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Protection against photoinhibition in the alpine plant *Geum montanum*

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Abstract *Geum montanum* L. is an alpine plant usually found at altitudes between 1700 and 2600 m. Its wintergreen leaves can be subjected to very low temperatures and at the same time receive high photon flux densities at the beginning of the growth season when the snow melts. We report results of a study, performed with classical methods of biophysics, showing that leaves of *G. montanum* were remarkably tolerant to sunlight even at low temperatures. This tolerance results from the interplay of photorespiration and CO_2 photosassimilation. When temperatures approach 0°C, responses include stomatal opening and CO_2 uptake even under desiccation stress. This permits linear electron transport that is sufficient to avoid the excessive reduction of the electron transport chain which is known to lead to photodamage. In addition, excitation energy was shifted from photosystem (PS)II to PSI which is a very efficient energy quencher. Sensitivity of P700 in PSI to oxidation by far-red light was decreased and rates of dark reduction of photooxidized P700 were increased by actinic illumination, suggesting activation of cyclic electron transport. Consistent with this, far-red light was able to decrease the quantum yield of PSII (measured by the F_v/F_m ratio of chlorophyll fluorescence). We suggest that cyclic electron transport decreases the lumenal pH under strong light. In the presence of zeaxanthin, this increases energy dissipation at the PSII level. At low temperatures, P700 remained strongly oxidized under high irradiation while the primary electron acceptor of PSII, Q_A, was largely reduced. This shows efficient control of electron transport presumably at the level of the cytochrome b/f complex and suggests formation of a protective transthylakoid proton gradient even when linear electron transport is much reduced in the cold. Thus, several mechanisms cooperate to effectively protect the photosynthetic apparatus of *G. montanum* from photodamage. We see no indication of destructive "photostress" in this species during the growth season under alpine low-temperature and drought conditions.

Key words Electron transport · Low temperature · Photosynthesis · Photosystems I and II · Water stress

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Introduction

Alpine plants may experience extremes in temperature, water availability and irradiation in different combinations and changing succession during a vegetation period. Temperatures close to or even below freezing may be combined with strong irradiation early in a spring or autumn day, and high temperatures may act on the same plant later during the same day. Particularly in summer and early autumn, drought may enforce stomatal closure and limit photosynthetic carbon uptake under high irradiation while temperatures may be variable.

Photosystem (PS)II of the chloroplast electron transport chain is known to be sensitive to high fluxes of photosynthetically active radiation particularly when a shortage of electron acceptors limits linear electron flow and leads to an excessive reduction of electron carriers between PSI and PSII. At least for elevated temperatures, such reduction is known to be damaging. Excessive reduction is likely to occur when low temperatures limit electron flow, or when stomata are closed during drought periods so that external CO_2 does not reach the photosynthetic apparatus. As shown

by Powles et al. (1983), high photosynthetically active photon flux densities (PPFDs) can lead to oxidative damage by active oxygen species when the capacity of enzymic systems capable of detoxifying active oxygen is overloaded in the cold. However, recent studies of three alpine plants, *Ranunculus glacialis*, *Homogyne alpina* and *Soldanella alpina*, have shown that PSII was particularly resistant to photoinhibition and that no correlation could be found between high light tolerance and the capacity of the antioxidant system, although the plants contained much higher concentrations of ascorbate and glutathione than lowland plants (Streb et al. 1997).

Photoinhibition of PSII may be prevented by mechanisms capable of dissipating excess absorbed energy as heat before active reaction centres are excited (Horton et al. 1996; Ruban et al. 1997). Dissipation requires protonation of membrane components on the inner side of the thylakoid membrane and the presence of zeaxanthin in the membranes (Demmig-Adams and Adams 1992; Björkman and Niyogi, in press). A low intrathylakoid pH activates the synthesis of zeaxanthin (Hager 1980). Neither electron flow to CO_2 nor electron flow to oxygen during photorespiration are likely to decrease the intrathylakoid pH sufficiently for the required protonation and synthesis reactions because, at an H^+/e ratio of three (Rich 1991; Kobayashi and Heber 1995) and an H^+/ATP ratio of four (Rumberg et al. 1990; Kobayashi et al. 1995), linear electron flow deposits as many protons in the intrathylakoid space as are needed for ATP production during photosynthesis or photorespiration. Main candidates for decreasing the intrathylakoid pH sufficiently for triggering protective mechanisms are oxygen reduction in the Mehler reaction and cyclic electron transport. In previous work, the Mehler reaction was shown to be unable to prevent photodamage to the photosynthetic apparatus of leaves when photoassimilation and photorespiration were inhibited while the Mehler reaction remained active (Wu et al. 1991; Heber et al. 1995a; Wiese et al. 1998). This suggested a role for cyclic electron transport in triggering photoprotective mechanisms by decreasing the intrathylakoid pH (Heber and Walker 1992; Heber et al. 1995b). However, cyclic electron flow requires oxidized electron carriers on the reducing side of PSII (Arnon and Chain 1979). At high temperatures, the interplay between photoassimilation and photorespiration can cause sufficient electron drainage even when stomata are closed to keep electron carriers partially oxidized. However, at low temperatures, photorespiration is low or absent. Only oxygen remains available as an electron acceptor, as its concentration in air, 21%, can produce gradients large enough to overcome stomatal barriers even when stomata are closed. In the present work, we show that the alpine plant *Geum montanum* is remarkably resistant to photodamage. We also provide evidence for the mechanisms which enable the plants to avoid the deleterious effects of high light under extreme environmental conditions.

Materials and methods

Plant material

Plants of *G. montanum* growing in an alpine meadow close to the experimental station at the Col du Lautaret (altitude 2100 m) were used for the experiments. Table 1 gives some characteristics of the light and temperature climates close to the experimental field as measured at the Lautaret climatic station from 1995 to 1997. High PPFDs were often associated with cold temperatures. Even in August, about 10% of the days had a mean temperature lower than 10°C when PPFDs were higher than 1000–1500 μmol m^{-2} s^{-1}. Moreover, during July, climatic conditions were highly variable at the high altitudes. Snow fell repeatedly and covered the ground for some time down to 2000 m above sea level. In this environment, *Geum* plants produced more than 50% of their leaves at the end of May and in early June, when the coldest temperatures of the growing season occurred.

Measurements were performed either in the field with attached or detached leaves or in the laboratory with detached leaves. In a few cases, plants were dug out and transfered to Orsay University close to Paris.

Gas exchange measurements

Net CO_2 assimilation (A) and transpiration (E) were measured using the infrared gas analysis system DA-1000. The CO_2 molar ratio in air was usually around 340 ppm. Some measurements were also made at Orsay (near Paris) over 2 days following transfer of *Geum* plants from the Col du Lautaret, using the gas exchange system described by Cornic and Ghashghaie (1991). Three days after transfer, the plants exhibited clear signs of acclimation by increasing the temperature optimum of photosynthesis and decreasing the extent of the Warburg effect at temperatures lower than 12°C.

State of PSII: chlorophyll fluorescence

Modulated chlorophyll fluorescence was measured in parallel to CO_2 uptake by leaves using the Mini-PAM equipment (Walz, Effeltrich, Germany). The light guide used had a diameter of little more than 1 mm. Chlorophyll fluorescence was also measured with the PAM 101 fluorometer (Walz) (Schreiber et al. 1986). The frequency of modulated measuring light was 1.6 kHz with darkened leaves and 100 kHz under actinic illumination. Saturating 1-s light pulses were provided by a KL 1500 light source (Schott, Wiesbaden, Germany). Another KL 1500 light source provided actinic illumination. Several fluorescence yields were measured routinely: (1) the fluorescence of dark-adapted leaves excited only by the measuring beam (F_o), (2) the maximal fluorescence of dark-adapted leaves induced by saturating 1-s light pulses (F_m). (3) the maximal

Table 1 Occurrence of days presenting a mean temperature lower than 10°C associated with a photosynthetically active photon flux density either higher than 1000 (A) or 1500 (B) μmol m^{-2} s^{-1} from June to September at the Lautaret meteorological station. Frequencies are calculated from observations made during 3 years (1995–1997)

Month	A (%)	B (%)
June	32.0	19.0
July	9.7	4.3
August	10.0	7.8
September	21.0	12.0

fluorescence of illuminated leaves induced by saturating 1-s light pulses (F'_m), (4) the steady-state fluorescence emitted from PSII under continuous illumination (F_s) and (5) the minimum fluorescence (F'_o) observed immediately after actinic illumination was turned off. Occasionally, far-red illumination was turned on at the F'_o level in order to control whether F'_o really indicated minimum fluorescence.

From the fluorescence parameters, the ratio $\Delta F/F'_m$, calculated from $(F'_m-F_s)/F'_m$, provided a relative measurement of the quantum yield of PSII photochemistry which is strongly correlated with the quantum yield of linear electron transport to photosynthetic acceptors (Genty et al. 1989). $(F_s-F'_o)/(F'_m-F'_o)$ was used as a relative indicator of the redox state of the primary quinone acceptor Q_A in the reaction centre of PSII. The quantum yield of PSII open traps was estimated under actinic light by the ratio $(F'_m-F'_o)/F'_m$ $(=F_v/F'_m)$, and also in the dark by the ratio $(F_m-F_o)/F_m$ $(=F_v/F_m)$.

Estimation of whole-chain photosynthetic electron transfer in intact leaves from chlorophyll fluorescence

When multiplied by PPFD, $\Delta F/F'_m$ values give information on relative rates of whole-chain electron transport. Simultaneous measurements of chlorophyll fluorescence and gas exchange in a nitrogen atmosphere with 700 ppm CO_2 permitted quantification of electron flow by fluorescence as described in Heber et al. (1995a). PPFDs were measured with a Li-Cor quantum meter (Li-Cor, Lincoln, Neb.).

State of PSI: oxidation of P700

The redox state of P700 was monitored in leaves as 820-nm absorption of the cation radical of P700 by the PAM 101 fluorometer using a suitable emitter-detector unit (Schreiber et al. 1988). Sunlight, or white or red light from a halogen source were used for actinic illumination. The half bandwidth of the red light (filters: RG 630, Schott; Calflex C, Balzers, Liechtenstein) ranged from 630 to 760 nm. Far-red light capable of exciting mainly PSI was provided by an RG9 filter (Schott). The half bandwidth was from 710 to 760 nm. Effective absorption of far-red was determined as follows. P700 was oxidized in leaves of spinach (as flash equipment was not available in the mountains and *G. montanum* was not available where flash equipment could be used) by the highest intensity of far-red light available. Single (ST) and multiple (MT) turnover flashes from the lamps XST 103 and XMT 103 (Walz) were fired to transiently reduce photooxidized P700 by electrons coming from PSII. They were capable of exciting both PSII and PSI. After the flashes, the background far-red reoxidized P700. Rates of reoxidation and areas formed by reduction and oxidation were measured as in Asada et al. (1992) and the area ratios were related to one another. The ratio of MT to ST areas represents the functional pool of intersystem electrons per reaction centre of PSII, and was between eight and nine electrons per PSII reaction centre. From this, the functional pool was calculated to be about 6.6 μmol m^{-2} leaf area. At the maximum rate of P700 oxidation, 15% of this was oxidized within 0.2 s. Thus, the highest intensity of far-red used in the spinach and *Geum* experiments was capable of oxidizing P700 at a rate close to 5 μmol m^{-2} s^{-1} in spinach. It is assumed that PSI activity is similar in spinach and *G. montanum*.

HCN and glycolaldehyde poisoning

Leaves of *G. montanum* were fumigated with 3.4% HCN (liberated from KCN by sulphuric acid) for 5 min or received 40 mM glycolaldehyde through the petiole with the transpiration stream.

Results and discussion

Linear electron flow to external CO_2 compared to total light-dependent electron flow and reduction of the quinone acceptor Q_A of PSII

The optimum temperature for maximum net CO_2 uptake by illuminated *G. montanun* leaves was about 18°C in air with 356 ppm CO_2. Owing to the inhibition of photorespiration, it was higher when the oxygen content of the atmosphere was decreased to 1% (Fig. 1A). Assimilation A was inhibited by 21% oxygen even at 5°C (Fig. 1B), indicating that ribulose bisphosphate carboxylase still had substantial oxygenase activity at this low temperature. This clearly indicates a plant adapted to cold condition (Cornic and Massaci 1996).

When a leaf, maintained above the optimal temperature under a PPFD of 1800 μmol^{-2} s^{-1}, was cut from the plant and allowed to wilt in air, rates of photosynthesis and transpiration sometimes increased for a few minutes (Ivanoff effect; Ivanoff 1928) before both rates decreased. Alternatively, the reductions occurred immediately, as shown in Fig. 2 where A and transpiration E reached about 20% and 30% of their initial value within 2 h. Total linear electron flow, estimated by the

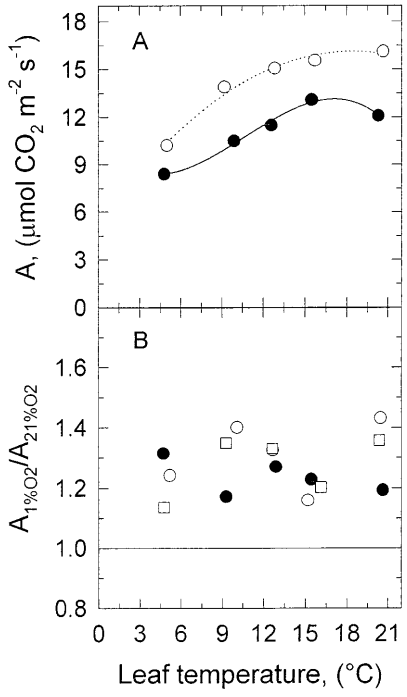

Fig. 1 **A** Net CO_2 uptake by leaves of *Geum montanum* as a function of leaf temperature in air with 356 ppm CO_2 (*closed circles*) and after the oxygen content of the atmosphere was adjusted to 1% with 356 ppm CO_2 (*open circles*). Data are the means of three independent experiments with different plants. Photosynthetically active photon flux density (PPFD) 1100 μmol m^{-2} s^{-1}; vapor pressure deficit 0.7 ± 0.2 kPa. **B** Ratio of A measured in 1% O_2 to A measured in 21% O_2. Calculations are made from data in **A.** *Different symbols* refer to measurements done with three different plants

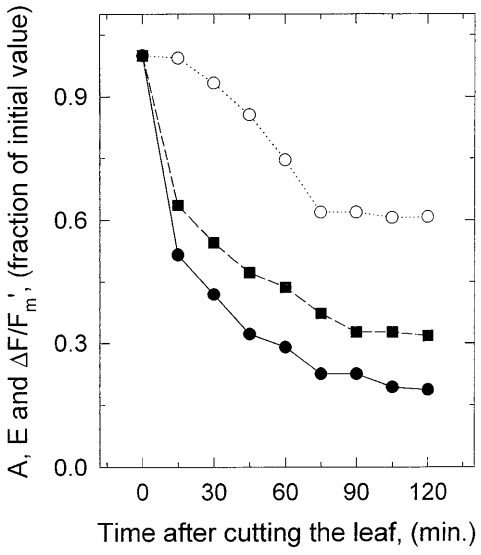

Fig. 2 Changes in net CO_2 uptake (*A*, *closed circles*), transpiration (*E*, *squares*) and quantum yield of photosystem (PS)II photochemistry $\Delta F/F'_m$ (open circles) measured during dehydration of a cut leaf of *G. montanum* in air. PPFD was 1800 µmol m^{-2} s^{-1}

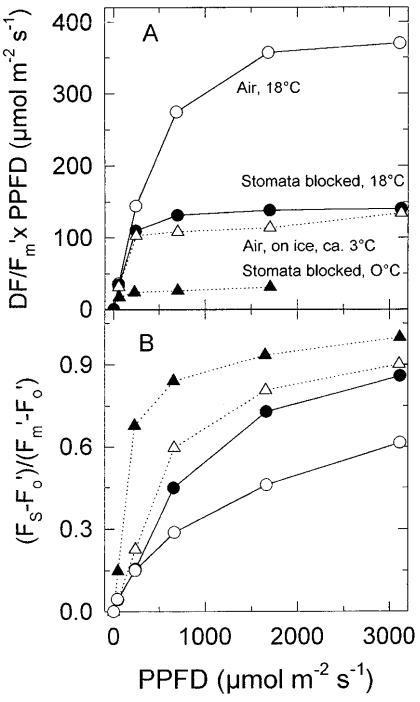

Fig. 3 **A** Linear electron flow as expressed by $\Delta F/F'_m \times$ PPFD of a detached leaf of *G. montanum* as a function of PPFD (*open circles* air, 18°C; *closed circles* stomata blocked under water, 18°C; *open triangles* air, on ice, ca 3°C; *closed triangles* stomata blocked under ice water, 0°C). **B** State of reduction of Q_A in the reaction centre of PSII as indicated by $(F_s-F'_o)/(F'_m-F'_o)$ of a detached leaf of *G. montanum* as a function of PPFD (symbols as in **A**)

quantum yield of PSII photochemistry, started to decline only 15 min after cutting when net CO_2 uptake was already inhibited by 50%. After little more than 1 h, electron flow had reached a steady state which was about 60% of the initial value. This shows that at this stage, most of the electrons reduced electron acceptors other than external CO_2. In this condition, O_2 was mainly reduced via the oxygenase activity of ribulose bisphosphate carboxylase (Wu et al. 1991; Heber et al. 1995a; Cornic and Massaci 1996). Two hours after cutting the leaf, electron flow supported by the interplay between photorespiratory CO_2 release and the refixation of released CO_2 was about six times higher than the electron flow used for the reduction of external CO_2.

In similar gas exchange experiments performed with detached leaves under controlled conditions, stomata also closed when the water supply was interrupted by cutting the petiole, as shown by decreased transpiration in Fig. 2. After leaves had lost about 40% of their water, the temperature of the cuvette was reduced to 12°C. Not surprisingly, lowering the temperature initially decreased transpiratory water loss. However, transpiration subsequently increased slowly indicating that closed stomata had started to reopen. Opening even continued in the light when the leaf temperature was further decreased to about 4°C. This behaviour of *Geum* was similar to that already reported by Cornic and Ghasghaie (1991) for water-stressed bean leaves which opened stomata when the temperature was decreased from 25 to 12°C.

In Fig. 3A, total electron transport of a leaf of *G. montanum* (as indicated by $(\Delta F/F'_m \times$ PPFD) is compared as a function of PPFD at 18°C and at a temperature not far above freezing point in two situations: (1) when open stomata permitted gas exchange, and (2) when gas exchange was blocked under water. Absence of gas exchange across the stomata had little effect on electron transport under low light but decreased electron transport by 60% under sunlight. Under light saturation, electron transport at 18°C with blocked stomata was similar to electron transport at 3°C with open stomata. When gas exchange across stomata was blocked at 0°C, linear electron transport was much reduced.

Figure 3B shows the fluorescence ratio $(F_s-F'_o)/(F'_m-F'_o)$ as a function of PPFD for leaves of *G. montanum* which were submitted to the conditions used to obtain the data decribed in Fig. 3A. $(F_s-F'_o)/(F'_m-F'_o)$ may be taken as a relative measure of the reduction of the quinone acceptor, Q_A, of PSII. Absolute numbers for Q_A reduction would require knowledge of the connectivity between the PSII reaction centres (Genty et al. 1995). Nevertheless, the values of $(F_s-F'_o)/(F'_m-F'_o)$ indicate large differences in Q_A reduction depending on whether or not gas exchange with surrounding air was possible. Even at PPFDs above sunlight, an appreciable percentage of Q_A remained oxidized at a temperature of 3°C as long as open stomata permitted gas exchange with air. Not unexpectedly, interruption of stomatal gas exchange, which eliminated external CO_2 as an electron acceptor, increased reduction of Q_A as indicated by fluorescence, but reduction was still incomplete at the photon flux density of full sunlight both at 18 and 0°C.

Oxidation of P700

Figure 4 compares photooxidation of P700 in the reaction centre of PSI at 18°C and at 3°C as indicated by absorption changes close to 820 nm. Although about 30% of light-induced absorption increases are known to be caused by the oxidation of plastocyanin (Klughammer and Schreiber 1991), this electron carrier donates electrons directly to P700. Its redox responses reflect those of P700. Leaves were illuminated first by red light (capable of exciting PSI and PSII), and then by far-red light (capable of exciting predominantly PSI). In both cases, the *Geum* leaf had been preilluminated at a PPFD of 1300 μmol m^{-2} s^{-1} for 5 min before the experiment. Turning on red light (PPFD 540 μmol m^{-2} s^{-1}: about 25% of full sunlight) first caused oxidation and then transient reduction of P700. Reduction is caused by electrons coming from PSII. Subsequently, P700 was oxidized in an oscillatory mode which was particularly noticeable at 3°C.

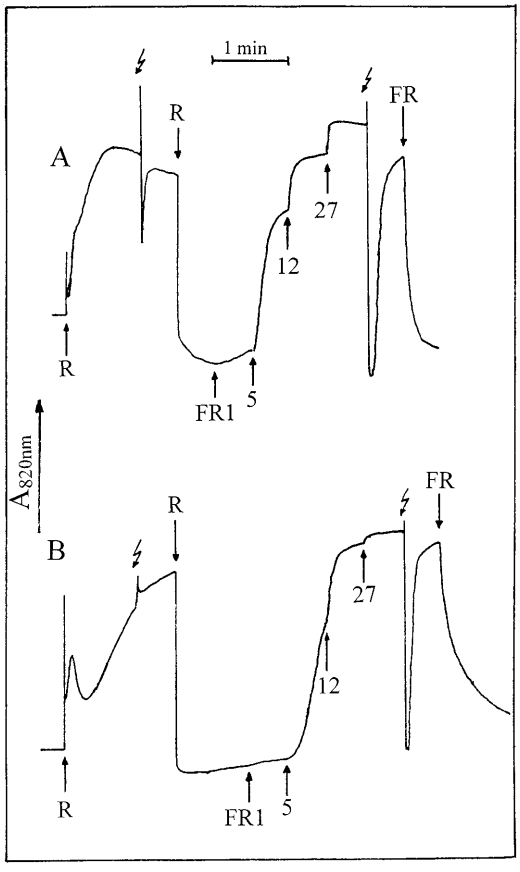

Fig. 4 Light-dependent redox changes of P700/plastocyanin as indicated by 820-nm absorption of a light-adapted leaf of *G. montanum*. The leaf was first illuminated with a broad band of red light capable of exciting both PSII and PSI. During illumination, a 1-s saturating pulse of white light was given. After darkening for about 1 min, far-red light of the relative intensities 1, 5, 12 and 27 was given in sequence to oxidize P700, and a 1-s saturating white flash was finally added to far-red 27. Compare the oxidative and reductive phases in the redox state of P700. **A** Air, 18°C. **B** Air, on ice, ca 3°C

At 18°C, a saturating flash of white light (1 s) first oxidized P700 and then reduced it transiently by electrons from PSII which had not been available to PSI before the flash (Fig. 4A). After the red light was turned off and photooxidized P700 had been reduced, stepwise increases of far-red light (up to 5 μmol m^{-2} s^{-1} absorbed far-red photons) oxidized P700 stepwise as well. At the lowest far-red intensities, sigmoid oxidation was apparent particularly at 3°C (Fig. 4B). A white flash given in the presence of far-red reduced photooxidized P700 transiently because electrons became available from PSII. Importantly, reoxidation was not slower but actually somewhat faster at 3°C than at 18°C. After the far-red light was turned off, reduction in the dark was much slower than reduction after red illumination because the far-red had exhausted the pool of electrons between PSII and PSI. In addition, reduction was slower at 3°C than at 18°C after far-red illumination.

There were general similarities in the responses of the leaf to red and far-red light at the two different temperatures. Important differences were the absence of the reductive phase observed after a saturating flash at 3°C in the presence of actinic illumination and the fast reoxidation of flash-reduced P700 under far-red illumination at the same temperature. The absence of the reductive phase at 3°C after a 1-s flash under actinic illumination indicates excellent control of electron flow to PSI. It was also observed that steady-state oxidation of P700 was very similar in leaves maintained under saturating far-red light either at 18 or 8°C (not shown). Reduction of Q_A as shown in Fig. 3B indicates that control of electron flow to PSI is exerted at a site between PSI and PSII, presumably by the cytochrome b/f complex. This control is known to require a large transthylakoid proton gradient or, at a controlled pH of the chloroplast stroma, a low intrathylakoid pH. Under far-red illumination and in the absence of a large proton gradient, electrons from PSII reached photooxidized P700 rapidly. They were even more rapidly transferred to the reducing side of PSI at 3°C than at 18°C. This is a remarkable observation in view of the temperature dependence of conventional chemical reactions involving partner collision.

Observations similar to those shown in Fig. 4 for *Geum* leaves kept in air were also made when the leaves were submerged in water in order to block gas exchange (data not shown).

State of PSI

Figure 5 shows recordings of modulated chlorophyll fluorescence of a sun-adapted leaf of *G. montanum* which, after cutting from the plant and slight desiccation in the sun to cause stomatal closure, was strongly illuminated at 18°C for 12 min (PPFD = 3000 μmol m^{-2} s^{-1}) and then placed in the dark. During illumination and after darkening, 1-s pulses of saturating white light (PPFD 13,000 μmol m^{-2} s^{-1}) were given every minute.

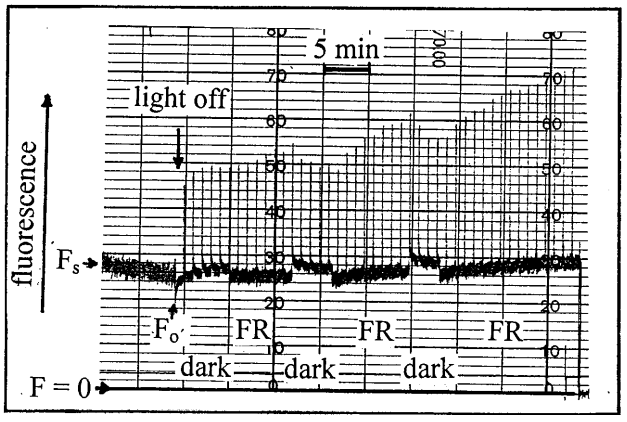

Fig. 5 Recovery in the dark, or under far-red light (*FR*), of modulated chlorophyll fluorescence of a detached leaf of *G. montanum* after some wilting in the sun and a subsequent 12-min illumination period at a PPFD of 3000 μmol m^{-2} s^{-1}. One-second pulses of saturating white light (13,000 μmol m^{-2} s^{-1}) were given every minute to probe for the extent of relaxation of non-photochemical fluorescence quenching which had occurred under actinic illumination. Absorption of far-red light was below 25 μmol m^{-2} s^{-1}

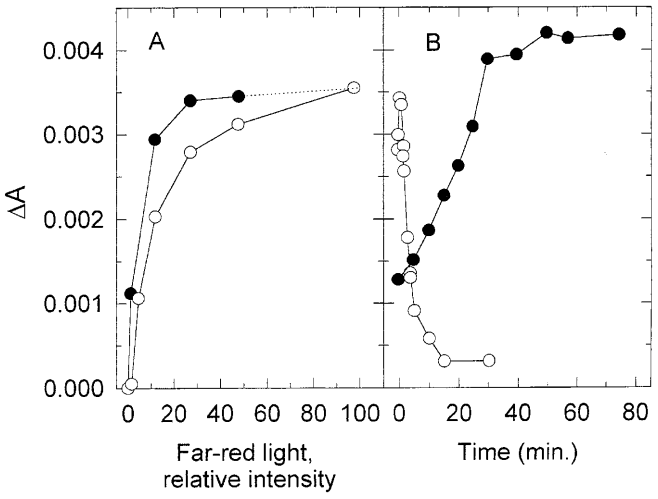

Fig. 6 **A** Oxidation by far-red light of P700/plastocyanin in a leaf of *G. montanum* (as indicated by 820-nm absorbance: ΔA) as a function of far-red light intensity, directly after a 30-min preillumination period with a PPFD of 2000 μmol m^{-2} s^{-1} (*open circles*), and at the same position of the same leaf after prolonged darkening (*closed circles*). **B** Changes in 820-nm absorbance caused by weak far-red light of constant intensity in a dark-adapted leaf of *G. montanum* immediately after exposure of the leaf to strong white light (PPFD = 3800 μmol m^{-2} s^{-1}) for the times indicated (*open circles*), and effects of the length of darkening on changes in 820-nm absorbance after a leaf had been light-adapted at a PPFD of 1800 μmol m^{-2} s^{-1} for the times indicated (*closed circles*)

In the light, they failed to cause transient fluorescence increases because of strong fluorescence quenching. After darkening caused fluorescence to decrease to the level F_o' owing to the oxidation of Q_A^-, basic fluorescence F_s recovered slowly and pulse-induced fluorescence spikes became large. After about 5 min of darkening, no further increase in the fluorescence spikes was seen. This suggests that energy-dependent quenching of chlorophyll fluorescence (q_E quenching) had relaxed at F_v/F_m = 0.45. When far-red was provided, basic fluorescence decreased owing to oxidation of Q_A^-. Simultaneously, recovery of pulse-induced fluorescence was stimulated. Turning off the far-red increased basic fluorescence and decreased the pulse-induced fluorescence spikes. These responses could be repeated. At the end of the experiment, F_v/F_m had increased under the influence of far-red to about 0.6.

The stimulating effect of far-red light on the recovery of F_v/F_m suggests that under illumination, excitation energy had been shifted from PSII to PSI (Bukhov et al. 1996). Far-red is known to reverse this so-called state shift.

If this interpretation is correct, one might expect a more sensitive oxidation of P700 by far-red light after light adaptation than in a predarkened leaf. However, the opposite was observed (Fig. 6A). After exposure of part of a leaf to 2000 μmol m^{-2} s^{-1} for 30 min, sensitivity of P700 to oxidation by far-red light was considerably decreased compared to a darkened control. Prolonged darkening reversed this effect. Figure 6B shows that about 4 min illumination with a very high PPFD (3800 μmol m^{-2} s^{-1}) was sufficient to produce a half-maximum decrease in sensitivity to P700 oxidation by far-red light. The figure also shows that, shortly after the onset of strong illumination, the sensitivity of P700 to oxidation is transiently increased as expected from a state shift such as that shown in Fig. 5. Only later does the sensitivity of P700 to oxidation decrease. This effect was reversible. As also shown in Fig. 6B (closed symbols), the sensitivity of P700 oxidation to far-red returned in the dark after preillumination with strong light. The half-time of return to the state of increased sensitivity to oxidation was about 20 min.

Decreased sensitivity of P700 to far-red oxidation can be explained by an increased rate of dark reduction of photooxidized P700 in leaves which had previously been exposed to strong white light. This is shown in Fig. 7. Semilog plots of dark reduction (not shown) revealed two reactions with different rate constants which contributed to the reduction of photooxidized P700. The slower one reduced P700 with a half-time of reduction ($t_{1/2}$) of 4 s in the predarkened leaf and of 0.75 s after light adaptation.

The data shown in Figs. 5, 6 and 7 suggest that after strong illumination, which shifts excitation energy from PSII to PSI, cyclic electron flow occurs around PSI. Electrons pumped by PSI from the intersystem chain to the reducing side of PSI are suggested to return to the intersystem chain, thereby decreasing the sensitivity of P700 to photooxidation by far-red light.

If this is true, coupled cyclic electron transport driven by far-red light is expected to form a transthylakoid proton gradient. Since protonation reactions in the thylakoid interior are known to decrease PSII activity, attempts were made to demonstrate pH-dependent control of PSII by cyclic electron transport around PSI. Such control should be seen as a far-red-dependent de-

crease in F_v/F_m. Although, in the experiment shown in Fig. 7, the opposite was observed, this was explained as a reversal of a state 1/state 2 transition which, by increasing F_v/F_m, would obscure a simultaneous decrease in F_v/F_m (Bukhov et al. 1996). By taking advantage of the observation that a state shift could not be demonstrated in dark-adapted leaves of *G. montanum*, we could indeed show a decrease in F_v/F_m by far-red light (Fig. 8). A dark-adapted leaf was briefly illuminated to induce non-photochemical fluorescence quenching which started to relax after darkening. At F_v/F_m = 0.68, a far-red beam (absorbed photons about 5 μmol m^{-2} s^{-1}) was turned on. Far-red not only interrupted but actually partially reversed relaxation of fluorescence to F_v/F_m = 0.64. Turning the far-red off rapidly brought F_v/F_m to 0.71. This observation is interpreted as control of PSII by cyclic electron flow around PSI. No control was exerted by weak red light (data not shown). Surprisingly, observations very similar to those shown in Fig. 8 for a leaf temperature of 18°C were also made when the leaf was kept on ice, even though lowering the temperature is known to reduce intersystem electron carriers (Fig. 3B).

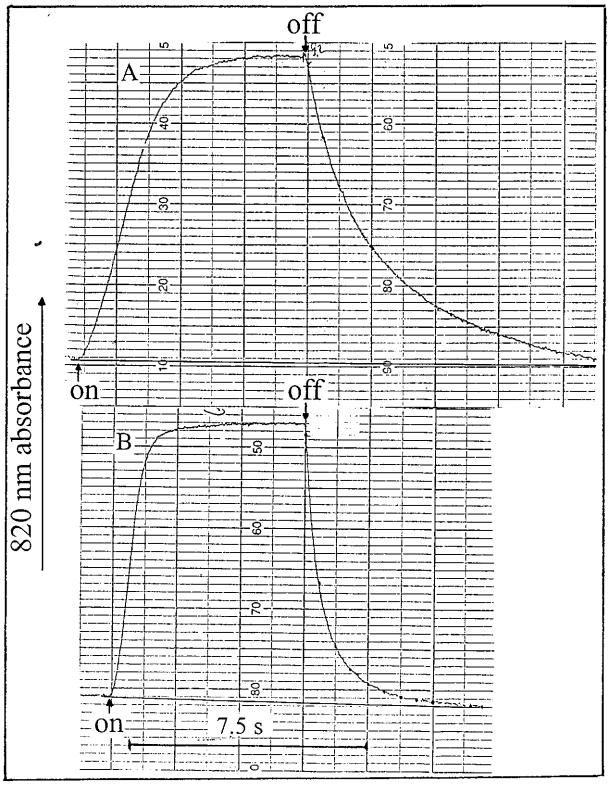

Fig. 7 Oxidation by far-red light and reduction in the dark of P700/plastocyanin in a dark-adapted leaf of *G. montanum* as indicated by 820-nm absorbance (**A**) and after a 6-min exposure of the leaf to 2000 μmol m^{-2} s^{-1} (**B**). The intensity of the far-red necessary to obtain comparable oxidation was higher in **B** than in **A**

Photoinhibition after exposure at different temperatures to high irradiances

Photoinhibition was estimated by measuring the decrease in the quantum yield of PSII photochemistry using the fluorescence parameter F_v/F_m. Maximum $\Delta F/F_m$ in dark-adapted healthy leaves is known to be close to 0.8 in many different plant species (Björkman and Demmig 1987). Values were close to 0.8 in the field during early mornings or late evenings after leaves had been darkened for several minutes. No evidence for photoinhibition could be obtained in the field.

To check the resistance to high-light stress of *Geum*, comparative experiments were performed in which leaves were exposed perpendicular to sunlight (1600–2000 μmol m^{-2} s^{-1}). Exposure time was 4 h. The following conditions were chosen (Table 2): (1) the leaves remained attached to the plant; (2) cut leaves were floated on water (temperature about 18 to 22°C); (3) cut leaves were immersed in water of about 20°C to block stomatal gas exchange; (4) cut leaves were placed on ice (due to radiant warming and insufficient contact to ice, the leaf temperature rose up to 13°C and wilting oc-

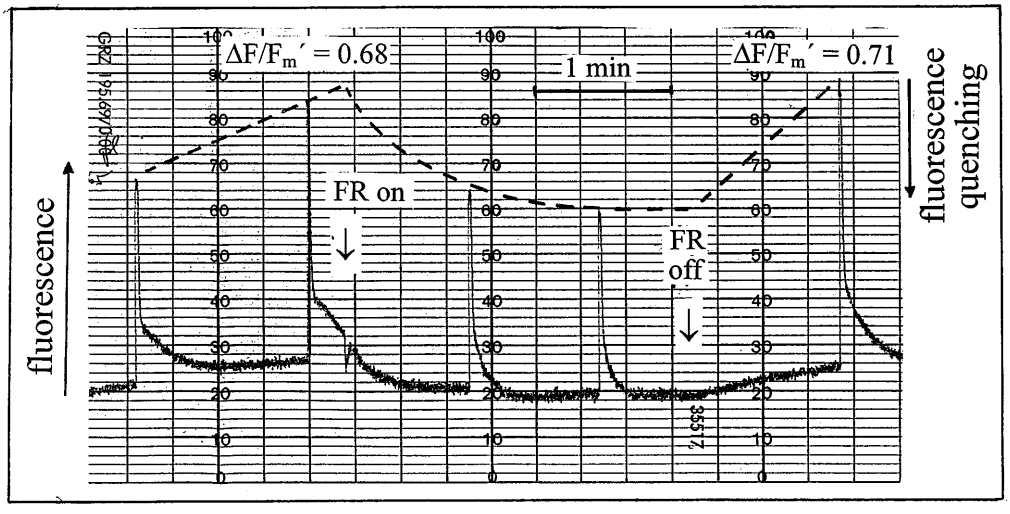

Fig. 8 Modulated chlorophyll fluorescence of an initially dark-adapted leaf of *G. montanum* shortly after the leaf had received a PPFD of 1500 μmol m^{-2} s^{-1} for 30 s and was subsequently darkened. One-second pulses of saturating light (13,000 μmol m^{-2} s^{-1}) were given as indicated to probe for recovery of maximum fluorescence F_m. Far-red (*FR*) was present during the second and third pulse

Table 2 Recovery of the quantum efficiency of electron flow through PSII as indicated by F_v/F_m after about 21 h in the dark (with or without 12.5 mM lincomycin) after a 4-h exposure period of leaves of *Geum montanum* to sunlight in different conditions. Data are the means of several independent measurements (±confidence interval: $\alpha = 5\%$). For full explanation, see text

Time in the dark after high-light treatment	F_v/F_m				
	Leaf attached to the plant	Leaf placed on water, 20°C	Submerged leaf, 20°C	Leaf placed on ice	Leaf in ice water, 0°C
0.5 h	0.737 ± 0.038	0.690 ± 0.056	0.569 ± 0.021	0.332 ± 0.034	0.477 ± 0.037
21 h	0.807 ± 0.024	0.767 ± 0.031	0.755 ± 0.022	0.631 ± 0.031	0.671 ± 0.030
21 h + lincomycin	0.784 ± 0.023	0.700 ± 0.051	0.700 ± 0.037	0.537 ± 0.049	0.627 ± 0.033

curred during sun exposure; (5) cut leaves were immersed in ice water to block stomatal gas exchange.

Care was taken that the same PPFD was incident on the submerged and the non-submerged leaves during the high-light treatment. After the 4-h sun exposure, the leaves were immediately transferred to darkness and left in the dark at room temperature, floating either on water or on water plus lincomycin at 2, 5 and 12.5 mM to inhibit protein synthesis in the chloroplasts (Aro et al. 1994). No appreciable differences in the lincomycin data were noted suggesting saturation of inhibition of protein synthesis already at the lower concentrations of the antibiotic.

The high-light treatment decreased F_v/F_m dramatically, particularly at the low temperatures (not shown, but see Fig. 5) but recovery as measured after 30 min of darkness was substantial in all cases. It was largest at the normal temperature and when wilting did not occur or leaf gas exchange was not blocked by submersion. After 21 h in the dark, F_v/F_m recovered to or close to control values when leaves were maintained at normal temperature. Values remained significantly lower at low temperature. As expected, lincomycin did not have much effect on recovery since the synthesis of those proteins which may be degraded during photoinhibition (mainly D1 protein) requires some light. It is concluded that, in *Geum* leaves, the decrease in F_v/F_m after high-light treatment involves little or no protein damage at normal temperature and was largely due to a down-regulation of PSII photochemistry. At low temperature, on the other hand, appreciable protein degradation had occurred.

Interestingly, the extent of photoinhibition seen after 21 h of darkness was not increased by blocking gas exchange across the stomata, either at normal or low temperature (Table 2), though whole-chain electron transport was much decreased (as shown in Fig. 3A) and reduction of Q_A was increased (Fig. 3B).

After leaves had been cut from the plant and left for 4 h in the sun without water supply, water loss amounted to 60% of the initial leaf weight. Depending on the extent of wilting, the leaf temperature rose up to 27°C in the sun. Full turgor always returned after the leaves had been placed on water. The F_v/F_m ratio measured after 21 h in the dark in the presence of lincomycin was 0.70 ± 0.038, a value very similar to that measured at about 20°C with non-dehydrated cut leaves.

To further test whether the relationship between photoinhibition and excessive reduction of the electron transport chain is as stringent as commonly assumed, leaves of *G. montanum* were poisoned with either HCN or glycolaldehyde. By inactivating ascorbate peroxidase in addition to ribulose bisphosphate carboxylase, HCN inhibits not only carbon assimilation and photorespiration, but also the detoxification of H_2O_2 which is generated by the Mehler reaction. In contrast, as an inhibitor of phosphoribulokinase (Gardeström and Wigge 1988), glycolaldehyde inhibits only assimilation and photorespiration, not the reactions of the Asada pathway (Wiese et al. 1998). Table 3 shows that strong illumination caused extensive damage to PSII only in wilted HCN-poisoned leaves. Less damage was observed when dehydration was either avoided or the temperature was reduced. Relative protection against strong illumination at reduced temperatures might be explained by lowered rates of the Mehler reaction (Havaux and Devaud 1994). Glycolaldehyde feeding was less effective in increasing the sensitivity of leaves to photoinhibition than HCN poisoning, although, by inhibiting electron flow, both inhibitors strongly increased reduction of the electron transport chain in illuminated leaves (data not shown).

Table 3 Recovery of the quantum efficiency of electron flow through PSII as indicated by $\Delta F/F_m$ about 20 h after a 4-h exposure period of HCN- or glycolaldehyde-poisoned leaves of *G. montanum* to sunlight. Gas exchange measurements performed before the stress experiments had revealed reduction of light-saturated CO_2 assimilation by 88% after HCN poisoning and by 82% after glycolaldehyde feeding. Average of four different leaves within one experiment. (The experiment was performed with and without lincomycin as in Table 1, but averages were taken of the lincomycin and the control data because lincomycin data were not noticeably different from the H_2O controls. Statistical treatment of the data was not possible because of insufficient replications.). Conditions as in Table 2. For full explanation, see text

	$\Delta F/F_m$	
	HCN fumigation	Glycolaldehyde feeding
Wilted leaf, left without water	0.05	0.58
Submerged leaf, 20°C	0.32	0.65
Leaf placed on ice	0.35	0.63
Leaf in ice water, 0°C	0.46	0.48

Conclusions

The alpine plant *G. montanum* is remarkably tolerant of strong illumination under widely differing temperature and dehydration conditions. When attached to the plant, leaves do not seem to suffer appreciable degradation of the D1 protein in the reaction centre of PSII at ambient temperatures (Table 2). Damage was still not very considerable even when light stress, heat stress and dehydration stress were combined in detached leaves. Likewise, restricting gas exchange across the stomata and lowering the temperature (Table 2) failed to appreciably increase photoinhibitory damage, even though reduction of the electron transport chain was considerably increased under such conditions (Fig. 3B).

It appears that several factors contribute to the high tolerance of light and resistance to photoinhibition of *Geum* leaves:

(1) The interplay of photorespiration and reassimilation of released CO_2 facilitates appreciable electron flow even when closed stomata do not permit assimilation of external CO_2, for example under drought stress (Figs. 1, 2, and 3A). When electron flow is restricted at low temperatures, stomata open in desiccation-stressed leaves permitting assimilation of external CO_2. This electron transport helps to prevent excessive reduction of the electron transport chain.

(2) Excess excitation energy is shifted from PSII to PSI as shown by the reversal of this shift under far-red light (Fig. 5). Oxidized P700 in the reaction centre of PSI (Fig. 4), which is an effective quencher of chlorophyll fluorescence, aids in the harmless radiationless dissipation of absorbed light energy.

(3) Under strong illumination, P700 changes its oxidation and reduction characteristics (Figs. 6, 7). This is interpreted as activation of cyclic electron transport which, by virtue of coupled transthylakoid proton transport, and through protonation of PSII proteins, can downregulate PSII activity (Fig. 8). Equilibration between photooxidized P700 and Q_A^- does not occur (Fig. 4) although Q_A is much reduced, particularly at low temperatures (Fig. 3B). The only possible explanation is the presence of a transthylakoid proton gradient capable of maintaining a large redox gradient at the level of the cytochrome b/f complex. It is particularly remarkable that control of PSII activity by far-red light was not only observed at room temperature (Fig. 8) but also when leaves were placed on ice, i.e. when reduction of the electron transport chain was much increased under strong light.

As has been demonstrated by Arnon and Chain (1979) for isolated thylakoids and later by Kobayashi and Heber (1994) and Heber et al. (1995b) for intact chloroplasts, cyclic electron transport is under strict redox control. Both the presence of oxidized electron carriers between PSII and PSI and electron availability at the reducing side of PSI are required for cyclic electron transport to operate around PSI. However, the present data for leaves throw some doubt on this seemingly established concept. Impressed by the observations presented here for low temperatures, it seems that in leaves PSI may be sufficiently separated from PSII not to interfere with cyclic electron transport even when Q_A of PSII is strongly reduced. This spatial separation may permit PSI to exert control over PSII by creating a proton gradient which extends its effect to PSII by the high mobility of protons while it is shielded from the influence of reduced membrane-located intersystem electron carriers such as plastoquinone.

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References

Arnon DI, Chain RK (1979) Regulatory electron transport pathways in cyclic photophosphorylation. FEBS Lett 102:133–138

Aro EM, Mccaffery S, Anderson JM (1994) Recovery from photoinhibition in peas (*Pisum sativum* L) acclimated to varying growth irradiances – role of D1 protein turnover. Plant Physiol 104:1033–1041

Asada K, Heber U, Schreiber U (1992) Pool size of electrons that can be donated to P_{700}^+ as determined in intact leaves: donation to P_{700}^+ from stromal components via the intersystem chain. Plant Cell Physiol. 33:927–932

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

Björkman O, Niyogi KK (in press) Xanthophylls and excess-energy dissipation: a genetic dissection in *Arabidopsis*. Proc XI Int Photosynth Congr, Budapest

Bukhov NG, Wiese C, Neimanis S, Heber U (1996) Effects of far-red light on the relaxation of chlorophyll fluorescence quenching and on light-induced conformational changes in the thylakoid membranes of leaves. Photosynth Res 50:181–191

Cornic G, Ghashghaie J (1991) Effect of temperature on net CO_2 assimilation and photosystem II quantum yield of electron transfer of French bean leaves (*Phaseolus vulgaris* L.) during drought stress. Planta 185:255–260

Cornic G, Massaci A (1996) Leaf photosynthesis under drought stress. In: Baker NR (ed) Photosynthesis and the environment. Kluwer, Dordrecht, pp 347–366

Demmig-Adams B, Adams III WW (1992) Photoprotection and other responses of plants to high light stress. Annu Rev Plant Physiol Plant Mol Biol 143:599–626

Gardeström P, Wigge B (1988) Influence of photorespiration on ATP/ADP ratios in the chloroplasts, mitochondria, and cytosol, studied by rapid fractionation of barley protoplasts. Plant Physiol 88:69–76

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

Genty B, Rizza F, Ruelle D (1995) The relationbship between the energy dependent quenching of chlorophyll fluorescence and the capacity of oxygen evolution at PSII in vivo. In: Mathis P

(ed) Photosynthesis from light to biosphere IV. Kluwer, Dordrecht, pp 283–286
Hager A (1980) The reversible light-induced conversion of xanthophylls in the chloroplasts. In: Czygan FC (ed) Pigments in plants. Fischer, Stuttgart, 57–79
Havaux M, Devaud A (1994) Photoinhibition of photosynthesis in chilled potato leaves is not correlated with a loss of photosystem II activity – preferential inactivation of photosystem I. Photosynth Res 40:75–92
Heber U, Walker D (1992) Concerning a dual function of coupled cyclic electron transport in leaves. Plant Physiol 100:1621–1626
Heber U, Bligny R, Streb P, Douce R (1995a) Photorespiration is essential for the protection of the photosynthetic apparatus of C3 plants against photoinactivation under sunlight. Bot Acta 109:307–315
Heber U, Gerst U, Krieger A, Neimanis S, Kobayashi Y (1995b) Coupled cyclic electron transport in intact chloroplasts and leaves of C3 plants: does it exist? If so, what is its function? Photosynth Res 46:269–275
Horton P, Ruban AV, Walters RG (1996) Regulation of light harvesting in green plants. Annu Rev Plant Physiol Plant Mol Biol 47:655–684
Ivanoff L (1928) Zur Methodik der Transpirationsbestimmung am Standort. Ber Dr Bot Ges 46:306–310
Klughammer C, Schreiber U (1991) Analysis of light-iduced absorption changes in the near-infrared region. I. Characterization of various components in isolated chloroplasts. Z Naturforsch 46c:233–244
Kobayashi Y, Heber U (1994) Rates of vectorial proton transport supported by cyclic electron flow during oxygen reduction by illuminated intact chloroplasts. Photosynth Res 41:419–428
Kobayashi Y, Heber U (1995) Coupling ratios $H^+/e = 3$ versus $H^+/e = 2$ in chloroplasts and quantum requirements of net oxygen exchange during the reduction of nitrite, ferricyanide or methylviologen. Plant Cell Physiol 36:1630–1620
Kobayashi Y, Kaiser W, Heber U (1995) Bioenergetics of carbon assimilation in intact chloroplasts: coupling of proton to electron transport at the ratio $H^+/e = 3$ is incompatible with $H^+/ATP = 3$ in ATP synthesis. Plant Cell Physiol 36:1629–1637
Powles SB, Berry JA, Björkman O (1983) Interaction between light and chilling temperature on the inhibition of photosynthesis in chilling-sensitive plants. Plant Cell Environment 6:117–123
Rich PR (1991) The osmochemistry of electron-transfer complexes. Biosci Rep 11:539–571
Ruban AV, Phillip D, Young AJ, Horton P (1997) Carotenoid-dependent oligomerization of the major chlorophyll a/b light harvesting complex of photosystem II in plants. Biochemistry 36:7855–7859
Rumberg B, Schubert K, Strelow F, Tran-Anh T (1990) The H^+/ATP coupling ratio at the H^+-ATP-synthase of spinach chloroplasts is four. In Baltscheffsky M (ed) Current research in photosynthesis III. Kluwer, Dordrecht, pp 125–128
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
Schreiber U, Klughammer C, Neubauer C (1988) Measuring P700 absorbance changes around 830 nm with a new type of pulse modulated system. Z Naturforsch 43c:686–698
Streb P, Feirabend J, Bligny R (1997) Resistance to photoinhibition of photosystemII and catalase and antioxidative protection in high mountain plants. Plant Cell Environ 20:1030–1140
Wiese C, Shi L, Heber U (1998) Oxygen reduction in the Mehler reaction is insufficient to protect photosystems I and II of leaves against photoinactivation. Physiol Plant 102:437–446
Wu J, Neimanis S, Heber U (1991) Photorespiration is more effective than the Mehler reaction to protect the photosynthetic apparatus against photoinhibition. Bot Acta 104:283–291