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Changes in photosynthesis, pigment composition and glutathione contents in two Antarctic lichens during a light stress and recovery

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Abstract

Over last decades, several studies have been focused on short-term high light stress in lichens under laboratory conditions. Such studies reported a strong photoinhibition of photosynthesis accompanied by a partial photodestruction of PSII, involvement of photoprotective mechanisms, and resynthetic processes into gradual recovery. In our paper, we applied medium [800 μmol(photon) m^{-2} s^{-1}] light stress to induce negative changes in PSII funcioning as well as pigment and glutathione (GSH) content in two Antarctic fruticose lichen species. Chlorophyll (Chl) fluorescence parameters, such as potential and effective quantum yield of photosynthetic processes and fast transients (OJIP) recorded during high light exposition and recovery, revealed that *Usnea antarctica* was less susceptible to photoinhibition than *U. aurantiaco-atra*. This might be supported by a more pronounced high light-induced reduction in Chl *a* and *b* contents in *U. aurantiaco-atra* compared with *U. antarctica*. In both experimental species, total GSH showed an initial increase during the first 30–40 min of high light treatment followed by a decrease (60 min) and an increase during dark recovery. Full GSH recovery, however, was not finished in *U. aurantiaco-atra* even after 5 h indicating lower capacity of photoprotective mechanisms in the species. OJIP curves showed high light-induced decrease in both species, however, the recovery of the OJIPs shape to pre-photoinhibitory values was faster and more apparent in *U. antarctica* than in *U. aurantiaco-atra.* The results are discussed in terms of sensitivity of the two species to photoinhibition and their photosynthetic performance in natural environment.

Additional key words: carotenoids; chlorophyll fluorescence; performance index; thallus.

Introduction

Lichens are capable to grow and photosynthesize in various conditions. They occur in many ecosystems around the world, *e.g.* in the most extreme environments, such as Arctic tundra, the Antarctica, deserts or rain forests. Lichen thalli consist of two partners: a fungal mycobiont and a green algal or cyanobacterial photobiont. Together, the partners form a functional unit (Kranner and Birtić 2005). The fungus forms the shape of the thalli and protects the algae/cyanobacteria from direct light by the layer of upper cortex. In algal lichens, algae produce specific compounds that protect them from negative effects of high light doses, especially highly oxidative oxygen species formation, harmful to the components of algal photosynthetic apparatus. They also produce sugar alcohols that help them to survive during very long dry periods. Ribitol, mannitol, and arabitol are compounds increasing osmotic potential of algal cells and represent protective mechanism exploited during drying (Colville and Kranner 2010). Lichens contain a great variety of chemical compounds (see *e.g.* Huneck 2006). Some of them exhibit antibiotic activity (Onofri *et al.* 2000), others are involved in the protection against oxidative stress (Colville and Kranner 2010, Del Hoyo *et al.* 2011). In lichens from mountainous, subpolar, and polar regions including Antarctica, numerous UV-B screening compounds have been identified, such as *e.g.* usnic acid

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Abbreviations: ABS/RC – absorption of light energy per reaction centre; DM – dry mass; ETo/RC – photosynthetic electron transport rate per reaction centre; Chl – chlorophyll; F_v/F_m – potential quantum yield of PSII; GSH – glutathione; GSSG – glutathione, oxidized form; OJIP – fast chlorophyll fluorescence transients; PI Abs – performance index; ROS – reactive oxygen species; TRo/RC – trapping rate per reaction centre; Φ_{PSII} – effective quantum yield of PSII.
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(Bjerke *et al.* 2004, Hauck *et al.* 2007, Singh *et al.* 2011). Lichens with high amounts of UV-B absorbing compounds have typically yellow and/or black thalli (Rikkinen 1995).

Lichens from polar habitats are considered tolerant to high light. Some studies showed that high light have in most cases negative effect on photosynthesis in lichens, on their PSII-related processes in particular (*e.g.* Barták *et al.* 2003, 2004). There are some protective mechanisms which lichens can exploit during the high light stress period. Among them, desiccation-related morphological changes, such as curling of the thalli, provide effective protection for shielded thalli parts (Barták *et al.* 2006). There are also some physiological mechanisms, such as disconnection of light-harvesting complexes, acidification of thylakoid lumen, dissipation of excess excitation energy by conversion of violaxanthin to zeaxanthin or other possible pathways (Heber *et al.* 2007), emission of energy by Chl fluorescence (*e.g.* Tretiach *et al.* 2012) or scavenging of reactive oxygen species by antioxidative activity (Vráblíková *et al.* 2005). When wet lichens are exposed to high intensities of light for a long time, increased concentrations of photoprotective pigments and of the antioxidant, α-tocopherol, are found in photobiont (Kranner *et al.* 2005).

Several enzymatic and nonenzymatic compounds are involved in photoprotection of lichens, such as water-soluble ascorbate, GSH, and membrane-bound α-tocopherol (Kranner *et al.* 2005). Among them, the tripeptide, GSH, represents an important compound because it is one of indispensable antioxidants, a part of ascorbate-glutathione cycle and serves also as a sulphur storage compound (Noctor *et al.* 2002). It is located in both algal and fungal cells unlike ascorbate and α-tocopherol (Kranner *et al.* 2005). The redox couple (GSH and GSSG) has many important functions, such as storage and transport of reduced sulfur (Rausch and Wachter 2005), detoxificaton of xenobiotics, scavenging of reactive oxygen species (ROS), and also an important function in the ascorbate-glutathione cycle (May *et al.* 1998, Tausz 2001, Müller *et al.* 2004). In plant cells, GSH is localized in cytosol and organelles (Müller *et al.* 2004). The capacity of the redox system is affected by the total amount of GSH in the cells and tissues, by the redox ratio of GSH/GSSG, and by the activity of the enzymes necessary for regenerating GSH (*i.e.* NADPH-dependent glutathione reductase; Tausz 2001).

In lichens, many species- (Vráblíková *et al.* 2005, Mrak *et al.* 2010) and hydration-dependent changes of GSH (Kranner 2002) have been reported. Only few studies focused on GSH content during a high light stress and recovery in lichens (*e.g.* Štepigová *et al.* 2007). They were, however, devoted mainly to a short-term, high light stress exploiting irradiances close to 2,000 μmol m^{-2} s^{-1} of PAR (*e.g.* Barták *et al.* 2004) that led to a typical decrease in total GSH content due to photodestruction to glutamyl-cysteine or cysteine-glycine. Moreover, resynthesis of glutathione and the restoration of the initial pool takes much more time (at least hours) than reversible inter-conversion of xanthophyll cycle pigments after strong photoinhibition and recovery (Vráblíková *et al.* 2005). Much less attention has been paid to the effects of moderate, light and/or long term light treatments on GSH contents and extent of photoinhibition in lichens. In this study, we focused on the short-term treatment of lichens by a moderate light intensity to show that glutathione contents and its redox state are not necessarily related to a decrease in functioning of lichen photosynthetic apparatus, PSII reaction centres in particular, during the early phase of photoinhibition. We hypothesized that, in contrast to previous studies exploiting high light and desiccation stress (Kranner and Birtić 2005), moderate light stress applied in hydrated lichens would lead rather to an increase in total glutathione content. Such response was found in higher plants (Burrit and MacKenzie 2003, Demmig-Adams *et al.* 1990), but it has not yet been evidenced in lichens. Therefore, we focused on the effects of moderate light on GSH in hydrated lichen. In this paper, we evaluated the extent and a time course of GSH in two fruticose lichen species treated by moderate light in order to quantify treatment- and species-specific changes in GSH. We expected interspecific differences in their sensitivity to photoinhibition and GSH response to light treatment. Therefore, we combined Chl fluorescence approach to evaluate the extent of photoinhibition in PSII and analyses of GSH contents as dependent on photo-inhibitory treatment and recovery.

Materials and methods

Sample collection and handling: In February 2013, lichen thalli of *Usnea antarctica* were collected at the James Ross Island (Antarctica) at several sites in Halozetes Valley (63°48′57″ S, 57°50′30″ W, 260 m a.s.l.). The collection sites were distributed within a 300 × 100 m area located at hyaloclastite breccia boulders forming an upper layer of ice-cored moraine. Hyaloclastite breccia boulders represent a typical lithotype of James Ross Island Volcanic Group (Košler *et al.* 2009). These rocks are approx. 5 million years old (Smellie *et al.* 2008) and have been transported by small glacier forming a moraine rim of a glacial cirque (Davies *et al.* 2013). High abundance of the species was possible due to local microclimate, availability of snow precipitation, and liquid water in particular (Láska *et al.* 2011). Thalli of *U. antarctica* formed a rich cover (90% of the area), individual thalli clusters were found predominantly on the upper surfaces and more or less vertically oriented. After the collection, the thalli were dried out naturally, stored at 5ºC, and transported to the laboratories in Brno, Czech Republic. The thalli were kept in the dark in the dry state at 5ºC. Before experiments, the thalli were rewetted for 48 h by spraying with

demineralized water and wet cellulose sheet located beneath the thalli. During rewetting, the thalli were exposed to a dim light (20 μmol m^{-2} s^{-1} of PAR) at 5ºC so that photosynthetic processes could be fully restored (tested by Chl fluorescence until maximum values of potential quantum yield of PSII (F_v/F_m) were reached, data not shown). Thalli of *Usnea aurantiaco-atra* were collected from a lichen-rich locality at the King George Island (62°12'20"S, 58°57'10"W, 50 m a.s.l.), Antarctica. The collection site was located on the top of pronounced elevation formed by basaltic, andesitic, and pyroclastic volcanic rocks. These rocks cropping out here are approx. 8 million years old. Clusters of *U. aurantiaco-atra* thalli were oriented in a range of angles over individual stones (below 50 cm), however, only vertically oriented ones were collected for further experiments. After transport to the Czech Republic, the thalli were stored and handled in the same way as *U. antarctica* before experiments. The two experimental species are representatives of lichens having fruticose morphology of the thallus. However, they differ in their thallus size and branching pattern (Fig. 1). *U. aurantiaco-atra* is typically much taller and branched than *U. antarctica*. Moreover, *U. aurantiaco-atra* possesses apothecia, *i.e.* fruiting bodies located at the tips of some thalli. Both experimental species have green alga *Trebouxia* sp. as a photosynthesizing partner.

Photosynthetic pigments: Chls and carotenoids (Car) were extracted from dry lichen powder by acetone with $MgCO_3$ and fine sand grains. Extracts were measured by a spectrophotometer (*Specord*, *Analytik*, Jena, Germany) following the procedure optimized for lichens (Štepigová *et al.* 2007). After extraction, contents of Chl *a*, Chl *b*, and Car were calculated using the formulae by Holm (1954).

Glutathione contents: Segments of lichens were taken from the control (before exposition to experimental light, *see* photoinhibitory treatment) and light-treated lichen thalli after 10, 20, 30 min exposition to light and consequent recovery (recovery time: 10, 30, 60, and 360 min). Content of total glutatione (GSH) was then determined in each segment (typically 5 segments per treatment and sampling time) according to Kranner (1998). Segments of lichen thalli were frozen in liquid nitrogen and lyophilized. Then they were homogenized using a ball mill (*Retsch MM 2000*, *Retsch*, Germany). Dry powder (0.06 g) was used for extraction of GSH. For determination of GSH, a method of labeling thiols with monobromobimane (mBBr) was used (Kranner 1998). Dry lichen powder was extracted with 2 ml 0.1 M HCl with addition of polyvinylpyrrolidone (PVP). It eliminates negative effects of phenolic compounds, which can interact with GSH during extraction. Disulphidic groups were reduced by dithiothreitol (DTT). Thiol groups were labeled with mBBr. Samples were incubated 15 min in the dark at room temperature. Labeling was stopped by 0.25% methanesulfonic acid. GSH was separated and quantified by a reverse-phase HPLC (Separation module *2690*, *Waters*, USA) with linear gradient using a fluorescence detector (*RZ-535*, *Shimadzu*, Japan) and C_{18} separation column. Solvent composition was water/methanol/acetic acid (1000/50/2.5) and water/methanol (1/9), respectively. Details on linear gradient and changes in solvent composition within a single run are summarized in Table 1.

Fig. 1. Silhouettes of typical thalli clump of *Usnea aurantiaco-atra* (*above*), and *Usnea antarctica* (*below*) showing different dimensions of thalli clumps. The thalli were photographed in fully hydrated state, thus, dehydration-dependent changes in size and structure were avoided. The bar represents 5 cm.

Photoinhibitory treatment: Before experiments, thalli of *U. antarctica* and *U. aurantiaco-atra* were handled as described above. To quantify the effect of medium irradiance on the GSH content in thalli, wet thalli were exposed to 800 μmol m^{-2} s^{-1} PAR for 1 h. As a source of homogenous light a LED source (*UTEE*, *Technical University Brno*, Czech Republic) composed of 17 superbright white warm LEDs (*Luxeon Warm-White*, *Philips Lumileds*, USA) providing a continuous spectrum was used. During the experiment, lichen thalli segments were placed in Petri dishes placed on ice to keep constant thalli temperature of 5.0 ± 0.4ºC (measured and recorded by a *HOBO thermometer*, *OnSet Computers*, USA). After photoinhibitory treatment, the thalli were moved into a place with light intensity of about 10 μmol m^{-2} s^{-1} PAR for the recovery phase (6 h). Segments of lichen thalli for the analysis were taken in 10 min intervals during the exposition and after 10, 30, 60, and 360 min of the recovery phase.

Quantum yields: Measurements of the below-specified Chl fluorescence parameters were made before and after the above-described, short-term photoinhibitory treatment. For the effective quantum yield of PSII (Φ_{PSII}) evaluation, actinic light was provided by a warm-white LED source (*UTEE*, Brno, Czech Republic). The light source was composed of 9 *Luxeon Rebel* warm-white LEDs (*Philips Lumileds*, USA) with continuous spectrum. Similarly to the photoinhibitory treatment, the lichen thalli were cooled

Table 1. HPLC linear gradient and particular steps of HPLC run used in GSH analyses. Solvent A consisted of H_2O/methanol/acetic acid (1000/50/2.5). Solvent B consisted of H_2O/methanol (1/9). For a single analysis, 26.3 and 9.8 ml of solvent A and B, respectively were used. In solvent A and B, pH was kept constant at 3.9 using a 10 M NaOH (HCl). Temperature of a column and a sample were maintained at 27 and 4°C, respectively.

HPLC run time [min]	Flow [ml min^{-1}]	Solvent A [ml]	Solvent B [ml]
0	1	95	5
20	1	85	15
26	1	0	100
34	1	95	5
36	1	95	5

by an ice jacket during Φ_{PSII} measurements. Sample temperature was kept constant (8ºC) and monitored by a thermocouple linked to a datalogger (*EdgeBox V8*, *Environmental Monitoring Systems*, Brno, Czech Republic) in 5-min interval. Chl fluorescence parameters were measured also after the light treatment, *i.e.* during recovery after 30, 60, and 270 min in the dark. First, dry thalli were rehydrated for 48 h in Petri dishes with a wet paper under dim light at 5ºC. After rehydration, the best places for fluorometric measurements within a branching structure of thalli were selected using Chl fluorescence imaging (*FluorCam HFC-010*, *Photon Systems Instruments*, Czech Republic). For further measurements, thalli parts exhibiting the highest Φ_{PSII} values were selected, typically at basal and/or intermediate thalli parts. Tiny, dark-pigmented, upper thalli parts were not taken into consideration since they showed lower Φ_{PSII} values than the rest of thalli. After the selection of measuring areas, thalli were predarken for 10 min and slow induction of Chl fluorescence supplemented with saturation pulses was measured according to Barták *et al.* (2008). A pulse-modulated fluorometer (*PAM 2000*, *Heinz Walz*, Germany) and *DA-2000* software were used to evaluate F_v/F_m and Φ_{PSII}. The F_v/F_m was calculated as $(F_m - F_0)/F_m$. For the Φ_{PSII} measurements, actinic light of 60 µmol m^{-2} s^{-1} PAR was used and combined with a saturation pulse applied after 5 min, when a steady-state Chl fluorescence level was reached. Then, Φ_{PSII} was calculated as $(F_m - F_s)/F_m'$. During experiment, the thalli were sprayed regularly by water to keep them moist.

Fast Chl fluorescence transients (OJIPs): To assess the effect of light treatment on PSII functioning, fast Chl fluorescence transients (OJIPs) were measured by a *FluorPen FL 1000* fluorometer (*Photon Systems Instruments*, Czech Republic) and analyzed according to Strasser *et al.* (2000). The measurements were taken repeatedly on each sample, *i.e.* before and after the photoinhibitory treatment, and then after 30, 60, and 270 min of dark recovery. From the OJIPs, parameters characterizing the yields and absorbed energy fluxes per reaction center of PSII and/or per cross section were calculated using a *FluorPen 1.0.4.0* software. The below parameters were selected: the performance index (PI Abs), absorption of light energy per reaction centre (ABS/RC), trapping rate per reaction centre (TRo/RC), and photosynthetic electron transport rate per reaction centre (ETo/RC).

Statistical analyses: The differences in pigment contents, glutathione, Chl fluorescence parameters as related either to species or the state before and after the photoinhibitory treatment were statistically analyzed using a one-way analysis of variance (*ANOVA*) and a LSD test (*STATISTICA v. 12* software package) to evaluate statistically significant differences (P=0.05).

Results

Pigments, UV-absorbing compounds and glutathione: *U. antarctica* had higher contents of Chl *a* than *U. aurantiaco-atra* (Table 2). Surprisingly, the opposite was true for Chl *b*. Thus, substantial difference in the ratio of Chl *a*/*b* was found between the species. In *U. antarctica*, the ratio was three times higher than that in *U. aurantiaco-atra*. There were not statistically significant differences in Car contents between the species. After the exposition to 800 µmol(photon) m^{-2} s^{-1}, a decrease of the Chl *a* concentration in *U. aurantiaco-atra* was apparent (Fig. 2) while changes in Chl *b* concentration were not statistically significant in both species. There were not any significant, light treatment-induced changes in the content of Car. However, the Car content was slightly higher in *U. antarctica* than in *U. aurantiaco-atra*. Total amount of GSH was twice as large in *U. antarctica* [78.9 nmol g^{-1} (DM)] than that in *U. aurantiaco- atra* [49.0 nmol g^{-1} (DM)] before the short-term, medium-light treatment. When exposed to the photoinhibitory treatment, the GSH content showed statistically insignificant changes exhibiting both an increase and decrease (Fig. 3) ranging within the interval of 60–108 nmol g^{-1}(DM) in *U. antarctica* and 19–90 nmol g^{-1}(DM) in *U. aurantiaco-atra*. With only one exception, no change in the GSH content

Table. 2. Natural content of photosynthetic pigments in *U. antarctica* and *U. aurantiaco-atra* (n = 5).

	Chl *a* [mg g^{-1}(DM)]	Chl *b* [mg g^{-1}(DM)]	Chl *a*/Chl *b*	Car [mg g^{-1}(DM)]
Usnea antarctica	0.0584 ± 0.006	0.0200 ± 0.009	3.6634 ± 0.807	0.0379 ± 0.006
Usnea aurantiaco-atra	0.0406 ± 0.005	0.0336 ± 0.009	1.2629 ± 0.232	0.0337 ± 0.007

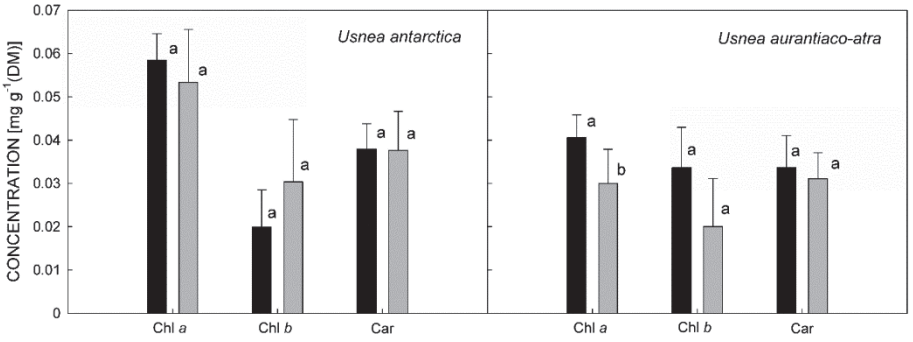

Fig. 2. Photosynthetic pigment contents in *Usnea antarctica* (*left*) and *Usnea aurantiaco-atra* (*right*). Bars denote SD (n = 5). The *black columns* indicate pigment contents evaluated before photoinhibitory treatment. *Grey columns* represent pigment contents evaluated immediately after photoinhibitory treatment. Characters (a, b) denote statistically significant difference (0.05) related to photoinhibitory treatment effect in a single lichen species.

was apparent in both species when GSH values recorded during photoinhibitory treatment were compared to those recorded during recovery (Fig. 2).

Effective quantum yield and Chl fluorescence parameters: Time courses of F_v/F_m and Φ_{PSII} showed light-dependent responses (Fig. 4). Immediately after the photoinhibitory treatment, a decrease in F_v/F_m was found in *U. antarctica* (45.4 and 53% of the initial values) and *U. aurantiaco-atra* (28.9 and 66.5%), respectively. Fast phase of recovery was completed 30 min after the end of the photoinhibitory treatment in *U. antarctica* species (Fig. 4, *cf.* F_v/F_m values recorded at 60 and 90 min) while it was slower in *U. aurantiaco-atra* (Fig. 4, *cf.* F_v/F_m values recorded at 60 and 120 min still did not differ significantly). The slow phase of recovery led to almost completed return of F_v/F_m values to pre-protoinhibitory values in *U. antarctica*, however, full recovery was not achieved since pre-photoinhitory F_v/F_m values differed significantly from those recorded at the end of the recovery period (270 min, Fig. 2). In *U. aurantiaco-atra*, the difference in F_v/F_m values recorded before the photoinhibitory treatment and at the end of the recovery period remained high indicating that the slow phase of PSII recovery was far from being completed. The time courses showed more pronounced photoinhibition in *U. aurantiaco-atra* than that in *U. antarctica* (F_v/F_m of 0.2 compared to 0.3, respectively, reached immediately after the photoinhibitory treatment) and less effective recovery.

In time courses of Φ_{PSII}, no photoinhititory effect was found in *U. antarctica* and *U. aurantiaco-atra.* Throughout the photoinhibitory and recovery periods, Φ_{PSII} values

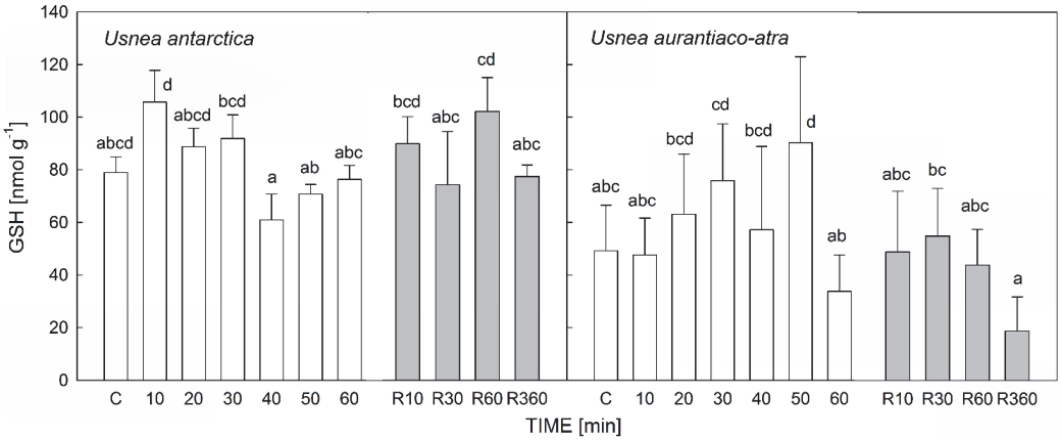

Fig. 3. Glutathione (GSH) contents in *Usnea antarctica* (*left*) and *Usnea aurantiaco-atra* (*right*) during photoinhibitory treatment (800 µmol m^{-2} s^{-1} PAR) and consequent recovery. The *white columns* indicate glutathione contents evaluated during photoinhibitory treatment, *grey columns* during recovery phase. Characters (a, b, c, d) denote statistically significant difference (0.05) in a single lichen species during photoinhibitory treatment and consequent recovery.

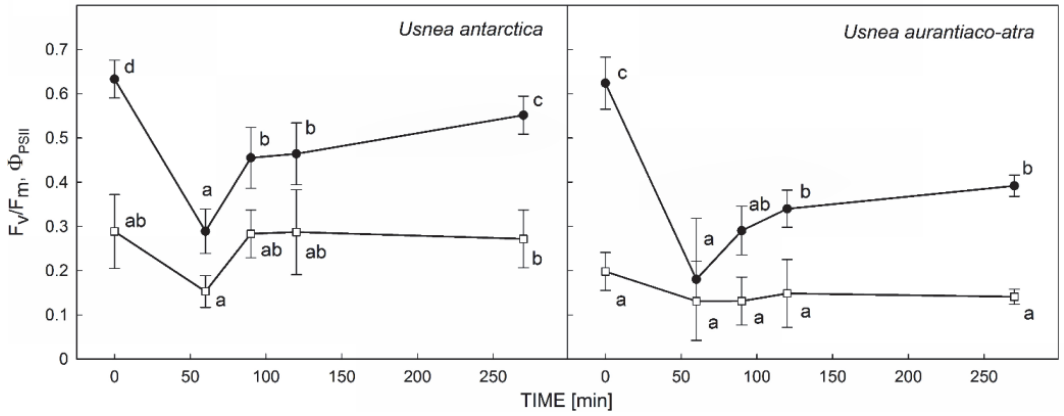

Fig. 4. Time courses of maximum quantum yield of PSII (F_v/F_m, *full symbols*) and effective quantum yield of PSII (Φ_{PSII}, *open symbols*) for *Usnea antarctica* (*left*) and *Usnea aurantiaco-atra* (*right*) exposed to high-light stress of 800 μmol m^{-2} s^{-1} PAR for 60 min followed by a 240 min-lasting recovery. Characters (a, b, c, d) denote statistically significant difference (0.05) for a single time course of individual lichen species.

changed only within a narrow interval and did not show any significant photoinhibitory treatment-dependent decrease.

Similarly to F_v/F_m and Φ_{PSII}, OJIPs showed the photoinhibition-dependent response. Compared to pre-photoinhibition control, both species exhibited a strong decrease in Chl fluorescence values throughout the OJIP curves recorded immediately after the photoinhibition treatment (Fig. 5). In *U. antarctica*, a decrease in F_P to 36.4% of the initial values was found. A less pronounced decrease was found in *U. aurantiaco-atra.* F_P reached 41.5% of pre-photoinhibition value. A photoinbitory treatment-dependent increase of particular levels of Chl fluorescence (F_0 = O, F_J, F_I, and F_D) was found in both experimental species. Here we reported only the increases of F_J and F_I that are attributed to the effectivity of photosynthetic electron transport from Q_A to plastoquinone pool. They reflect the redox state of the electron carriers located in a close proximity to PSII. Immediately after the photoinhibitory treatment, the F_I/F_P ratio increased from 0.818 to 1.000 in *U. antarctica.* The same parameters reached 0.849 and 0.965 in *U. aurantiaco-atra.* High light treatment led to flattening of the OJIP shapes in both species which resulted in $F_J = F_P$ in photoinhition-treated samples. Therefore, photoinhibition brought a change in F_I/F_P from positive to negative values. With the time of recovery, OJIP shapes and absolute values of Chl fluorescence showed a partial return to pre-photoinhibition values. In both species, however, the recovery did not reach the initial values (F_P of 74.3% in *U. antarctica*, 77.2% in *U. aurantiaco-atra*). While gradual increase of Chl fluorescence values (OJIP shapes) was apparent in *U. antarctica*, *U. aurantiaco-atra* showed a rapid partial recovery during the first 30 min followed by a much slower recovery within the next 60 min. The effect of the photoinhibition treatment on OJIP-derived parameters characterizing functioning of energy conversion in PSII is shown in Table 3. Immediately after the photoinhibitory treatment (60 min, Table 3), an increase/decrease was found in PI Abs values in *U. aurantiaco-atra*/*U. antarctica*, respectively. ABS/RC and TRo/RC showed an increase/decrease after 60 min of photoinhibitory treatment in *U. antarctica*/*U. aurantiaco-atra*, respectively. ETo/RC showed photoinhibition-induced decrease in *Usnea antarctica*, while remained unchanged in *U. aurantiaco-atra*. In both lichen species, the OJIP-derived parameters showed completed recovery to pre-photoinhibitory values after 4.5 h of recovery. Statistically significant interspecific differences were apparent in all the parameters only immediately after the photoinhibitory treatment (60 min), while no statistically significant interspecific difference was found during recovery.

Discussion

Total Chl *a* content expressed per dry mass unit was found lower in *U. aurantiaco-atra* than in *U. antarctica.* The photoinhibitory treatment brought the significant decrease in Chl *a* content only in *U. aurantiaco-atra*, while Chl *b* content was unaffected in both species. This might indicate the negative effect of high light on LHCs in *U. aurantiaco-atra* while *U. antarctica* remained unchanged. Such a change in Chl *a* might be attributed to a photodestruction of Chl molecules in *U. aurantiaco-atra* due to ROS formed during a high light treatment (Del Hoyo *et al.* 2011).

Surprisingly, total Chl contents, irrespective whether evaluated before or after the photoinhibitory treatment, showed somewhat lower values in both species than that reported for lichens of *Usneaceae* family: *Usnea hirta*

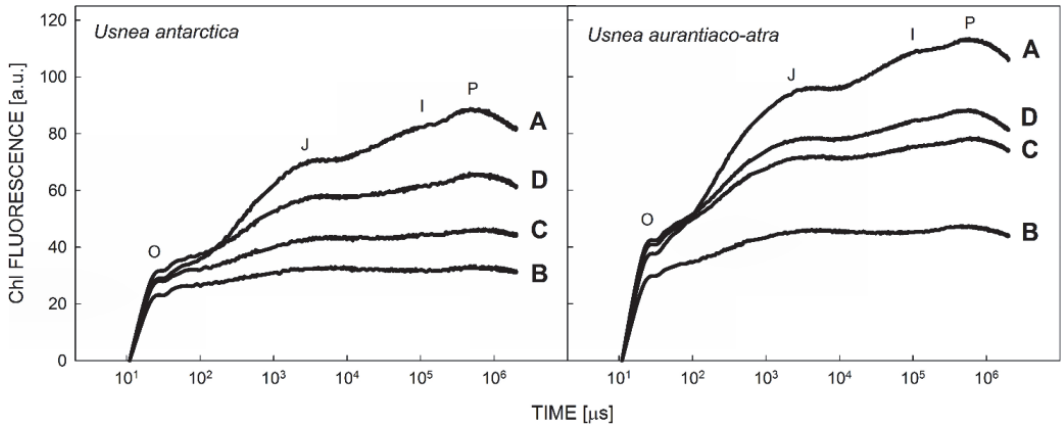

Fig. 5. Effect of photoinhibitory treatment (800 μmol m^{-2} s^{-1} for 60 min) on a decrease of chlorophyll fluorescence values forming OJIP transients and their consequent recovery in dark. At individual curve, O, J, I, and P points represent increasing chlorophyll fluorescence values indicating an increased number of reduced quinone molecules during exposition to short-term light pulse. The curves represent the OJIPs recorded before (A) and immediately after (B) photoinhibitory treatment, and after 30 (C) and 90 min (D) of consequent recovery.

Table. 3. Chlorophyll fluorescence parameters derived from OJIPs recorded in lichens before (control), and after photoinhibitory treatment (800 μmol m^{-2} s^{-1} for 60 min). The parameters calculated during following dark recovery are reported as the values recorded at the following time: 90, 120, and 330 min. Values are means of at least 3 replicates ± SD. Statistically significant differences are indicated as *different letters* in the upper case (LSD test).

OJIP parameters	Control	60 min	90 min	120 min	330 min
Usnea antarctica					
PI Abs	0.212 ± 0.083^{d}	0.015 ± 0.010^{a}	0.036 ± 0.027^{ab}	0.106 ± 0.059^{bc}	0.145 ± 0.073^{cd}
ABS/RC	3.481 ± 0.583^{a}	7.765 ± 1.375^{b}	6.341 ± 1.786^{b}	4.641 ± 0.949^{a}	4.151 ± 0.690^{a}
TRo/RC	2.338 ± 0.343^{a}	3.153 ± 0.391^{b}	2.926 ± 0.569^{b}	2.647 ± 0.411^{ab}	2.599 ± 0.327^{ab}
ETo/RC	0.645 ± 0.138^{b}	0.370 ± 0.076^{a}	0.608 ± 0.315^{b}	0.613 ± 0.139^{b}	0.623 ± 0.158^{b}
Usnea aurantiaco-atra					
PI Abs	0.145 ± 0.073^{b}	0.284 ± 0.131^{c}	0.032 ± 0.026^{a}	0.087 ± 0.076^{ab}	0.147 ± 0.095^{b}
ABS/RC	4.151 ± 0.690^{ab}	3.444 ± 0.409^{a}	6.440 ± 1.845^{c}	5.009 ± 0.944^{b}	4.371 ± 0.725^{ab}
TRo/RC	2.599 ± 0.327^{ab}	2.454 ± 0.250^{a}	2.784 ± 0.315^{b}	2.643 ± 0.215^{ab}	2.551 ± 0.249^{ab}
ETo/RC	0.623 ± 0.158^{ab}	0.631 ± 0.088^{b}	0.418 ± 0.179^{a}	0.487 ± 0.262^{ab}	0.640 ± 0.164^{b}

[0.8–2.8 mg g^{-1}(DM), Riddell *et al.* 2012], *U. amblyoclada* [1.47 mg g^{-1}(DM), Carreras *et al.* 2005], *U. aurantiaco-atra* [0.2–1.8 mg g^{-1}(DM), Palmqvist *et al.* 2002]. The cause of such a difference is unknown, however, several reasons might be considered such as *e.g.* interspecific, intrathalline, age- and light regime of the site of collection-related differences. Even samples of a single lichen species collected at the same localities in Antarctica may vary substantially in Chl content. In our study, the samples had generally low total Chl contents (Table 2) while other samples from the same localities (*U. antarctica* – James Ross Island, *U. aurantiaco-atra* – King George Island), collected and analyzed in our laboratory earlier, showed higher values: 0.75 mg g^{-1}(DM) in *U. antarctica* (Bohuslavová 2012), and 0.23 mg g^{-1}(DM) in *U. aurantiaco-atra* (Krábková 2013).

The studied species differed in the total GSH pool, however, they did not show significant changes of GSH pool during the high light treatment. Moreover, during the recovery period, the GSH pool in *U. antarctica* and *U. aurantiaco-atra* was almost the same as the initial value before the photoinhibitory treatment. This shows a high constitutive capacity of GSH (Kranner 2002) in both species because photoinhibitory treatment [800 μmol (photon) m^{-2} s^{-1} for 1 h] did not lead to light-induced GSH decrease as reported for higher PAR doses (Barták *et al.* 2003). Thus, light doses of about 800 μmol m^{-2} s^{-1} PAR might be considered as moderate and causing no reduction in the GSH content and its redox state. Under such light conditions, the concentration of glycine (GSH substrate) increases (Tausz 2001) and degradation of GSH is slower than synthesis (Szalai *et al.* 2009). Therefore, changes in the GSH content during the high light treatment found in our study might be considered a dynamic response of the GSH pool to mild or moderate light stress. This is comparable to the evidence from higher plants. Noctor and Foyer (1998) reported that the amount of GSH increased during or after experimental light treatment. However,

Burrit and MacKenzie (2003) showed a decrease in GSH concentration in shaded *Begonia* leaves transferred to full sunlight which did not cause a dynamic change of GSH but a decrease in response to acute high light stress. The same response might be found in lichens. If a light dose is larger than that causing dynamic response of GSH, an acute short-term photoinhibition occurs in lichens and leads to a rapid destruction of GSH to glutamylcystein and glycin (Vráblíková *et al.* 2005, Štepigová *et al.* 2007). Such destructive changes of GSH lead to resynthesis of GSH during dark or low light-recovery of lichens from strong photoinhibitory stress. In our study, a slow increase during the medium light stress treatment was observed. This increase could be explained as a light-dependent synthesis of GSH due to the fact that light stress was probably not strong enough to cause degradation of GSH. The changes in the GSH pool might be therefore attributed to dynamic changes in response to medium light stress.

The short-term exposition to 800 μmol m^{-2} s^{-1} PAR led to a decrease in F_v/F_m and Φ_{PSII} as well as fast recovery in *U. antarctica*. The result is comparable to the earlier study made on the same species (Barták *et al.* 2003) in which 70 and 60% recovery was found for F_v/F_m and Φ_{PSII} after 240 min of dark recovery from strong photoinhibition (2,000 μmol m^{-2} s^{-1} of PAR). More recent study (Barták *et al.* 2011) using the same photoinhibory treatment, but Chl fluorescence imaging approach, led to similar conclusions that the fast phase of recovery (up to 70% of pre-photoinhibition values) occurs within the first 30 min after the photoinhibitory treatment indicating the high resistance of *U. antarctica* to photoinhibition. This might be attributed to effective photoprotective mechanisms, such as quenching of excitation energy *via* zeaxanthin (Demmig-Adams *et al.* 1990) or the state 1-2 transition (Wendler and Holzwarth 1987, Veerman *et al.* 2007). In our study, significant recovery of Φ_{PSII} was apparent after 30 min in *U. antarctica.* It indicates the sufficient capacity of photoprotective mechanisms to cope with photoinhibition. Similarly, Singh *et al.* (2013) reported a 15–20 min time range comprising the fast phase of recovery of Φ_{PSII} and nonphotochemical quenching in photoinhibition resistant lichen, *Stereocaulon foliosum*. In our study, *U. aurantiaco-atra*, however, showed more pronounced decrease than that in *U. antarctica* and the uncompleted recovery. These differences support an idea of higher sensitivity of *U. aurantiaco-atra* to photoinhibition than that of *U. antarctica.*

It is well established that Chl fluorescence values forming OJIP transient decrease with photoinhibitory stress in PSII leading to a flattening of the OJIP curves. Such response is reported both in higher plants and lichens (Barták 2014). In our study, F_0 (*i.e.* Chl fluorescence at "O" point) decreased when measured immediately after the photoinhibitory treatment followed by an F_0 increase during recovery. Some studies, however, reported only an increase in F_0 caused by photoinhibitory treatment (Manrique *et al.* 1993). Such apparently contradictory results might be explained by differences in exposition time. Short-term high light treatments lead to F_0 decrease in majority of algal lichens (*see e.g.* Barták *et al.* 2011) reflecting fast and dynamic changes happening in photosynthetic apparatus, in LHC in particular, such as detachment of LHCII from PSII core (Bilger *et al.* 1989), aggregation of LHCs and/or partial photodestruction of LHCII. Long-term photoinhibitory treatments, however, lead to acclimatory changes which are typically manifested as F_0 increase compared to untreated controls. OJIP-based analysis of functional effectivity of LHCII and core of PSII under radiation stress has been studied recently (Stirbet 2013).

Recovery of OJIP transient, *i.e.* changes in OJIP shape towards pre-photoinhibitory status, was faster and more pronounced in *U. antarctica* than in *U. aurantiaco-atra* documenting higher sensitivity of photosynthetic apparatus to photoinhibition in the latter one. This conclusion might be supported also by the photoinhibition-induced changes of ABS/RC and TRo/RC, which were positively affected in *U. antarctica*, while the negative effect was apparent in *U. aurantiaco-atra* immediately after the photoinhibitory treatment. During recovery, both experimental species showed similar values and trends in OJIP-derived parameters. Thus, interspecific differences in PSII sensitivity to photoinhibition were apparent only just after photoinhibition. ABS/RC and TRo/RC are specific fluxes in PSII evaluating effectivity of energy flow in PSII before Q_A, while ETo/RC evaluates photosynthetic performance after Q_A (Strasser *et al.* 2000).

In our study, *U. aurantiaco-atra* showed higher sensitivity to photoinhibition of photosynthetic processes than *U. antarctica*. In field studies from Antarctica (Kappen *et al.* 1991), different extent of photoinhibition in gasometrically-measured photosynthesis is reported for *U. spacelata*. The authors attributed photoinhibition to the light quantity available at the site of lichen growth. For thalli grown at sunny habitats, lesser sensitivity to photoinhibition was found than that from shaded sites. In our study, the site of collection should not be a reason for the difference found in sensitivity to photoinhibition since both species were collected from the sunny sites. However, austral summer light regimen differs between the sites of collection because James Ross Island and King George Island are located in two distinguished biogeographical regions (Terauds *et al.* 2012). Therefore, differences in regional and local light climate might be considered as possible reasons for interspecific differences. Among intrinsic factors, affecting sensitivity to photoinhibition in the two studied species, intrathalline distribution of symbiotic algal cells, colour of thalli, thickness of upper cortex, and complexity of thallus branching might be other reasons for the differences found in their sensitivity to photoinhibition. In this concept, individual "branches" of shrub-like thalli may exhibit different sensitivity to photoinhibition due to self-shading effect when thallus is in a natural orientation in the field. Such analysis,

however, has not yet been made and seems to be a target for future studies focused on modelling of light interception of *Usnea* sp. clumps under different radiation and hydration regimen. Apart from a thallus dimension, the two *Usnea* species used in our study differed in their colour. In the field, many "ecotypes" exist showing a wide range of colours from pale to black according to prevailing light environment. To avoid variability in sample colour and its possible effect on sensitivity to photoinhibition, the typical, yellow thalli showing uniform colour with minimum black parts were selected in both species. However, the experimental species, when compared, showed the different extent of black thalli parts. In *U. antarctica*, upper and middle thalli parts showed much more black microareas and/or spots due to the presence of secondary metabolites located in the upper cortex than the same thalli parts in *U. aurantiaco-atra*, which exhibited rather yellowish than black colour. Therefore, less pronounced sensitivity to photoinhibition in *U. antarctica* might be attributed to more effective photoprotection provided by secondary compounds.

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