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Prediction of photoinhibition of photosynthesis from measurements of fluorescence quenching components

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Abstract. Photoinhibition of photosynthesis in willow (*Salix* sp.) leaves was investigated by measuring the ratio of variable (F_V) to maximal (F_M) chlorophyll fluorescence. The use of this parameter was justified as it showed similar kinetics of recovery from photoinhibition as did the light-limited rate of gross photosynthesis. When leaves of different status were exposed to different environmental conditions for 1 h, the large variation in photoinhibition subsequently observed was not correlated with the incident photosynthetic photon flux density. However, it was positively correlated with the fraction of closed reaction centres of photosystem (PS) II, and negatively correlated with the magnitude of the high-energy fluorescence quenching, both of which were measured during the treatment. The best correlation was obtained when the two parameters were taken together in a multiple regression model. Young high-light-acclimated leaves were the most resistant to photoinhibition at a given fraction of closed PSII centres, which was explained by these having the most prominent high-energy quenching. Leaves illuminated in the presence of dithiothreitol and the uncoupler nigericin were found at the other end of the correlation. However, the markedly lowered high-energy quenching induced by dithiothreitol was not fully matched by the expected increase in photoinhibition. The fraction of closed PSII centres provided a relative not an absolute measure of excessive excitation, since the correlation model varied depending on the length of the treatment. In a separate experiment the response to fluctuating light under otherwise constant conditions was studied. It was found that under these more restricted conditions photoinhibition could be predicted from the integrated light dose.

Key words: Chlorophyll fluorescence – Fluorescence quenching – Photoinhibition of photosynthesis – Photosynthesis (photoinhibition) – *Salix*

Abbreviations and symbols: DTT = dithiothreitol; F_0 = minimum fluorescence; F_V = variable fluorescence; F_M = maximum fluorescence; PPFD = photosynthetic photon flux density; PS = photosystem; QA = primary stable electron acceptor of PSII

Introduction

It is well known that exposure of leaves to excessive light may cause photoinhibition of photosynthesis (Powles 1984). This is typically manifested as sustained decreases in the quantum yields of photosynthesis and of photosystem (PS)II photochemistry, the latter being probed by the fluorescence ratio of F_V/F_M (e.g. Demmig and Björkman 1987). Photoinhibition may also be expressed in the intermediate light range, in which case it results from a decrease in the convexity of the photosynthetic light-response curve (Leverenz et al. 1990; Ögren and Sjöström 1990). The sensitivity of the photosynthetic apparatus to light is influenced by other environmental factors, for instance temperature (Ludlow and Björkman 1984; Öquist and Ögren 1985), and availability of water (Björkman and Powles 1984) and nitrogen (Osmond 1983). Not only the concurrent but also the preceding environment is important, especially with respect to light (Powles and Critchley 1980). Thus, the extent of photoinhibition cannot generally be predicted from the instantaneous light level, except under strictly defined conditions. An attempt to find a more general predictor may take advantage of the fluorescence-modulation technique (Ögren and Baker 1985; Schreiber et al. 1986), as this allows determination of the energization of the photosynthetic apparatus. Two components of the fluorescence quenching are typically resolved (Schreiber et al. 1986): photochemical quenching, which is an approximate measure of the fraction of open PSII reaction centres, and non-photochemical, or high-energy quenching which mainly reflects the transthylakoid proton gradient (Krause et al. 1982). Both of these may play a role in the protection against photoinhibition, as indicated by experiments with spinach leaves exposed to light at subzero temperatures (Somersalo and Krause 1990). The main objective of the present study was to determine the generality of this fluorescence approach. Does it provide the information that enables the different situations of photoinhibition to be described within one general mechanism?

Photoinhibition under natural conditions is difficult to predict not only because light interacts with other factors, but also because light in itself is a variable factor. Nevertheless, in the dynamic light environment of a willow canopy photoinhibition could still be predicted from light measurements (Ögren and Sjöström 1990). It was found that the light dose, integrated and weighted over the preceding 6 h, explained the major part of the variation in photoinhibition observed in upper leaves. The present work further addresses the question as to what extent the photoinhibitory response is based on integration of light when other biological and environmental conditions are held constant.

Material and methods

Plant material. Plants of a single clone of *Salix* sp. were grown in a growth room and in the field, as described previously (Ögren 1988a, 1988b, respectively). The third to the sixth uppermost fully expanded leaves were used. The leaves from the field were considered to be high-light-acclimated as they were situated on peripheral shoots and facing the sector SE–SW. They were collected in the morning before they had been exposed to direct sunlight. Unless otherwise stated leaves from the growth room were used. These were considered to be low-light-acclimated as they were developed at a photosynthetic photon flux density (PPFD) of 200–240 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (17 h photoperiod). Attached leaves were used except for experiments with nigericin, dithiothreitol (DTT) and with leaves from the field in which cases the petioles were cut and put into the desired solution and water, respectively.

Treatments with DTT and nigericin. The leaf petiole was cut under water and transferred to a small vessel containing 1 mM DTT (Sigma, St. Louis, Mo., USA) or 1 μM nigericin (Boehringer-Mannheim, FRG). The leaf was then kept in the growth room at a low PPFD (20 $\mu mol \cdot m^{-2} \cdot s^{-1}$) to avoid photoinhibition. Uptake of the chemicals was facilitated by increasing the rate of transpiration using a fan. An amount of solution corresponding to roughly twice the leaf fresh weight was taken up after 2 h. Thereafter the leaf was kept in darkness for 1 h before it was subjected to the photoinhibitory treatment.

Photoinhibitory treatment. This was done in a temperature-controlled gas-exchange cuvette that accommodated the central section of leaf. The rest of the leaf was held between wet paper tissues. The cuvette window was penetrated by a four-branched optical fibre bundle with its terminal end fixed at a distance of 2 mm from the upper leaf surface as defined by a spacer. By this means a uniform PPFD at the leaf surface was obtained, which was found to be essential. The photoinhibitory PPFD, provided by a projector lamp, was guided through a fibre branch and measured at the position of the leaf using a quantum sensor (Model Li-190SB; Li-Cor Inc., Lincoln, Neb., USA). In the experiment of Fig. 4 a periodic light beam was added by a another fibre branch connected to a projector lamp equipped with a computer-controlled magnetic shutter (model 1016 017; Prontor magnetic O, Prontor-Werk, FRG). The leaf section enclosed in the cuvette but external to the fibre was held in dim light, except for the experiment of Fig. 1 where a general PPFD was provided by a mercury-halide lamp (HQI 1000 W; Osram, Berlin, FRG) matching the PPFD of the fibre. Dark-adapted leaves were used except for the experiments of Figs. 1 and 4 where the leaves were pre-treated at a PPFD of 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 15 min. When the influence of altered temperature and atmospheric CO_2 content was studied the cuvette was preconditioned for 5 min before the light treatment was started.

Measurement of fluorescence. Fluorescence was measured using a modulation fluorometer (PAM 101 and 103 with a Schott lamp; H. Walz, Effeltrich, FRG). Data sampling and control was served by a Macintosh SE equipped with data-acquisition card (Model ACSE–12; Strawberry Tree Computers, USA).

The variable fluorescence/maximum fluorescence (F_V/F_M) ratio was measured before and after the photoinhibitory treatment. In the former case after dark-adaptation for 60 min and in the latter case after far-red illumination for 30 min unless otherwise stated. It was measured at normal CO_2 level, at a leaf temperature of 25° C, and in the presence of a far-red irradiance of 10 $W \cdot m^{-2}$, provided by a fibre branch connected to a projector lamp with a cut-off filter (RG 715; Schott AG, Mainz, FRG). Far-red illumination was used to ensure complete reoxidation of the primary stable electron acceptor of PSII (Q_A) in between the light flashes. The minimal fluorescence level, F_0, was sensitized by an irradiance of 0.2 $W \cdot m^{-2}$ (modulated at 100 kHz) and averaged over 10-s intervals. The maximal fluorescence, F_M, was obtained in either of two ways. In the studies of Figs. 2 and 3 a single flash was applied (5200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 3 s in length), while in the studies of Figs. 1 and 4 repetitive flashes were applied (3600 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 1 s in length) at intervals of 5 min and 100 s, respectively. The energy of the repetitive flashes was lower in order to avoid any flash-induced energization. As a result, the absolute value of F_M and hence of F_V/F_M was slightly underestimated (about 5%). However, this can be ignored as the same procedure was used before and after the treatment, and only relative changes were of interest.

The fraction of closed PSII centres was measured during the treatments of Figs. 2 (at 10 min intervals) and 3. The maximal fluorescence in the presence of the background light, F_M', was determined using an extrapolation method suggested by Markgraf and Berry (1990). Three flashes of increasing PPFD (2700, 3600 and 5200 $\mu mol \cdot m^{-2} \cdot s^{-1}$; 2 s in length) were applied. The intervening periods were long enough to allow the return of the quasi steady-state fluorescence, F_S. The peak signals were plotted versus the reciprocal value of PPFD. The straight line obtained, $r^2 > 0.98$ in all cases, extrapolated to infinite PPFD (the intercept on the y-axis) was taken as the true F_M'. This procedure was adopted as the flash lamp was not saturating in the presence of a high photosynthetic rate. Within 20 s after the series of flashes a far-red cut-off filter (specified above) was rapidly inserted into the photoinhibitory light beam to give a far-red irradiance in the range of 10–70 $W \cdot m^{-2}$. The fluorescence minimum observed within the first few seconds was taken as F_0', corresponding to the state where the PSII acceptor Q_A is re-oxidized. The measurement was repeated at a higher far-red irradiance to assure that the true minimum was observed. The reduction state of Q_A, or the fraction of closed PSII centres, was estimated from $Q_{red}/Q_{total} = (F_S\text{-}F_0')/(F_M'\text{-}F_0')$. At the end of the treatments of Fig. 2 the high-energy quenching was calculated as $(1\text{–}F_M'/F_M)$. Thus, the extent of high-energy quenching was measured with respect to the maximal rather than, what is common practice, the variable fluorescence (Schreiber et al. 1986). This was necessary in order to include the data of the anaerobically treated leaves which were devoid of variable fluorescence. (From theoretical considerations the two methods are in principal the same, and indeed, when they were compared critically, similar data were generated.) The control value (F_M) taken was that measured 30 min after the treatment. Thus, the high-energy quenching calculated here comprises the rapidly relaxing components including state transition but not photoinhibition (Horton and Hague 1988).

Measurement of photosynthesis. The recovery of gross photosynthesis from photoinhibition was studied using an open gas-exchange system described elsewhere (Ögren 1988a). Rates were calculated according to von Caemmerer and Farquhar (1981). The experiment could not be done in a single run as this had required alternating measurements of dark-respiration and net photosynthesis. This was impossible as the steady-state rate of photosynthesis was reached too slowly (> 40 min) even after short periods of darkness. Instead the two processes were followed separately in two consecutive experiments using adjacent sections of the same leaf. The experimental conditions were the same except for the intermittent 5-min periods of darkness when respiration was measured. The validity of

this protocol was justified by the fluorescence data that were indistinguishable in the two experiments.

Results

The chlorophyll fluorescence ratio of F_V/F_M as a probe for photoinhibition of photosynthesis. When photoinhibition was induced in a willow leaf by exposure to high light this was associated with quantitatively similar inhibitions of the light-limited rate of gross photosynthesis and of the ratio of F_V/F_M. A close relationship between these two parameters was also maintained during the subsequent period of recovery at a low PPFD (Fig. 1), confirming that the F_V/F_M ratio can be used as a probe for photoinhibition.

The dependence of photoinhibition on PPFD, Q_A reduction and high-energy quenching. Can contrasting situations of photoinhibition be explained on the basis of one mechanism? To address this question willow leaves were subjected for 1 h to various conditions of PPFD, temperature as well as atmospheric CO_2 content. The leaves used were of different status, as affected by age and light prehistory, or by transpirational uptake of DTT and the uncoupler nigericin. For the data set as a whole the variation in photoinhibition observed 30 min after termination of the treatment, ranging from nil up to nearly 40%, did not correlate with the applied PPFD (Fig. 2A). However, when individual data subsets were compared, one consistent pattern was revealed, namely a higher resistance to photoinhibition in high-light- as compared to low-light-acclimated leaves.

The inhibition of the F_V/F_M ratio was the result of a decrease in F_M (data not shown) and generally, a rise in F_0 (Fig. 2B). Some of the high-light-acclimated leaves differed from this by showing a slight decrease in F_0. However, in these leaves the F_V/F_M ratio was not or only marginally affected.

Clearly, the incident PPFD could not explain the variation in photoinhibition. A much better predictor was found in the fraction of closed PSII reaction centres, i.e. centres with reduced Q_A. With an increased fraction of closed centres, above a threshold in the range of 30–45%, there was a roughly linear increase in the extent of photoinhibition (Fig. 2C). A closer look reveals that data subsets are somewhat shifted relative to each other. In comparison with leaves held at 25° C, leaves held at 12° C were more resistant to photoinhibition. So also were leaves acclimated to high light, especially the young ones. Less resistant at a given redox state of PSII were leaves fed with DTT or nigericin.

An explanation for these differences is offered by Fig. 2D, which shows that photoinhibition was negatively correlated with the rapidly relaxing high-energy quenching. Thus, the high-light-acclimated leaves were more resistant at a given fraction of closed PSII centres, because of their more pronounced high-energy quenching. This essentially also applied in the low-temperature situation, though the data overlap somewhat. The opposite situation, nigericin- and DTT-treated leaves being less resistant, can be explained by the loss of high-energy quenching associated with these treatments.

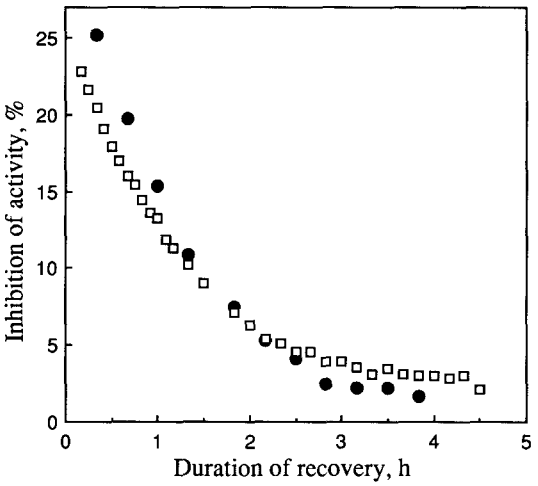

Fig. 1. The recovery of the light-limited rate of gross photosynthesis (●) and of the ratio of F_V/F_M (□) in low-light-acclimated willow leaves that had been exposed to a PPFD of 2000 μmol · m^{-2} · s^{-1} at 25° C for 2 h. The PPFD during recovery was 100 μmol · · m^{-2} · s^{-1}, except for short periods of far-red illumination (10 s) and of darkness (5 min) associated with the fluorescence and the respiration measurements, respectively

Table 1. The coefficients of determination (r^2) of the linear regression models developed to explain the variation in photoinhibition of the data in Figs. 2C, D. The reduction state of Q_A and the high-energy quenching were used as independent variables, either separately in simple models or together in a multiple model. Modelling was repeated without the apparent outliers, the DTT pretreated leaves

Model	r^2	
	All data	DTT data excluded
Simple (Q_A reduction)	0.545	0.590
Simple (High-energy quenching)	0.389	0.529
Multiple	0.842	0.915

This dual nature of photoinhibition was further supported by a linear regression analysis using photoinhibition as the dependent variable. When the independent variables, the fraction of closed PSII centres and the high-energy quenching, were used in separate regression models the r^2 values obtained were 0.55 and 0.39, respectively (Table 1). When they were used together in a multiple regression model the correlation was strengthened considerably ($r^2 = 0.84$). The correlation was further improved when the data of DTT-treated leaves were excluded ($r^2 = 0.92$). The DTT-treated leaves were excluded as they seem to be outliers, being more resistant to photoinhibition than expected in view of their markedly lowered high-energy quenching (Fig. 2D). The strong correlation found in the multiple regression model was not the consequence of any correlation between the

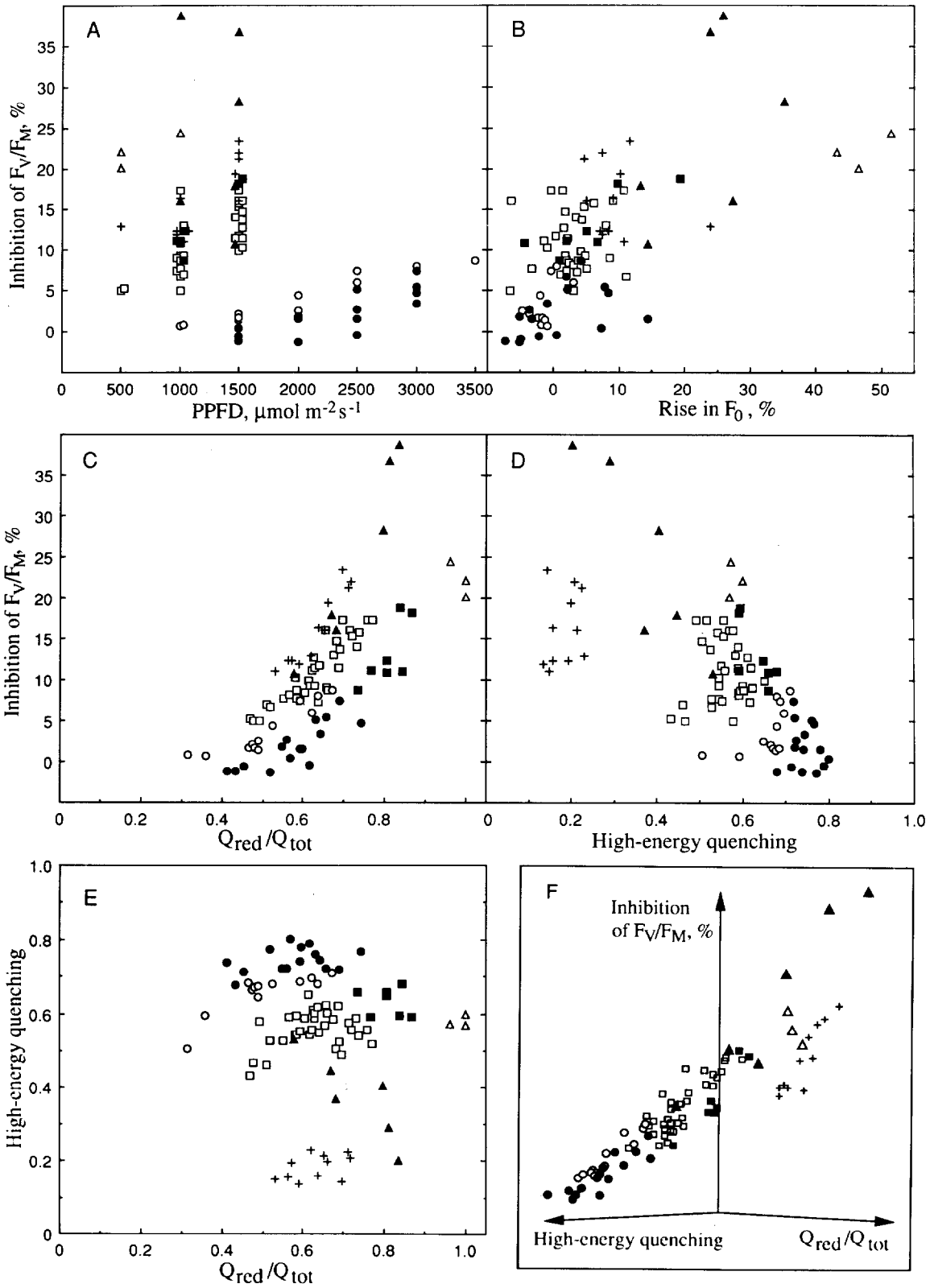

Fig. 2A–F. Results obtained with willow leaves subjected to various PPFD situations for 1 h. The extent of inhibition of the F_V/F_M ratio as a function of PPFD during treatment **A**, of the concurrent change in F_0 **B**, of the average reduction state of Q_A – i.e. the fraction of closed PSII centres – during treatment **C**, and of the high-energy quenching (minus photoinhibitory quenching) at the end of the treatment **D**. Also, the high-energy quenching as a function of the reduction state of Q_A **E**, and the extent of inhibition of the F_V/F_M ratio as a function of both the reduction state of Q_A and the high-energy quenching **F**. The treatments were carried out at a temperature of 25° C, at 5% and normal CO_2 levels using low-light-acclimated leaves (□), or under conditions different from these: at a temperature of 12° C (■), in the presence of nigericin (▲) or DTT (+), in an atmosphere of pure nitrogen (△), and using young (●) and mature (○) high-light-acclimated leaves

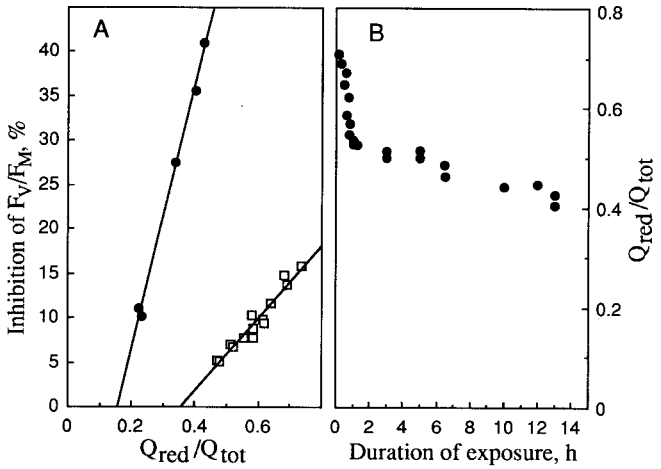

Fig. 3A, B. The extent of inhibition of the F_V/F_M ratio in willow leaves following exposure to a PPFD of 1000–1500 μmol · m^{-2} · · s^{-1} at 25° C for 1 h (□) and 13 h (●) as a function of the average (1 h) or the final (13 h) reduction state of Q_A during treatment **A**, and the time course of the reduction state of Q_A during exposure to 1500 μmol · m^{-2} · s^{-1} PPFD **B**. The data in **B** are collected from two runs

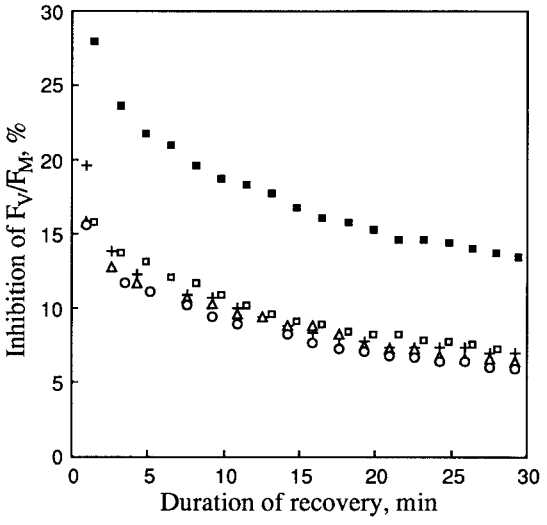

Fig. 4. The recovery of the F_V/F_M ratio under far-red illumination following exposure of different spots of a single willow leaf for 1 h to the following PPFDs in units of μmol · m^{-2} · s^{-1}: alternating 1500/150 with shift every 4 s (□), 32 s (△) and 5 min (+), and, constant 825 (○) and 1500 (■)

two independent variables, as evidenced by Fig. 2E and the associated r^2 of 0.01 (0.05 without DTT subset). A visual representation of the three-dimensional data set is shown in Fig. 2F.

The time dependence of photoinhibition. In the above study all treatments were of equal duration (1 h). When leaves were subjected to a much longer treatment (13 h) the linear relationship between the fraction of closed PSII centres during treatment and the subsequent photoinhibition still remained (Fig. 3A). However, it was shifted to higher degrees of photoinhibition, demonstrating that the time of exposure also must be considered. This time dependence is partly explained by a drift with time in the fraction of closed PSII centres; from 62%, the average during the first hour, to 42% at the end of the 13-h period (Fig. 3B).

Photoinhibition in dynamic light. The occurrence of photoinhibition in fluctuating light was studied under highly controlled conditions. For instance, the biological variation was minimized by using different spots of the same leaf. Exposure to alternating 150 and 1500 μmol · m^{-2} · s^{-1} PPFD for 1 h resulted in the same extent of photoinhibition as did the constant average PPFD (825 μmol · m^{-2} · s^{-1}) (Fig. 4). Furthermore, the time characteristics of the light variation was not critical, at least not within the range of seconds to minutes. Photoinhibition is thus based on the integration of light, provided all other conditions are constant. The generality of this conclusion was confirmed in other experiments where the total exposure time and the amplitude of the PPFD cycle was varied. (data not shown).

Discussion

The F_V/F_M ratio was found to be a good diagnostic probe for photoinhibition of photosynthesis (Fig. 1), in agreement with many studies (e.g. Demmig and Björkman 1987), including those using willow plants growing in the field (Ögren 1988b; Ögren and Sjöström 1990). Although photoinhibition was assayed under limiting light only, photoinhibition of photosynthesis in the field was expressed even when assayed under moderate up to full daylight. This was attributed to a decrease in the convexity of the photosynthetic light-response curve (Ögren and Sjöström 1990). In the same study, evidence was also presented that photoinhibition occurs at light levels below what is required to saturate photosynthesis. This is further supported here using a protocol that allows small effects to be resolved. For instance, a small but significant photoinhibition occurred after 1 h at a PPFD of 500 μmol · m^{-2} · s^{-1} (Fig. 1A), which was well below that which saturated photosynthesis in these leaves (> 1500 μmol · m^{-2} · s^{-1}, data not shown). Also, since the assay was done 30 min after termination of the treatment the initial photoinhibition is expected to be even higher.

When willow leaves were exposed to alternating high and low PPFD at regular intervals of seconds or minutes, the subsequent photoinhibition was the same as when the average PPFD was applied constantly (Fig. 4). This finding that photoinhibition is a function of the integrated PPFD, provided that other environmental and biological factors are constant, gives support to a previous observation: in the dynamic light environment of a willow canopy, photoinhibition varied in response to the weighted light dose over the preceding hours (Ögren and Sjöström 1990). The reason that the light dose had to be weighted was presumably because recovery from photoinhibition occurred when the light level was low for prolonged periods.

Despite the variation in the experimental conditions applied and in the photoinhibition subsequently found,

the latter was accurately predicted by two factors taken together: the fraction of closed PSII centres and the extent of high-energy quenching (Fig. 2F, Table 1). The redox state of PSII centres thus provides a general probe for excessive excitation, presumably because it reflects the balance between absorption of light and its dissipation through electron transfer. A comparison of low- and high-light-acclimated leaves at the mature stage may illustrate this point. The high-light-acclimated leaves showed much less photoinhibition at a given PPFD (Fig. 2A), in agreement with the literature (Powles and Critchley 1980), and with the knowledge that such leaves have a higher capacity for electron transport per leaf area and PSII unit (Anderson et al. 1988). However, when the leaves were compared at the same fraction of closed PSII centres (Fig. 2C) the difference in sensitivity was much reduced. The difference that still remained was explained by a higher level of the protective high-energy quenching in the high-light-acclimated leaves (Fig. 2D; discussed below).

The relationship between closure of PSII centres, i.e. Q_A reduction, and photoinhibition may be indirect as discussed above, or causal. A causality can be envisaged in view of some results indicating a link between photoinhibition and a damage at Q_B, the secondary electron acceptor in PSII (Ohad et al. 1984); a connection to Q_A is conceivable as the two quinones are physically and chemically close to each other. A second possibility can be derived from more theoretical considerations. Many workers have suggested that photoinhibition may result from the continued operation of charge separation in centres with reduced Q_A, leading to the hypothetical effects of double-reduced Q_A (Styring et al. 1989) and-or creation of a relatively long-lived $P680^+$, the primary donor in its oxidized state. Both these species have the potential to trigger detrimental processes.

However, such mechanisms invoking a cause-effect relationship are in some aspects difficult to reconcile with the data. First, photoinhibition was correlated with the reduction state of Q_A per se, irrespective of the actual PPFD. This finding appears to be in conflict with particularly the second of the above mechanisms. Even though reduction of Q_A is considered to be a primary event, the subsequent processes leading to the photoinhibitory damage are assumed to be driven by light and should therefore depend on the prevailing PPFD. Second, the reduction state of Q_A associated with incipient photoinhibition varied depending on the duration of treatment (Fig. 3), indicating that this is only a relative measure of excessive excitation.

The damage concept of photoinhibition has been questioned before. It has been suggested (for instance Demmig and Björkman 1987), that the initial decrease in the photochemical efficiency is under physiological control. Thereby the accumulation of fully competent but closed PSII centres in high light is avoided. This situation could otherwise lead to the hypothetical second stage of photoinhibition, a sustained photodamage of centres. It has been suggested that the first stage is attained by the creation of a quencher of excitation energy in the PSII antenna (Demmig and Björkman 1987), which possibly is identical with zeaxanthin (Demmig et al. 1987). However, when a rise in F_0 is observed, as in the present study (Fig. 2B), an alteration of the PSII reaction centre itself appears more plausible (Ögren and Sjöström 1990). An alteration of the PSII centres has also been invoked to explain a related phenomenon, namely the light-induced down regulation of the quantum yield of PSII (Weis and Berry 1987). This effect may resemble photoinhibition, but is different for mainly two reasons. First, it is rapidly reversible on return to low light. Second, it is increased with increased high-energy quenching, whilst the reverse was found for photoinhibition.

The last point may indicate that the high-energy quenching confers protection to photoinhibition by effecting a down regulation of the PSII efficiency. As expected, photoinhibition was accelerated when the high-energy quenching was decreased by an uncoupler (Fig. 2D), in agreement with previous experiments with isolated chloroplasts (Krause and Behrend 1986). The high-energy quenching was decreased even more by DTT (Fig. 2D). This effect has been thoroughly investigated before (Bilger et al. 1989). However, photoinhibition in the willow leaves was not accelerated by DTT as much as one would expect from the general relationship. Although the reason for this is unknown, it could indicate that part of the high-energy quenching is neutral with respect to photoinhibition. Alternatively, the reducing ability of DTT may augment photoprotective processes. As for the in-situ situation, it is interesting to note that the most pronounced high-energy quenching and the least photoinhibition was observed in the uppermost (young) leaves grown under full daylight. Thus the most exposed leaves are also the most protected ones. Similarly though less evident, an increased high-energy quenching rendered low temperatures less photoinhibitory than one would expect from the low-temperature induced closure of PSII centres (Fig. 2D).

In conclusion, the present study indicates that the considerable variability of photoinhibition in response to physiological and environmental variation is only apparent. At the mechanistic level photoinhibition is highly predictable and related to the redox state of PSII and the energization level of the photosynthetic membrane.

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References

Anderson, J.M., Chow, W.S., Goodchild, D.J. (1988) Thylakoid membrane organisation in sun/shade acclimation. Aust. J. Plant Physiol. **15,** 11–26

Bilger, W., Björkman, O., Thayer, S.S. (1989) Light-induced spectral absorbance changes in relation to photosynthesis and the epoxidation state of xanthophyll cycle components in cotton leaves. Plant Physiol. **91,** 542–551

Björkman, O., Powles, S.B. (1984) Inhibition of photosynthetic reactions under water stress: interaction with light level. Planta **161,** 490–504

Demmig, B., Björkman, O. (1987) Comparison of the effect of excessive light on chlorophyll fluorescence (77K) and photon

yield of O_2 evolution in leaves of higher plants. Planta **171,** 171–184

Demmig, B., Winter, K., Krüger, A., Czygan, F.-C. (1987) Photoinhibition and zeaxanthin formation in intact leaves. A possible role of the zanthophyll cycle in the dissipation of excess light energy. Plant Physiol. **84,** 218–224

Horton, P., Hague, A. (1988) Studies on the induction of chlorophyll fluorescence in isolated barley protoplasts. IV. Resolution of non-photochemical quenching. Biochim. Biophys. Acta **932,** 107–115

Krause, G.H., Behrend U. (1986) ΔpH-dependent chlorophyll fluorescence quenching indicating a mechanism of protection against photoinhibition of chloroplasts. FEBS Lett. **200,** 298–302

Krause, G.H., Vernotte, C., Briantais, J.-M. (1982) Photoinduced quenching of chlorophyll fluorescence in intact chloroplasts and algae. Resolution into two components. Biochim. Biophys. Acta **679,** 116–124

Leverenz, J.W., Falk, S., Pilström, C.-M., Samuelsson, G. (1990) The effects of photoinhibition on the photosynthetic light-response curve of green plant cells *(Chlamydomonas reinhardtii)*. Planta **182,** 161–168

Ludlow, M.M., Björkman, O. (1984) Paraheliotropic leaf movement in *Siratro* as a protective mechanism against drought-induced damage to primary photosynthetic reactions: damage by excessive light and heat. Planta **161,** 505–518

Markgraf, T., Berry, J. (1990) Measurement of photochemical and non-photochemical quenching: correction for turnover of PS2 during steady-state photosynthesis. In: Current research in photosynthesis, vol. IV, pp. 279–282, Baltscheffsky, M., ed. Kluwer Academic Publishers, Dordrecht

Ögren, E. (1988a) Suboptimal nitrogen status sensitizes the photosynthetic apparatus in willow leaves to long term but not short term water stress. Photosynth. Res. **18,** 263–275

Ögren, E. (1988b) Photoinhibition of photosynthesis in willow leaves under field conditions. Planta **175,** 229–236

Ögren, E., Baker, N.B. (1985) Evaluation of a technique for the measurement of chlorophyll fluorescence from leaves exposed to continuous white light. Plant Cell Environ. **8,** 539–547

Ögren, E., Sjöström, M. (1990) Estimation of the effect of photoinhibition on the carbon gain in leaves of a willow canopy. Planta **181,** 560–567

Ohad, I., Kyle, D.J., Arntzen, C.J. (1984) Membrane protein damage and repair: removal and replacement of inactivated 32 kilodalton polypeptides in chloroplast membranes. J. Cell Biol. **99,** 481–485

Öquist, G., Ögren, E. (1985) Effects of winter stress on photosynthetic electron transport and energy distribution between the two photosystems of pine as assayed by chlorophyll fluorescence kinetics. Photosynth. Res. **7,** 19–30

Osmond, C.B. (1983) Interactions between irradiance, nitrogen nutrition, and water stress in the sun-shade responses of *Solanum dulcamara*. Oecologia **57,** 316–321

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

Powles, S.B., Critchley, C. (1980) Effect of light intensity during growth on photoinhibition of intact attached bean leaflets. Plant Physiol. **65,** 1181–1187

Schreiber, U., Schliwa, U., 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

Somersalo, S., Krause, G.H. (1990) Effects of freezing and subsequent light stress on photosynthesis of spinach leaves. Plant Physiol. Biochem. **28,** 467–475

Styring, S., Virgin, I., Jegerschöld, C., Andersson, B. (1989) The effects of photoinhibition on the structure and function of photosystem II. Physiol. Plant. **76,** A27

Von Caemmerer S., Farquhar, G.D. (1981) Some relationships between the biochemistry of photosynthesis and the biochemistry of leaves. Planta **153,** 376–387

Weis, E., Berry, J. (1987) Quantum efficiency of photosystem II in relation to "energy"-dependent quenching of chlorophyll fluorescence. Biochim. Biophys. Acta **894,** 198–208