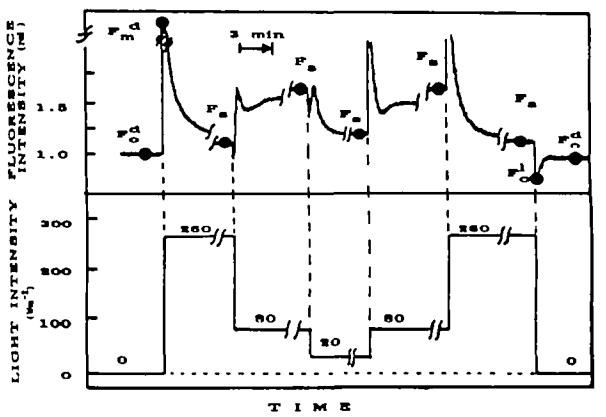

Fig. 2 The effect of three different light intensities (AL) (20, 80 and 260 W m^{-2}) on the relative levels of steady state chlorophyll fluorescence (F_s/F_o^d) of *Nerium oleander* leaf. At each actinic light intensity (AL), the F_s level was evaluated at steady state illumination (about 7–8 min equilibration between successive light intensities). Note that the level of F_s was low at very low (20 W m^{-2}) and very high (260 W m^{-2}) light intensities but it was higher at moderate light intensity (80 W m^{-2}). All the symbols are as in Figure 1.

P700 absorbance change measurements—In parallel with the fluorescence measurements, light-induced absorbance changes around 820 nm were measured with a Hansatech P700$^+$ measuring system. This was done by adding two separate arms of the fiber optics through two of the seven holes of the leaf chamber. Leaves were illuminated through one arm with 820 nm light pulsed at 800 Hz (820 LED). Note that the steady state 820 nm signal was measured simultaneously along with the two fluorescence measurements. Reciprocal disturbances of the signals can be avoided by using different modulated frequencies (4.8 kHz for fluorescence and 800 Hz for reflection of 820 nm). The reflected light by the leaves was monitored by a photodiode screened by a 820 nm interference filter through the other fiber optic arm. The signal from the detector was recorded with a chart recorder (Goerz Metrawatt, 120). The level of P700$^+$ under steady state conditions was measured and the percentage of P700$^+$ at any given steady state condition was determined after obtaining maximum P700$^+$ (100%) by giving a strong saturating far red light (2 W m^{-2}, 715 nm interference filter B40, Bird Atomic Co., in combination with a red cut off RG 690 Schott glass filter). The P700$^+$ redox state was estimated as described by Weis and Lechtenberg (1988).

Results and Discussion

Apparent contradictory phenomena—When many samples were measured under steady state conditions, and the data were averaged, a homeostatic level of steady state fluorescence as a function of light intensity was observed (Havaux et al. 1991b). However, a careful analysis of many individual samples reveals that every sample examined exhibits an optimum curve for the steady state Chl *a* fluorescence yield versus light intensity. The amplitude of the fluorescence yield and its position with respect to light intensity vary considerably from one sample to the other even in the same plant or within the same leaf (data not shown). A typical experiment done with *Nerium oleander* leaf is presented in Fig. 2. The optimum of the steady state fluorescence yield (F_s) always appeared at moderate (~80 W m^{-2}) light intensities independently of the preilluminating (low or high light) conditions of the plant. Lower level of steady state fluorescence was observed when leaves were irradiated with either high (260 W m^{-2}) or low (20 W m^{-2}) light inten-

sities. A similar type of optimum steady state fluorescence yield was reported earlier by Genty et al. (1992) in bean leaves.

Since the changes in fluorescence yield and its position with respect to light intensity vary from sample to sample it is possible that the above described individual behavior of each sample might got lost after the data of many parallel experiments were averaged. This leads to the homeostatic behavior reported by Havaux et al. (1991b).

A heterogeneous behavior between and within the leaf samples—Figure 3 shows the light intensity dependence of Chl *a* fluorescence yields at steady state (F_s) in single leaves of three different plants (*Ficus* sp. *Nerium oleander, Pisum sativum*) at 690 and at 730 nm. The steady state fluorescence yield (F_s) was clearly higher at moderate light intensities than at the low or the high light intensities in all these three plants at both 690 and 730 nm. The optimum level of steady state fluorescence for *Ficus* sp., *Nerium oleander* and *Pisum sativum* was around 25, 100 and 110 W m^{-2} respectively. The maximum increase in the steady state fluorescence yield in *Nerium oleander* was more than 1.5 times the fluorescence yield in the dark but in *Pisum sativum*, a typical value for a mature green healthy leaf was mostly found to be around 1.3 times the fluorescence yield in the dark. A small but constantly higher level of F_s was recorded in *Ficus* sp. The optical properties of these leaves are very different in different plants. For example the chlorophyll content per area unit is higher in *Ficus* than in *Pisum*. So the observed differences may be influenced by reabsorption effects.

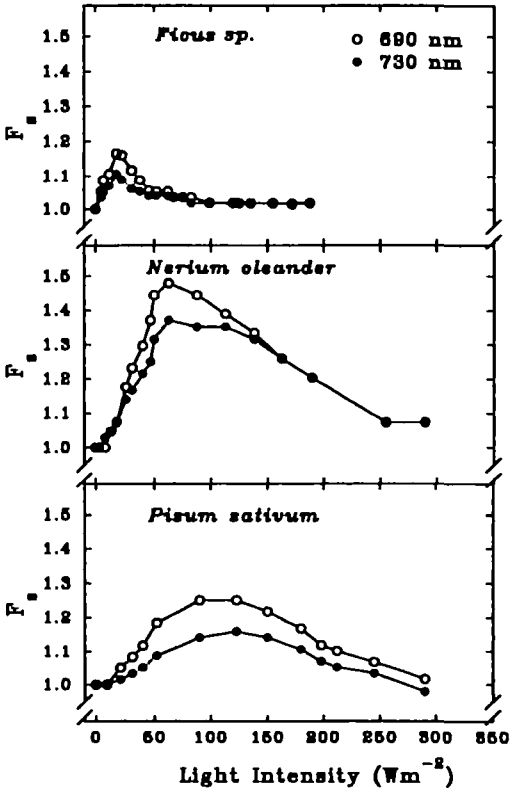

Fig. 3 The effect of different light intensities (AL) on the relative level of steady state fluorescence (F_s/F_o^d) of *Ficus* sp., *Nerium oleander* and *Pisum sativum*, monitored simultaneously at two different wavelengths. At each light intensity, the F_s level was evaluated at steady state illumination (about 7–8 min equilibration between successive light intensities).

The above-mentioned pattern was confirmed in many samples (n=12). The measured fluorescence yields appeared at somewhat different light intensities for different leaf samples of all the three plants. There were larger differences from one plant sample to another than from one leaf to another leaf of the same plant (data not shown). This variation in the steady state fluorescence yield depends not only on the actinic light intensities, but also on the wavelength of the florescence emission. But always an optimum steady state fluorescence level was observed on both wave-lengths. The normalized steady state fluorescence yield (F_s/F_o^d) at 730 nm is always lower than at 690 nm. This may be partly due to lower variable fluorescence at 730 nm than at 690 nm since 730 nm emission may contain contribution of photosystem I fluorescence (Strasser et al. 1987, Genty et al. 1990, Krause and Weis 1991).

A detailed comparison of the simultaneously measured modulated (F_s(mod)) and direct (F_s(dir)) Chl *a* fluorescence signals are shown in Fig. 4. Figure 4(A) shows the fluorescence yield from modulated measurement, Fig. 4(B) the direct fluorescence and Fig. 4(C) the yield obtained from F_s(dir)/I where I is the light intensity shown in Fig 4(D). Direct fluorescence (F_s(dir)) (Fig. 4B) is approximately proportional to the light intensity (Fig. 4D). However, an examination of fluorescence yields (Fig. 4A, C) shows that at low and high light intensities they are low and very close or equal to the value obtained in the dark (F_o^d). On the other hand, highest yields were observed at medium light intensities. Although the trend for the changes in the fluorescence yield by changing the light intensity was the same measured either by direct or by modulated light, the amplitude of fluorescence yield was different measured with modulated and direct lights. Since the modulated light intensity is very low it measures the fluorescence yield only on the upper surface of the leaf. On the other hand fluorescence yield measured by direct light is a mixture of all the cell layers of the leaf which can add the optical scattering effects of leaves (Uz and Saygin 1994).

The physiological state of the sample—Since the steady state fluorescence yield has similar values at low and at high light intensities, additional information are necessary to distinguish between the physiological state of the sample at high and low light intensities. The technique of the modulated fluorescence offers the possibility to measure at each steady state the fluorescence yield of the system

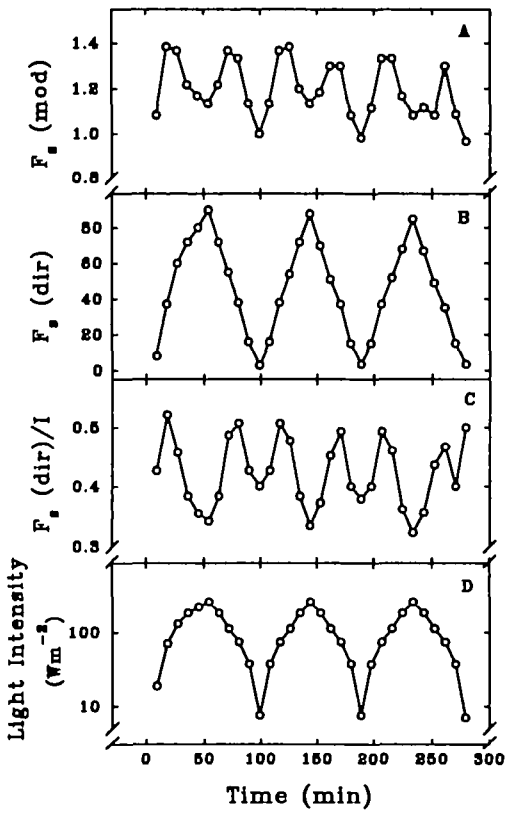

Fig. 4 The time course changes in the steady state level of fluorescence yield from modulated measurement; F_s (A), direct fluorescence; F_s (dir) (B) and the yield (C) obtained from F_s (dir)/I where I is the light intensity (D). Leaves were irradiated to one light intensity for about 7–8 min until it reached to the steady state (F_s or F_s (dir)) level. At that moment the light was increased or decreased step wise and a new F_s or F_s (dir) for that light intensity was recorded.

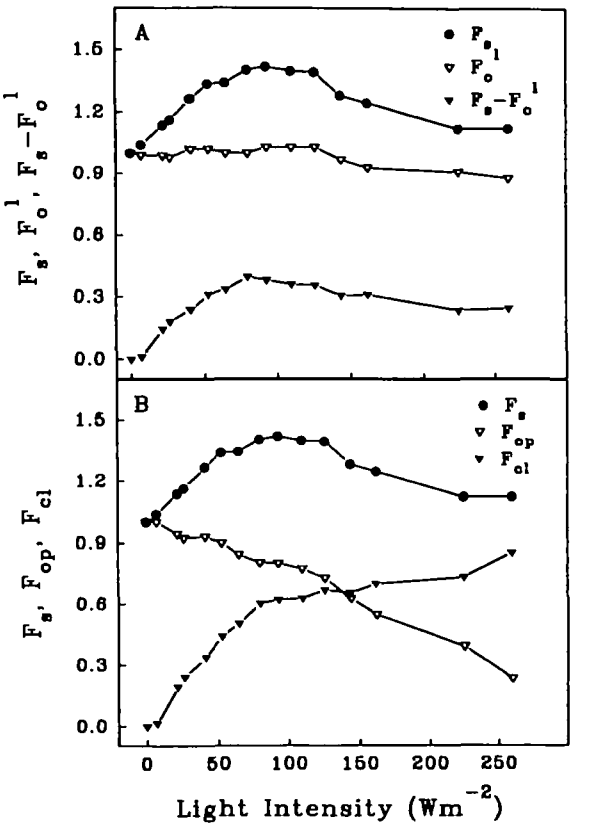

Fig. 5 (A) Changes in steady state (F_s), dark (F_o^l) and variable ($F_s-F_o^l$), fluorescence of *Nerium oleander* leaf at different light intensities. All the values are normalized to F_o^d. (B) Fluorescence due to open, $F_{op}=((1-V)\cdot F_o^l)$ and closed, $F_{cl}=(V_s\cdot F_m^l)$ reaction centers at different light intensities where $V_s=(F_s-F_o^l)/(F_m^l-F_o^l)$. Data were obtained from the same experiment as in (A). For a better comparison, F_s normalized with F_o^d values were replotted in figure (B). At each light intensity, the fluorescence values were obtained at steady state illumination (about 10 min equilibration between successive light intensities).

with all closed (F_m^l, by applying a strong flash) or open reaction centers (F_o^l, by turning off all actinic light sources). Thus we can measure three independent signals F_o^l, F_s and F_m^l. Figure 5A shows the light intensity dependence of the steady state Chl *a* fluorescence; F_s, the state with all reaction centers open; F_o^l and the so called variable fluorescence; $(F_s-F_o^l)$. The fluorescence signal at any time is a mixture of the fluorescence due to antenna chlorophylls with open and closed reaction centers. We have furthermore separated F_s into the fluorescence of open and closed PSII according to Strasser (1978) as described below:

$$F=F_{open}+F_{closed} \tag{1}$$

As open units are converted into closed units while the fluorescence rises from the initial level F_o to the maximum level F_m, we can follow the fluorescence being emitted by the open units only. When all units are open, the fluorescence is F_o, when all units are closed the fluorescence is F_m. Therefore, we can write for the fluorescence emission of antenna Chl being linked to an open reaction center as

$$F_{open}^l=F_o^l\cdot(1-V) \tag{2}$$

therefore, the fluorescence of antenna with closed reaction center is

$$F_{closed}^l=F_m^l\cdot V \tag{3}$$

where V is empirically the relative variable fluorescence at any time. For steady state conditions, we write

$$V_s=\frac{(F_s-F_o^l)}{(F_m^l-F_o^l)} \tag{4a}$$

In terms of the fraction of closed reaction centers B (labelled here as $B=[Q_A^-]/[Q_A]_{total}$), the expression for V_t has been derived as

$$V=\frac{B}{1+\frac{F_v}{F_o}\cdot p\cdot(1-B)} \quad (4b)$$

with p being the overall probability of excitation energy transferred between photosynthetic units (Strasser 1978).

Therefore $F_t=F_o^l+(F_m^l-F_o^l)\cdot V$ (5)

F_t, F_{open} (F_{op}) and F_{closed} (F_{cl}) as a function of light intensity is shown in Fig. 5B. With increasing light intensity, the fraction of closed and open reaction center increased and decreased respectively. With increasing light intensity the steady state fluorescence yield first increased and reached a maximum at around 100 W m^{-2} and then declined, as noted earlier. The fluorescence yield of open units (F_{open}) is decreased nearly linearly as a function of light intensity while the fluorescence yield of closed units (F_{closed}) exhibits a kind of a light saturating curve, which levels off after about 100 W m^{-2} (Fig. 5).

In order to understand better the optimum in F_t, we have measured and plotted the light-intensity dependence of several other parameters in *Nerium oleander* leaf which characterize the physiological state of a sample (Fig. 6): (1) The fraction of oxidized reaction center of PSI indicated as $P700^+$; (2) the fraction of closed reaction centers of PSII (V) which corresponds to the normalized relative variable fluorescence (Eq. 4a), if no energy transfer from one PSII unit to another is considered (p=0 in equation 4b); (3) ΦP_o (maximum quantum efficiency) of PSII$=1-(F_o^l/F_m^l)$; and (4) ΦP (actual quantum efficiency)$=1-(F_t/F_m^l)$ or $\Phi P=\Phi P_o\cdot(1-V_t)$. With increasing light intensity the fraction of closed reaction centers increased. However, the maximum quantum efficiency as well as the actual quantum yield of photochemistry decreased. An insignificant difference in the $P700^+$ was recorded at lower light intensities which increased linearly by further increasing light intensity. It is important to note here that the shift of $P700^+$ does not simply close PSI centers. Because of its absorbance properties, $P700^+$ traps excited states from the antenna as efficiently as photochemically active centers but converts the absorbed energy by a fast internal decay (Nuijs et al. 1986). So, the formation of $P700^+$ helps to de-excite the extra energy by non-photochemical pathways there by adjusting the photochemical activity to the rate of biochemical reactions. In this way it also protects PSI against photoinhibition.

The correlation of the fluorescence expressions and electron transport to the state of the reaction center— Leaves of other higher plants examined showed the data (not shown) similar to that shown in Fig. 6 for *Nerium*

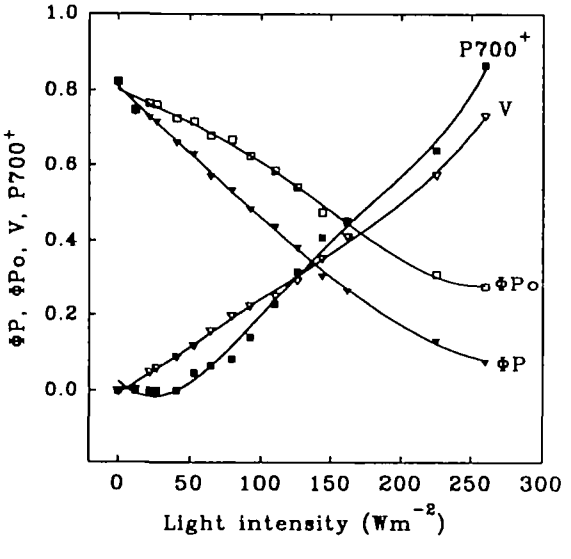

Fig. 6 Light intensity dependency of ΦP ($=1-(F_t/F_m^l)$), $\Phi P_o=1-(F_o^l/F_m^l)$, $V_t=(F_t-F_o^l)/(F_m^l-F_o^l)$, and $P700^+$ in *Nerium oleander* leaf. $P700^+$ changes were obtained by measuring the absorption changes at 820 nm simultaneously along with the fluorescence measurements on the same leaf using two different modulation frequencies (0.8 and 4.8 kHz). All the modulated fluorescence data were obtained from the experiment shown in figure 5.

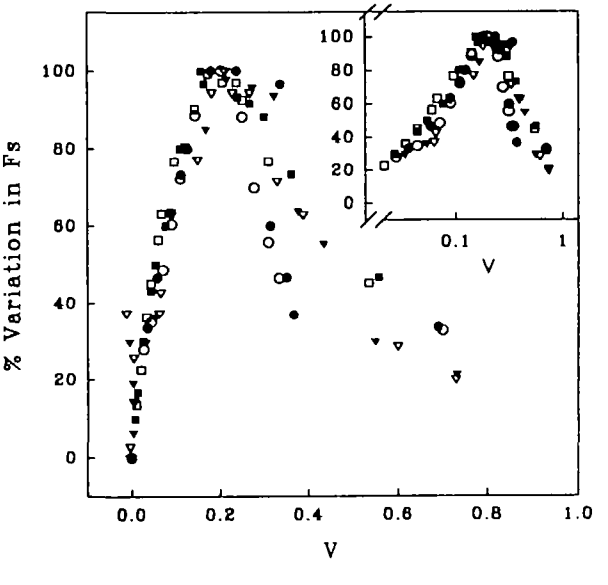

Fig. 7 Dependency of the steady state fluorescence, F_t on the fraction of closed PSII centers ($V_t=(F_t-F_o^l)/(F_m^l-F_o^l)$). 100% F_t level corresponds to the maximum change in F_t level of each leaf. Values were obtained from 3 different leaves of *Nerium oleander*. Different type of symbols correspond to different leaves. Closed and open symbols of the same type of symbol represent the fluorescence changes at 690 nm and 730 nm respectively. Insert shows the same graph but on logarithmic time scale.

oleander. All samples respond somewhat differently to light intensity as implied from results in Fig 3. If the changes in the optima of the steady state fluorescence intensity from the data of different experiments are normalized to the maximum values obtained for the optima, and then plotted as a function of the fraction of closed reaction centers ($V_s=(F_s-F_o')/(F_m'-F_o')$, see Eq. 4a), they superimpose nicely as shown in Figure 7. The F_s peak is always at V_s of about 0.2. This result establishes that there is a common steady state Chl *a* fluorescence behavior of higher plants that reflects the internal state of PSII.

A common quenching mechanism for all samples—We have shown above that the steady state fluorescence yield (F_s) is low at both low and high light intensities and that this phenomenon is independent of the sample used. The question now arises if like the F_s signals, the extreme fluorescence yields (F_o' and F_m') can also show a common pattern among different samples. We define an expression for ΦP_o (relative) and ΦP (relative) which always cover a range from zero to one:

$$\Phi P_o(\text{rel})=\frac{1-\frac{F_o'}{F_m'}}{1-\frac{F_o^d}{F_m^d}}=\frac{\Phi P_o(\text{light})}{\Phi P_o(\text{dark})} \quad (6)$$

$$\Phi P(\text{rel})=\frac{1-\frac{F_s'}{F_m'}}{1-\frac{F_o^d}{F_m^d}}=\frac{\Phi P(\text{light})}{\Phi P_o(\text{dark})} \quad (7)$$

Since $V_s=(F_s-F_o')/(F_m'-F_o')$, equation (7) is related to equation (6) as follows:

$$\Phi P(\text{rel})=\frac{\Phi P_o(\text{Light})}{\Phi P_o(\text{Dark})}\cdot(1-V) \quad (8a)$$

$$\text{or } \Phi P(\text{rel})=\Phi P_o(\text{rel})\cdot(1-V) \quad (8b)$$

Fig. 8 shows that ΦP(relative) and ΦP_o(relative) calculated from many different samples follow a similar trend when they are plotted against V_s, the fraction of the closed reaction centers always under steady state conditions. Thus a common quenching mechanism which determines the fluorescence properties under steady state conditions may play a role in all the samples examined.

It is evident that under steady state conditions the regulation of the Calvin cycle or reductant consuming reactions have very strong impact on the yield of in vivo Chl *a* fluorescence (Briantais et al. 1986, Weis and Lechtenberg 1989). Under weak light conditions the delta pH dependent quenching may be low whereas under conditions close to light saturation it may contribute very strongly (Quick and Horton 1984, Weis and Lechtenberg 1989, Ruban and Horton 1994). The decrease in F_s level at higher light intensities may be explained by photoinhibition (Powles 1984, Demmig-Adams and Adams 1992, Long et al. 1994), or by mechanism of light dependent chlorophyll quenching by the aggregation of light harvesting protein pigment complex of PSII (LHCII) (Ruban et al. 1992, Mullineaux et al. 1993). The $P700^+$ radical is also a highly efficient quencher of singlet excited state (Weis and Lechtenberg 1989).

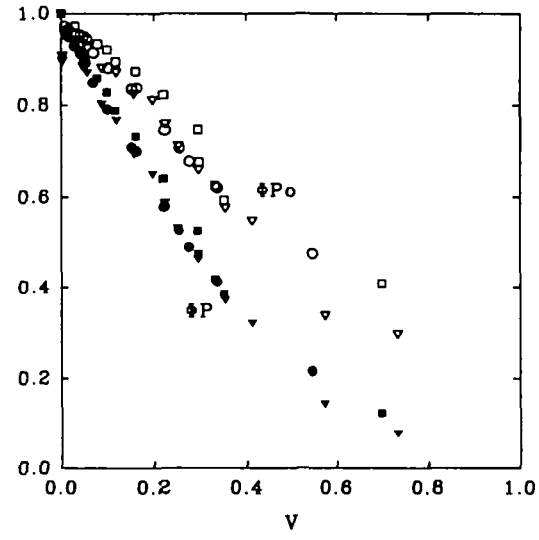

Fig. 8 Dependency of ΦP ($=1-(F_s/F_m')$) and ΦP_o ($=1-(F_o'/F_m')$) on V_s ($=(F_s-F_o')/(F_m'-F_o')$). Values were obtained from experiments done with three different leaves. Different type of symbols correspond to different leaves. Open and closed symbols shown are the averaged values obtained from 690 nm and 730 nm measurement values for ΦP_o and ΦP respectively.

The simulation of the steady state fluorescence intensity—A common quenching mechanism may be responsible for observed fluorescence phenomenon under steady state conditions. Here, we present a very simple model to simulate the typical experimental curves. This model is for sure still far away from reality, however, it shows that quite a complex fluorescence behavior can be simulated by introducing only one assumption to the usual description of a photosynthetic model. We consider usually the following rate constants for the deexcitation of a excited antenna pigment: k_F for fluorescence, k_P for photochemistry and k_N for the sum of all non-photochemical (including k_F) rate constants. Now we introduce an additional deexcitation rate constant k_X for an additional radiationless deexcitation flux. This flux is stated as proportional to the fraction of closed RCs labelled as B. Therefore, k_X is considered as a second order rate constant. The appearance of an additional deexcitation flux is linked to the appearing of a quencher X. The concentration or the quenching capacities of X are assumed to be a function (here simplified as proportional) to the steady state fraction of closed RCs.
We can write for the fluorescence of all open RCs

$$F_o=\frac{J\cdot k_F}{(k_N+k_P+k_X\cdot B)} \quad (9)$$

and for all closed RCs

$$F_m = \frac{J \cdot k_F}{(k_N + k_X \cdot B)} \tag{10}$$

where J is the photon flux density absorbed by the PSII. If we normalize F_o and F_m with the initial fluorescence of a dark adapted sample (with all RCs being open B=0) we can write:

$$\frac{F_s}{F_o^d} = \frac{J \cdot k_F/(k_N + k_P + k_X \cdot B)}{J \cdot k_F/(k_N + k_P)}$$

$$\frac{F_s}{F_o^d} = \frac{1}{1 + \frac{k_X}{k_N + k_P} \cdot B}$$

$$\frac{F_s}{F_o^d} = \frac{1}{1 + K_2 \cdot B} \tag{11}$$

$$\frac{F_m}{F_o^d} = \frac{J \cdot k_F/(k_N + k_X \cdot B)}{J \cdot k_F/(k_N + k_P)}$$

$$\frac{F_m}{F_o^d} = \frac{1}{\frac{k_N}{(k_N + k_P)} + \frac{k_X}{(k_N + k_P)} \cdot B}$$

$$\frac{F_m}{F_o^d} = \frac{1}{K_1 + K_2 \cdot B} \tag{12}$$

where $K_1 = k_N/(k_N + k_P)$ and $K_2 = k_X/(k_N + k_P)$

Considering the relative variable fluorescence V as an indication of the fraction of closed reaction centers we can formulate and simulate the expression F_{open}/F_o^d according to equation (2) and (11) and the expression F_{closed}/F_o^d according to equation (3) and (12) as

$$\frac{F_{open}}{F_o^d} = \frac{F_o \cdot (1 - V)}{F_o \cdot (1 + K_2 \cdot B)} = \frac{1 - V}{1 + K_2 \cdot V} \text{ if } B = V \tag{13}$$

$$\frac{F_{closed}}{F_o^d} = \frac{F_m \cdot V}{F_m \cdot (K_1 + K_2 \cdot B)} = \frac{V}{K_1 + K_2 \cdot V} \text{ if } B = V \tag{14}$$

The summation of equation (13) and (14) leads to the normalized steady state fluorescence emission F_s/F_o^d according to the equation (1) as

$$\frac{F_s}{F_o^d} = \frac{F_{open}}{F_o^d} + \frac{F_{closed}}{F_o^d} = \frac{1 - V}{1 + K_2 \cdot V} + \frac{V}{K_1 + K_2 \cdot V} \tag{15}$$

Equation (15) shows a function with a more or less pronounced maximum according to the choice of constants K_1 and K_2.

Figure 9A shows the experimental data points for the steady state fluorescence and it has been separated into fluorescence components related to open and closed centers versus the fraction of closed reaction centers by a given light intensity. Figure 9B shows the simulation of the same expression using equation (13), (14) and (15) and the same fraction of closed RCs as in Fig. 9A. The constants used were $K_1 = 0.06$ and $K_2 = 1.10$. The similarity of the experimental to the theoretically predicted curves on the basis of the chosen quenching mechanism is evident.

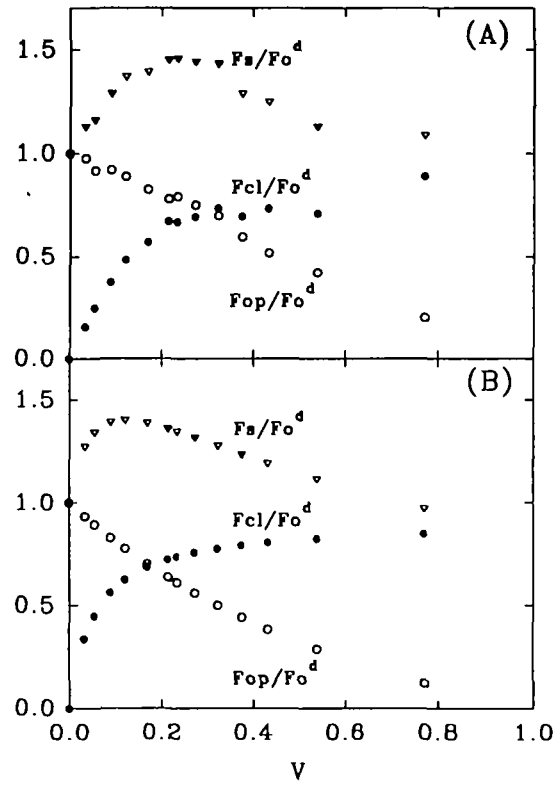

Fig. 9 (A) Changes in the steady state fluorescence (F_s) and the fluorescence components of units with open (F_{op}) and closed (F_{cl}) centers at different fractions of closed RCs; $V_s = (F_s - F_o^l)/(F_m - F_o^l)$. All the modulated fluorescence data were obtained from the experiment shown in figure 5. (B) Simulated data according to equation (13), (14) and (15) at different fraction of closed RCs. The constants used are $K_1 = 0.06$ and $K_2 = 1.10$.

Although we don't know yet the full mechanism responsible for the fluorescence emission yield of higher plants under steady state conditions but on the phenomenological basis we can conclude as: (1) The fluorescence yield under steady state condition is equal or very similar whether the steady state is established under very low light intensities (nearly all RC being open) or under high light conditions (nearly all RC being closed). (2) The value of this common fluorescence yield of the sample (which is physiologically in steady state) is equal or very similar to the fluorescence yield of a dark adapted sample with all RC being open. (3) A deviation of this homeostatic behavior with the appearance of higher fluorescence yields is observed generally under moderate light intensities when about 25% of the RC are closed. (4) The steady state fluorescence yield (F_s) as well as the yield of steady state variable fluorescence ($F_s - F_o^l$) exhibit an optimum as a func-

tion of the light intensity. However, the fraction of closed reaction centers (which can be considered as the electron donors for PSI and the Calvin-cycle) appears as a typical light saturation curve without any intermediate optimum. Therefore, we conclude that the observed fluorescence behavior reflects the energetic preconditions which guarantee the well known monotonically increasing photosynthetic activity upon increasing light intensity which levels off at about 100 to 200 W m^{-2}.

Only plants in an apparent optimal physiological state behave as reported above. However under sub-optimal conditions (e.g., stress), the steady state fluorescence is mostly higher than F_o^d. Such a behavior is as well predicted by equation (15) when a higher value for K_2 is chosen. We are convinced that a common energetic concept can be developed which can accommodate the four empirical findings mentioned above for physiological conditions.

We are convinced that fluorescence measurements are a helpful tool in environmental research. On a global scale, canopy and ocean observations will be developed by using satellite remote sensing. The goal is to develop techniques which allow the detection of vitality in plants, which is very difficult under steady state conditions. The fluorescence emission in vivo as a function of light intensity, as reported here, could be detected by remote sensing using the naturally different light intensities which occurs during the day. The reported function (steady state fluorescence versus the light intensity) which exhibits a typical optimum could be used as a marker function indicating the vitality of a photosynthetic sample. Normal and abnormal behavior of the vegetation due to e.g., pollution, stress and different diseases in critical areas on our globe could be detected and analyzed.

Authors thank Professor Govindjee for his valuable suggestions. This work was supported by Swiss National Foundation grant (#31-33678-92) to R.J.S.

References

Briantais, J.M., Vernotte, C., Krause, G.H. and Weis, E. (1986) Chlorophyll *a* fluorescence of higher plants: chloroplasts and leaves. *In* Light Emission by Plants and Bacteria. Edited by Govindjee, Amesz, J. and Fork, D.C. pp. 539–583. Academic Press, New York.

Demmig-Adams, B. (1990) Carotenoids and photoprotection in plants: a role of the xanthophyll cycle. *Biochim. Biophys. Acta* 1020: 1–24.

Demmig-Adams, B. and Adams III, W.W. (1992) Photoprotection and other response of plants to high light stress. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 43: 599–626.

Duysens, L.N.M. and Sweers, H.E. (1963) Mechanism of the two photochemical reactions in algae as studied by means of fluorescence. *In* Studies on Microalgae and Photosynthetic Bacteria. Edited by The Japanese Society of Plant Physiologists. pp. 353–372. University of Tokyo Press, Tokyo.

Genty, B., Briantais, J.M. and Baker, N.R. (1989) Relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim. Biophys. Acta* 990: 87–92.

Genty, B., Goulas, Y., Dimon, B., Peltier, G., Briantais, J.M. and Moya, I. (1992) Modulation of efficiency of primary conversion in leaves, mechanisms involved at PS2. *In* Research in Photosynthesis. Edited by Murata, N. Vol. IV, pp. 603–610. Kluwer Academic Press, Netherlands.

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

Govindjee and Satoh, K. (1986) Fluorescence properties of chlorophyll *b*- and chlorophyll *c*-containing algae. *In* Light Emission by Plants and Bacteria. Edited by Govindjee, Amesz, J. and Fork, D.C. pp. 497–537. Academic Press, New York.

Havaux, M., Strasser, R.J. and Greppin, H. (1991a) A theoretical and experimental analysis of qP and qN coefficients of chlorophyll fluorescence quenching and their relation to photochemical and nonphotochemical events. *Photosyn. Res.* 27: 41–55.

Havaux, M., Strasser, R.J. and Greppin, H. (1991b) Effect of incident light intensity on the yield of steady-state chlorophyll fluorescence in intact leaves. An example of bioenergetic homeostasis. *Environ. Exp. Bot.* 31: 23–32.

Heber, U., Neimanis, S., Dietz, K.J. and Viil, J. (1986) The assimilatory power as driving force in photosynthesis. *Biochim. Biophys. Acta* 852: 144–155.

Holmes, J.J., Weger, H.G. and Turpin, D.H. (1989) Chlorophyll *a* fluorescence predicts total photosynthetic electron flow to CO_2 or NO_3^-/NO_2^- under transient condition. *Plant Physiol.* 91: 331–337.

Kautsky, H. and Franck, U. (1943) Chlorophyllfluoreszenz und Kohlensäureassimilation. *Biochem. Z.* 315: 139–232.

Kautsky, H. and Hirsch, A. (1931) Neue Versuche zur Kohlensäureassimilation. *Naturwissenschaften* 48: 964.

Krause, G.H. and Weis, E. (1991) Chlorophyll fluorescence and photosynthesis: the basics. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 42: 313–349.

Long, S.P., Humphries, S. and Falkowski, P.G. (1994) Photoinhibition of photosynthesis in nature. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 45: 633–662.

Mullineaux, C.W., Pascal, A.A., Horton, P. and Holzwarth, A.R. (1993) Excitation-energy quenching in aggregates of the LHC II chlorophyll-protein complex: a time resolved fluorescence study. *Biochim. Biophys. Acta* 1141: 23–28.

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

Papageorgiou, G. (1975) Chlorophyll fluorescence: an intrinsic

probe of photosynthesis. *In* Bioenergetics of Photosynthesis. Edited by Govindjee. pp. 319–371. Academic Press, New York.

Peterson, R.B., Sivak, M.N. and Walker, D.A. (1988) Relationship between steady-state fluorescence yield and photosynthetic efficiency in spinach leaf tissue. *Plant Physiol.* 88: 158–163.

Powles, S.B. (1984) Photoinhibition of photosynthesis induced by visible light. *Annu. Rev. Plant Physiol.* 35: 15–44.

Quick, W.P. and Horton, P. (1984) Studies on the induction of chlorophyll fluorescence in barley protoplasts. II. Resolution of fluorescence quenching by redox state and the transthylakoid pH gradient. *Proc. R. Soc. Lond.* B220: 371–382.

Ruban, A.V. and Horton, P. (1994) Spectroscopy of non-photochemical and photochemical quenching of chlorophyll fluorescence in leaves; evidence for a role of the light harvesting complex of photosystem II in the regulation of energy dissipation. *Photosyn. Res.* 40: 181–190.

Ruban, A.V., Rees, D., Pascal, A.A. and Horton, P. (1992) Mechanism of pH-dependent dissipation of absorbed excitation energy by photosynthetic membranes. II The relationship between LHCII aggregation in vitro and qE in isolated thylakoids. *Biochim. Biophys. Acta* 1102: 39–44.

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. *Photosyn. Res.* 10: 51–62.

Strasser, R.J. (1978) The grouping model of plant photosynthesis. *In* Chloroplast Development. Edited by Akoyunoglou, G. and Argyroudi-Akoyunoglou, J.H. pp. 513–542. Elsevier/North-Holland Biomedical Press, Amsterdam.

Strasser, R.J. (1984) Thermodynamically forced state changes in chloroplasts. *In* Advances in Photosynthesis Research, Vol III. Edited by Sybesma C. pp. 267–270. Martinus Nijhoff, The Hague.

Strasser, R.J., Schwarz, B. and Bucher, J.B. (1987) Messung der chlorophyll fluoreszenz-kinetik bei verschiedenen wellenlängen als rasches verfahren zur frühdiagnose von immissionsbelastungen an waldbaumen: ozoneinwirkungen auf buchen un peppelin. *Eur. J. Forest Pathol.* 17: 149–157.

Trissl, H.W., Gao, Y. and Wulf, K. (1993) Theoretical fluorescence induction curves derived from coupled differential equations describing the primary photochemistry of photosystem II by an exciton-radical pair equilibrium. *Biophys. J.* 64: 974–988.

Uz, m. and Saygln, O. (1994) Determination of the light path-length elongation in leaves by measuring P700 quantitatively. *Photosyn. Res.* 40: 175–179.

Weis, E. and Lechtenberg, D. (1988) Steady state photosynthesis in intact plants as analyzed by chlorophyll fluorescence and far-red spectroscopy. *In* Applications of Chlorophyll Fluorescence. Edited by Lichtenthaler, H. pp. 71–76. Kluwer Academic Publisher, Dordrecht.

Weis, E. and Lechtenberg, D. (1989) Fluorescence analysis during steady-state photosynthesis. *Phil. Trans. R. Soc. London* B323: 253–268.

(Received December 31, 1994; Accepted May 1, 1995)