PHOTOSYNTHETICA 35 (2): 191-203, 1998

Heat emission as a protective mechanism against high-irradiance stress in sugar maple leaves

K. VEERANJANEYULU and R.M. LEBLANC*

Department of Chemistry, University of Miami, Coral Gables, Florida 33124, U.S.A.

Abstract

High-irradiance (HI) induced changes in heat emission, fluorescence, and photosynthetic energy storage (ES_T) of shade grown sugar maple (*Acer saccharum* Marsh.) saplings were followed using modulated photoacoustic and fluorescence spectroscopic techniques. HI-treatment at 900-4400 μmol m^{-2} s^{-1} for 15 min caused an increase in heat emission and a decrease in ES_T. In some leaves, HI-treatment of 900 μmol m^{-2} s^{-1} for 1 min induced a rapid increase in heat emission with a marginal decrease in ES_T. Parallel to the increase in heat emission, there was a decrease in fluorescence, and this phenomenon was reversible in darkness. Quenching of thermal energy dissipation and a recovery in ES_T were observed during the first 15 min after the HI-treatment. This down-regulation of photochemical activity and its recovery may be one of the photoprotective mechanisms in shade grown sugar maple plants. The increase in thermal energy dissipation was greater in the red absorbing long wavelength (640-700 nm) region than in the blue absorbing short wavelength region of photosynthetically active excitation radiation. The photochemical activity was affected more in short wavelengths (400-520 nm) than in the long wavelength region of the spectrum. This can be due to the migration of light-harvesting chlorophyll (Chl) *a/b* protein complex from photosystem (PS) 2 to PS1 and/or to the disconnection of carotenoid pool from Chls in the pigment bed of photosynthetic apparatus.

Additional key words: *Acer saccharum;* fluorescence; photoacoustic spectroscopy; photoinhibition; photoprotection; photosystems

---

Received 23 April 1997, *accepted* 8 October 1997.

*Author for correspondence; fax: (305)-284-4371, e-mail: mmodrono@umiami.miami.edu

Abbreviations: Chl, chlorophyll; ES_{PS1}, energy storage of photosystem 1; ES_{PS2}, energy storage of PS2; ES_T, energy storage of PS1 and PS2; HI, high irradiance; LHC2, light-harvesting chlorophyll *a/b* protein complex of photosystem 2; PA, photoacoustic; PAR, photosynthetically active radiation; PS, photosystem; Q_m, PA signal in the absence of any background radiation; Q_{ma}, PA signal in the presence of background "white light"; Q_{mfrl}, PA signal in the presence of background far-red radiation.

Acknowledgement: This work was financially supported by the Natural Sciences and Engineering Research Council of Canada, the Fonds pour la Formation de Chercheurs et l'Aide à la Recherche du Québec, Canada, and the University of Miami, Coral Gables, Florida. K.V. acknowledges the postdoctoral research fellowship from the University of Miami.

Introduction

Exposure of leaves to irradiance higher than that required to induce maximum photochemical activity causes a decline in the photon efficiency of photosynthesis, a phenomenon termed photoinhibition. Photoinhibition has been attributed primarily to the reduction in photochemical efficiency of PS2 due to its inactivation and/or degradation of its D1 polypeptide by the HI-stress (Powles 1984, Critchley 1988, Aro *et al.* 1993, Long *et al.* 1994). Photoinhibition is accompanied by biophysical changes such as fluorescence quenching and increase in heat emission. These changes are partly reversible and are considered to be the regulatory processes of energy dissipation that provide some protection to the PS2 reaction center. Thermal dissipation of excitation energy by non-radiative deexcitation of pigments is probably one of the protective mechanisms against HI-stress (Fork *et al.* 1986, Krause 1988, Krause and Weis 1991, Demmig-Adams and Adams 1993). This is confirmed by measuring non-photochemical energy quenching of fluorescence and heat emission using photoacoustic spectroscopy (Buschmann 1987, Krause 1988, Krause and Weis 1991, Demmig-Adams and Adams 1993).

Despite heat emission being considered as a protective mechanism, several studies, which demonstrated an increase in heat emission and fluorescence quenching, also showed an inhibition in photochemical activity (Buschmann 1987, Krause 1988, Bilger and Björkman 1990, Krause and Weis 1991, Demmig-Adams and Adams 1993). This inhibition can be due to the decreased photon efficiency of photosynthesis, and not in the maximal rate. However, it is not clear whether there is any protection afforded to the photosynthetic apparatus (as evidenced by no decrease in photochemical activity) by the changes in biophysical processes or by a reversible decrease in photochemical activity, which are considered as protective mechanisms. The down regulation of PS2 activity without any degradation of the D1 polypeptide of the reaction center and the increase in thermal energy dissipation may be protective mechanisms against HI-stress (Aro *et al.* 1993, Gilmore and Björkman 1994, Leitsch *et al.* 1994). Since fluorescence, heat emission, and photochemistry are competitive processes for the absorbed radiant energy, and the quantum yield of fluorescence is low when compared to heat emission and photochemistry, the magnitude of fluorescence quenching-related heat emission may be low and difficult to isolate. Hence, there is no information on the extent of increased heat emission when there is no inhibition of photochemical activity after HI-treatment. In the present study, using photoacoustic and modulated fluorescence techniques, we show that some degree of protection to the photosynthetic apparatus in the shade grown sugar maple saplings is done by increased heat emission.

Furthermore, in the literature there is not much information on the changes in thermal energy dissipation of pigments in the spectral range of photosynthetically active radiation (PAR) between 400 and 700 nm. A significant increase in absorbance at around 510 nm in HI-treated leaves was demonstrated by Bilger *et al.* (1989) and Bilger and Björkman (1994). Horton *et al.* (1991, 1994) have shown *in vitro* that HI-treatment causes aggregation of LHC2 resulting in the formation of an efficient pathway for non-radiative dissipation of excitation energy, and demonstrated the

appearence of new absorption bands at 510, 660, and 690 nm, and of a long-wavelength fluorescence band at 700 nm. Hence, there may also be some variations in the amplitude of the increased heat emission in the spectral range of PAR.

We also examined the changes in heat emission in the spectral range of PAR and the changes in amplitude of HI-induced decrease in photochemistry in the spectral range of PAR by measuring photochemical energy storage using the PA spectroscopy.

Materials and methods

Plants: 5 to 7 years-old sugar maple (*Acer saccharum* Marsh.) saplings were collected from a maple stand near Berthierville (Québec, Canada) during May. Plants were dug from the forest floor and transplanted into pots containing soil taken from the same location. Pots were brought to the university campus and kept under the shade of a pine tree. Plants were watered twice a week with tap water. Experiments were done during July and August.

Photoacoustic spectroscopy detects periodic heat emission from a sample irradiated with a modulated radiation beam. The periodically evolved heat causes pressure fluctuations in the closed cell, which are detected by a microphone. In green leaves, besides heat emission, photosynthetic O_2 exchange also contributes to the signal in the low frequency range (Bults *et al.* 1982, Malkin and Canaani 1994). Heat emission alone can be measured at a frequency, where there is no O_2 contribution, and photosynthetic energy storage can be evaluated using saturating non modulated background radiation, *i.e.*, at a frequency of 80 Hz in sugar maple saplings (Veeranjaneyulu *et al.* 1991).

PA measurements were done with a laboratory-built PA spectrometer as described by Carpentier *et al.* (1983). A radiation beam from a xenon arc lamp (*Canrad-Hanova*, Newark, NJ, USA) passing through a monochromator (*Schoeffel Instruments Corp.*, Westwood, NJ, USA) was modulated by a mechanical chopper. The modulated beam was focussed into the PA cell containing leaf disk (18 mm diameter upper surface towards radiation beam) by lens and mirror. Non-modulated saturating background radiation (tungsten-halogen; *Sylvania*) was directed onto the sample through a fiber optic light guide. The acoustic signals were detected by a microphone (*General Radio*, Bolton, MA, USA), preamplified, and analyzed by a lock-in amplifier (model *393, Ithaco Dynatrac*, Ithaca, NY, USA). The signals were either displayed on a chart recorder or stored on diskettes in a computer (*Apple IIe*). Background far-red radiation (>715 nm) was provided by placing filters (*Schott GG 427, OG 560, RG 645,* and *RG 715* nm) in the path of "white light". Oxygen and thermal signals at 25 Hz were separated according to Poulet *et al.* (1983). Energy storage was measured at a frequency of either 80 or 100 Hz by recording the signal in the presence (Q_{ma}) and absence (Q_m) of background "white light". ES_T was calculated by dividing ($Q_{ma} - Q_m$) with Q_{ma}. PS1 and PS2 activities were determined as described by Veeranjaneyulu *et al.* (1991). PS1 energy storage was measured by recording the signal in the presence of background far-red radiation (Q_{mfrl}), and

ES_{PS1} was calculated by dividing ($Q_{mfrl} - Q_m$) with Q_{ma}. ES_{PS2} was derived from $ES_T - ES_{PS1}$. State 1-state 2 transitions were studied as described in Veeranjaneyulu *et al.* (1991).

Spectra were recorded between 700 and 400 nm in the absence or presence of the background "white light" with a scan speed of 0.417 nm s^{-1} (25 nm min^{-1}), and a time constant of 4 s in the lock-in amplifier. Spectral band width was 8 nm. Values were stored in the *Apple IIe* computer, and corrected for lamp irradiance with a carbon black reference. Energy storage values in the spectral range were divided by wavelength.

Fluorescence was measured using a trifurcated fiber optic light guide with one arm to deliver excitation radiant beam (470 nm, 29 μmol m^{-2} s^{-1}), a second arm for background saturating radiation, and a third arm to collect fluorescence from the sample. Fluorescence detection system consisted of a *RG 715* filter, a 730 nm band-pass interference filter, and a photodetector (*United Detector Technology*, Santa Monica, CA, USA, model *PIN-10D*). The signal from the photodetector was analyzed by a lock-in amplifier, and displayed on a chart recorder.

Fluorescence excitation spectra were recorded by means of a *Spex Fluorolog 2* (Metuchen, NJ, USA) spectrofluorometer equipped with *Datamate DM1* data acquisition system and a water cooled *Hamamatsu* model *R928* photomultiplier tube. Fluorescence was detected in frontal geometry. The spectra were corrected for irradiance (Gruszecki *et al.* 1991b).

HI-treatment (tungsten-halogen lamps, *Sylvania*) was given through a fiber optic light guide to the leaf disk (18 mm diameter) kept in the closed PA cell. A thermal filter transmitting about 50 % at 670 nm and about 5 % at 700 nm was kept in the path of radiant beam to reduce heating of the sample. Measurements were made on the same leaf disk before (control) and after the HI-treatment, without disturbing the sample position in the PA cell. This enabled us to determine the changes in Q_{ma} and ΔQ correctly. But this HI-treatment can be at the CO_2 compensation concentration, as the volume of the PA cell is small, and the CO_2 concentration may be depleted rapidly.

Results and discussion

The signal recorded in the absence of any background radiation (Q_m) represents the thermal deactivation of pigments at a state when the reactions are performing modulated photochemistry. The signal recorded at 100 Hz in the presence of background non-modulated "white light" (Q_{ma}) (Fig. 1) indicated the thermal deactivation of pigments (heat emission) at a state when all the reaction centers of PS1 and PS2 are completely closed. The difference between Q_{ma} and Q_m indicates the fraction of absorbed energy stored as chemical energy and is referred to as photosynthetic energy storage of the sample. This is normalized with Q_{ma} to obtain the relative quantum yield of energy storage ($ES_T = \Delta Q/Q_{ma}$) and to eliminate any stress induced changes in the heat diffusion characteristics of the leaf.

It is important to use the correct irradiance by non-modulated background "white light", at which it saturates the photochemistry of photosystems and releases all the absorbed modulated radiation. For sugar maple saplings, this irradiance was between 300 and 670 μmol m^{-2} s^{-1}. Below or above this range the background irradiance decreased ΔQ, which was due to the loss of PA signal at Q_{ma} and not to any inhibition of photochemical efficiency of the leaf.

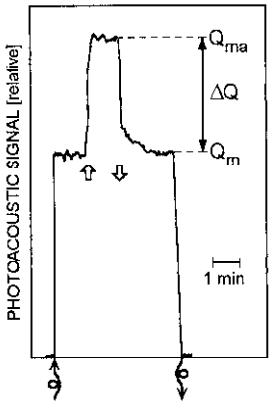


Fig. 1. PA signal of sugar maple leaf at 100 Hz where there is no O_2 contribution to the signal. Modulated radiation, 650 nm; 27 μmol m^{-2} s^{-1}; ⇧, ⇩ on and off, respectively. Background "white light", 480 μmol m^{-2} s^{-1}; ⇧, ⇩ on and off, respectively. Time constant 1.25 s.

ES_T decreased drastically and Q_{ma} increased significantly by about 45 % at irradiances greater than 2000 μmol m^{-2} s^{-1} (Fig. 2). The large decline in ES_T at HI-treatment is primarily due to the reduction in photochemical efficiency of PS2 (Powles 1984, Critchley 1988, Aro *et al.* 1993, Long *et al.* 1994). This may be due to

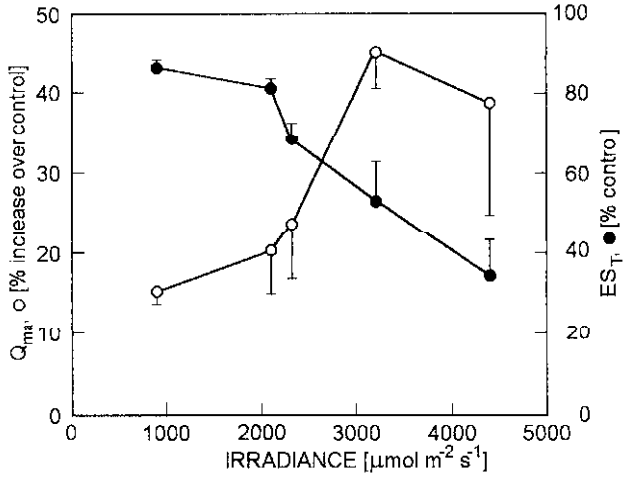


Fig. 2. Changes in photochemical activity (ES_T, ●) and heat emission (Q_{ma}, ○) with an increase in the irradiance during treatment (15 min). Vertical bars represent SD (n = 4). Other conditions are as in Fig. 1.

inactivation of reaction center and/or degradation of D1 protein of PS2 (Kyle *et al.* 1984, Cleland *et al.* 1986, Critchley 1988, Krause 1988, Eckert *et al.* 1991, Aro *et al.* 1993). The large increase in heat emission at HI (>3000 μmol m^{-2} s^{-1}), although reported in the literature (Havaux 1989), is difficult to account for, since fluorescence quenching related energy dissipation and thermal deactivation are the only two

processes contributing to Q_{ma} (as modulated photochemistry is saturated by non-modulated background radiation), and fluorescence quenching alone can not explain such a large increase in heat emission. Hence, there could be some changes in the heat diffusion characteristics of the leaf under these HI-conditions (Malkin and Canaani 1994).

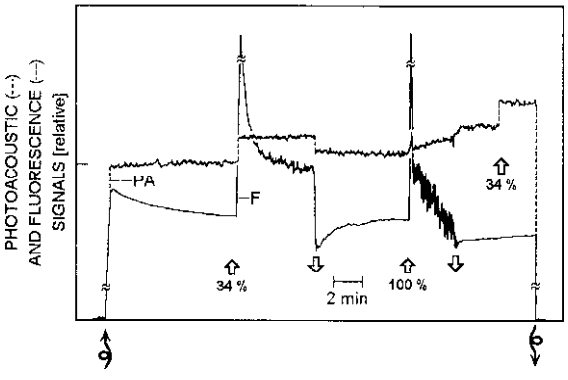


Fig. 3. Photoacoustic signal from sugar maple leaves in the absence and in the presence of two different background irradiances. Modulated radiation 470 nm; 29 μmol m^{-2} s^{-1}. 100 % corresponds to 900 μmol m^{-2} s^{-1}. Other conditions are as in Fig. 1.

In some leaves (Fig. 3), the increase in heat emission was noticed immediately after even 1-min exposure to moderately increased irradiance (900 μmol m^{-2} s^{-1}), and Q_{ma} increased by about 8 %. At the end of this experiment (Fig. 3), heat emission increased by about 16 %, and ES_T decreased by about 13 %. Non-photochemical fluorescence quenching during the HI-treatment can lead to increased thermal deactivation of pigments (Krause and Weis 1991, Demmig-Adams and Adams 1993). Thus, the 8 % increase in heat emission (assuming that heat diffusion characteristics of the leaf were not changed by 1 min exposure to moderate HI) could be from fluorescence quenching due to HI-stress. If one accepts this view, the observed inhibition in ES_T can be overestimated, as ES_T is calculated by dividing ΔQ with Q_{ma}, which includes a fluorescence quenching related heat emission. Hence at this treatment the photochemical activity of the leaf is only marginally affected, and the increased heat dissipation of the excess radiant energy may offer some protection to the photosynthetic apparatus. The rapid increase in heat emission was also observed in the low frequency range at 25 Hz when O_2 and heat emission signals were separated vectorially.

An increase in non-photochemical fluorescence quenching can indicate the increase in thermal deactivation of pigments (Krause and Weis 1991, Demmig-Adams and Adams 1993). In order to understand this phenomenon, we simultaneously measured heat emission and fluorescence quenching. Fig. 4 supports the concept that fluorescence quenching can indicate the increased thermal deactivation of pigments. In the presence of saturating non-modulated background irradiance (34 % or 306 μmol m^{-2} s^{-1}), fluorescence rose initially to the maximum, and decreased to a steady state. The amplitude of PA signal also increased due to the release of stored energy as heat. Upon turning off the background "white light", both fluorescence and PA signals decreased and reached a steady state. This steady state

fluorescence signal was marginally smaller and PA signal was marginally greater than that before the application of background radiation. In the presence of high background irradiance (100 % or 900 μmol m^{-2} s^{-1}), fluorescence reached its maximum initially and then decreased drastically. The PA signal increased, but the increase was less than that in the presence of saturating iradiance (34 %). As mentioned earlier, this was due to the loss of PA signal in the presence of 100 % background irradiance. After removing the background radiation, fluorescence signal decreased by about 15 %, and the PA signal (Q_{ma} measured in the presence of 34 % background radiation) increased by about 12 % of the initial level in this example.

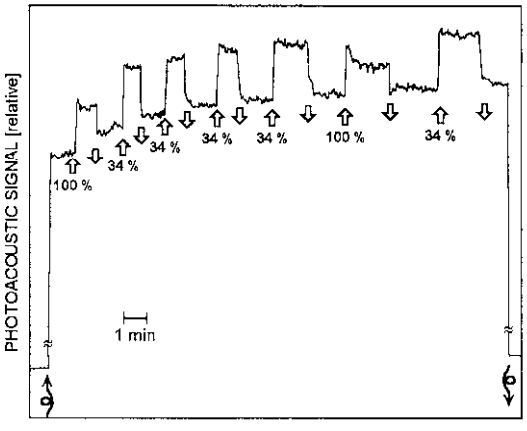
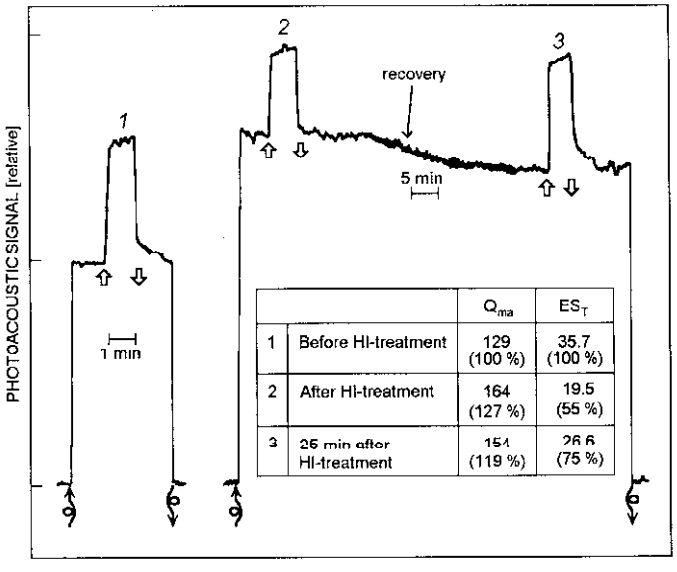


Fig. 4. Fluorescence (F, $\lambda_{730\ nm}$) and photoacoustic signals at 80 Hz from sugar maple leaves. Modulated radiation, 470 nm, 29 μmol m^{-2} s^{-1}. 100 % corresponds to 900 μmol m^{-2} s^{-1}. Other conditions are as in Fig. 1.

This experiment shows a decrease in fluorescence and an increase in heat emission. As the heat emission (Q_{ma}) was measured in the presence of saturating (34 %) background irradiance, only fluorescence and heat emission accounted for the absorbed modulated radiant energy. Hence any change in one dissipation pathway should reflect in the other. Considering the reported low quantum yields of fluorescence, one may argue that the amplitude of fluorescence decrease (15 %) may not account for the extent of increase in rate constant of thermal deexcitation (12 %). Under these conditions, the increased heat emission may not only be from fluorescence quenching but also from the changes in heat diffusion characteristics of the leaf. But it is difficult to accept that a brief exposure (1 to 2 min) of the leaf to moderate HI could cause a change in leaf morphology altering its heat diffusion characteristics. The reported fluorescence quantum yields were largely obtained with growth room raised "model" plants, and limited information is available on the plants growing on forest floor. In sugar maple saplings, we always recorded fluorescence signal of about 2 to 3 times greater amplitude than that recorded with growth room pea plants.

Sugar maple saplings are shade-tolerant and capable to persist long periods in a closed canopy (Lei and Lechowicz 1990). On the forest floor, the understory plants receive usually low photon flux densities with intermittent sunflecks. The irradiance and duration of these sunflecks depend on the holes in the canopy, and leaves may experience photoinhibition (Pearcy 1990) of unclear extent. CO_2 exchange

measurements under natural sunfleck regimes show no convincing evidence for photoinhibition (Pearcy 1987, 1990, Pfitsch and Pearcy 1989). But some low temperature fluorescence measurements done in the laboratory suggest the occurrence of photoinhibition in the understory plants (Powles and Björkman 1981, Le Gouallec *et al.* 1991). The observed rapid regulation of thermal energy dissipation in sugar maple saplings may be an important protective mechanism in these forest understory plants to maintain efficient use of low irradiance and at the same time to minimize the photoinhibitory damage during sunflecks. Nevertheless, if one still believes that the increased heat emission may partly be due to a change in heat diffusion characteristics of the leaf, this physical change may also be considered as a rapid regulatory mechanism to dissipate the absorbed energy during sunflecks, because the forest understory plants experience sunflecks of such high irradiance.



		Q_{ma}	ES_T
1	Before HI-treatment	129 (100 %)	35.7 (100 %)
2	After HI-treatment	164 (127 %)	19.5 (55 %)
3	25 min after HI-treatment	154 (119 %)	26.6 (75 %)

Fig. 5. Photoacoustic signal from sugar maple leaf before (*1*), immediately after (*2*), and (*3*) 25 min after HI-treatment (4000 μmol m^{-2} s^{-1}, 15 min). Other conditions are as in Fig. 1.

This phenomenon of increased heat emission is reversible in the dark. It decreased by about 8 % after 10 min in darkness when compared to its amplitude immediately after the HI-treatment. A leaf treated with 4000 μmol m^{-2} s^{-1} for 15 min showed an inhibition of 45 % in ES_T with an increase in heat emission by 27 % (Fig. 5). In the presence of modulated radiation, one can notice the quenching of thermal energy dissipation due to the recovery of photochemical activity of the leaf. The well documented recovery of photochemical activity in photoinhibited leaves and cells may be due to the synthesis of chloroplast proteins and/or the conversion of $PS2_\beta$ to Q_B-reducing forms (Ohad *et al.* 1984, Greer *et al.* 1986, Neale and Melis 1990). In the present study, recovery was fast in the first 15 min after the HI-treatment. This is consistent with the observations of Neale and Melis (1990) who have noticed a fast recovery phase of O_2 evolution, insensitive to chloroplast protein translation inhibitors. They suggest that this fast recovery phase is due to the conversion of $PS2_\beta$ to Q_B-reducing form in the initial period. As dicussed (Aro *et al.* 1993, Gilmore and

Björkman 1994, Leitsch *et al.* 1994), the down regulation of and rapid recovery in photochemical activity could be one of the protective mechanisms against high intense sunflecks in the shade grown sugar maple plants. Thus, this experiment demonstrates that the thermal deactivation process and photochemistry are competitive processes for the excitation energy.

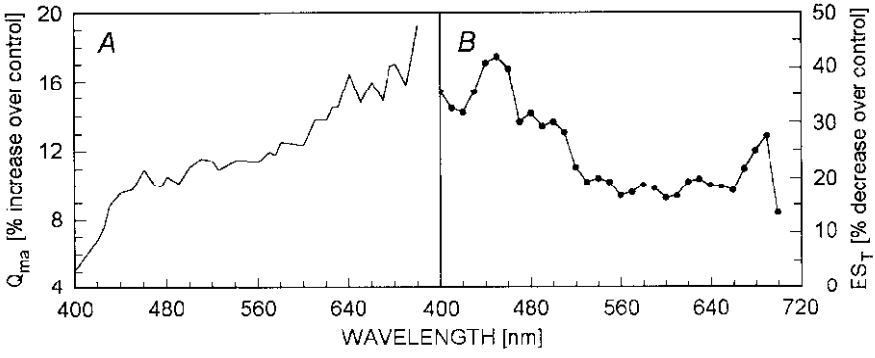


Fig. 6. Increase (*A*) in heat emission (Q_{ma}) or decrease (*B*) in ES_T [%] over control in HI-treated (2000 μmol m^{-2} s^{-1}, 15 min) sugar maple leaves in the spectral range between 400 and 700 nm. Other conditions are as in Fig. 1.

We also examined the changes in amplitude of increased heat emission due to thermal deactivation of pigments after HI-treatment in the spectral range between 400 and 700 nm (Fig. 6*A*). The extent of increase was greater in the long wavelength (640-700 nm, red) region than that in the short wavelength region of the spectrum. This greater increase in heat emission in the red region can be assigned to the thermal deactivation of long wavelength absorbing Chl *a* species, which show absorption maxima in this region. This may further support the concept that fluorescence quenching related energy is dissipated through thermal deactivation, as fluorescence is maximum in the long wavelength red region. Furthermore, energy absorbed by various accessory pigments is transferred to Chl *a* molecules and finally to the reaction centers. Under HI, this energy transfer could be at maximum, and an enhanced dissipation of energy in the form of heat (not as fluorescence, since fluorescence can be reabsorbed by pigments in an intact leaf) by Chl *a* molecules, before it is being transferred to reaction centers, may be considered as a protective mechanism. Excess radiation causes the aggregation of light-harvesting complexes of PS2 resulting in the formation of an efficient pathway for non-radiative dissipation of excitation energy (Horton *et al.* 1991, 1994). This is demonstrated by measuring energy quenching of fluorescence in isolated LHC2. LHC2 aggregation is associated with quenching of fluorescence and appearance of absorption bands at 510, 660, and 690 nm, and a long wavelength fluorescence band at 700 nm. However, some reports indicate no significant absorbance changes in the red region (Bilger *et al.* 1989, Bilger and Björkman 1994) and no changes in the relative fluorescence quantum yield for the Chl forms absorbing between 650 and 690 nm upon aggregation of LHC2 (Jennings *et al.* 1994). Consistent with the observations of Horton *et al.*, present study

demonstrates a greater increase in heat emission in long wavelength (640-700 nm) than short wavelength region of the spectrum (Fig. 6*A*). The principle that the physical properties of bound pigments are modulated by the apoprotein in pigment-protein complexes is well accepted. Hence, the above phenomenon could be due to HI-induced molecular changes in pigment-protein complexes, which may lead to increased thermal deactivation of pigments (Horton *et al.* 1994).

Since we observed a variation in the amplitude of increased heat emission in the spectral range of PAR, we were interested to measure the HI-induced changes in the photochemical activity of leaf in the spectral range 400-700 nm. We have recently demonstrated that migration of LHC2 from PS2 to PS1 (transition to state 2) causes a decrease in total photochemical activity of a leaf in the region 400-580 nm (Veeranjaneyulu and Leblanc 1994). LHC2 may dissociate from PS2 and couple to PS1 during HI-treatment (Horton and Lee 1985, Fork and Satoh 1986, Veeranjaneyulu and Leblanc, unpublished). Hence, we measured ES_T between 400 and 700 nm, and the spectrum in control plants (before HI-treatment) was similar to the spectra reported earlier (Veeranjaneyulu and Leblanc 1994). In HI-treated leaves, we noticed significantly greater decrease of ES_T in the region of 400-520 nm than in the long wavelength region (Fig. 6*B*). This is possibly due to the following reasons:

(*1*) During the HI-treatment, LHC2 dissociation from PS2 and coupling to PS1 could have lead to a greater decrease of ES_T in the short wavelength region. The values of Table 1 confirm the occurrence of state 1-state 2 transitions in HI-treated leaves. When the leaf preadapted to state 1 was HI-treated, there was a clear increase in ES_{PS1} indicating the coupling of LHC2 to PS1. This LHC2 coupling to PS1 can cause a decrease in total photochemical activity in the short wavelength region as demonstrated in control plants (Veeranjaneyulu and Leblanc 1994). This is due to the decrease in energy transfer efficiency of carotenoids to Chls. A detailed study of state 1-state 2 transitions and *in vivo* PS1 and PS2 activities during HI-treatment was submitted for publication elsewhere.

Table 1. Chanes in ES_T, ES_{P1}, and ES_{P2} in state 1 and state 2 before and after high-irradiance (400 μmol m^{-2} s^{-1}) treatment in sugar maple leaves. The treatment was given to leaf disk preadapted to state 1. Means of four replicates ± S.D.

	Before treatment			After treatment			
	state 2	state 1	state 1 – state 2	state 2	state 1	state 1 – state 2
ES_T	33.9 ± 1.3	33.6 ± 0.9		14.8 ± 4.1	21.0 ± 4.9	22.4 ± 4.1	
ES_{PS1}	5.8 ± 0.5	2.3 ± 1.1	−3.5	4.7 ± 0.3	4.8 ± 1.4	2.9 ± 1.0	−1.9
ES_{PS2}	28.1 ± 1.4	31.4 ± 1.2	+3.3	10.2 ± 4.2	15.9 ± 4.2	19.6 ± 3.9	+3.7
ES_{PS2}/ES_{PS1}	4.85	13.82		2.18	3.30	6.84	

(*2*) Another possible explanation may be the "disconnection" of carotenoid pool from Chls in the pigment bed of photosynthetic apparatus as reported in HI-treated pea plants (Gruszecki *et al.* 1991a). The ratio of fluorescence intensity in the

excitation spectrum at 470 nm (where both Chls and carotenoids absorb) to that at 600 nm (only Chls absorb) represents the participation of carotenoids in Chl fluorescence (Gruszecki *et al.* 1991a,b). The ratio in control (1.94 ± 0.42) sugar maple plants decreased after HI-treatment (1.42 ± 0.1) which indicated possible disconnection of singlet-singlet energy transfer from the carotenoids to Chls. Hence, such an interruption in energy transfer to Chls and to the reaction center can cause a greater decrease in the photochemical activity between 400 and 520 nm.

In conclusion, the present study provides experimental evidence for the rapid increase in heat emission during HI-stress in shade-grown sugar maple saplings. This heat emission is reversible in darkness and the rapid response may be a protective mechanism against high-irradiance sunflecks. This increase is greater in the long wavelength (640-700 nm) than in the short wavelength region of PAR, which can be assigned to the increased thermal deactivation of long wavelength absorbing Chl *a* species. The leaf photochemical activity is affected more in the short wavelength region (400-520 nm) than in the long wavelength region of PAR, possibly due to the migration of LHC2 from PS2 to PS1 and to the disconnection of carotenoid pool from Chls in the pigment bed during HI-treatment. The migration of LHC2 from PS2 to PS1 is supported by the increase in PS1 activity and occurrence of state 1-state 2 transitions after HI-treatment. The disconnection of carotenoid pool from Chls in the pigment bed is evidenced by the decrease in the ratio of intensities of fluorescence excitation at 470/600 nm indicating the decreased energy transfer from carotenoids to Chls.

References

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

Bilger, W., Björkman, O.: Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. - Photosynth. Res. **25**: 173-185, 1990.

Bilger, W., Björkman, O.: Relationships among violaxanthin deepoxidation, thylakoid membrane conformation, and nonphotochemical chlorophyll fluorescence quenching in leaves of cotton (*Gossypium hirsutum* L.). - Planta **193**: 238-246, 1994.

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

Bults, G., Horwitz, B.A., Malkin, S., Cahen, D.: Photoacoustic measurements of photosynthetic activities in whole leaves. Photochemistry and gas exchange. - Biochim. biophys. Acta **679**: 452-465, 1982.

Buschmann, C.: Induction kinetics of heat emission before and after photoinhibition in cotyledons of *Raphanus sativus*. - Photosynth. Res. **14**: 229-240, 1987.

Carpentier, R., LaRue, B., Leblanc, R.M.: Photoacoustic spectroscopy of *Anacystis nidulans*. I. Effect of sample thickness on the photoacoustic signal. - Arch. Biochem. Biophys. **222**: 403-410, 1983.

Cleland, R.E., Melis, A., Neale, P.J.: Mechanism of photoinhibition: Photochemical reaction center inactivation in system II of chloroplasts. - Photosynth. Res. **9**: 79-88, 1986.

Critchley, C.: The molecular mechanism of photoinhibition - facts and fiction. - Aust. J. Plant Physiol. **15**: 27-41, 1988.

Demmig-Adams, B., Adams, W.W., III: Light stress and photoprotection related to the xanthophyll cycle. - In: Foyer, C.H., Mullineaux, P.M. (ed.): Causes of Photooxidative Stress and Amelioration of Defense Systems. Pp. 105-126. CRC Press, Boca Raton 1993.

Eckert, H.-J., Geiken, B., Bernarding, J., Napiwotzki, A., Eichler, H.J., Renger, G.: Two sites of photoinhibition of the electron transfer in oxygen evolving and Tris-treated PS II membrane fragments from spinach. - Photosynth. Res. **27**: 97-108, 1991.

Fork, D.C., Bose, S., Herbert, S.K.: Radiationless transitions as a protection mechanism against photoinhibition in higher plants and a red alga. - Photosynth. Res. **10**: 327-333, 1986.

Fork, D.C., Satoh, K.: The control by state transitions of the distribution of excitation energy in photosynthesis. - Annu. Rev. Plant Physiol. **37**: 335-361, 1986.

Gilmore, A.M., Björkman, O.: 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, 1994.

Greer, D.H., Berry, J.A., Björkman, O.: Photoinhibition of photosynthesis in intact bean leaves: role of light and temperature, and requirement for chloroplast-protein synthesis during recovery. - Planta **168**: 253-260, 1986.

Gruszecki, W.F., Veeranjaneyulu, K., Leblanc, R.M.: Qualitative changes in the fluorescence spectra of intact pea leaves after photoinhibition. - Biochem. Cell Biol. **69**: 399-403, 1991a.

Gruszecki, W.F., Veeranjaneyulu, K., Zelent, B., Leblanc, R.M.: Energy transfer process during senescence: fluorescence and photoacoustic studies of the intact pea leaves. - Biochim. biophys. Acta **1056**: 173-180, 1991b.

Havaux, M.: Increased thermal deactivation of excited pigments in pea leaves subjected to photoinhibitory treatments. - Plant Physiol. **89**: 289-292, 1989.

Horton, P., Lee, P.: Phosphorylation of chloroplast membrane proteins partially protects against photoinhibition. - Planta **165**: 37-42, 1985.

Horton, P., Ruban, A.V., Rees, D., Pascal, A.A., Noctor, G., Young, A.J.: Control of the light-harvesting function of chloroplast membranes by aggregation of the LHCII chlorophyll-protein complex. - FEBS Lett. **292**: 1-4, 1991.

Horton, P., Ruban, A.V., Walters, R.G.: Regulation of light harvesting in green plants. Indication by nonphotochemical quenching of chlorophyll fluorescence. - Plant Physiol. **106**: 415-420, 1994.

Jennings, R.C., Zucchelli, G., Bassi, R., Vianelli, A., Garlashi, F.M.: The relation between the minor chlorophyll spectral forms and fluorescence quenching in aggregated light harvesting chlorophyll *a/b* complex II. - Biochim. biophys. Acta **1184**: 279-283, 1994.

Krause, G.H.: Photoinhibition of photosynthesis. An evaluation of damaging and protective mechanisms. - Physiol. Plant. **74**: 566-574, 1988.

Krause, G.H., Weis, E.: Chlorophyll fluorescence and photosynthesis: The basics. - Annu. Rev. Plant Physiol. Plant mol. Biol. **42**: 313-349, 1991.

Kyle, D.J., Ohad, I., Arntzen, C.J.: Membrane protein damage and repair: Selective loss of a quinone-protein function in chloroplast membranes. - Proc. nat. Acad. Sci. USA **81**: 4070-4074, 1984.

Le Gouallec, J.-L., Cornic, G., Briantias, J.-M.: Chlorophyll fluorescence and photoinhibition in a tropical rainforest understory plant. - Photosynth. Res. **27**: 135-142, 1991.

Lei, T.T., Lechowicz, M.J.: Shade adaptation and shade tolerance in saplings of three *Acer* species from eastern North America. - Oecologia **84**: 224-228, 1990.

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

Long, S.P., Humphries, S., Falkowski, P.G.: Photoinhibition of photosynthesis in nature. - Annu. Rev. Plant Physiol. Plant mol. Biol. **45**: 633-662, 1994.

Malkin, S., Canaani, O.: The use and characteristics of the photoacoustic method in the study of photosynthesis. - Annu. Rev. Plant Physiol. Plant mol. Biol. **45**: 493-526, 1994.

Neale, P.J., Melis, A.: Activation of a reserve pool of photosystem II in *Chlamydomonas reihardtii* counteracts photoinhibition. - Plant Physiol. **92**: 1196-1204, 1990.

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

Pearcy, R.W.: Photosynthetic gas exchange responses of Australian tropical forest trees in canopy, gap and understory micro-environments. - Funct. Ecol. **1**: 169-178, 1987.

Pearcy, R.W.: Sunflecks and photosynthesis in plant canopies. - Annu. Rev. Plant Physiol. Plant mol. Biol. **41**: 421-453, 1990.

Pfitsch, W.A., Pearcy, R.W.: Daily carbon gain by *Adenocaulon bicolor* (*Asteraceae*), a redwood forest understory herb, in relation to its light environment. - Oecologia **80**: 465-470, 1989.

Poulet, P., Cahen, D., Malkin, S.: Photoacoustic detection of photosynthetic oxygen evolution from leaves. Quantitative analysis by phase and amplitude measurements. - Biochim. biophys. Acta **724**: 433-446, 1983.

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

Powles, S.B., Björkman, O.: Leaf movement in the shade species *Oxalis oregana*. II. Role in protection against injury by intense light. - Carnegie Inst. Washington Year Book **80**: 63-66, 1981.

Veeranjaneyulu, K., Charland, M., Charlebois, D., Leblanc, R.M.: Photoacoustic study of changes in the energy storage of photosystems I and II during state 1-state 2 transitions. - Plant Physiol. **97**: 330-334, 1991.

Veeranjaneyulu, K., Leblanc, R.M.: Action spectra of photosystems I and II in state 1 and state 2 in intact sugar maple leaves. - Plant Physiol. **104**: 1209-1214, 1994.