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BRIEF COMMUNICATION

Irradiance prior to and during desiccation improves the tolerance to excess irradiance in the desiccated state of the old forest lichen *Lobaria pulmonaria*

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Abstract

Hydrated thalli of the lichen *Lobaria pulmonaria* were either preconditioned to dim irradiance (DI, 5 μmol m^{-2} s^{-1}) or medium irradiance (MI, 200 μmol m^{-2} s^{-1}) for 6 h. After this 6 h period, the thalli were allowed to desiccate under the two respective irradiances. Thereafter, these dry lichens were exposed to high irradiance (HI, 1 000 μmol m^{-2} s^{-1}) for 60 h. After this HI treatment, the maximal photochemical quantum yield (F_V/F_M) and the de-epoxidation state of xanthophyll cycle pigments (DEPS) were highest in thalli preconditioned to MI. Hence irradiance in the last hydrated period before sampling is significant for the physiological state of lichens. A standardized irradiance pre-treatment before start of experiments is recommended.

Additional keywords: chlorophyll fluorescence; photosynthetic quenching; photoinhibition; xanthophyll cycle.

A lichen is a symbiotic association between one or more autotroph photobionts and the species-specific heterotroph fungal partner, the mycobiont. These symbiotic organisms possess various mechanisms to protect themselves against excessive radiant energy. The mycobiont produces various pigments in the upper cortex that screen excess photons and/or UV-B for the photobiont situated in the upper part of the medulla, like melanins (Gauslaa and Solhaug 2001), parietin (Solhaug and Gauslaa 1996, 2004), and usnic acid (McEvoy *et al.* 2006, Nybakken and Julkunen-Tiitto 2006). Furthermore, during desiccation, the fungal tissues curl, causing shade and thereby reduced photoinhibition in the photobiont (Barták *et al.* 2006). Among the photoprotective traits of the photobiont, xanthophyll cycle is important (Adams *et al.* 1993). The xanthophyll cycle is an irradiance-triggered conversion of violaxanhin (V) *via* antheraxanthin (A) to zeaxanthin (Z). Z is considered to be a thermal dissipater of excess photon energy (Frank *et al.* 1994). The reverse conversion from Z to V takes place when the radiant energy is no more in excess.

Given some time, lichens acclimate to the environment both on spatial (*e.g.* Gauslaa *et al.* 2006) and temporal scales (Gauslaa and McEvoy 2005, Vráblíková *et al.* 2006). Also short-term conditions during the last previous desiccation event in the field may possibly influence the physiological state of lichens. We do not usually know the irradiance and heat doses experienced during the last desiccation cycles preceding the collection. Nevertheless, laboratory experiments dealing with lichen physiology are often based on samples collected in the field with unknown irradiance during the last desiccation cycles. Standardized pre-conditioning before the start of any experiment aims at minimizing the influence of previous field conditions on the physiological state. Preconditioning the thalli in wet state and low irradiance for *e.g.* 24 (Barták *et al.* 2003, 2004, Vráblíková *et al.* 2005) or 48 h (*e.g.* Gauslaa and Solhaug 2000, 2004, Solhaug and Gauslaa 2004) is common. However, there is a need to analyze effects of such preconditioning practices. We aim to do so by studying thalli that have experienced different irradiances during the last desiccation cycle.

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We focused on the epiphytic foliose old forest lichen *Lobaria pulmonaria* that is susceptible to high irradiance, HI (*e.g*. Gauslaa and Solhaug 1999, 2000). Tschermak-Woess (1995) observed HI susceptibility of *Dictyochloropsis reticulata,* the green algal photobiont of *L. pulmonaria*. Despite of photosynthetic inactivation in desiccated thalli (Bilger *et al.* 1989), photoinhibition can be strong also in desiccated thalli of *L. pulmonaria* (Gauslaa and Solhaug 1996, Barták *et al.* 2006). Our objective was to study how irradiance prior to and during the last desiccation cycle influences the susceptibility to HI in the desiccated state in *L. pulmonaria*.

L. pulmonaria was collected on March 11, 2006 from *Quercus petraea* at Husåskollen, Froland, AustAgder, S. Norway (58°35'N, 8°32'E, 360 m a.s.l.). Sampled thalli were dried at room temperature before they were put in a freezer at –20 °C until experiment took place (within 8 weeks). Thalli were mixed, and 40 thalli (each 2–4 cm^2 large) were randomly selected for experiment. They were randomly divided in two sample sets: (*1*) 10 thalli for chlorophyll (Chl) fluorescence measurements, (*2*) 30 thalli for xanthophyll and Chl extraction, 10 of them for control, 10 for 6-h treatment, and 10 for 60-h treatment (see Fig. 1). All thalli were sprayed with de-ionized water and kept moist on wet filter paper under a dim irradiance (DI) for 24 h to allow for relaxation of short term down-regulation of photosystem 2 (PS2). Afterwards, each thallus of both sample sets was divided from tip to basis into two similar parts and sprayed with excess water. These thallus pairs were kept hydrated on wet filter paper for another 6 h during which one of each pair was treated with 200 µmol m^{-2} s^{-1} (MI pre-treatment) and repeatedly sprayed with water. The MI treatment is close to saturation irradiance for photosynthesis in *L. pulmonaria* (MacKenzie *et al.* 2001). Furthermore, irradiance up to this level is presumably representative for lichen photosynthesis in the field, because strong direct sun causes a nearly instant desiccation. The other pairs were kept at DI of less than 5 µmol m^{-2} s^{-1} (DI-pre-treatment). No spraying was required to avoid desiccation in DI. After the 6-h wet period, the thalli were placed on dry filter paper on which they naturally dried to an air-dried state within less than 2 h in each of the two pre-treatments (MI and DI). Finally, the dry MI and DI samples were exposed to 1 000 µmol m^{-2} s^{-1} for 60 h. A plexiglass tray with a 10-cm layer of water was inserted between the lamp and the lichens to reduce heat radiation during the HI treatment. A mercury metal halide lamp (*Philips HPI* 400 W), that does not produce UV-B or UV-C (unpublished data), was used for all treatments. The whole experiment was done in a growth chamber at 12 °C and 75 % air humidity.

After standard preconditioning, the initial (control) state of sample set *1* was assessed by measuring F_V/F_M. The Chl fluorescence parameters resulting from quenching analysis were measured after 6-h pre-treatments (MI and DI) and after the subsequent 60-h HI treatment (see Fig. 1). Chl fluorescence measurements were done in hydrated state. Before the last measurement, the dry thalli after 60-h HI treatment were hydrated for 24 h under DI to allow for relaxation of short term down-regulation of PS2.

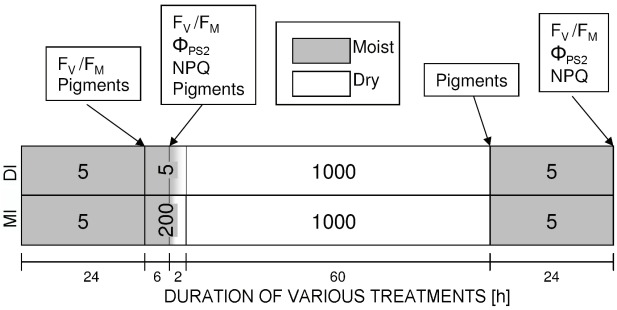

Fig. 1. Time schedule (*x-axis*) of irradiance treatments (*numbers in boxes*) [µmol m^{-2} s^{-1}] in the dim irradiance (DI) and medium irradiance (MI) pre-treatments. *Arrows* show the time when chlorophyll fluorescence was measured, and/or samples were taken for pigment analysis. *Shaded boxes* represent periods of continuous hydration, *open boxes* show periods in which the lichens were desiccated.

We used subsequent protocol for quenching analysis (Barták *et al.* 2003): After 10 min darkness, a saturating pulse of sufficient intensity (tested prior to experiments) was applied to determine F_M (maximal Chl fluorescence of a dark adapted sample). Then, actinic irradiance (160 µmol m^{-2} s^{-1}) was turned on. Steady-state Chl fluorescence (F_S) was reached after 5 min. Finally, another saturating pulse was applied to determine F_M' (maximal Chl fluorescence of an irradiance-adapted sample). We used obtained data for calculation of following Chl fluorescence parameters: (*1*) Maximal photochemical quantum yield of PS2, F_V/F_M; (*2*) irradiance-adapted quantum yield of PS2, $\Phi_{PS2} = (F_M' - F_S)/F_M'$ (Genty *et al.* 1989); (*3*) non-photochemical quenching, $NPQ = (F_M - F_M')/F_M'$ (Bilger and Björkman 1990). Chl fluorescence measurements were done with portable, modulated fluorometer (*PAM-2000*, *Walz*, Effeltrich, Germany) operated by a laptop computer.

Sample set *2* was treated as sample set *1*, but after the 60 h of irradiance treatment sample set *2* thalli were collected when still dry (Fig. 1). The desiccated thalli were frozen in liquid nitrogen immediately after collection. They were stored at –80 °C for not more than 2 weeks. Samples were removed from freezer and placed in a desiccator with silica gel for 1 h in dark. Then, dry mass of thalli was measured. Thalli were hydrated for 30 s and submerged in 3 cm^3 dimethylformamide (DMF) for 96 h at 4 °C in darkness. Extracts were analyzed on HPLC according to Niinemets *et al.* (1998). Pigments were quantified and the de-epoxidation state of xanthophyll cycle pigments (DEPS) was calculated as (Z+A)/(V+A+Z), where Z = zeaxanthin, A = antheraxanthin, and V = violaxanthin.

The control value of F_V/F_M measured after 24 h relaxation in DI before any irradiance treatment was 0.68±0.10. There was no significant difference between the two samples before the treatments were applied, meaning that the experiment should be well suited to detect differences due to treatments. Thalli exposed to 200 µmol m^{-2} s^{-1} for 6 h showed lower F_V/F_M values compared to those kept at DI (p=0.0270; Fig. 2*A*). Since thalli were dry during the 60 h HI exposure, it was not possible to measure Chl fluorescence directly. However, after the final 24 h relaxation period at DI in the hydrated state the difference in F_V/F_M between the MI- and DI-pre-treated thalli became reversed (Fig. 2*A*). Photoinhibition, measured as a decrease of F_V/F_M, was significantly less for MI thalli than for DI thalli (p=0.0022). No significant difference in yield values occurred between the MI and DI pre-treatments (Fig. 2*B*). Non-photochemical quenching (NPQ) was significantly highest directly after the MI pre-treatment for 6 h in the wet state (p<0.0001), but not after 60 h (Fig. 2*C*).

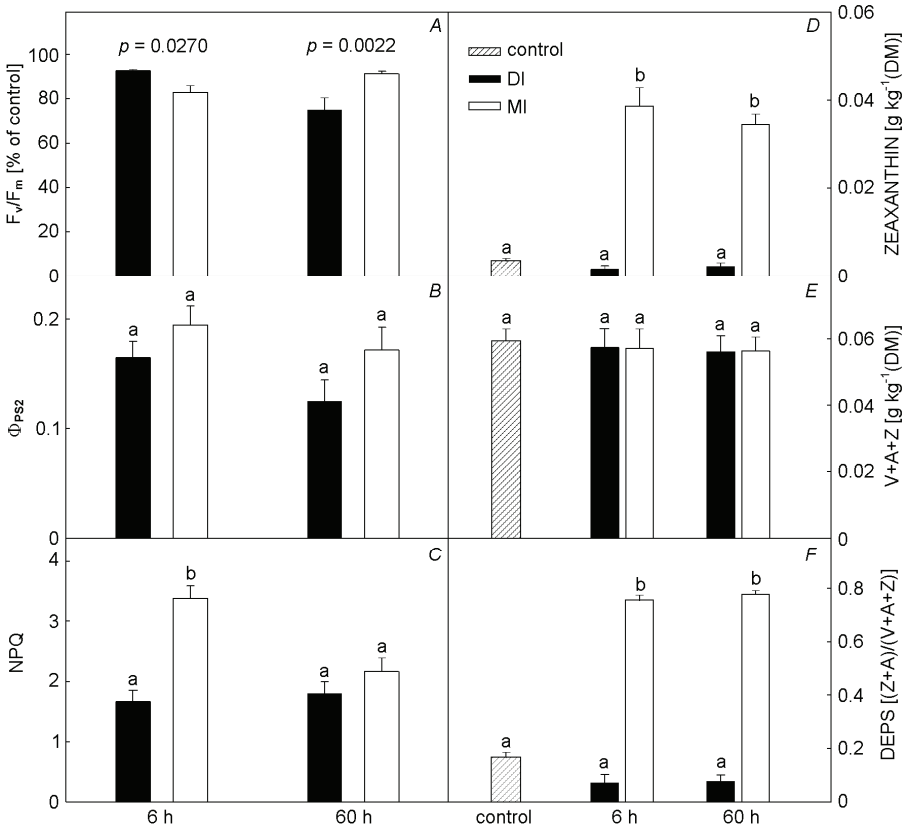

Fig. 2. (*A*) Optimal quantum yield of photosystem 2 (PS2, F_V/F_M), (*B*) PS2 yield (Φ_{PS2}), (*C*) non-photochemical quenching (NPQ), (*D*) zeaxanthin content, (*E*) total xanthophyll cycle pigments' pool, and (*F*) DEPS for the MI-thalli (*white columns*) and DI-thalli (*black columns*) measured immediately after the pre-treatments (6 h) or directly after the 60-h high irradiance (HI) exposure in the desiccated state (*D–F*) or followed by 24-h hydration at DI (*A–C*). F_V/F_M and pigments were also measured for control thalli. The significance levels for the difference in F_V/F_M between MLP and DLP are shown above the columns (Mann-Whitney Rank Sum Test), significant differences in Φ_{PS2} and NPQ (p<0.05, Student-Newman-Keul method), and in zeaxanthin, VAZ, and DEPS (p<0.05). All pair-wise multiple comparison procedures (Dunn's method)) are indicated with small letters above the columns. *Bars* show one standard error (n = 9–10).

Content of Z considerably increased after 6 h at 200 µmol m^{-2} s^{-1} compared to the control (Fig. 2*D*), while Z in DI-thalli did not change. The 60 h final exposure at 1 000 µmol m^{-2} s^{-1} showed that no significant conversion or degradation of Z occurred in the dry state. DEPS in MI-thalli reached almost 0.8, indicating high conversion of V to Z and A (Fig. 2*F*). Total amount of xanthophyll cycle pigments did not change during the experiment (Fig. 2*E*).

Our results show that the irradiance experienced during the latest desiccation cycle influences *L. pulmonaria*'s ability to cope with HI during a following desiccation period. In an annual cycle, lichens experience highly contrasting climatic conditions during numerous hydration-desiccation cycles (*e.g.* Lange 2002, 2003a,b). According to Lange (2003a), 74 continuous rainy days with no desiccation during sunshine hours produced only 4 % of the annual carbon gain, whereas 105 d a year with dew that normally forms in clear weather with subsequent drying in the sun, accounted for 40 % of the annual

C gain. Also the 57 d with nocturnal rain and subsequent drying during daylight caused a substantial C gain (28.5 %). Like our experimental data, the field data of Lange and Green (2006) suggest that desiccation under irradiation is optimal. One reason may be caused by the supra-saturation depression of net photosynthesis during full hydration that disappears during desiccation (*e.g*. Lange and Green 1996). There seems to be an additional beneficial response of desiccation under irradiation due to increased ability to cope with excess photon energy in the following desiccation period. The mechanism for the increased photoprotection in the desiccated state is, however, unclear. Although the content of Z and DEPS were much higher in thalli desiccated in MI compared with thalli desiccated in DI, this mechanism was probably not the cause of increased HI resistance. According to Heber *et al.* (2006), photoprotection in the desiccated state does not require Z-dependent energy dissipation. Z may instead possibly act as an antioxidant and lipid stabilizing compound as proposed by Müller-Moulé *et al.* (2003).

Lichen growth depends on the irradiance received during wet periods, as well as on the duration of these periods (*e.g*. Palmqvist 2000). The high Z content and DEPS are stable throughout the HI-period in the desiccated state (Fig. 2*D,F*). The high Z content and DEPS status throughout the desiccation period will enable the thalli to dissipate excess energy in the next wet period. Since most of the carbon gain in lichens occurs during periods with numerous hydration-desiccation cycles, the acclimation to HI achieved in the preceding wet period is probably important for the thalli to rapidly cope with HI in the following wet period. However, most of the difference in NPQ had disappeared, although not completely, after 24 h in DI (Fig. 2*C*). Thus, in addition to a high stability of both Z content and DEPS during the desiccated period, the thalli exhibit a high flexible ability to dissipate energy in the wet state. As in higher plants, this flexible NPQ is probably important for an optimal use of photon energy in the wet state during changing irradiances that occurs for *L. pulmonaria* in old forests characterized by high frequency of canopy gaps.

The irradiance in the preceding wet period clearly influences the ability of lichens to cope with HI in subsequent experiments (Fig. 2). It is difficult to sample lichens with a well-defined hydration and irradiation prehistory for physiological experiments; especially epiphytic forest lichens experience micro-site variation in moisture and irradiance due to canopy-dependent sunflecks. In our earlier experiments we have used 24–48 h acclimation in DI to relax short term photoinhibition. However, because the HI susceptibility of *L. pulmonaria* relative to other species is documented both in lab experiments (*e.g*. Gauslaa and Solhaug 1996) and in field experiments (*e.g*. Gauslaa *et al.* 2001), we believe that the main conclusions in these earlier studies are still valid. Nevertheless, we found that preconditioning of lichens influences subsequent responses, and should therefore be given attention in future studies. Some preconditioning to reduce the effect of time- and habitat-specific environmental factors is required in many types of studies. However, a standard pre-treatment may not be suitable for all experiments, and specific preconditioning may be needed for specific experiments.

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