Planta (1995) 197: 583–591

Planta

High susceptibility to photoinhibition of young leaves of tropical forest trees

G. Heinrich Krause,* Aurelio Virgo, Klaus Winter

Smithsonian Tropical Research Institute, Apartado 2072, Balboa, Republic of Panama

Received: 31 December 1994/Accepted: 28 April 1995

Abstract. Photoinhibition of photosynthesis was studied in young (but almost fully expanded) and mature canopy sun leaves of several tropical forest tree species, both under controlled conditions (exposure of detached leaves to about 1.8 mmol photons·m^{-2}·s^{-1}) and in the field. The degree of photoinhibition was determined by means of the ratio of variable to maximum chlorophyll (Chl) fluorescence emission (F_V/F_M) and also by gas-exchange measurements. For investigations in situ, young and mature leaves with similar exposure to sunlight were compared. The results show a consistently higher degree of photoinhibition in the young leaves. In low light, fast recovery was observed in both types of leaves in situ, as well as in the laboratory. The fluorescence parameter $1 - F_S/F'_M$ (where F_S = stationary fluorescence and F'_M = maximum fluorescence during illumination) was followed in situ during the course of the day in order to test its suitability as a measure of the photosynthetic yield of photosystem II (PSII). Electron-transport rates were calculated from these fluorescence signals and compared with rates of net CO_2 assimilation. Measurements of diurnal changes in PSII 'yield' confirmed the increased susceptibility of young leaves to photoinhibition. Calculated electron transport qualitatively reflected net CO_2 uptake in situ during the course of the day. Photosynthetic pigments were analyzed in darkened and illuminated leaves. Young and mature leaves showed the same Chl *a*/*b* ratio, but young leaves contained about 50% less Chl *a* + *b* per unit leaf area. The capacity of photosynthetic O_2 evolution per unit leaf area was decreased to a similar extent in young leaves. On a Chl basis, young leaves contained more α-carotene, more xanthophyll cycle pigments and, under strong illumination, more zeaxanthin than mature leaves. The high degree of reversible photoinhibition observed in these young sun leaves probably represents a dynamic regulatory process protecting the photosynthetic apparatus from severe damage by excess light.

Key words: Chlorophyll fluorescence – Photoinhibition (photosynthesis) – Photosynthetic gas exchange – Photosystem II – Tropical forest species – Xanthophyll cycle

Introduction

There is growing evidence that photoinhibition of photosynthesis occurs under a variety of conditions in many plant species in nature (see Long et al. 1994 for a recent review). Effects of excess light in combination with other environmental stress factors, in particular low temperatures (see Krause 1994) have been well documented. However, in full sunlight substantial photoinhibition may take place in situ even if other stress factors are absent (yet leaf temperatures may be high), as shown, for example, in studies on leaves of *Salix* sp. (Ögren 1988; Ögren and Rosenqvist 1992), *Eucalyptus* sp. (Ögren and Evans 1992), several Crassulacean acid metabolism plants (Adams 1988; Adams et al. 1988), the mediterranean shrub *Arbutus unedo* (Demmig-Adams et al. 1989) and cultured herbaceous plants (Bolhàr-Nordenkampf et al. 1991). Little is known about photoinhibition in canopy sun leaves of the tropical rain forest. These leaves have to endure extremely high sunlight, yet should be capable of efficient photosynthesis in low light during extended periods of shading by clouds.

In the present study, photoinhibition and recovery in canopy sun leaves of several tree species in a seasonally dry rain forest of Central Panama was investigated. The term 'photoinhibition' is defined here in a broad sense as a slowly reversible light-induced inhibition of photosynthesis, which comprises both regulatory protective and damaging processes in photosystem (PS)II (see Krause 1988; Ögren and Evans 1992; Leitsch et al. 1994). The decrease in the ratio of maximum variable to maximum

**Permanent address*: Institute of Plant Biochemistry, Heinrich Heine University Düsseldorf, Universitätsstrasse 1, D-40225 Düsseldorf, Germany

Abbreviations: Chl = chlorophyll; F_O = initial chlorophyll fluorescence; F_M = maximum total fluorescence; F_V = maximum variable fluorescence (= $F_M - F_O$); J_F = rate of PSII-driven electron transport Φ_{PSII} = photosynthetic yield of PSII; PFD = photon flux density (400–700 nm); PS = photosystem

Correspondence to: G.H. Krause; FAX: 49 (211) 311 3706

total fluorescence (F_V/F_M) was used as a reliable and well-reproducible measure of photoinhibition. A close correlation between F_V/F_M ratio and optimum quantum yield of photosynthetic O_2 evolution has been found in many cases (see Krause and Weis 1991). According to model calculations (Giersch and Krause 1991), not a strictly linear, but a quasi-linear relationship – suitable for practical use – between F_V/F_M and activity of PSII can be expected. In addition, the fluorescence parameter $1 - F_S/F'_M$ (where F_S = stationary fluorescence and F'_M = maximum fluorescence during illumination) was determined, which has been suggested to represent the actual quantum yield of PSII at the time of measurement (Genty et al. 1989).

In the canopy of semideciduous Panamanian rain forests, flushes of young leaves, distinguished by their light-green color, are found in the wet season (e.g. *Castilla elastica*, *Antirrhoea trichantha*), late rainy and early dry season (*Anacardium excelsum*) or throughout the year (*Ficus insipida*). These young leaves are equally or even more sun-exposed than the dark-green mature leaves. In all cases tested, the young leaves proved to be significantly more susceptible to photoinhibition, but were capable of recovering rapidly in low light, both in situ and under controlled conditions in the laboratory. To assess the photoprotective role of the xanthophyll cycle (see Demmig-Adams 1990; Demmig-Adams and Adams 1992), we compared the composition of photosynthetic pigments between dark-adapted and strongly illuminated young and mature leaves, respectively. A high xanthophyll-cycle activity in canopy sun leaves has been described recently (Königer et al. 1995). We observed an increased pool size of xanthophyll-cycle pigments per chlorophyll (Chl) molecule and, during illumination, a higher zeaxanthin level per Chl in young leaves. These features may be involved in a strong reversible down-regulation of PSII activity in young leaves in response to excess light.

Materials and methods

Plant material and study site. Canopy leaves of the following tree species growing in a semideciduous tropical lowland forest in Parque Natural Metropolitano near Panama City, Panama, were studied: *Castilla elastica* Sessé (Moraceae), *Ficus insipida* Willd. (Moraceae), *Antirrhoea trichantha* (Grieseb.) Hemsl. (Rubiaceae), *Anacardium excelsum* (Bertero & Balb.) Skeels (Anacardiaceae). The area receives an average annual rainfall of 1740 mm with a dry season from about mid-December to mid-April. Mean annual air temperature is 28 °C. Leaves in the forest canopy were accessible by means of a construction crane. In addition, young (1–1.5 m tall) trees of *Calophyllum longifolium* Willd. (Clusiaceae), *Swietenia macrophylla* G. King (Meliaceae) and *Ficus insipida* cultivated in pots outdoors were used. Data were obtained in the late rainy and early dry season (November to January 1993/94). Leaf samples were harvested early in the morning, or measurements were done in situ during the course of the day. Sun-exposed light-green, almost fully expanded young leaves and dark-green, mature leaves were chosen for the study.

Photoinhibition and recovery under controlled conditions. Detached leaves were placed into a controlled-environment chamber (EGC, Chagrin Falls, Ohio, USA) equipped with two 2000-W metal-halide lamps (HRI-T; Radium-Elektrizitäts-Ges., Wipperfürth, Germany). The upper leaf surface was exposed to a photon flux density (PFD) of 1.7–1.9 mmol·m^{-2}·s^{-1}. Air temperature was 20 °C and relative humidity 66 ± 5%. Leaf petioles were immersed in water. The PFD was measured with a quantum sensor (LI 189; Li-Cor, Lincoln, Neb. USA). Leaf temperatures, recorded with a thermocouple attached to the lower leaf surface were between 27 and 29 °C. Discs of 1.8 cm^2 area were punched from the leaf blades after specified times of high-light exposure and placed on moist filter paper in Petri dishes (temperature 25–27 °C). The leaf discs were darkened for 10 min before the degree of photoinhibition was determined by fluorescence measurement. For recovery from photoinhibition the leaf discs were illuminated at a PFD of 25–30 μmol·m^{-2}·s^{-1}.

Chlorophyll fluorescence. Chlorophyll *a* fluorescence was recorded with a PAM 2000 portable fluorometer (Walz, Effeltrich, Germany). The instrument was equipped with a leaf clip holder (2030-B; Walz) including a micro-quantum sensor to monitor PFD and a thermocouple to measure temperature at the lower leaf surface. For data acquisition, a Poqet PC (Poqet Computer Corp., Santa Clara, Calif., USA) was used with the Data Aquisition Software DA-2000 (Walz).

The following fluorescence parameters served to elucidate the activity of PSII during photoinhibition and recovery periods: (i) The F_V/F_M ratio was obtained after 10 min dark adaptation of leaf discs (or only 2 min darkness during recovery of detached leaf segments). The dark period is required for relaxation of energy-dependent quenching (q_E) and of possible quenching related to state transition (q_T) (Somersalo and Krause 1990). Initial fluorescence, (F_O) was recorded in weak modulated 'measuring' light after 3 s weak far-red illumination to fully reoxidize the electron-transport chain between PSII and PSI. For accurate calculation of F_V/F_M by use of the DA-2000 software, the intensity of the saturating pulse was chosen to reach F_M as a plateau line of the induction signal at least 0.16 s before termination of the pulse. Total pulse time was 0.8 s. (ii) Steady-state values of photosynthetic 'yield' (Genty et al. 1989), $\Phi_{PSII} = 1 - F_S/F'_M$ (where F_S = stationary level of fluorescence emission and F'_M = maximum fluorescence during illumination) were determined at different PFDs during the course of the day in situ. The parameter F'_M was measured using the same procedure as for F_M (above). (iii) From Φ_{PSII}, rates of PSII-driven electron transport (J_F) were calculated according to Krall and Edwards (1992) as

$$J_F = \Phi_{PSII} \cdot PFD \cdot a \cdot f \quad \text{(Eq. 1)},$$

where a is the fractional light absorptance of the leaf, and f the fraction of absorbed photon energy distributed to PSII. For the calculation, an equal distribution of excitation energy between PSII and PSI was assumed, i.e. $f = 0.5$. Fractional light absorptance in the range 400–700 nm of leaves was determined using an integrating sphere (LI-1800–12; Li-Cor) connected to a spectroradiometer (LI-1800; Li-Cor).

Photosynthetic gas exchange. Photosynthetic O_2 evolution was measured at saturating CO_2 (5%) and 30 °C with a leaf-disc oxygen-electrode system (Hansatech, King's Lynn, Norfolk, UK). The CO_2 assimilation was monitored in situ in ambient air using the CO_2-measuring system CPQ 130 (Walz).

Dry weight of leaves. Leaf discs were dried at 60 °C for at least 48 h to determine the dry weight per unit area.

Pigment analyses. Leaf discs of 4.5 cm^2 area were immediately frozen and stored in liquid nitrogen. After quantitative extraction with 100% acetone, samples were membrane-filtered and analyzed immediately with a Waters HPLC system (Waters Millipore, Milford, Mass, USA). For separation of pigments, a Spherisorb ODS-1 5 U column (250 mm long, 4.6 mm i.d.; Alltech, Deerfield, Ill, USA) with a μBondapak C_{18} Guard-Pak pre-column (Waters Millipore) was used. Solvents were degassed with an ERC-3312 Degasser (Erma, Tokyo, Japan). Elution occurred at a flow rate of 2 ml·min^{-1} with acetonitrile:methanol:Tris-HCl buffer (0.1 M, pH. 8.0), 72:12:7 (by vol.) isocratically for 6 min followed by a linear gradient for 10 min to methanol:hexane, 7:1 (v:v) and a further 4-min isocratic elution with this medium. The detector was set to 440 nm. The HPLC system was calibrated with purified pigments. The peak height

of bands was used to quantify the pigments. The HPLC method used here is described in more detail elsewhere (Königer et al. 1995).

Results

Photoinhibition of detached leaves under controlled conditions. In order to compare the susceptibility to photoinhibition between different species and leaf ages, detached leaves were subjected to a standard procedure of high-light exposure in a controlled-environment chamber, followed by a recovery treatment in low light. Figure 1A–D illustrates that young leaves responded more sensitively to high light than mature leaves, as indicated by the more pronounced decrease in F_V/F_M ratios of young leaves. Recovery during low-light exposure was remarkably fast, so that after 40 min only small differences in F_V/F_M ratios between young and mature leaves remained (Fig. 1A–D). Susceptibility to photoinhibition differed least between young and mature leaves of *Anacardium* (Fig. 1C), probably because the mature leaves were already in a state of senescence at the time of the experiment (29th Nov.).

The strongest and fastest response to excess light was observed in young leaves of *Castilla* (Fig. 1A), showing a marked decrease in F_V/F_M during the first minutes of illumination, as depicted in more detail in Fig. 2 (left panel). The kinetics of recovery in low light was biphasic

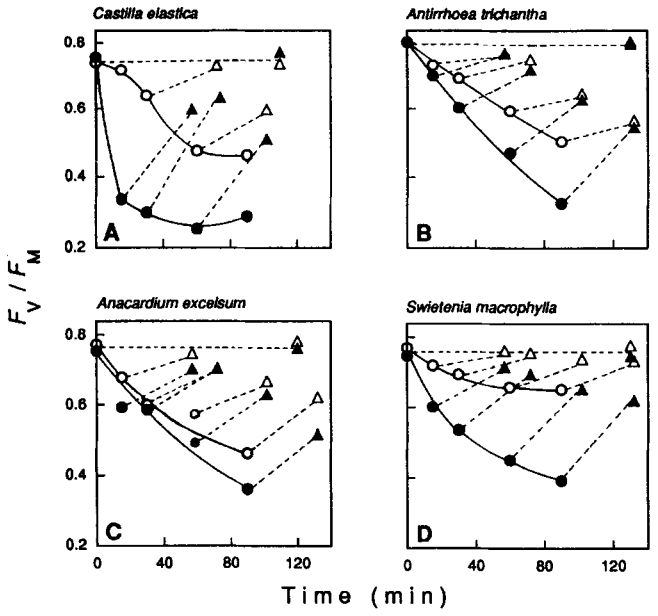

Fig. 1A–D. Photoinhibition and partial recovery of detached canopy leaves under controlled conditions, as indicated by F_V/F_M ratios. Young and mature detached leaves were subjected to standard high-light exposure in a controlled-environment chamber followed by recovery treatment in low light (25–30 $\mu mol \cdot m^{-2} \cdot s^{-1}$). ● ▲, young leaves; ○ △, mature leaves; ○ ●, leaves after high-light treatment for different times; ▲ △, leaf sections after 40 min recovery (or controls). Light absorptance in young/mature leaves was 0.807 ± 0.011/0.894 ± 0.005 (*Castilla*), 0.763 ± 0.016/0.872 ± 0.005 (*Antirrhoea*), 0.819 ± 0.019/0.882 ± 0.004 (*Anacardium*) and 0.819 ± 0.008/0.870 ± 0.005 (*Swietenia*), respectively (means and SD for $n = 4$)

(Fig. 2, right panel). From linear extrapolation of the slow part of the semilogarithmic plot of ln (F_V/F_M) vs. time, the contribution of the slow phase to total recovery was estimated. The fast phase of recovery with a halftime of about 30 min (Fig. 2, triangles) was resolved by subtraction of slow from total recovery. This fast phase accounted for roughly half of the extent of photoinhibition. Extrapolation of the slow phase to the original F_V/F_M ratio of 0.76 gave a halftime of about 2.5 h. In preliminary experiments with *Castilla* and *Anacardium* (data not shown), incubation of leaves with streptomycin, an inhibitor of chloroplast protein synthesis, enhanced photoinhibition but only partially affected recovery during 40 min in low light.

The relationship between decrease in F_V/F_M and photoinhibition of photosynthesis was investigated with leaves of *Swietenia*. Figure 3 depicts light saturation curves of gross O_2 evolution under optimal (high CO_2) conditions for a young and a mature control leaf, harvested in the morning prior to high sunlight exposure. Optimal quantum yields calculated from the linear parts of the curves (legend to Fig. 3) were somewhat lower than expected for non-stressed leaves (cf. Björkman and Demmig 1987), which may indicate a sustained photoinhibition (see below). Young leaves exhibited only about half the photosynthetic capacity of mature leaves. After 90 min standard photoinhibition treatment, optimum CO_2-dependent O_2 evolution measured in limiting light had declined more strongly in young than in mature leaves. The F_V/F_M ratio was decreased to the same extent as O_2 evolution for both types of leaves (Table 1). When measured in saturating light (670 $\mu mol \cdot m^{-2} \cdot s^{-1}$), much less inhibition of O_2 evolution was found (data not shown); this is expected because in high light carbon metabolism rather than electron transport limits gross photosynthesis.

Significant changes in F_O related to photoinhibition were not observed.

Photoinhibition and recovery in situ. Determination of F_V/F_M ratios in situ during the course of the day (Figs. 4–6) revealed that strong photoinhibition took place under natural conditions during exposure to full sunlight (Figs. 4, 5). The decline in F_V/F_M in situ was of similar extent or somewhat less than that for detached leaves

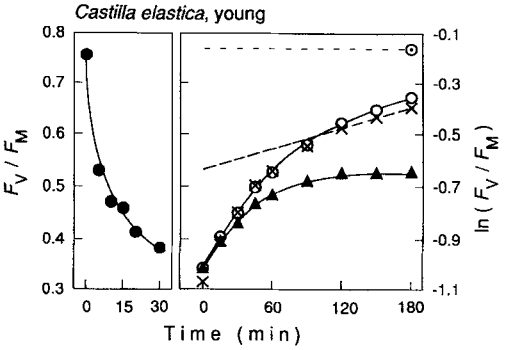

Fig. 2. Kinetics of photoinhibition and recovery in detached young leaves of *Castilla elastica*. *Left*: ●, time course of photoinhibition (as for Fig. 1); *right*: ○, time course of recovery; ×, semilogarithmic plot of ln (F_V/F_M) vs. time; ▲, calculated fast recovery phase (halftime about 30 min)

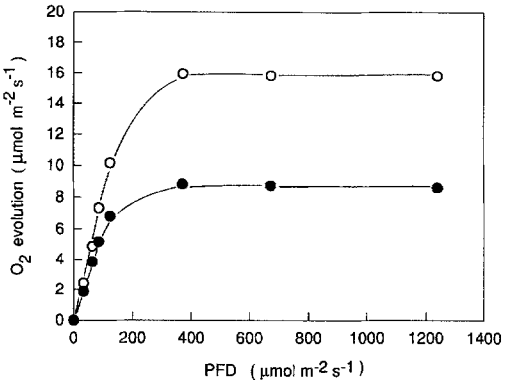

Fig. 3. Gross CO_2-dependent photosynthetic O_2 evolution in young (●) and mature (○) leaves of *Swietenia macrophylla* (Mahogany) as a function of PFD. Rates were determined at saturating CO_2 concentrations. Optimal quantum yields of O_2 evolution (considering absorptance values given in the legend to Fig. 1) were 0.077 and 0.097 for young and mature leaves, respectively

Table 1. Gross O_2 evolution and F_V/F_M ratio of detached leaves of *Swietenia macrophylla* after 90 min standard photoinhibition treatment. O_2 evolution was measured at saturating [CO_2] in limiting light (33 µmol·m^{-2}·s^{-1}). Data are presented as a percentage of the control (kept in low light) value (means ± SD; n = number of determinations using different leaves). Control values: young leaf, 2.9 µmol O_2·m^{-2}·s^{-1}, $F_V/F_M = 0.72$; mature leaf, 2.5 µmol O_2·m^{-2}·s^{-1}, $F_V/F_M = 0.78$

Leaf type	O_2 evolution (% of control)	F_V/F_M (% of control)	n
Young	49.7 ± 14.2	50.2 ± 7.6	4
Mature	72.1 ± 5.9	69.6 ± 2.7	3

under a comparable light regime in the controlled-environment chamber. The relatively weak response of *Antirrhoea* (Fig. 4B) is probably due to self-shading effects of the undulated leaf surface. For *Anacardium*, data from young leaves only are shown (Figs. 4D, 6B), because of senescence of the mature leaves at the end of the rainy season (6th Dec.). An almost identical photoinhibition in situ was observed later (21st Jan.) with young *Anacardium* leaves developed in the early dry season (data not shown).

Fast recovery was observed in situ when the PFD declined in the afternoon (Figs. 5, 6). Usually, the control values of F_V/F_M observed in the morning were approached again at sunset. A rather fast response of partial recovery could also be observed during shading by clouds, e.g. in young leaves of *Castilla*, *Ficus* and *Anacardium* (Figs. 4A, C and 6B) and in both types of leaves of *Swietenia* and *Calophyllum* (Fig. 5A, B). It should be noted that morning values of F_V/F_M were often below 0.8 (particularly in young leaves, e.g. in Fig. 4A and 5A, B; see also Fig. 1). This seems to indicate sustained photoinhibition with no recovery overnight. Such an effect was not seen when high-light stress was absent during preceding days.

Whereas in the controlled-environment chamber (air temperture, 20 °C), the leaf temperature was kept below 30 °C during illumination, leaf tempeatures in situ often rose to 40 °C or higher. We therefore assessed whether heating the leaf tissue alone induced a decrease in F_V/F_M

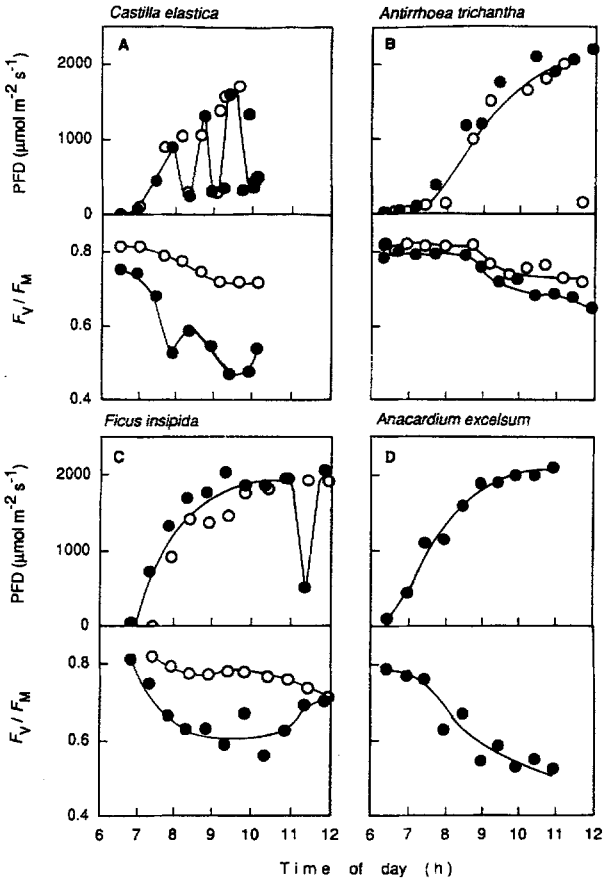

Fig. 4A–D. Photoinhibition as indicated by the F_V/F_M ratio in young (●) and mature (○) leaves in situ (lower panels) compared with changes in PFD from morning to noon (upper panels)

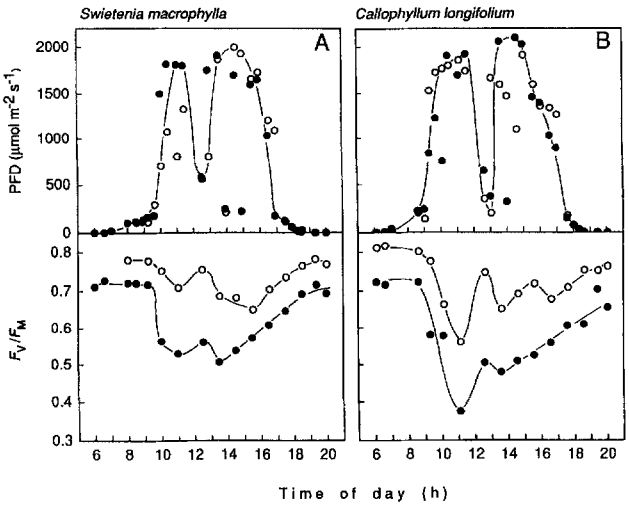

Fig. 5A, B. Course of photoinhibition and recovery during 1 d in young (●) and mature (○) leaves of *Swietenia macrophylla* (**A**) and *Calophyllum longifolium* (**B**). Potted young trees were used. *Upper panels* depict the diurnal course of PFD, *lower panels* the diurnal course of F_V/F_M

in detached leaves of *Swietenia* and *Anacardium*. Exposure of young and mature leaves in the dark for 1 h to 34 °C followed by 1 h at 39 °C did not have any effect on the F_V/F_M ratio (Table 2). In high light, the effects on the

F_V/F_M ratio were similar at temperatures below 30 °C and around 40 °C (see Fig. 1 and Figs. 4–6).

Photosynthetic yield of PSII and electron transport in situ. The fluorescence parameter $1 - F_S/F'_M$, designated as 'yield' of PSII (Φ_{PSII}, Genty et al. 1989), obtained with canopy leaves in situ between early morning and noon, is plotted as a function of PFD in Fig. 7A–C. As expected, a general decline in Φ_{PSII} with increasing PFD was found, but at high PFD, the Φ_{PSII} remained substantially higher in mature than in young leaves. This effect probably results from both the lower photosynthetic capacity (cf. Fig. 3) and the higher degree of photoinhibition in the young leaves. Photoinhibition became apparent from the Φ_{PSII} data, when, after full sunlight exposure, leaves were shaded by clouds. Under these conditions, Φ_{PSII} values were considerably lower than expected from the original curve of Φ_{PSII} vs. PFD, as clearly seen in Fig. 7 (triangles).

From measurements of Φ_{PSII}, PFD and light absorptance by leaves, rates of PSII-driven electron transport (J_F) can be calculated (Krall and Edwards 1992). In Figs. 8 and 9, such rates are compared with rates of net CO_2 assimilation measured in situ during the course of the day. It can be seen that changes in CO_2 assimilation related to changing PFD, as well as a typical mid-day depression of net CO_2 exchange, are reflected by the fluorescence parameter, J_F. At low, strictly limiting PFD, the relationship between J_F and PFD was linear (data not shown), but – probably due to sustained photoinhibition – absolute values of J_F were slightly (10–20%) lower than those expected for optimum photochemical utilization of absorbed photons (cf. Björkman and Demmig 1987). At high PFD, calculated values of J_F were often higher than expected, even if contributions of photorespiration and O_2 reduction (Mehler reaction) are considered (see Discussion).

Pigments. Detailed analyses of photosynthetic pigments in canopy leaves of several tree species are given in Table 3 and Fig. 10. Young leaves showed less dry weight and Chl per unit area than mature leaves. However, the two leaf types had the same Chl *a*/Chl *b* ratio. Also the ratios lutein/Chl and neoxanthin/Chl were not different. Despite the markedly different Chl contents, light absorptance was only a few percent lower in young than mature leaves (see legend to Fig. 1).

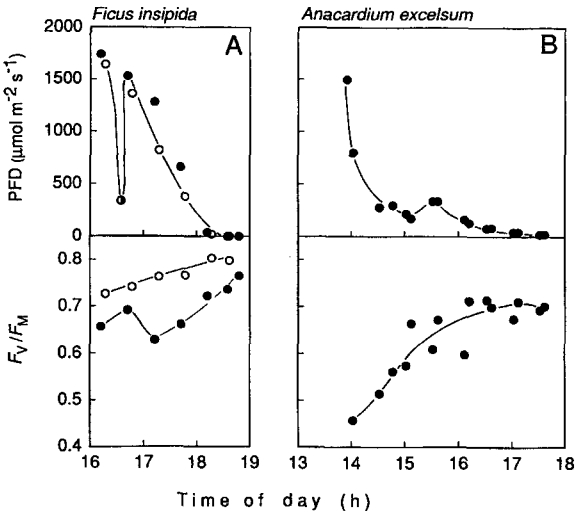

Fig. 6A, B. Recovery of young (●) and mature (○) leaves from photoinhibition (*lower panels*) during decrease in PFD (*upper panels*) in situ. **A** Canopy leaves of *Ficus insipida* recovering after exposure to full sunlight when the PFD declined towards sunset. **B** Canopy leaves of *Anacardium excelsum* recovering when the PFD was reduced by clouds

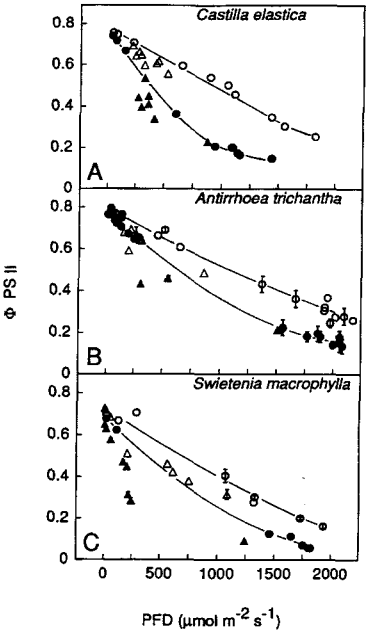

Fig. 7A–C. Photosynthetic yield of PSII in canopy leaves in situ as a function of PFD. Yield (Φ_{PSII}) was determined from fluorescence signals (Genty et al. 1989) during the course of the day from morning (6.30 h) to noon (**A, B**) or evening (**C**). ● ▲, young leaves; ○ △, mature leaves; ▲ △, measurements done when leaves were shaded by clouds following high light exposure, or PFD declined towards sunset. **A** Means of double determinations or single measurements are given. **B, C** Means ± SD are given (if larger than symbols) when three to five measurements were done at similar PFDs (SD of PFD was not more than ± 7%). Otherwise, data from double or single determinations are shown

Table 2. Effect of high temperatures in the dark on the F_V/F_M ratio of young (Y) and mature (M) leaves of *Anacardium excelsum* and *Swietenia macrophylla*. Detached leaves were heated in darkness in a controlled-environment chamber with ventilated moistened air for 1 h at 34 °C followed by 1 h at 39 °C (air temperature). Data are means from two leaves

Species	Leaf type	F_V/F_M (controls)	1 h 34 °C		1 h 39 °C	
			Leaf T (°C)	F_V/F_M	Leaf T (°C)	F_V/F_M
Anacardium excelsum	Y	0.748	31	0.736	37	0.760
	M	0.814	33	0.810	39	0.806
Swietenia macrophylla	Y	0.736	31	0.729	39	0.716
	M	0.806	33	0.799	39	0.799

The epoxidation state of the xanthophyll-cycle pigments determined after dark adaptation or 30 min strong illumination was similar in young and mature leaves (Table 3). On a Chl basis, the xanthophyll-cycle pool size (sum of zeaxanthin, antheraxanthin and violaxanthin) was increased in young leaves, as illustrated in Fig. 10A–C, although considerable variations in xanthophyll contents between individual leaves were observed. This resulted in higher levels of zea-plus antheraxanthin and of zeaxanthin per Chl present in young leaves in high light, while higher violaxanthin/Chl ratios in young than in mature leaves were seen in the dark. Notably, α-carotene contents (per Chl) were considerably lower in young leaves. In *Castilla* and *Antirrhoea*, the β-carotene levels were accordingly higher, so that the total carotene content was about the same in both leaf types (Fig. 10). As expected, the 30-min preillumination did not change the levels (per Chl) of carotenes and lutein, nor the sum of the xanthophyll-cycle pigments (data not shown).

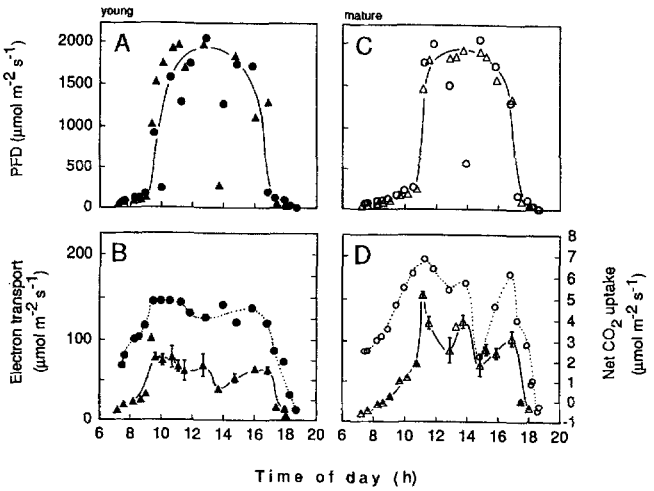

Fig. 8A–D. Comparison of photosynthetic net CO_2 assimilation and PSII-driven electron transport (calculated from fluorescence measurements) in young (**A, B**) and mature (**C, D**) leaves of *Swietenia macrophylla* during the course of a day. A potted tree cultivated outdoors, height about 1.5 m, was used. *Upper panels* give the incident light (PFD) during measurements; *lower panels* denote rates of net CO_2 assimilation (● ○) and PSII electron transport (▲ △). Electron-transport data represent means of three to five measurements and are based on a light absorptance of 0.82 for young leaves and 0.88 for mature leaves (see legend to Fig. 1); SD values are shown if larger than symbols (SD of PFD was not more than ± 7%). Rates of net CO_2 uptake are from single determinations done in parallel with fluoresence recording on the same leaves

Discussion

Photoinhibition. At first sight, the increased susceptibility of young sun leaves to photoinhibition compared with mature leaves (Figs. 1, 2, 4, 5) is surprising. Young leaves are similarly or even more exposed to sun than mature leaves and should not be less acclimated to high light. Studies on sun leaves of *Salix* (Ögren 1991) showed *less* photoinhibition in young than in older leaves. The characteristics of pigment composition, light absorptance and photosynthetic performance of the tropical forest canopy leaves may at least in part explain the differences in susceptibility to photoinhibition: (i) The young leaves contained around 50% less Chl per unit leaf area than the mature ones (Table 3), but light absorptance was only slightly lower in the young leaves (legend to Fig. 1). The Chl *a*/Chl *b* ratio, however, was the same in the two leaf types, indicating similarly developed antenna systems but

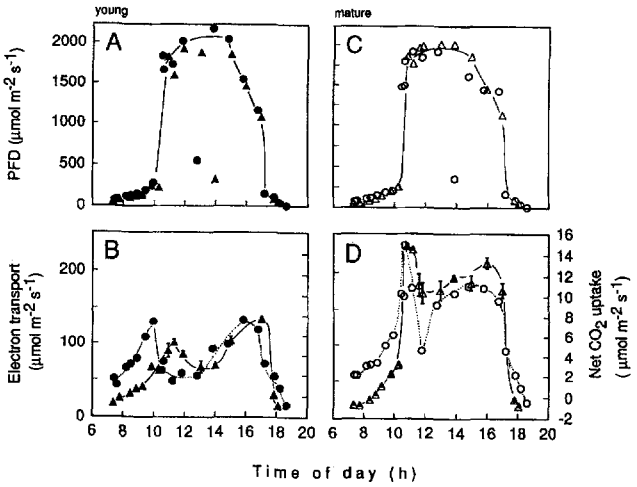

Fig. 9A–D. Comparison of photosynthetic net CO_2 assimilation and PSII-driven electron transport in leaves of *Ficus insipida* during the course of a day. Conditions and symbols as for Fig. 8. For calculation of electron transport an average light absorptance of 0.84 (Demmig and Björkman 1987) was assumed

Table 3. Specific dry weight, content of lutein (L), neoxanthin (N), and epoxidation state of xanthophyll-cycle pigments, (V + 0.5A)/(Z + A + V), of young (Y) and mature (M) canopy leaves. Mean ± SD are given for $n = 6$ (Chl, L, N) or $n = 3$ (other data); n.d. = not determined. For pretreatment of leaves see legend to Fig. 10. A, antheraxanthin; V, violaxanthin; Z, zeaxanthin

Species	Leaf type	specific dry weight ($g \cdot m^{-2}$)	Chl $a + b$ ($\mu mol\, m^{-2}$)	Chl a/Chl b ($mol \cdot mol^{-1}$)	L/Chl $a + b$ ($mmol \cdot mol^{-1}$)	N/Chl $a + b$ ($mmol \cdot mol^{-1}$)	Epoxidation state	
							Dark	Light
Castilla	Y	62 ± 8	448 ± 135	2.74 ± 0.22	151 ± 20	80 ± 13	0.90 ± 0.01	0.29 ± 0.03
elastica	M	101 ± 7	878 ± 87	2.86 ± 0.21	145 ± 5	68 ± 2	0.94 ± 0.04	0.28 ± 0.02
Antirrhoea	Y	59 ± 7	555 ± 56	2.82 ± 0.10	127 ± 8	71 ± 3	0.87 ± 0.04	0.24 ± 0.04
trichantha	M	80 ± 8	1009 ± 114	2.67 ± 0.18	124 ± 14	74 ± 5	0.90 ± 0.04	0.24 ± 0.04
Anacardium	Y	50 ± 6	432 ± 21	2.61 ± 0.08	124 ± 4	68 ± 4	0.88 ± 0.00	0.21 ± 0.02
excelsum	M	129 ± 13	1029 ± 195	2.70 ± 0.15	134 ± 7	70 ± 4	0.87 ± 0.01	0.19 ± 0.01
Swietenia	Y	28 ± 0.6	474 ± 110	2.61 ± 0.47	n.d.	n.d.	n.d.	n.d.
macrophylla	M	80 ± 4	807 ± 107	2.35 ± 0.23				

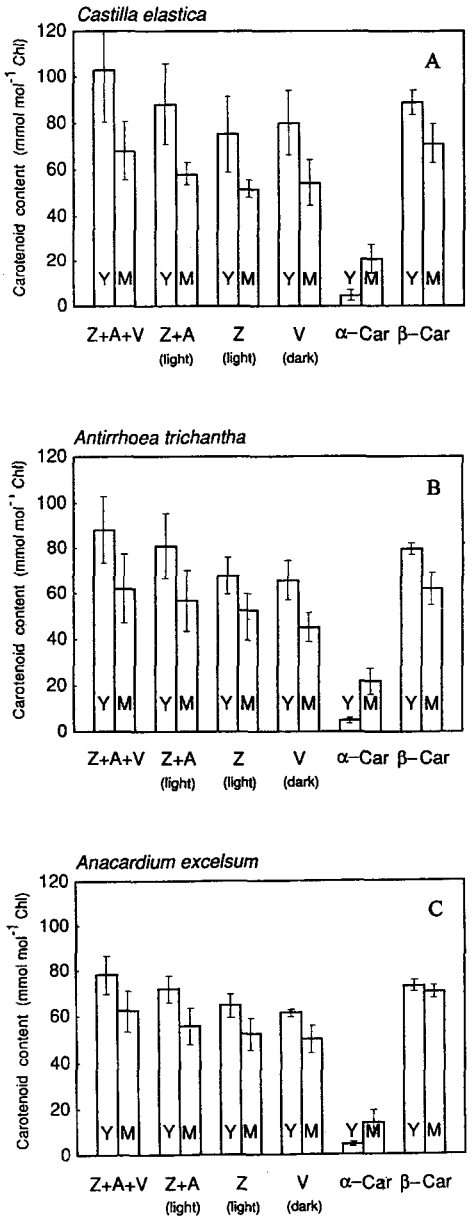

Fig. 10A–C. Xanthophyll-cycle pigments (*A*, antheraxanthin, *V*, violaxanthin; *Z*, zeaxanthin) and carotenes (*Car*) in young (*Y*) and mature (*M*) canopy leaves in dark- and light-adapted states. Pigments were analyzed in detached leaves either after 16 h dark adaptation or after 30 min illumination (*light*, 1.7–1.9 mmol photons $m^{-2} \cdot s^{-1}$) following the dark period. Leaf temperatures were about 27 °C during dark adaptation and 28 °C during illumination. For the sum Z + A + V and for carotenes, light was without effect, and data from dark- and light-adapted leaves were averaged. Values are means ± SE ($n = 6$ for Z + A + V and Car; others, $n = 3$)

fewer photosynthetic units per unit leaf area in young than in old leaves. (ii) Young leaves exhibited much lower capacities of photosynthetic O_2 evolution (about 50% less in the case of *Swietenia*; Fig. 3) and net CO_2 assimilation (Figs. 8, 9), which roughly corresponds to the lower Chl content. Due to these two factors, the same light exposure would result in a much higher fraction of excess light and higher average Chl excitation in the young leaves.

Recovery. The fast recovery from photoinhibition at low PFD in both leaf types, particularly in the young leaves (Figs. 1, 2, 5, 6), is remarkable. In *Eucalyptus* sun leaves, Ögren and Evans (1992) observed one exponential recovery phase with a halftime of 45 min. In the tropical leaves studied here, recovery appeared to occur in (at least) two phases. In the experiment depicted in Fig. 2, sections of young leaves of *Castilla* exhibited a fast recovery (halftime about 30 min), which accounted for about 50% reversion of photoinhibition. This was clearly distinct from a much slower phase (halftime several hours). In situ, shading by clouds led to fast partial recovery (Figs. 5, 6), whereas the slow phase became apparent towards sunset. Often full recovery was not reached, and a remaining sustained photoinhibition was indicated by lowered F_V/F_M ratios in the morning, particularly in young leaves (Figs. 4A, 5, 6). Similarly, in leaves of plants growing in forest gaps, strong photoinhibition caused by full sun exposure in situ during mid-day periods was followed by two distinct recovery phases in response to shading by the surrounding canopy (data not shown).

In a recent study on detached spinach leaves (Leitsch et al. 1994), using streptomycin as an inhibitor of chloroplast protein synthesis, it was shown that the first fast recovery phase was independent of turnover of the PSII reaction-center protein, D1. In contrast, the slow phase depended on protein synthesis in the chloroplasts and thus can be attributed to degradation and replacement of photoinactivated D1 protein. It was postulated that these two recovery phases reflect two steps of photoinhibition, the first one being directly reversible, whereas in the second step the D1 protein becomes affected and eventually degraded. It was also shown that in presence of streptomycin, photoinhibition in spinach (at 20 °C) is enhanced (Schnettger et al. 1994), indicating an important role of D1 turnover in maintaining a population of active PSII in high light (see Aro et al. 1993 for a recent review on D1 turnover in relation to photoinhibition and recovery). Similar effects to those observed in spinach were seen in preliminary tests with the tropical canopy leaves. From the experiment of Fig. 2 (*Castilla*) it can be estimated that about 50% of the full recovery represented D1-independent reversion of photoinhibition. The other 50% could be due to replacement of D1 protein in the PSII reaction center. The sustained photoinhibition not reversed overnight in situ probably results from the light requirement of D1 protein synthesis.

Carotenoids. The three tested species show the same tendency of a higher ratio of xanthophyll-cycle pigments to Chl in young than in mature leaves (Fig. 10A–C). As a result, a higher zeaxanthin/Chl ratio was obtained in high light (Fig. 10), even though the epoxidation state (EPS) was similar in young and mature leaves (Table 3). The very low EPS after 30 min strong illumination shows that in both leaf types most violaxanthin is available to the de-epoxidase, confirming the high xanthophyll-cycle activity of tropical canopy leaves in the Panamanian forest reported by Königer et al. (1995).

An interesting feature is the significantly lower α-carotene content per Chl in young leaves than in mature leaves (Fig. 10). It is unknown, whether a decrease in the ratio of

α- to β-carotene plays a role in photoprotection. However, this ratio is strikingly lower in sun leaves than in shade leaves of the same species (Thayer and Björkman 1990) and in leaves of sun plants compared with shade plants (Demmig-Adams and Adams 1992; Königer et al. 1995). Both the relatively lower α-carotene content and the higher xanthophyll-cycle pool size found in the young tropical forest canopy leaves indicate that these experienced higher excitation of photosynthetic pigments than the mature sun leaves.

The increased zeaxanthin level in excess light and, at the same time, the higher susceptiblity to photoinhibition of the young leaves seems to contradict the concept of a photoprotective function of zeaxanthin (Demming et al. 1987). Recent experiments with spinach, as well as with young and mature leaves of *Castilla elastica* and *Anacardium excelsum* (data not shown), show a close correlation of the fast phase of recovery from photoinhibition (discussed above) and epoxidation of zeaxanthin in low light. Thus, the first, rapidly reversible phase of photoinhibition might be related to de-epoxidation of viola- to zeaxanthin. We speculate that this component of photoinhibition represents a long-lived form of the faster (within a few minutes) relaxing 'energy-dependent' fluorescence quenching (q_E) mechanism, which is promoted by zeaxanthin and requires a trans-thylakoid proton gradient (Horton et al. 1994; Thiele and Krause 1994). Such a 'photoinhibitory' quenching (q_I) mechanism would facilitate thermal dissipation of excess energy in PSII (Demmig and Börkman 1987; Somersalo and Krause 1989, 1990; Öquist et al. 1992a; Krause 1994) and minimize inactivation of the D1 protein, in addition to, or as an alternative to the fast-relaxing q_E. This hypothesis would be consistent with the stronger photoinhibition and pronounced fast component of recovery in the young sun leaves (Figs. 1, 2, 4–6). Gilmore and Björkman (1994) suggested that substantial q_E may be maintained after light stress in darkness by a proton flux into the thylakoids driven by ATP hydrolysis. Such a mechanism cannot be excluded here but would appear to be a highly wasteful process, particularly under strongly fluctuating PFD in the forest canopy.

Photosystem II yield and electron transport calculated from fluorescence data. The general decline in the steady-state 'yield' of PSII (Φ_{PSII}) with increasing PFD (Fig. 7A–C), calculated according to the model of Genty et al. (1989), reflects the decrease in the fraction of absorbed light energy utilized in photosynthesis. Particularly at high PFD, less light is utilized in young leaves than in mature leaves (cf. Figs. 3, 8, 9), which results in lower Φ_{PSII} in young leaves. The Φ_{PSII} measurements generally become less accurate with increasing PFD due to lower signal-to-noise ratios. Thus, the contribution of photoinhibition to the decrease in Φ_{PSII} at extremely high PFD is difficult to deduce from these measurements. However, upon return from high to low PFD, e.g. by shading, comparison with the original Φ_{PSII} (seen at the same low PFD prior to high-light exposure) will give an estimate of photoinhibition in situ, similar to a measurement of maximum photosynthesis in low, limiting light. Such an indication of photoinhibition can be seen in Fig. 7, most pronounced again with the young leaves, when the PFD in situ was reduced by clouds.

The calculated electron-transport rate, J_F, was a rather reliable measure of true PSII-driven electron transport in low, strictly limiting light. From the data in Figs. 8 and 9, ratios of J_F to net CO_2 assimilation (J_F/A_{net}) between 6 and 10 were obtained in low light, similar to ratios reported by Krall and Edwards (1992) for several C_3 plants. These ratios, exceeding the theoretical value of $4e^-$ transported through PSII per CO_2 fixed, are reasonable if an estimate of electron transport related to photorespiration (at 30 °C) is considered (Krall and Edwards 1992). At very high PFD, the calculated J_F increased to higher values than expected, the data of Figs. 8 and 9 giving J_F/A_{net} ratios between 15 and 23. There could be a failure of the model, but part of the strong increase in J_F might be explained by a shift to relatively faster rates of photorespiration at the higher leaf temperatures (close to 40 °C or above) caused by the high levels of incident sunlight (cf. Oberhuber and Edwards 1993). In part the calculated high rates of J_F might also result from an overestimation of the factor f in Eq. 1 due to increased partitioning of excitation energy to PSI under photoinhibitory conditions (Ögren and Öquist 1984; Somersalo and Krause 1989). In several previous studies, deviations from a linear relationship between Φ_{PSII} calculated from fluorescence signals and quantum yield of photosynthesis was observed for C_3 plants under photorespiratory (Harbinson et al. 1990) or non-photorespiratory conditions (Seaton and Walker 1990; van Wijk and van Hasselt 1990; Öquist and Chow 1992; Oberhuber et al. 1993). While further investigations are needed to clarify these discrepancies, the changes in calculated J_F reflected qualitatively the diurnal changes in CO_2 assimilation (Figs. 8, 9). The data indicate that during the mid-day depression of CO_2 assimilation (probably related to stomatal closure), PSII electron transport strongly declined owing to photoinhibition and down-regulation by the energy-dependent quenching mechanism. Thus, J_F calculations are a useful means of monitoring, at least qualitatively, the trend of electron transport in situ by simple and fast fluorescence tests.

Conclusions. The high degree of photoinhibition in the young canopy sun leaves indicated by a strong decrease in the F_V/F_M ratio probably reflects a dynamic regulatory response of the photosynthetic system to excess absorbed light energy. The sustained quenching of Chl fluorescence is based on an increase in the rate constant of thermal deactivation, in addition to, or as an alternative to, the faster-relaxing 'energy-dependent' fluorescence quenching. The observed photoinhibition is possibly associated with some loss of productivity (cf. Ögren and Sjöström 1990) but might protect photosynthetic pigments and electron-transport apparatus from severe destruction. In low light, a large part of the photoinhibitory quenching relaxes within less than 1 h. This fast recovery probably does not require D1 protein synthesis and may be related to xanthophyll-cycle activity, which is increased in the young leaves. The hypothesis that energy-dependent (q_E) and photoinhibitory (q_I) quenching in part follow a similar mechanism, i.e. that both depend on zeaxanthin formation, needs to be verified.

The part of photoinhibition which reverses slowly in the course of hours, and which after severe light stress

during the diurnal cycle can still be detected the following morning, is probably related to impairment of the D1 protein in the PSII reaction center. Centers with affected D1 protein presumably contribute to thermal energy dissipation. Degradation of the 'marked' D1 protein seems to take place only as fast as it can be replaced by synthesis (Schnettger et al. 1992; 1994; Aro et al. 1993). Thus the damaged centers probably play a protective role, too. High D1 protein turnover assumed for sun leaves (Öquist et al. 1992b) should also be viewed as a regulatory process, as it obviously functions to maintain an active PSII population under conditions of high irradiation and to restore full PSII activity during prolonged periods of low light.

The authors thank Catherine Lovelock, Smithsonian Tropical Research Institute, Panama City, Panama, for valuable comments on the manuscript and Milton García, Smithsonian Tropical Research Institute, for assistance in leaf absorptance measurements. The study was supported by a Visiting Fellow award of the Smithsonian Institution to G.H.K. and grants of the Mellon Foundation and the Deutsche Forschungsgemeinschaft (SFB 189).

References

Adams III WW (1988) Photosynthetic acclimation and photoinhibition of terrestrial and epiphytic CAM tissues growing in full sunlight and deep shade. Aust J Plant Physiol 15: 123–134

Adams III WW, Terashima I, Brugnoli E, Demmig B (1988) Comparison of photosynthesis and photoinhibition in the CAM vine *Hoya australis* and several C_3 vines growing on the coast of eastern Australia. Plant Cell Environ 11: 173–178

Aro E-M, Virgin I, Andersson B (1993) Photoinhibition of photosystem II. Inactivation, protein damage and turnover. Biochim Biophys Acta 1143: 113–134

Björkman O, Demmig B (1987) Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. Planta 170: 489–504

Bolhàr-Nordenkampf HR, Hofer M, Lechner EG (1991) Analysis of light-induced reduction of the photochemical capacity in field-grown plants. Evidence for photoinhibition? Photosynth Res 27: 31–39

Demmig B, Björkman O (1987) Comparison of the effect of excessive light on chlorophyll fluorescence (77 K) 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 xanthophyll cycle in the dissipation of excess light energy. Plant Physiol 84: 218–224

Demmig-Adams B (1990) Carotenoids and photoprotection in plants: A role for the xanthophyll zeaxanthin. Biochim Biophys Acta 1020: 1–24

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

Demmig-Adams B, Adams III WW, Winter K, Meyer A, Schreiber U, Pereira JS, Krüger A, Czygan F-C, Lange OL (1989) Photochemical efficiency of photosystem II, photon yield of O_2 evolution, photosynthetic capacity, and carotenoid composition during the midday depression of net CO_2 uptake in *Arbutus unedo* growing in Portugal. Planta 177: 377–387

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

Giersch C, Krause GH (1991) A simple model relating photoinhibitory fluorescence quenching in chloroplasts to a population of altered photosystem II reaction centers. Photosynth Res 30: 115–121

Gilmore AM, Björkman O (1994) Adenine nucleotides and the xanthophyll cycle in leaves. II. Comparison of the effects of CO_2- and temperature-limited photosynthesis on photosystem II fluorescence quenching, the adenylate energy charge and violaxanthin de-epoxidation in cotton. Planta 192: 537–544

Harbinson J, Genty B, Baker NR (1990) The relationship between CO_2 assimilation and electron transport in leaves. Photosynth Res 25: 213–224

Horton P, Ruban AV, Walters RG (1994) Regulation of light harvesting in green plants. Indication by nonphotochemical quenching of chlorophyll fluorescence. Plant Physiol 106: 415–420

Königer M, Harris GC, Virgo A, Winter K (1995) Xanthophyll-cycle pigments and photosynthetic capacity in tropical forest species: a comparitive field study on canopy, gap and understory plants. Oecologia, in press

Krall JP, Edwards GE (1992) Relationship between photosystem II activity and CO_2 fixation in leaves. Physiol Plant 86: 180–187

Krause GH (1988) Photoinhibition of photosynthesis. An evaluation of damaging and protective mechanisms. Physiol Plant 74: 566–574

Krause GH (1994) Photoinhibition induced by low temperatures. In: Baker NR, Bowyer JR (eds) Photoinhibition of photosynthesis. From molecular mechansisms to the field. BIOS Scientific Publishers, Oxford, pp 331–348

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

Leitsch J, Schnettger B, Critchley C, Krause GH (1994) Two mechanisms of recovery from photoinhibition in vivo: Reactivation of photosystem II related and unrelated to D1-protein turnover. Planta 194: 15–21

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

Oberhuber W, Edwards GE (1993) Temperature dependence of the linkage of quantum yield of photosystem II to CO_2 fixation in C_4 and C_3 plants. Plant Physiol 101: 507–512

Oberhuber W, Dai Z-Y, Edwards GE (1993) Light dependence of quantum yields of photosystem II and CO_2 fixation in C_3 and C_4 plants. Photosynth Res 35: 265–274

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

Ögren E (1991) Prediction of photoinhibition of photosynthesis from measurements of fluorescence quenching components. Planta 184: 538–544

Ögren E, Evans JR (1992) Photoinhibition of photosynthesis in situ in six species of *Eucalyptus*. Aust J Plant Physiol 19: 223–232

Ögren E, Öquist G (1984) Photoinhibition of photosynthesis in *Lemna gibba* as induced by interaction between light and temperature. III. Chlorophyll fluorescence at 77 K. Physiol Plant 62: 193–200

Ögren E, Rosenqvist E (1992) On the significance of photoinhibition of photosynthesis in the field and its generality among species. Photosynth Res 33: 63–71

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

Öquist G, Chow WS (1992) On the relationship between the quantum yield of photosystem II electon transport, as determined by chlorophyll fluorescence and the quantum yield of CO_2-dependent O_2 evolution. Photosynth Res 33: 51–62

Öquist G, Chow WS, Anderson JM (1992a) Photoinhibition of photosynthesis represents a mechanism for the long-term regulation of photosystem II. Planta 186: 450–460

Öquist G, Anderson JM, McCaffery S, Chow WS (1992b) Mechanistic differences in photoinhibition of sun and shade plants. Planta 188: 422–431

Schnettger B, Leitsch J, Krause GH (1992) Photoinhibition of photosystem 2 in vivo occurring without net D1 protein degradation. Photosynthetica 27: 261–265

Schnettger B, Critchley C, Santore UJ, Graf M, Krause GH (1994) Relationship between photoinhibition of photosynthesis, D1 protein turnover and chloroplast structure: effects of protein synthesis inhibitors. Plant Cell Environ 17: 55–64

Seaton GGR, Walker DA (1990) Chlorophyll fluorescence as a measure of photosynthetic carbon assimilation. Proc R Soc London Ser B 242: 29–35

Somersalo S, Krause GH (1989) Photoinhibition at chilling temperature. Fluorescence characteristics of unhardened and cold-acclimated spinach leaves. Planta 177: 400–416

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

Thayer S, Björkman O (1990) Leaf xanthophyll content and composition in sun and shade determined by HPLC. Photosynth Res 23: 331–343

Thiele A, Krause GH (1994) Xanthophyll cycle and thermal energy dissipation in photosystem II: Relationship between zeaxanthin formation, energy-dependent fluorescence quenching and photoinhibition. J Plant Physiol 144: 324–332

van Wijk KJ, van Hasselt PR (1990) The quantum efficiency of photosystem II and its relation to non-photochemical quenching of chlorophyll fluorescence; the effect of measuring light and growth temperature. Photosynth Res 25: 233–240