Sensitivity of photosystem 2 of Antarctic lichens to high irradiance stress: Fluorometric study of fruticose (*Usnea antarctica*) and foliose (*Umbilicaria decussata*) species

M. BARTÁK*, H. VRÁBLÍKOVÁ, and J. HÁJEK

Department of Plant Physiology and Anatomy, Faculty of Science, Masaryk University, Kotlářská 2, CZ-61137 Brno, Czech Republic

Abstract

Two lichen species collected in maritime Antarctica (King George Island) were exposed under laboratory conditions to excess irradiance to evaluate the response of photosystem 2 (PS2). The response was measured on fully hydrated lichen thalli at 5 °C by means of a modulated fluorometer using chlorophyll (Chl) fluorescence induction curve supplemented with analysis of quenching mechanisms. Chl fluorescence parameters [*i.e.* ratio of variable to maximum Chl fluorescence (F_V/F_M), quantum yield of PS2 photochemical reactions (Φ_2), quenching coefficients] were evaluated before and several times after exposition to high irradiance in order to characterise the extent of photoinhibition, fast and slow phase of recovery. Strong irradiance (2 000 µmol m^{-2} s^{-1}) caused high degree of photoinhibition, particularly higher in fruticose (*Usnea antarctica*) than in foliose (*Umbilicaria decussata*) lichen species. Fast phase of recovery from photoinhibition, corresponding to regulatory mechanisms of PS2, was more apparent in *U. decussata* and Φ_2 than in *U. antarctica* and F_V/F_M and Φ_2 within 40 min after photoinhibitory treatment. It was followed by a slow phase lasting several hours, corresponding to repair and re-synthesis processes. After photoinhibitory treatment, recovery of non-photochemical quenching (NPQ) was faster and more pronounced in *U. decussata* than in *U. antarctica.* Significant differences were found between the two species in the rate of recovery in fast- (q_E) and slow-recovering (q_{T+I}) component of NPQ.

Additional key words: chlorophyll fluorescence; low temperature; photoinhibition; photosynthesis; non-photochemical quenching; recovery.

Introduction

Lichens can survive in extreme ecosystems due to their ability to cope with unfavourable factors of the environment, such as drought, extremes and fast fluctuations in temperature, long-lasting snow cover, *etc*. During their lifetime, some lichen must also cope with both episodic and long-term exposure to high irradiance. High irradiance may induce regulatory mechanisms and, especially when a lichen thallus is fully hydrated, negative changes in photosynthetic apparatus of lichen photobiont. The negative effects are more apparent in lichen species having green alga as photobiont than in cyanobacterial lichen species (Demmig-Adams *et al.* 1990a,b). During the last few decades, the attention of lichen physiologists has been focused on the question whether high irradiance may cause photoinhibition of photosynthesis in lichens *in situ.* There are two contradictory opinions on photoinhibition in lichens that possess green algae as photobionts. According to the first one, photoinhibition is a common phenomenon in the field at least in shade-adapted species (Gauslaa and Solhaug 2000), or species with a limited pool of xanthophyll cycle pigments (Valladares *et al.* 1997). The other opinion is that photoinhibition is very rare in the field (Kappen *et al.* 1998).

Generally, lichens possess several mechanisms to avoid photoinhibition. In the field, high irradiance is obviously accompanied by increased temperature of thallus.

Received 5 June 2003, *accepted* 17 September 2003.
*Corresponding author; fax: (+420) 5 41211214, e-mail: mbartak@sci.muni.cz
Symbols and abbreviations: A – antheraxanthin; Chl – chlorophyll; NPQ – non-photochemical quenching of Chl fluorescence; LHC – light-harvesting complex; P_N – net photosynthetic rate; PS2 – photosystem 2; q_0 – quenching of background Chl fluorescence; q_E – energy-dependent quenching; q_P – photochemical quenching of Chl fluorescence;; q_{T+I} – state transition and photoinhibition-related quenching of Chl fluorescence; V – violaxanthin; Z – zeaxanthin.
Acknowledgements: The authors acknowledge a kind determination of lichen specimens made by Dr. Vězda (*Herbarium Lichenicorum*, Brno, CZ). This work was supported by the grant No. J07/98: 143100007 given by the Czech Ministry of Education.

Lichens, being poikilohydric organisms, desiccate rapidly with increasing temperature and become photosynthetically inactive when water content in thallus decreases under 15 % of fully hydrated state, see, *e.g.*, Hájek *et al.* (2001). Desiccating or dry thallus is less influenced by high irradiance, because desiccation leads to functional disconnection of antennae complexes (LHC) of PS2, which might lead to further photoprotection at high irradiance (Bilger *et al.* 1989). On the other hand, high hydration of thallus increases the probability of photoinhibition, because it reduces availability of CO_2 for photosynthesis due to hydration-induced resistance to CO_2 diffusion into the thallus (Lange *et al.* 1999). Under low temperature, full hydration of a thallus may, due to reduced rate of evaporation, last substantially longer than under high temperature. Therefore, the probability of photoinhibition may increase at low temperatures.

The reflectance of the cortex layer of a lichen thallus represents another protective mechanism against high irradiance effect. The spectral reflectance of lichen thallus changes according to its hydration status (Solheim *et al.* 2000). The presence of photoprotective pigments and compounds like, *e.g.*, flavonoids (UV screens) in lichen thallus may substantially reduce the probability of irradiance-induced negative effects on photosynthetic apparatus of photobiont. In the cells of photobiont, the presence of xanthophyll cycle pigments and especially the capacity of conversion of violaxanthin (V) to zeaxanthin (Z) represents another photoprotective mechanism because Z is involved in quenching of excitation energy in LHCs. In spite of the photoprotective mechanism, photoinhibition is reported in lichens both when induced in laboratory experiments simulating outdoor conditions (Gauslaa and Solhaug 1996) and in the field in low irradiance-adapted lichens (Gauslaa and Solhaug 2000).

In the present paper we hypothesised that photoinhibition in lichens is a common phenomenon that might be pronounced at low temperature. Globally, lichens face low temperature to the largest extent in alpine zones of high mountains and polar regions. Since our recent research in Brno is focused on eco-physiology of lichens and mosses from maritime Antarctic ecosystems, we have chosen two Antarctic lichen species to study low-temperature photoinhibition. In our experiments, we focused on the following questions: (*1*) Does photoinhibition occur in green-algae lichens under laboratory-simulated physical conditions of Antarctic environment? (*2*) Is photoinhibition reversible or under which physical conditions does recovery from photoinhibition occur? (*3*) Is there any difference between two lichen species with contrasting anatomy (foliose *vs.* fruticose thallus) in the extent of photoinhibition and in the capacity of non-photochemical quenching mechanisms to protect from excess irradiance energy?

Materials and methods

Lichen collection and handling: Thalli of *Usnea antarctica* and *Umbilicaria decussata* were collected in close vicinity of the Peruvian Antarctic Station (Machu Picchu) situated at the King George Island (Admiralty Bay, 62°S, 58°W) in maritime Antarctica. Thalli of *U. decussata,* foliose lichen with typical umbilicate anatomy, were collected from rock surfaces at the altitude of 150 m a.s.l. and the distance of approximately 200 m from the coastline. Clumps of thalli of *U. antarctica,* a fruticose lichen with typical miniature shrub morphology, were collected in the neighbourhood of the station from stone blocks forming a moraine. After collection, the thalli were desiccated under natural outdoor conditions, then stored in dark at 5 °C, and transferred to the Masaryk University, Brno (CZ) where they were stored under dim irradiation at 2 °C. 24 h before measurements, the thalli were re-wetted at 5 ^{o}C by de-mineralised water supplied to a sheet of filter paper placed in close contact with lichen thalli. For measurements, thalli of *U. antarctica* were divided into upper (more dark-pigmented) and lower (less pigmented) parts and measured separately because we expected different sensitivity to photoinhibition in the two thallus parts.

Chlorophyll (Chl) fluorescence measurements: After re-wetting, the thalli in fully hydrated state were exposed to photoinhibitory irradiance (2 000 μmol m^{-2} s^{-1} for 30 min at 5 °C) denoted as photoinhibitory treatment in the following text. The irradiance provided by a halogen lamp passed a water filter in order to cut off infra-red radiation. Fully hydrated lichen thalli (*U. decussata)* or their fragmented parts (*U. antarctica)* were placed on the surface of an aluminium block that was cooled to 5 °C by cold water circulating between the thermostat and the block. Surface temperature of exposed lichen thalli was measured every 5 s using a contact temperature sensor and a data logger *HOBO* (*Onset Computers*, USA). To avoid interfering effects of thalli desiccation during the exposure to high PPFD, the lower surface of thalli was in permanent contact with a moist paper supplied with cold (5 ^{o}C) water. Before, and several times after the photoinhibitory treatment, typically at 30-60 min intervals, a set of Chl fluorescence parameters (specified in Table 1) was measured using a fluorometer *PAM-2000* (*H. Walz*, Effeltrich, Germany). After photoinhibitory treatment, recovery was measured in samples placed in the dark at 5 °C. Photoinhibition and recovery were monitored as time-dependent changes of the following Chl fluorescence parameters. Basic parameters (F_V/F_M, $\Phi_2 = \Delta F/F_M'$, where $\Delta F = F_M' - F_S$) and quenching coefficients (q_P, NPQ, q_0—see Table 1) were determined from analysis of slow kinetics of Chl fluorescence supplemented with saturation pulses using the following sequence. On dark-adapted (10 min) thalli of lichens, weak irradiance was

Table 1. Quenching coefficients of the chlorophyll (Chl) fluorescence used in experiments. F_M – maximum Chl fluorescence on dark-adapted thalli, F_M' – maximum Chl fluorescence on light-adapted thalli (under actinic irradiance), F_M'' – maximum Chl fluorescence measured 40 s after turning off the actinic radiation, F_0 – background Chl fluorescence before the induction of photosynthesis, F_0' – background Chl fluorescence during actinic radiation, F_s – steady-state Chl fluorescence during actinic irradiation. For calculations of quenching coefficients in which F_M is an input parameter, pre-photoinhibition F_M values were used.

Abbreviation	Definition	Reference
Photochemical quenching, q_P	$(F_M' - F_S)/(F_M' - F_0')$	Bilger and Schreiber (1986)
Non-photochemical quenching, NPQ	$(F_M - F_M')/F_M'$	Bilger and Björkman (1990)
State transition + photoinhibitory quenching, q_{T+I}	$(F_M - F_M'')/(F_M - F_0')$	Roháček and Barták (1999)
Quenching of background Chl fluorescence, q_0	$(F_0 - F_0')/F_0'$	Bilger and Schreiber (1986)
Energy dependent quenching, q_E	$[1 - (F_M' - F_0')/(F_M - F_0)] - q_{T+I}$	Krause and Weis (1991)

first applied in order to determine background Chl fluorescence (F_0). Then a saturation pulse of 5 000 μmol m^{-2} s^{-1} allowed to calculate maximum capacity of PS2 (F_V/F_M). Actinic radiation (35 μmol m^{-2} s^{-1}) was then applied for 5 min until steady-state Chl fluorescence (F_S) was reached. A saturation pulse was then applied in order to measure F_M'. Actinic radiation was then turned off and a final saturation pulse was applied after 40 s in order to evaluate fast-relaxing (q_E, dosens of seconds) and slow-relaxing (q_{T+I}, dosens of minutes) components of non-photochemical quenching (the peak was denoted as F_M'').

Results

Photoinhibitory treatment induced a strong decrease in F_V/F_M and Φ_2 values (Fig. 1). The time course of the decrease and following recovery was similar in both species studied. However, the extent of the decrease differed between species. In *U. decussata*, F_V/F_M was reduced to 78 % of initial value in contrast to more sensitive *U. antarctica* that showed a F_V/F_M decrease to 38 % of initial value. Upper and lower thallus parts of *U. antarctica* did not differ in the reduced value of F_V/F_M. Fast phase of recovery was apparent, especially in *U. antarctica*, as a steep rise of F_V/F_M values within the first 40 min after the end of photoinhibitory treatment. Slow phase of recovery was characterised by less pronounced F_V/F_M rise ongoing within the following 4 h. There was a large difference between the two species studied in the time required for full recovery of F_V/F_M. While full recovery was completed in *U. decussata* after 3 h, the F_V/F_M reached only about 75 % of initial values

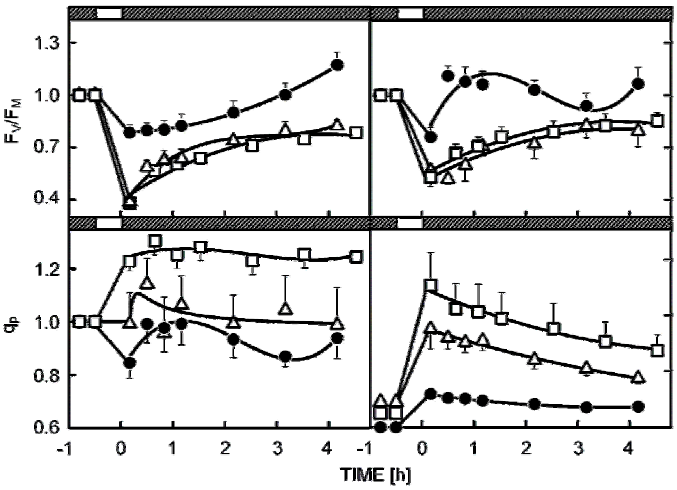

Fig. 1. Photoinhibition and recovery of Chl fluorescence parameters expressed as time courses of photochemical efficiency of photosystem 2, PS2 (F_V/F_M), quantum yield of PS2 (Φ_2), photochemical quenching (q_P), and the quenching of background Chl fluorescence (q_0, calculated as $F_0 - F_0'/F_0'$). The time courses are recorded for *Umbilicaria decussata* (●) and *Usnea antarctica* lower (□) and upper thalli parts (Δ), respectively, before, and immediately after high irradiance treatment at 2 000 μmol m^{-2} s^{-1} for 30 min, and during subsequent recovery in dark. Thalli were fully hydrated during the measurements and thalli temperature was maintained at 5 °C. Standard errors of means (n = 6 for *U. antarctica* lower part, n = 5 for *U. antarctica* upper part and *U. decussata*) are indicated by *vertical bars* when larger than symbol. Except q_0, relative values are presented, *i.e.* absolute values of individual Chl fluorescence parameters were divided by initial pre-photoinhibiton absolute value. Normalisation was necessary for the comparison of the two species because absolute values of Chl fluorescence parameters were substantially lower in *U. decussata* than *U. antarctica.*

in *U. antarctica* after 4 h of recovery. Slight difference in the rate of F_V/F_M recovery was found between the upper (faster-recovering) and lower (slower-recovering) thalli parts. In both species, strong irradiance induced a decrease of Φ_2 values, much more pronounced in *U. antarctica* (55 % of initial value) than in *U. decussata* (92 % of initial value). Subsequent recovery of Φ_2 was extremely fast in the latter species and completed 20 min after the end of photoinhibitory treatment. *U. antarctica*, on the contrary, showed uncompleted recovery even after 4 h, when the thalli exhibited only about 80 % of initial value of Φ_2.

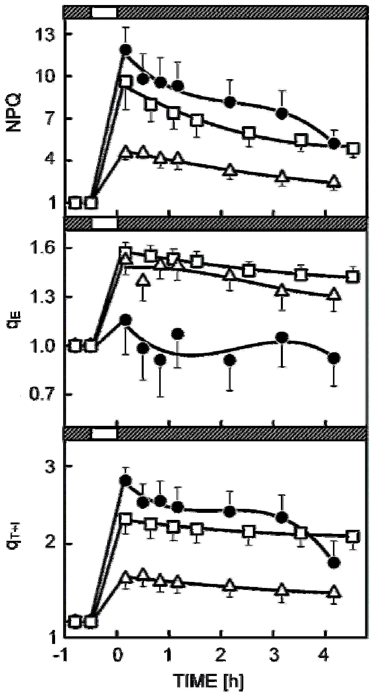

Fig. 2. Photoinhibition and recovery of Chl fluorescence parameters expressed as time courses of non-photochemical quenching (NPQ), energy-dependent quenching (q_E), and state transition and photoinhibition-related quenching (q_{T+I}). The time courses are recorded for *Umbilicaria decussata* (●) and *Usnea antarctica* [lower (□) and upper (Δ) thalli parts, respectively] before, and immediately after high irradiance treatment at 2 000 μmol m^{-2} s^{-1} for 30 min, and during subsequent recovery in dark. Thalli were fully hydrated during the measurements and thalli temperature was maintained at 5 °C. Standard errors of means ($n = 6$ for *U. antarctica* lower part, $n = 5$ for *U. antarctica* upper part and *U. decussata*) are indicated by *vertical bars* when larger than symbol. Data points are presented as relative values, *i.e.* absolute values of individual Chl fluorescence parameters were divided by initial pre-photoinhibiton absolute value. Normalisation was necessary for the comparison of the two species because absolute values of Chl fluorescence parameters were substantially lower in *U. decussata* than *U. antarctica.*

After photoinhibitory treatment, photochemical quenching (q_P) showed different time course in each species. In *U. decussate*, it slightly decreased immediately after exposure to high irradiance, recovered fast (within 30 min), and became more or less constant with slight fluctuations throughout next 4 h. *U. antarctica*, in contrast, exhibited an increase in q_P values immediately after the photoinhibitory treatment. The increase was more pronounced (by a factor of about two) in lower than in upper thallus part. Upper part showed full recovery of q_P to initial value after 2 h. In the lower part, however, no recovery of q_P was apparent even after 4 h.

The time course of non-photochemical quenching and its components during photoinhibitory treatment and subsequent recovery are summarised in Fig. 2. NPQ increased after photoinhibitory treatment by a factor of 4.0–12.0, most apparently in *U. decussata* and the lower thallus part of *U. antarctica.* The following recovery was typical by a gradual decrease of NPQ values recorded within next 4 h. Fast phase of NPQ recovery was apparent within the first 30 min after the end of photoinhibitory treatment in *U. decussata*, while it was not distinguishable in *U. antarctica.* In both species, full recovery of NPQ was not reached after the following 4 h. Time course of q_{T+I}, a dominant component of non-photochemical quenching in photoinhibited thalli, was similarly shaped to NPQ time course. The increase of q_{T+I} was

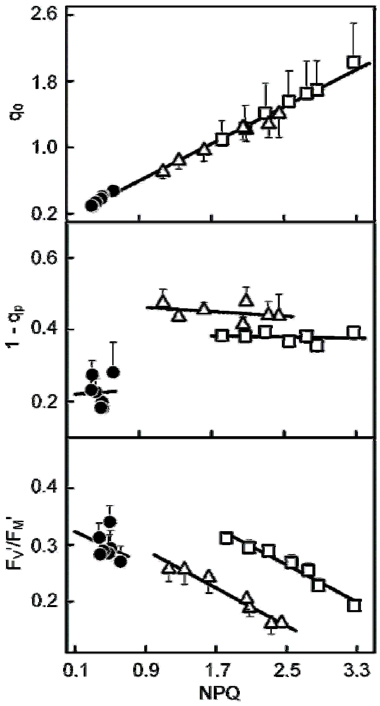

Fig. 3. Dependences of background Chl fluorescence quenching (q_0, *upper panel*), closure of photosystem 2 (PS2) reaction centres ($1 - q_P$, *central panel*), and non-radiative excitation energy dissipation in PS2 antennae (F_V'/F_M', *lower panel*) on non-photochemical quenching (NPQ). Data points are means of at least 5 replicates ± standard error of mean indicated by a *bar*. Data were recorded after photoinhibitory treatment during recovery. ● – *U. decussata*, Δ – *U. antarctica* (upper thallus part), □ – *U. antarctica* (lower thallus part).

more pronounced in *U. decussata* (by the factor of 3.0) than in *U. antarctica* (by the factor of 1.5–2.2).

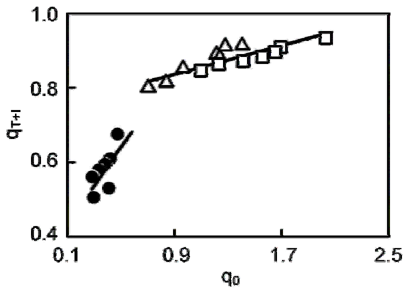

Fig. 4. Dependence of state transition and photoinhibition-related quenching (q_{T+I}) on background Chl fluorescence quenching (q_0). Data points are means of at least 5 replicates. Data were recorded after photoinhibitory treatment during recovery. ● – *Umbilicaria decussata*, Δ – *Usnea antarctica* (upper thallus part), □ – *U. antarctica* (lower thallus part).

Similarly to NPQ, significant difference was found between thallus parts in q_{T+I} values after photoinhibitory treatment: lower thallus parts exhibited higher q_{T+I} than upper parts. Fast phase of q_{T+I} recovery was apparent only in *U. decussata* within the first 30 min of recovery, while it lacked in *U. antarctica*. Increase in q_E induced by strong irradiation was more pronounced in both upper and lower parts of *U. antarctica* (factor of 1.6) than in *U. decussata* (factor of 1.2). The latter species showed full recovery of q_E after 1 h in dark, while in *U. antarctica*, only partial q_E recovery was apparent after 4 h in dark.

Species-specific differences in the involvement of quenching mechanisms are demonstrated in Fig. 3. During recovery, quenching of background Chl fluorescence (q_0) was strongly positively correlated to NPQ. Both upper and lower thalli parts of *U. antarctica* reached much higher q_0, and NPQ values than the less sensitive *U. decussata*. Pooled data showed that the q_0 to NPQ relationship was fairly linear with a high slope indicating involvement of antenna-type quenching. Parameter $1 - q_P$, when plotted against NPQ, remained more or less constant throughout the whole recovery period indicating that the parameter is not dependent on decreasing NPQ during recovery. Between the two species studied, however, substantial differences were found in $1 - q_P$ values. *U. antarctica* exhibited much higher values than *U. decussata* demonstrating a higher photoinhibition. The extent of PS2 reaction centre closure in *U. antarctica* differed between the upper and lower thalli parts. It was significantly higher in upper than lower thallus part. Linear relation was found between q_0 and q_{T+I} (Fig. 4). For *U. antarctica*, q_0 and q_E reached higher values but the slope of the relation was somewhat lower than in *U. decussata.* For both species, q_E reached substantially lower values (below 0.07) than q_{T+I} and thus the q_E data are not shown in Fig. 4.

Discussion

F_V/F_M, quantum yield of PS2: In this study, photoinhibition of PS2 in two contrasting lichen species was examined with a special respect to excess energy dissipation by non-photochemical quenching and its components. Both species studied showed decrease of F_V/F_M and Φ_2 after photoinhibitory treatment and consequent recovery towards initial values. Extent of photoinhibition, however, differed between species. *U. decussata* exhibited a lower irradiance-induced decrease of F_V/F_M than *U. antarctica*. F_V/F_M fully recovered in *U. decussata* after 3 h (the F_V/F_M value found after 4 h of recovery is an artefact), while it did not in *U. antarctica.* The inter-specific difference in extent of photoinhibition was also apparent in Φ_2. We may, therefore, suggest that both potential and actual photosynthetic processes might be more photoinhibited in the lichen with fruticose rather than foliose anatomy when exposed to high irradiance under full thallus hydration. This idea might be supported by several field and laboratory experiments that reported different level of photoinhibition in fruticose (Gauslaa and Solhaug 1996) and foliose species (Gauslaa *et al.* 2001, MacKenzie *et al.* 2002). Within a single thallus of *U. antarctica,* however, the upper and lower thallus parts do not apparently differ in the extent of F_V/F_M and Φ_2 decrease and consequent recovery. This might indicate that dark colour of the upper hairy thallus part has no effect on the sensitivity of the species to photoinhibition. In the field, however, the upper part of the thallus desiccates more rapidly than the rest of thallus, becomes physiologically inactive, and thus protects the lower parts of the thallus by effective photon absorption and reflectance (Solheim *et al.* 2000). For more comments *see* Ecophysiological consequences.

In our experiments, the decrease in F_V/F_M after photoinhibitory treatment was caused by a decrease of F_M rather than by an increase of F_0 (data not shown). In higher plants, for photoinhibition a decrease of F_M and, in most cases, an increase of F_0 is typical (*e.g.* Xu and Wu 1996, Hong and Xu 1999). In lichens, in contrast, F_0 decreases in most cases after photoinhibitory treatment as found by Heber *et al.* (2000, 2001) and in our earlier observations (Barták *et al.*, unpublished). In our experiment, F_M decreased to a larger extent than F_0 after photoinhibition that numerically resulted in a decreased F_V/F_M. The reason for F_0 drop after photoinhibitory treatment in poikilohydric mosses and lichens is not yet satisfactorily elucidated. It might be attributed to a high irradiance-induced detachment of antennae, re-arrangement of chloroplast, and resulting increased re-absorption of Chl fluorescence as shown earlier for a poikilohydric moss (Bartošková *et al.* 1999).

Quenching parameters: High irradiance-induced non-photochemical quenching represents involvement of non-radiative excitation energy dissipation processes. They comprise: (*1*) Trans-thylakoid ΔpH-dependent inter-conversion of xanthophyll-cycle pigments (q_E). (*2*) Structural changes in PS2 related to photoinhibition (q_{T+I}). Since NPQ recovered much faster and in more extent in *U. decussata* than *U. antarctica*, we may consider that the capacity of the above protective mechanisms (q_E, q_{T+I}) is higher in the former species. In *U. antarctica*, NPQ is mostly due to photoinhibited PS2 centres because q_E (*see* Fig. 2) was less efficient than in *U. decussata*. An increase of NPQ was, irrespective to lichen species and phase of recovery, accompanied by an increase in q_0 (see Fig. 3), which is typical for antennae-type quenching (Bukhov *et al.* 2001). Recent studies focused on inter-relation between NPQ, q_E, and zeaxanthin-dependent quenching (*e.g.* Li *et al.* 2002) during recovery from photoinhibition revealed that q_E is fast-recovering while de-epoxidation state of xantophyll-cycle pigments is slow-recovering. Fast-recovering q_E is located in PS2 antennae (Laisk and Oja 2000) and lasts several minutes (Müller *et al.* 2001) while xanthophyll de-epoxidation state recovers in dosens of minutes (Li *et al.* 2002). Li *et al.* (2002) suggest that the rate of NPQ recovery strongly depends on proportion between q_E and xanthophyll de-epoxidation state at the end of photoinhibitory treatment. If the photoinhibition is moderate or short-term, q_E forms major part of quenching and therefore recovery of NPQ is fast, in terms of minutes. Our data obtained on lichens suggest that severe or long-term photoinhibition leads to a higher involvement of xanthophyll-dependent quenching and, consequently, to a slow recovery of NPQ after photoinhibitory treatment. This opinion is supported by the slow recovery of de-epoxidation state of xanthophyll-cycle pigments reported by Li *et al.* (2002) on PsbS-lacking mutants and wild plants of *Arabidopsis thaliana.* Other supporting item is a high correlation of NPQ (q_{T+I} in particular) to de-epoxidation state of xanthophyll-cycle pigment pool during recovery from severe photoinhibition found in our previous study in a green alga photobiont isolated from a foliose lichen species *Umbilicaria hirsuta* (unpublished). Similar studies (Gilmore *et al.* 1996, Färber *et al.* 1997) made on higher plants also revealed positive relation between NPQ and Z+A pool during photoinhibitory treatment. In higher plants, V to Z conversion in PS2 antennae during photoinhibition is q_E- and also q_I-dependent (Färber *et al.* 1997).

Different shapes of q_P time courses found in this study for *U. decussata* and the upper thalli parts of *U. antarctica* may suggest that fraction of open, *i.e.* Q_A reducing PS2 centres, either decreases or increases but recovers quickly (1 h). Increase in q_P and no recovery found in lower thalli parts of *U. antarctica* shows the complexity of post-photoinhibition response. Non-uniform response of q_P might be supported by the interpretation of q_P data presented in the study of *Parmelia quercina* (Calatayud *et al.* 1997) showing non-uniform response in gradually desiccating thallus treated by medium irradiance. When measured under full hydration, q_P showed rather gradual increase similarly to the upper part of *U. antarctica* in this study. In photoinhibition studies, rather increase of q_P (*e.g.* Demmig-Adams *et al.* 1990a) than decrease is reported. The reason why both positive and negative effects of photoinhibitory treatment on q_P were found within the same thallus in this study is not clear. We may attribute it either to the seemingly different optical properties of the upper part of *U. antarctica* caused by dark pigmentation of the hairy tips, to the method of F_0' evaluation at the steady state Chl fluorescence, or to another unknown reason. The value of F_0' is, however, essential for q_P calculation (see Table 1).

Low temperature effects: In our experimental set-up, we measured lichen response to high PPFD under low temperature (5 ºC) in order to simulate the likely outdoor Antarctic conditions during Austral summer. We may, therefore, expect low temperature effect on components of non-photochemical quenching and V to Z conversion. In lichens, however, the effect is unclear. In higher plants, it was shown previously that a short-term drop to low temperature induces increase in non-photochemical quenching (*e.g.* Król *et al*. 1999) and reduces de-epoxidation rate and V to Z conversion (Arvidsson *et al*. 1997, by 50 % at 4 ºC). When higher plants pass cold acclimation prior to the high-irradiance stress, they exhibit higher rates and contents of xanthophyll-cycle pigment conversion as shown by Jung *et al*. (1998), Verhoeven *et al*. (1999), Koroleva *et al*. (2000), *etc*. To our best knowledge, similar study on lichens is lacking. We can thus only speculate about the low temperature effect on the capacity of xanthophyll-cycle pigment pool and non-photochemical quenching mechanisms, and their exploitation under low temperature photoinhibition. However, our previous findings (Hájek *et al*. 2001) showing long-term acclimation of Φ_2, and non-photochemical quenching to low temperature and the Chl fluorescence data presented in this study, indicate that the photoprotective capacity of *U. antarctica* and *U. decussata* might be sufficient to cope with a strong photoinhibition under low temperature.

Ecophysiological consequences: We found that photoinhibition might be induced in Antarctic lichen species under the close-to-reality external conditions. We also demonstrated sufficient capacity of photoprotective mechanisms of the two species studied. It is, however, still disputable how frequently these responses may occur in the field. We still do not have enough data describing long-term (seasonal) variation in local microclimate and physiological state of a lichen thallus. Available field studies (Schroeter *et al*. 1995, 1997) estimated the time of lichen thallus metabolic activity to about 50 % of total annual time. Majority of the active time is, however, characteristic by prevailing respiration, particularly due to long-lasting snow cover and winter conditions. In spite of the fact that some lichen species, especially in maritime Antarctica, may be physiologically active under snow cover (Pannewitz *et al*. 2003), the time interval of *in situ* physiological activity with positive lichen net photosynthesis (P_N) is rather short. Nevertheless, photoinhibition may theoretically happen any time when the lichen is physiologically active, no matter whether exhibiting positive or negative P_N. It is questionable how large portion of the time may cover situations when low-temperature photoinhibition is probable. We can, however, consider that photoinhibitory conditions might happen during the beginning of Austral summer, when lichen thalli are well hydrated either from melting snow or snowfall episodes together with high irradiance. The duration of such situations probably does not exceed tens of minutes due to progressive desiccation of thalli but, according to our results presented in this paper and field measurements (Gloser 2001), may be sufficiently long to induce *in situ* photoinhibition. The probability of photoinhibition might be higher in fruticose rather than foliose lichens, because the latter desiccate more rapidly in the maritime Antarctic climate due to their anatomy (Huiskes *et al*. 1997). We may, therefore, conclude that *in situ* photoinhibition is likely in Antarctic lichens, especially in those from maritime regions. To confirm this idea needs further long-term field measurements because the studies made so far (Kappen *et al*. 1991, 1998) are not sufficient for any conclusion about *in situ* photoinhibition.

References

Arvidsson, P.-O., Carlsson, M., Stefánsson, H., Albertsson, P.-Å., Åkerlund, H.-E.: Violaxanthin accessibility and temperature dependency for de-epoxidation in spinach thylakoid membranes. – Photosynth. Res. **52**: 39-48, 1997.

Bartošková, H., Nauš, J., Výkruta, M.: The arrangement of chloroplasts in cells influences the reabsorption of chlorophyll fluorescence emmission. The effect of desiccation on the chlorophyll fluorescence spectra of *Rhizomnium punctatum* leaves. – Photosynth. Res. **62**: 251-260, 1999.

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., Rimke, S., Schreiber, U., Lange, O.L.: Inhibition of energy-transfer to photosystem II in lichens by dehydration: Different properties of reversibility with green and blue-green phycobionts. – J. Plant Physiol. **134**: 261-268, 1989.

Bilger, W., Schreiber, U.: Energy-dependent quenching of dark-level chlorophyll fluorescence in intact leaves. – Photosynth. Res. **10**: 303-308, 1986.

Bukhov, N.G., Heber, U., Wiese, C., Shuvalov, V.A.: Energy dissipation in photosynthesis: Does the quenching of chlorophyll fluorescence originate from antenna complexes of photosystem II or from the reaction center? – Planta **212**: 749-758, 2001.

Calatayud, A., Deltoro, V.I., Barreno, E., del Valle-Tascon, S.: Changes in *in vivo* chlorophyll fluorescence quenching in lichen thalli as a function of water content and suggestion of zeaxanthin-associated photoprotection. – Physiol. Plant. **101**: 93-102, 1997.

Demmig-Adams, B., Adams, W.W., III, Green, T.G.A., Czygan, F.-C., Lange, O.L.: Differences in the susceptibility to light stress in two lichens forming a phycosymbiodeme, one partner possessing and one lacking the xanthophyll cycle. – Oecologia **84**: 451-456, 1990a.

Demmig-Adams, B., Máguas, C., Adams, W.W., III, Meyer, A., Kilian, E., Lange, O.L.: Effect of high light on the efficiency of photochemical energy conversion in a variety of lichen species with green and blue-green phycobionts. – Planta **180**: 400-409, 1990b.

Färber, A., Young, A.J., Ruban, A.V., Horton, P., Jahns, P.: Dynamics of xanthophyll-cycle activity in different antenna subcomplexes in the photosynthetic membranes of higher plants. – Plant Physiol. **115**: 1609-1618, 1997.

Gauslaa, Y., Ohlson, M., Solhaug, K.A., Bilger, W., Nybakken, L.: Aspect-dependent high-irradiance damage in two transplanted foliose forest lichens, *Lobaria pulmonaria* and *Parmelia sulcata*. – Can. J. Forest Res. **31**: 1639-1649, 2001.

Gauslaa, Y., Solhaug, K.A.: Differences in the susceptibility to light stress between epiphytic lichens of ancient and young boreal forest stands. – Funct. Ecol. **10**: 344-354, 1996.

Gauslaa, Y., Solhaug, K.A.: High-light-intensity damage to the foliose lichen *Lobaria pulmonaria* within a natural forest: The applicability of chlorophyll fluorescence methods. – Lichenologist **32**: 271-289, 2000.

Gilmore, A.M., Hazlett, T.L., Debrunner, P.G., Govindjee: Photosystem II chlorophyll *a* fluorescence lifetimes and intensity are independent of the antenna size differences between barley wild-type and chlorina mutants: Photochemical quenching and xanthophyll cycle-dependent nonphotochemical quenching of fluorescence. – Photosynth. Res. **48**: 171-187, 1996.

Gloser, J.: Ecophysiological study of Antarctic Lichens and Mosses. Technical report. – ANTAR XII Peruvian Antarctic Expedition, Masaryk University, Brno 2001.

Hájek, J., Barták, M., Gloser, J.: Effects of thallus temperature and hydration on photosynthetic parameters of *Cetraria islandica* from contrasting habitats. – Photosynthetica **39**: 427-435, 2001.

Heber, U., Bilger, W., Bligny, R., Lange, O.L.: Phototolerance of lichens, mosses and higher plants in an alpine environment: analysis of photoreactions. – Planta **211**: 770-780, 2000.

Heber, U., Bukhov, N.G., Shuvalov, V.A., Kobayashi, Y., Lange, O.L.: Protection of the photosynthetic apparatus against damage by excessive illumination in homoiohydric leaves and poikilohydric mosses and lichens. – J. exp. Bot. **52**: 1999-2006, 2001.

Hong, S.-S., Xu, D.-Q.: Light-induced increase in initial chlorophyll fluorescence F_o level and the reversible inactivation of PS II reaction centers in soybean leaves. – Photosynth. Res. **61**: 269-280, 1999.

Huiskes, A.H.L., Gremmen, N.J.M., Francke, J.W.: Morphological effects on the water balance of Antarctic foliose and fruticose lichens. – Antarct. Sci. **9**: 36-42, 1997

Jung, S., Steffen, K.L., Lee, H.J.: Comparative photoinhibition of a high and a low altitude ecotype of tomato (*Lycopersicon hirsutum*) to chilling stress under high and low light conditions. – Plant Sci. **134**: 69-77, 1998.

Kappen, L., Breuer, M., Bölter, M.: Ecological and physiological investigations in continental Antarctic cryptogams. 3. Photosynthetic production of *Usnea sphacelata*: diurnal courses, models, and the effect of photoinhibition. – Polar Biol. **11**: 393-401, 1991.

Kappen, L., Schroeter, B., Green, T.G.A., Seppelt, R.D.: Chlorophyll *a* fluorescence and CO_2 exchange of *Umbilicaria aprina* under extreme light stress in the cold. – Oecologia **113**: 325-331, 1998.

Koroleva, O.Y., Krause, G.H., Bruggemann, W.: Effects of long-term chilling under excessive light on xanthophyll cycle activity and non-photochemical fluorescence quenching in *Lycopersicon* genotypes. – J. Plant Physiol. **156**: 341-349, 2000.

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

Król, M., Ivanov, A.G., Jansson, S., Kloppstech, K., Hunner, P.A.: Greening under high light or cold temperature affects the level of xanthophyll-cycle pigments, early light-inducible proteins, and light-havesting polypeptides in wild-type barley and the *Chlorina f2* mutant. – Plant Physiol. **120**: 193-203, 1999.

Laisk, A., Oja, V.: Alteration of photosystem II properties with non-photochemical excitation quenching. – Phil. Trans. roy. Soc. London B **355**: 1405-1418, 2000.

Lange, O.L., Leisner, J.M.R., Bilger, W.: Chlorophyll fluorescence characteristics of the cyanobacterial lichen *Peltigera rufescens* under field conditions. 2. Diel and annual distribution of metabolic activity and possible mechanisms to avoid photoinhibition. – Flora **194**: 413-430, 1999.

Li, X.-P., Müller-Moulé, P., Gilmore, A.M., Niyogi, K.K.: PsbS-dependent enhancement of feedback de-excitation protects photosystem II from photoinhibition. – Proc. nat. Acad. Sci. USA **99**: 15222-15227, 2002.

MacKenzie, T.D.B., Król, M., Huner, N.P.A., Campbell, D.A.: Seasonal changes in chlorophyll fluorescence quenching and the induction and capacity of the photoprotective xanthophyll cycle in *Lobaria pulmonaria*. – Can. J. Bot. **80**: 255-261, 2002.

Müller, P., Li, X.-P., Niyogi, K.K.: Non-photochemical quenching. A response to excess light energy. – Plant Physiol. **125**: 1558-1566, 2001.

Pannewitz, S., Schlensog, M., Green, T.G.A., Sancho, L.G., Schroeter, B.: Are lichens active under snow in continental Antarctica? – Oecologia **135**: 30-38, 2003.

Roháček, K., Barták, M.: Technique of the modulated chlorophyll fluorescence: basic concepts, useful parameters, and some applications. – Photosynthetica **37**: 339-363, 1999.

Schroeter, B., Olech, M., Kappen, L., Heitland, W.: Ecophysiological investigations of *Usnea antarctica* in the maritime Antarctic. I. Annual microclimatic conditions and potential primary production. – Antarct. Sci. **7**: 251-260, 1995.

Schroeter, B., Schulz, F., Kappen, L.: Hydration-related spatial and temporal variation in photosynthetic activity in antarctic lichens. – In: Bataglia, B., Valencia, J., Walton, D.W.W. (ed.): Antarctic Communities, Species, Structure and Survival. Pp. 221-225. Cambridge University Press, Cambridge 1997.

Solheim, I., Engelsen, O., Hosgood, B., Andreoli, G.: Measurement and modeling of the spectral and directional reflection properties of lichen and moss canopies. – Remote Sens. Environ. **72**: 78-94, 2000.

Valladares, F., Sancho, L.G., Chico, J.M., Manrique, E.: Differences in the photosynthetic utilization of high irradiance by co-occurring lichen and vascular plants in the maritime Antarctica. – Bol. R. Soc. Esp. Hist. Nat. (Sec. Biol.) **93**: 119-125, 1997.

Verhoeven, A.S., Adams, W.W., III, Demmig-Adams, B., Croce, R., Bassi, R.: Xanthophyll cycle pigment localization and dynamics during exposure to low temperatures and light stress in *Vinca major*. – Plant Physiol. **120**: 727-737, 1999.

Xu, D.Q., Wu, S.: Three phases of dark-recovery course from photoinhibition resolved by the chlorophyll fluorescence analysis in soybean leaves under field conditions. – Photosynthetica **32**: 417-423, 1996.