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Duration of irradiation rather than quantity and frequency of high irradiance inhibits photosynthetic processes in the lichen *Lasallia pustulata*

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Abstract

Lichen thalli were exposed to 4 regimes differing in irradiance and duration of irradiation. Photosynthetic efficiency of thalli was monitored by chlorophyll fluorescence parameters and xanthophyll cycle analysis. Maximal quantum yield of photosystem 2 (F_V/F_M) decreased gradually with time in long-term treatment. The effect of additional short-term high irradiance (HI) treatment applied each 24 h was not significant. Nevertheless, short-term HI applied repeatedly on thalli kept in the dark led to a significant decrease of F_V/F_M. Non-photochemical quenching recorded during the long-term treatment corresponded to the content of zeaxanthin (Z). In short-term treatment, however, proportion of Z (and antheraxanthin) to total amount of xanthophyll cycle pigments recovered to the initial values every 24 h after each repeated short-term HI event in thalli kept in dark. Thus duration of irradiation rather than irradiance and frequency of HI events is important for a decrease in primary photosynthetic processes in wet thalli of *Lasallia pustulata.* Rapidly responding photoprotective mechanisms, such as conversion of xanthophyll cycle pigments, are involved mainly in short-term irradiation events, even at HI.

Additional key words: chlorophyll fluorescence; non-photochemical quenching; photoinhibition; photochemical quenching; xanthophyll cycle; zeaxanthin.

Introduction

Lichens are typical pioneers of the environment because of their ability to survive in extreme and inhospitable conditions, such as drought, low/high temperature, low/high irradiance, *etc*. Response of lichens to high irradiance (HI) has been investigated within last decade. Several authors monitored the response of lichens to long-term HI exposure (Gauslaa and Solhaug 2000); also the influence of short-term HI is well documented (Barták *et al.* 2003, 2004, Vráblíková *et al.* 2005). Seasonal acclimation of lichen thalli to irradiance and/or other conditions is well described (Leisner *et al.* 1996, Lange 2002, 2003, MacKenzie *et al.* 2002), as well as seasonal photoacclimation of photoprotective compounds formed by the mycobiont (Gauslaa and McEvoy 2005) or photobiont (Vráblíková *et al.* 2006). Mechanisms of lichen photoprotection and the dynamics of the compounds involved has recently been studied (Solhaug and Gauslaa 1996, Gauslaa and Solhaug 2001, 2004).

In lichens containing green algae as the photobiont, the photoprotective xanthophyll cycle is of great importance (Heber *et al*. 2006). It consists of reversible conversion of violaxanthin (V) *via* antheraxanthin to zeaxanthin (Z) (Adams *et al.* 1993, Demmig-Adams and Adams 1996). Z might be involved in dissipation of excess radiant energy either directly (Frank *et al.* 1994**)** or by conformational changes of light-harvesting complexes (LHCs) and resulting energy dissipation (Gilmore 1997). Another functions are protection against lipid peroxid-

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Abbreviations: A – antheraxanthin; CMI – continuous medium irradiance; Chl – chlorophyll; DEPS – de-epoxidation state of xanthophyll cycle pigments; DM – dry mass; F_V/F_M – dark-adapted maximal quantum yield; HI – high irradiance; LHC – light-harvesting complex; MI – medium irradiance; NPQ – non-photochemical quenching; q_P – photochemical quenching; PS2 – photosystem 2; RHI_M – repetitive high irradiance applied simultaneously with continuous medium irradiance; RHI_D – repetitive high irradiance applied in the dark; V – violaxanthin; Z – zeaxanthin; Φ_2 – quantum yield of photochemical reactions in PS2.

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ation (Jin *et al.* 2003) and de-excitation of singlet chlorophyll (Havaux and Niyogi 1999). The correlation between energy dissipation measured as non-photochemical quenching (NPQ) and the amount of Z has been confirmed for higher plants (Demmig-Adams 1990, Demmig-Adams and Adams 1996, Gilmore 1997), green algae (Masojídek *et al.* 2004), and lichens (MacKenzie *et al.* 2002). The increase in NPQ after HI treatment is caused by a number of energy-consuming processes. Those are photosystem 2 (PS2) back reaction (Krieger *et al.* 1992), cyclic electron transport around PS2 (Nedbal *et al.* 1992), LHC quenching (Horton *et al.* 1992), Z-mediated quenching (Demmig-Adams 1990), conformational changes in the reaction centre (Litvin *et al.* 2005), *etc.*

Only a few studies have focused on different aspects of photoinhibition in lichens so far. Their design varied substantially. Some studies exploited a long-term approach. Field experiments comprised "transplantation" (Gauslaa and Solhaug 2000, Gauslaa *et al.* 2006) providing lichens with more irradiance after transfer from low irradiance to HI natural conditions. Other studies focused on diurnal/seasonal courses of irradiance and dynamics of photoinhibition (Valladares *et al.* 1995, Schroeter *et al.* 2000, MacKenzie *et al.* 2001, Vráblíková *et al.* 2006). In a laboratory, both long-term (several days lasting) and short-term (typically tens of minutes to several hours) HI experiments have been done. In long-term experiment comparing HI susceptibility in desiccated and hydrated thalli (Gauslaa and Solhaug 2004), hydrated sun-adapted lichens were more susceptible to photoinhibition of photosynthesis than desiccated thalli. In another long-term experiment (Gauslaa and Solhaug 1999), the damaging effect of HI on desiccated *Lobaria pulmonaria* thalli seemed to be an additive effect of HI and high temperature. In these long-term studies, mainly fluorescence parameter F_V/F_M was used as a marker of photoinhibition. It gradually decreased as a function of the length of exposure to constant HI.

Studies of short-term effects of HI on photosynthetic processes in fully hydrated lichen thalli were focused mainly on the rate of inhibition and dark recovery (Demmig-Adams *et al.* 1990a,b, Barták *et al.* 2003, Vráblíková *et al.* 2005). Most of these short-term studies confirmed good ability of lichen thalli to recover from excess irradiance effects and involvement of Z- and antioxidant-related photoprotection.

The above short-term experiments exploited mainly design of a single HI event with recovery during/following dark in which F_V/F_M and NPQ were determined. Such approach is important for studying the underlying mechanisms. It has, however, some disadvantages, *e.g.* it does not reflect natural situations. In the field, lichens do not always have the possibility to recover fully from a single short-term HI stress. In the majority of cases, they are exposed to other excess irradiance events during recovery period due to *e.g.* canopy gaps or intermittent direct sunshine caused by movements of clouds during a windy weather. Unfortunately, only scarce attempts to establish laboratory-based experimental design reflecting natural irradiance fluctuations have yet been made in photoinhibition studies (Yin and Johnson 2000). For this reason, we used experimental design that considers repeated HI events with different irradiance conditions during recovery. We used combination of long- and short-term HI events to study the response of chlorophyll (Chl) fluorescence parameters and the dynamics of xanthophyll cycle pigments. To simplify and avoid the interactions of the above parameters with varying water potential during thallus desiccation (typical in the field), we worked with constantly hydrated thalli at fixed temperature. The aim of our study was to evaluate the short-term and long-term irradiance effects, as well as their combination, on photochemical processes of photosynthesis in *L. pustulata*. We hypothesized that cumulative effect of repeated HI events affects Chl fluorescence parameters and xanthophyll cycle pigment contents more than the exposure to continuous irradiation. We also expected that recovery time would prolong with repetition of short-term HI-treatment.

Materials and methods

Lichen collection and handling: Foliose thalli of *L. pustulata*, containing the green alga *Trebouxia* sp., were collected from sun-exposed south-facing rock slopes near the village of Ketkovice (49°12'N, 16°14'E, 35 W of Brno, Czech Republic) shortly before the experiments. Immediately after collection, the thalli were transported to a laboratory in Brno. They were naturally air-dried and stored under dim irradiance (about 10 μmol m^{-2} s^{-1}, checked by a *Li-1600* device) at 5 °C in a temperature controlled room. The thalli were rewetted 24 h before the beginning of the experiments. Full hydration was maintained by placing the thalli between two sheets of paper supplied with distilled water.

Irradiation treatments: Four different irradiances were used (see Fig. 1). We chose typical but contrasting treatments. To study differences in *L. pustulata* response to short- and long-term HI, the irradiances were as follows: HI was 1 800 μmol m^{-2} s^{-1}, medium irradiance (MI) was 600 μmol m^{-2} s^{-1}. To induce a short-term HI stress, the thalli were exposed to HI for 30 min. Consequent recovery was at a dim irradiance contrasting to a majority of photoinhibitory studies (Barták *et al.* 2003) in which dark recovery was a standard (see Discussion). Moreover, 10 μmol m^{-2} s^{-1} is a value close to compensation irradiance (see *e.g.* Smith and Griffiths 1998). In the 2nd treatment (CMI), thalli were exposed to continuous MI for 5 d. The 3rd treatment (RHI_M) was a combination of the above two: thalli were simultaneously exposed to the continuous MI for 5 d and each 24 h to HI for 30 min (repetitive short-term HI stress). The fourth treatment (RHI_D) excluded long-term continuous irradiation: thalli of RHI_D were kept in the dark for 5 d and exposed each 24 h to HI for 30 min to study repetitive short-term HI stress *per se*. In all irradiation treatments, 1 000 W halogen lamps were used as a source. Any infrared irradiation was cut-off using a water filter with circulating, temperature-controlled water. The photosynthetic photon flux densities (PPFDs) of the HI and MI treatments were kept constant throughout the experiment. Photosynthetically active radiation (PAR) was checked regularly by a *Li-1400* radiometer (*Li-COR*, USA). Fully hydrated thalli were placed onto temperature-controlled (18 °C) metal plate of a cultivator (*LABIO*, Czech Republic) covered with continuously watered sheets of gauze. To assure full thalli hydration throughout the treatments, the upper sides of thalli were regularly sprayed with distilled water (typically each 2 h). The air temperature (T), air humidity (RH), and thalli temperature (Tt) were continuously recorded by sensors connected to a HOBO data logger (*Onset Computers*, USA). Throughout the experiment, the above parameters were kept constant: T = 20±1 °C, RH = 70±5 %, Tt = 19±1 °C).

Chl *a* fluorescence parameters were measured using a fluorometer *PAM-2000* (*H. Walz*, Germany). The slow kinetics of Chl fluorescence supplemented with saturation pulses was measured and analysed using the below procedure which had been routinely used in our laboratory and described in Barták *et al.* (2003) or Vráblíková *et al.* (2005). We determined basic fluorescence (F_0), maximum fluorescence (F_M) on dark-adapted sample (10 min, sufficiently long as tested using F_M, data not shown), steady-state fluorescence (F_s), and maximum fluorescence (F_M') measured under actinic irradiance. We used the above Chl fluorescence levels in calculations of basic Chl fluorescence parameters (maximum quantum yield of PS2 photochemistry F_V/F_M, where $F_V = F_M - F_0$; effective quantum yield of PS2 photochemistry $\Phi_2 = \Delta F/F_M'$, where $\Delta F = F_M' - F_s$) and quenching coefficients [$q_P = (F_M' - F_s)/(F_M' - F_0')$, $NPQ = (F_M - F_M')/F_M'$)] (Roháček 2002). In all calculations of NPQ, we used F_M values recorded prior to the irradiation treatments on optimally hydrated thalli.

Pigment determination: Contents of Chl *a*, Chl *b*, violaxanthin (V), antheraxanthin (A), and Z were determined several times during exposure to particular irradiance (see Fig. 1). Fresh thalli samples (typically 100 mg of DM) taken for pigment analysis were frozen in liquid nitrogen and freeze-dried. Secondary metabolites that may negatively interact with the pigments during extraction were removed from dry intact thalli by 100 % acetone (Brown and Hooker 1977). Acetone did not enter living cells in dry thalli, but dissolved the lichen compounds that were located on the outer surface of hyphae. Afterwards, the thalli were dried in a desiccator and then ground in a ball mill (*Retsch MM 2000*, Germany). The dry powder was stored at –20 °C until the pigments were extracted by 100 % acetone containing $CaCO_3$ using the protocol optimized for lichens (Pfeifhofer *et al.* 2002). Pigments were analysed by HPLC (*Waters*, USA) using a UV-Vis detector. The content of xanthophyll cycle pigments was expressed as a de-epoxidation state of xanthophyll cycle pigments (DEPS), calculated as (Z+A)/(Z+A+V).

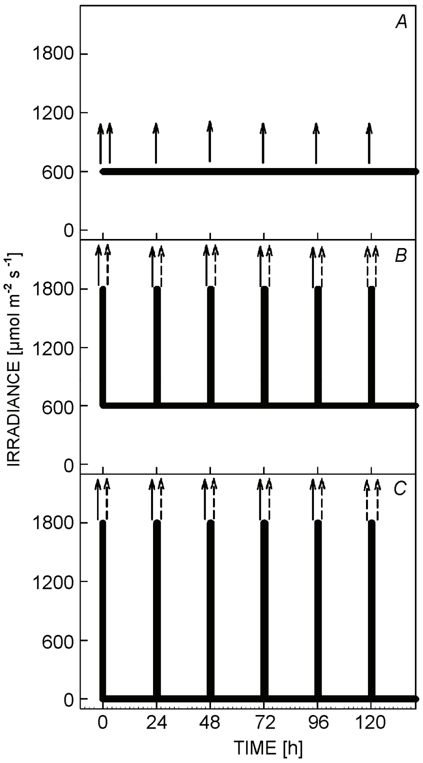

Fig. 1. The three long-term irradiation regimes used in this study. Lichen thalli were exposed to (*A*) 600 μmol m^{-2} s^{-1} (CMI), (*B*) 600 μmol m^{-2} s^{-1} with a 30-min irradiance of 1 800 μmol m^{-2} s^{-1} (HI) every 24 h (RHI_M), (*C*) were kept in dark with 30-min HI every 24 h (RHI_D). *Dashed arrows* indicate time of Chl fluorescence measurements, *simple arrows* indicate both time of Chl fluorescence measurements and thalli sampling for pigment analysis. Additional (not shown here) irradiance regime consisted of 30-min lasting HI and following recovery under dim irradiance. Chl fluorescence measurements and thalli sampling for pigment analysis in this last regime was done before, immediately after, and 1.0, 1.5, 2.5, 5.5, and 10.5 h after the end of irradiation.

Statistical analysis: *Statistica 6* software was used (*StatSoft*®). Data were tested by one-way ANOVA (p=0.05). Statistical differences in Chl fluorescence parameters and pigment contents caused by different irradiation treatments were tested by factorial ANOVA and Tukey HSD test.

Results

Different irradiances caused different extent of decrease in maximal quantum yield of PS2 (F_V/F_M). Single short term HI (1 800 µmol m^{-2} s^{-1}) caused F_V/F_M decrease to 0.56 (75 % of the initial values, Fig. 2*A*), while medium irradiance (600 µmol m^{-2} s^{-1}, MI) of the same duration caused a less apparent decrease (0.62, Fig. 3*A*). In the short-term HI, F_V/F_M exhibited a fast phase of recovery within the first hour after the termination of HI (Fig. 2*A*). Then a slow phase followed within 2–10 h of recovery in a dim light.

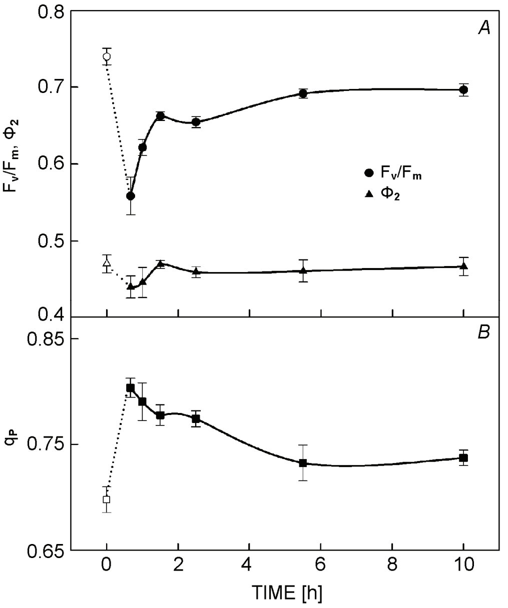

Fig. 2. (*A*) Maximal quantum yield (F_V/F_M, *circles*) and potential quantum yield ($Φ_2$, *triangles*) and (*B*) photochemical quenching (q_P), measured before (*empty symbols*), immediately after 30 min lasting short-term high irradiance treatment with 1 800 µmol m^{-2} s^{-1} and several times during recovery under dim irradiance (*full symbols*). Means of 10 replicates ± standard errors.

In long-term treatments, final F_V/F_M values were lower than those found after short-term treatment. During CMI exposure, F_V/F_M exhibited a biphasic decrease. First, a rapid decrease to 0.38 was found after 24-h exposure followed by much slower decrease to 0.24 found after 120-h exposure (Fig. 3*A*). Additional application of short-term HI (treatment RHI_M) caused small decrease in F_V/F_M after each short-term HI event (Fig. 3*C*). However, the HI-induced decrease in F_V/F_M values was statistically insignificant (p>0.05) and fully recovered within the following 24 h. In treatment RHI_D, contrastingly, short term HI events led to much more pronounced decrease in F_V/F_M and incomplete recovery within the following 24 h (Fig. 3*E*). Moreover, repetitive HI application led to a progressive overall decrease of F_V/F_M in an exponential manner (equation not shown) in RHI_D treatment. It resulted in the final F_V/F_M value of 0.44 found after 120 h. The influence of irradiation treatments on quantum yield of PS2 ($Φ_2$) (Fig. 3*B,D,F*) was very similar to F_V/F_M.

All treatments led to a gradual increase of NPQ. There is no obvious influence of HI when applied on thalli treated with CMI (Fig. 4*C*). The NPQ values of both CMI and RHI_M exposures reached the same maximum (approx. 2.7) at the end of the experiment (Fig. 4*A,C*). However, repetitive HI treatment of thalli placed in dark (RHI_D, Fig. 4*E*) led to step-wise increase of NPQ, reaching 73 % of the CMI or RHI_M treatments at the end of the experiment (after 120 h).

Photochemical quenching (q_P) showed specific responses for individual treatments. In CMI treatment, q_P showed an initial increase to 0.80 followed by a slow decrease (Fig. 4*B*). Contrastingly, q_P in RHI_D treatment exhibited an increase after each HI event accompanied with a generally increasing trend with time. The HI-induced increase in q_P was rather small with time (Fig. 4*F*). Exposure of lichens to RHI_M treatment, however, did not bring any general increase/decrease (Fig. 4*D*). The data were rather scattered showing only a non-significant HI-induced increase in q_P values.

Due to photon energy-induced conversion of V to Z, DEPS increased in the short-term HI, CMI, and RHI_M treatments. Surprisingly, a 30 min exposure to HI led to the same DEPS increase as reached after the first 30 min of CMI treatment (Fig. 5*A*). In CMI treatment, however, DEPS increased with time of exposure reaching the final value of 0.74. Additional application of short-term HI did not lead to significant DEPS increase compared to CMI (Fig. 5*B*).

Discussion

Optimally wetted lichen thalli exposed to continuous irradiation tend to decrease their efficiency of photochemical processes (*e.g.* Valladares *et al.* 1995). In our study, gradual exponential decrease of F_V/F_M and $Φ_2$ was caused by continuous irradiation (CMI). The decrease involves cumulative effect of long-lasting exposure of wet thalli and acclimation to such irradiance. Such a decrease reflects ongoing inhibition of photochemical processes in PS2 due to the negative effects of excess energy caused by irradiance doses absorbed by antennae Chls. When repetitive HI was added to the CMI treatment (RHI_M treatment), both the above parameters decreased in the short term but recovered fast (in terms of minutes) to the values of comparable to CMI (see Fig. 3*A–D*). The HI-induced decrease was more pronounced in F_V/F_M than $Φ_2$ and reached lower values during CMI exposure. Such response might be explained as a consequence of partial inhibition of PS2 function caused by a long-term CMI treatment (tens of hours). Then, any additional HI event leads to a more pronounced decrease and slowed-down recovery of F_V/F_M and Φ_2 with CMI time. It might be hypothesized that a short-term HI event in the field (such as *e.g.* sun fleck occurrence) may lead to a severe inhibition of photochemical processes of photosynthesis in hydrated lichens, when a period of irradiation by doses saturating photosynthesis precedes the HI event. The effect of cumulative and excess irradiation on F_V/F_M and Φ_2 was recently reported for particular parts of *Xanthoria parietina* during the growing season (Vráblíková *et al.* 2006). In our experiment, repetitive HI stress applied on dark-treated thalli (RHI_D) led to short-term decrease in F_V/F_M and Φ_2 and incomplete recovery within 24 h following each HI event. This may indicate that even a single HI event, when happening in a fully hydrated lichen thallus that is adapted to low irradiance, may lead to PS2 inhibition that is not fully recovered by consecutive re-arrangement and re-synthesis of pigment-protein components of PS2 within 24 h.

Non-photochemical processes were involved into energy dissipation much less in the dark (RHI_D) than under irradiation (CMI, RHI_M). Thus V to Z conversion is an irradiance-dependent process involved in photoprotection of poikilohydric autotrophs (Pfündel and Bilger 1994, Heber *et al.* 2006). It indicates that the majority of NPQ increase in CMI and RHI_M treatments was formed by energy-dependent quenching (q_E), a component of NPQ associated with conversion of V to Z. It is, however, surprising, that NPQ did not fully recover within 24 h in dark because our earlier studies (Vráblíková *et al.* 2005) showed full recovery under low irradiance. In irradiated lichens (CMI, RHI_M) NPQ increased with time of exposure (Fig. 4*A,C*). Additional HI, however, did not lead to a substantial increase in NPQ. It might be explained as a sufficient and/or almost fully exploited capacity of non-photochemical mechanisms activated by a long-term CMI treatment of hydrated lichens. Therefore, the pool of Z formed by CMI treatment might be sufficient to quench the excess energy input into PS2 during a short-HI event and no additional increase in NPQ is required. Such interpretation might be supported by a rapid increase of NPQ in *Turgidisculum complicatulum* when treated by irradiance up to 400 μmol m^{-2} s^{-1} followed by much less apparent NPQ increase at irradiance over that value (Smith and Gremmen 2001).

Photochemical quenching generally reflects the openness of PS2 reaction centres and redox state of the primary quinone acceptor. It responds sensitively to changes in individual physical factors, such as *e.g.* irradiance (Barták *et al.* 2003), water content in a thallus (Calatayud *et al.* 1997), and overall environmental conditions (Schlensog and Schroeter 2001). In our study, dynamics of q_P in CMI-treated thalli indicated that the fraction of open reaction centres of PS2 was, after an initial increase, constant. The same was true for RHI_M treatment, in which, however, each short-term HI events led to a short-term increase in q_P (see Fig. 4*D*). Such response was documented in our earlier study (Barták *et al.* 2003) where both an increase (*Usnea antarctica*) and a decrease (*Umbilicaria decussata*) in q_P was found

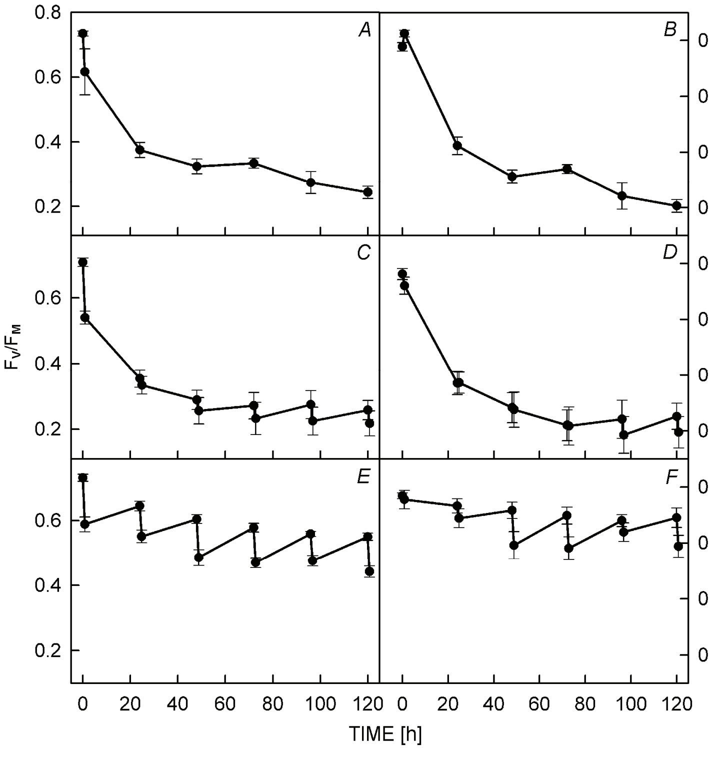

Fig. 3. Maximal quantum yield (F_V/F_M) (*A, C, E*) and potential quantum yield (Φ_2) (*B, D, F*) measured before and during different irradiation treatments. Thalli were exposed to (*A, B*) 600 μmol m^{-2} s^{-1} (CMI), (*C, D*) 600 μmol m^{-2} s^{-1} with a 30-min short-term HI-treatment (1 800 μmol m^{-2} s^{-1} every 24 h; RHI_M), and (*E, F*) were kept in dark with 30-min HI-treatment every 24 h (RHI_D). Means of 10 replicates ± standard errors.

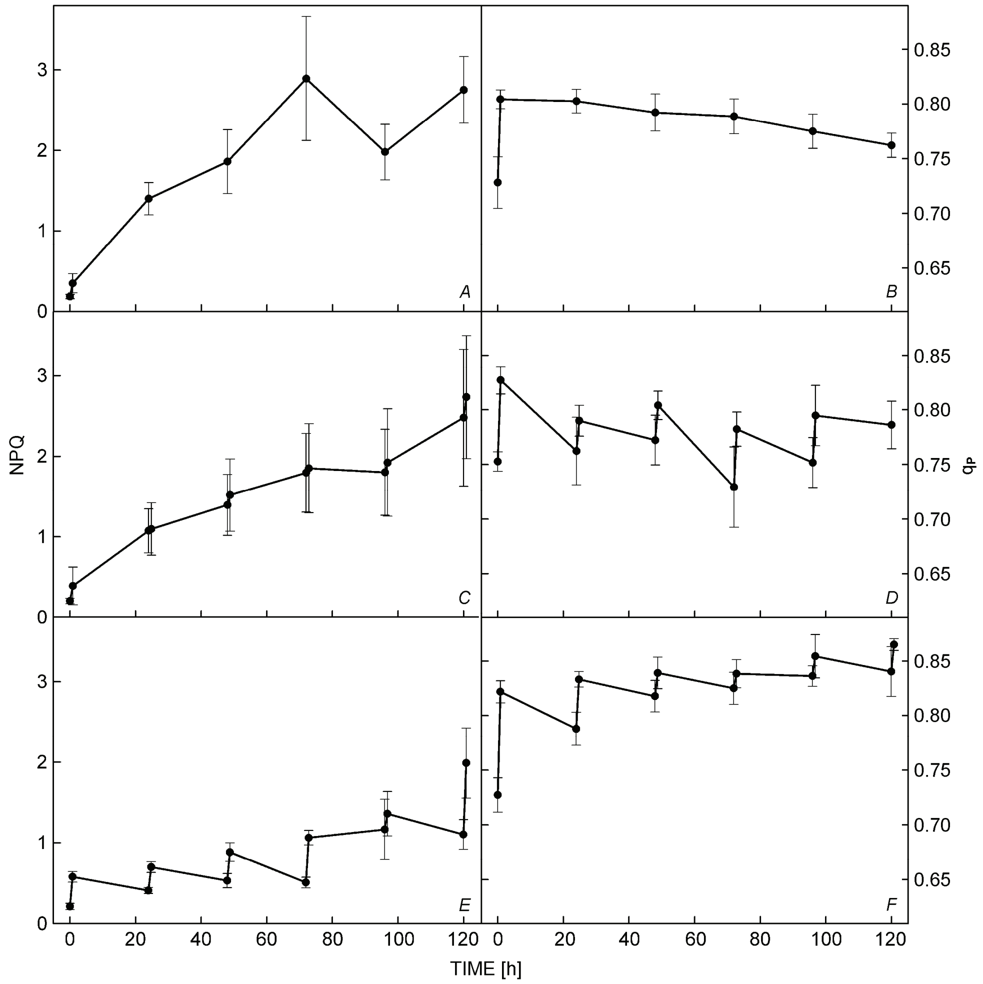

Fig. 4. (*A, C, E*) Non-photochemical (NPQ) and (*B, D, F*) photochemical (q_P) quenching of chlorophyll fluorescence measured during different irradiation treatments. Thalli were exposed to (*A, B*) 600 μmol m^{-2} s^{-1} (CMI), (*C, D*) 600 μmol m^{-2} s^{-1} with a 30-min short-term HI-treatment (1 800 μmol m^{-2} s^{-1} every 24 h; RHI_M), and (*E, F*) were kept in dark with 30-min HI every 24 h (RHI_D). Means of 10 replicates ± standard errors.

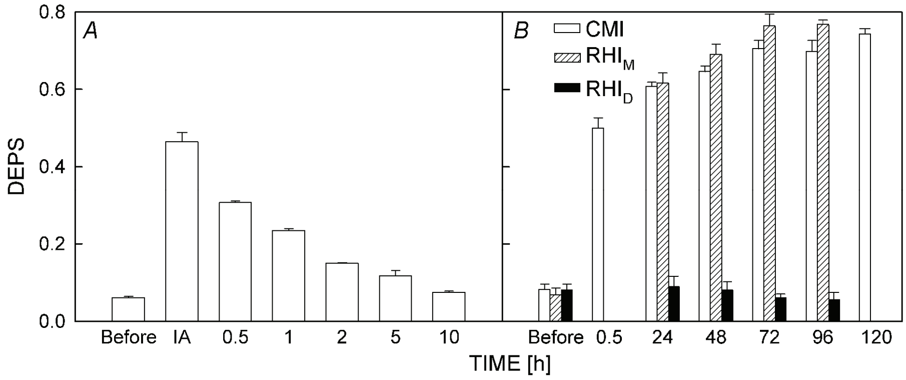

Fig. 5. De-epoxidation state of xanthophyll cycle pigments (DEPS) measured (*A*) before, immediately after 30 min lasting short-term high irradiance treatment with 1 800 μmol m^{-2} s^{-1}, and several times during recovery under dim irradiance, and (*B*) during different irradiation regimes as described in Fig. 1. Means of 10 replicates ± standard errors.

after a short-term HI. During dark treatment (RHI_D), q_P generally increased with time (Fig. 4*F*). Short-term HI, however, induced an increase in q_P, probably exploiting the same mechanism as suggested above. In our experiment, q_P calculation was based on traditional equation (assuming that independent antennae are functionally related to only a single reaction centre of PS2) (Bilger and Schreiber 1986). Recently, a shared antennae model was introduced assuming connection of neighbour antennae supplying absorbed radiant energy to several reaction centres of PS2. In the new model, Chl fluorescence parameters evaluating photochemical conversion of solar energy absorbed in PS2 are re-examined (Kramer *et al.* 2004). Thus a new equation evaluating the fraction of open reaction centres of PS2 (q_L) is introduced. To address this, we used the equation for q_L (Kramer *et al.* 2004) and re-calculated original data [$q_L = q_P (F_0'/F_s)$, not shown here]. Obtained q_L values were somewhat lower than q_P, the trends, however, remained the same. For *L. pustulata*, the trends in photochemical quenching were therefore not affected by connectivity of LHCs.

Similar to earlier studies on HI-stressed hydrated foliose lichen species (Barták *et al.* 2004, Vráblíková *et al.* 2006), DEPS increased after a single short-term HI treatment. During the following recovery there was an exponential DEPS decrease (Fig. 5*A*) accompanied by recovery of F_V/F_M and Φ_2 (Fig. 2*A*) exhibiting fast and slow phases related to regulatory and repair processes of PS2 components, respectively (Krause and Weis 1991). Since 10 h in dark did not lead to full recovery to the initial F_V/F_M and Φ_2 values, the dark interval was insufficient for re-synthesis and re-arrangement processes of HI-injured PS2 protein components, D1 protein in particular, as reported *e.g.* for algae (Etienne and Kirilovsky 1992, Zsiros *et al.* 2006). This might be supported by our earlier finding (Vráblíková *et al.* 2005) showing uncomplete recovery after 10 h in low irradiance. Also the RHI$_D$ data (Fig. 3*E*) and earlier observations (Barták *et al.* 2004) showed even more limited recovery of F_V/F_M compared to this study. DEPS values, however, recovered much faster both in this study (after 10 h – Fig. 5*A*, also Fig. 5*B* where RHI$_D$ is fully recovered after 24 h) and our earlier experiments (3 h). Fast DEPS recovery in lichen is also reported from a field study (Valladares *et al.* 1995). Rapidity of DEPS recovery is caused by fast biochemical reactions of the xanthophyll cycle, *i.e.* rate constants of majority of the reactions are under 0.5 min^{-1} (Frommolt *et al.* 2001). In long-term irradiation treatments, DEPS increased with the time of exposure (CMI) reaching a constant value after 48 h. Additional HI applied each 24 h caused DEPS increase above the CMI value but not before 48 and 72 h. Therefore, we suggest that 24 h recovery under moderate irradiance is sufficient for DEPS to return to initial, pre-HI values. After each HI event, however, DEPS increases dramatically as shown in Fig. 5*A* for a single HI event. These findings may have importance for understanding DEPS dynamics in natural systems. It seems that duration of irradiation (even if low irradiance is considered) rather than short-term HI plays an important role in control of DEPS increase when other interacting factors, such as *e.g.* fluctuating temperature (Latowski *et al.* 2003) and desiccation (Calatayud *et al.* 1997) are excluded.

Involvement of V to Z conversion into photoprotection of irradiated *L. pustulata*, its photobiont in particular, is documented by the DEPS to NPQ relation (Fig. 6). The slopes of the relation, however, differ between CMI and RHI$_M$ treatments. Since NPQ was nearly two fold higher in CMI than in RHI$_M$ for the same DEPS of about 0.7, more effective involvement of NPQ mechanisms might be expected in long-term treated *L. pustulata* thalli. HI-induced DEPS increase is obviously related to a reduced effect of PS2 photochemistry (Gilmore and Yamamoto 2001) and increased NPQ. For analysis of DEPS to NPQ relation, we point out also the importance of experimental conditions under which the data are taken. Clear difference exists *e.g.* in the slope of DEPS to NPQ relation when data are taken during the irradiation or during recovery (see Fig. 6).

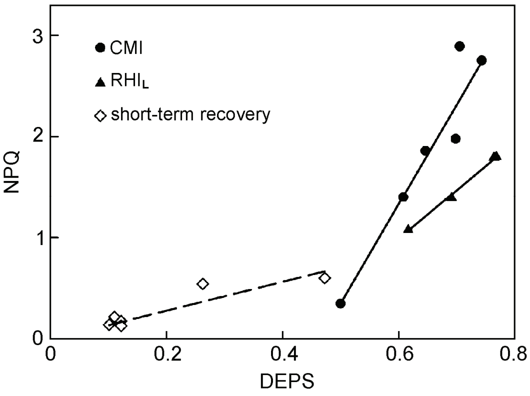

Fig. 6. Relation of NPQ to DEPS values. *Circles* represent 600 μmol m^{-2} s^{-1} treatment (CMI) (see Fig. 4*A* and 5*B*), *triangles* represent 600 μmol m^{-2} s^{-1} with a 30-min short-term treatment with 1 800 μmol m^{-2} s^{-1} every 24 h (RHI$_M$, see Fig. 4*C*), and *diamonds* represent short-term high irradiance treatment (data are from Vráblíková *et al.* 2005).

Our data indicate that rather medium irradiance lasting for several hours than short-term HI causes reduction of primary photosynthetic efficiency in hydrated thalli of *L. pustulata*. Rapidly responding photoprotective mechanisms, such as *e.g.* V to Z conversion, are exploited during short-term irradiation, even if irradiance is high. However, a complex response of several photoprotective mechanisms with different time scales must be considered when evaluating non-photochemical quenching that increases as a consequence of both short- and long-term irradiance stress imposed on wet thalli.

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