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Ultraviolet radiation reduces lichen growth rates

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Abstract We quantified relative growth rates (RGR) in shade-adapted and melanin-deficient thalli of *Cetraria islandica* and *Lobaria pulmonaria* cultivated in short-term growth chamber experiments with and without UV-B radiation. In the first experiment done under optimal PAR (125 μmol m^{-2} s^{-1}), but high UV-B radiation (1 W m^{-2}), UV-B radiation significantly reduced RGR ($P < 0.001$). The second experiment with higher PAR, but more natural ratios between wavelength ranges (PAR: 500 μmol m^{-2} s^{-1}; UV-A: 7 W m^{-2}; UV-B: 0.4 W m^{-2}), caused a reduction in mean RGR in *L. pulmonaria* to just 45% of rates in experiment 1. *Lobaria pulmonaria* screened from UV-B radiation had 1.9 and 1.6 times higher RGR than non-screened thalli in experiment 1 and 2, respectively. UV-B radiation significantly induced melanin synthesis in the second experiment only, causing significantly less photoinhibition than in thalli receiving just PAR. This is consistent with PAR-protective roles of melanins. Chlorophylls were not affected by UV-B radiation in any experiment. Because UV-B radiation affected RGR more than pure photobiont responses, the mycobiont is likely the more UV-B-susceptible partner. Apart from reduced RGR, we found little evidence for adverse UV-B effects.

Keywords *Cetraria islandica* · Light screening · *Lobaria pulmonaria* · Melanin · Relative growth rate · UV-B

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1 Introduction

Lichens are stable, self-supporting symbiotic associations of a mycobiont and one or more photosynthetic partners (photobiont), where the photobiont fixes carbon and the mycobiont provides shelter and mineral nutrients. Lichens are most dominant in exposed habitats at higher latitudes and/or elevations, where they often experience high light and UV-B (Bjerke et al. 2002; Leppik et al. 2015; Lutsak et al. 2016). UV-B, reported to increase due to chlorofluorocarbon emissions inducing stratospheric ozone layer depletion (Aphalo et al. 2012), influences the biosphere and can cause direct damage to cellular processes (Buffoni Hall et al. 2003). Plants have protective mechanisms to avoid excess UV-B radiation (Caldwell et al. 1998). They synthesize UV-B absorbing phenolic compounds in the epidermal tissue acting as sunscreen and preventing UV-B-radiation to penetrate (Jenkins 2009; Burchard et al. 2000). Over the last years, there has been a shift of interest from UV-B radiation as a stressor to UV-B as a regulatory factor (Robson et al. 2015; Hideg et al. 2013). Effects of UV-B are better known in plants than in lichens, although lichen-dominated habitats are open, solar radiation-exposed areas with much UV-B.

Compared to plants, lichens are slow-growing, long-lived organisms (Armstrong and Bradwell 2011). However, recent studies (Bidussi et al. 2013a; Eaton and Ellis 2014; Alam et al. 2015) have showed that lichens can grow relatively fast. The synthesis of secondary lichen compounds, some of them induced by UV-B radiation, can also be investigated in short-time studies (Solhaug and Gauslaa 2004). Among the few published experimental studies on UV-B radiation effects, Sonesson et al. (1995) showed that UV-B enhanced photosystem II (PSII) efficiency, whereas Larsson et al. (2009) found no adverse effect of UV-B on lichens' growth rate. UV-B radiation has also been reported to slightly reduce or not to

affect PSII efficiency (Bjerke et al. 2005; Solhaug et al. 2003). Thus, the few studies on impacts of UV-B radiation on lichen growth are hardly conclusive and more data are needed.

The synthesis of various solar radiation-absorbing compounds in the upper lichen cortex (Solhaug and Gauslaa 2012), like melanins in some species (Solhaug et al. 2003; Matee et al. 2016) and carbon-based secondary lichen compounds, like usnic acid and parietin in others (Bjerke et al. 2002; Solhaug and Gauslaa 2004; Buffoni-Hall et al. 2002; McEvoy et al. 2006; Nybakken and Julkunen-Tiitto 2006), can be induced by UV-B radiation. These abundant fungal pigments screen UV radiation and PAR (Solhaug and Gauslaa 1996; Gauslaa and Solhaug 2001; Nybakken et al. 2004; McEvoy et al. 2007; Solhaug et al. 2010) by forming a layer above the photobionts that protects them from excess solar radiation (as reviewed by Solhaug and Gauslaa 2012). The concentration of cortical pigments increases seasonally with solar height (Gauslaa and McEvoy 2005) and differs between lichen species (Bjerke et al. 2002) in ways consistent with a solar radiation-protective function. Melanins representing a complex group of chemical compounds, occur in a variety of organisms in various kingdoms (Riley 1997), including humans (Routaboul et al. 1999; Brenner and Hearing 2008) and non-lichenized fungi (Grishkan and Nevo 2010). The Peltigeralean lichens capable of fixing nitrogen are more likely to produce L-DOPA melanins rich in N, whereas e.g. Parmelioid lichens likely produce melanins of the DHN-type (Matee et al. 2016).

The presence of melanins complicates measurements of chlorophylls because recommended chlorophyll extraction methods for lichens (Palmqvist and Sundberg 2002) simultaneously extract melanins (Meeßen et al. 2013) with partly overlapping absorbance. Extraction of chlorophylls without melanin contamination is possible in acetone or ethanol (Meeßen et al. 2013). However, DMSO is a better solvent for chlorophyll extraction, because grinding is not necessary and there is less loss of solvent due to much lower vapor pressure than for acetone. Thus, we need rapid methods that can extract chlorophylls and melanins, and measure each of them.

Here, we aim to quantify how UV-B radiation influences lichen growth and formation of brown pigments in two common, melanin-producing lichens with contrasting ecology. None of these have UV-B-absorbing cortical carbon-based lichen compounds. However, they have UV-B absorbing medullary lichen compounds, but the intrathalline location of such compounds is less consistent with a UV-B screening role. Our main objective is to quantify UV-B-effects on relative growth rate (RGR) as well as on pure photobiont viability parameters like chlorophylls and chlorophyll fluorescence. Growth rate is a robust and ecologically relevant measure of fitness integrating various functions of all partners in the symbiotic consortium, whereas photosynthetic parameters are exclusive photobiont responses. A second objective is to develop a new protocol for quantifying melanic pigments formed in the experiments, and to use this method to assess these pigments in lichens with and without exposure to UV radiation.

2 Material and methods

2.1 Lichen material

Many separate colonies of the fruticose chlorolichen *Cetraria islandica* (L.) Ach and the foliose cephalolichen *Lobaria pulmonaria* (L.) Hoffm. were collected in southeastern Norway August–September 2014; *C. islandica* from mixed open birch/pine forests in Sørmarka, Ski, Akershus (59°48′54 N, 10°54′34 E, 170 m a.s.l.); *L. pulmonaria* from open oak forests in Langangen, Porsgrunn, Telemark (59°06′43″N, 9° 50′ 05″E, 140 m a.s.l.). Attached bryophytes and debris were removed before lichens were air dried Then one to three (depending on total colony size) full-size branched lobes (hereafter called thalli) were cut from each colony. In total, a batch with close to 100 thalli of each species were prepared. The mean weight of the *L. pulmonaria* thalli used in experiments was 127 ± 6 mg; 58–270 mg (mean ± 1SE; min-max); in thallus area these figures were 14 ± 1.0 cm^2; 6.5–35.9 cm^2. Dry mass (DM) of *C. islandica* was 162 ± 7 mg; 80–200 mg; area was not measured due to its three-dimensional fruticose structure. All thalli were then stored at −18°C before start of the experiment. At start of each experiment, the number of randomized thalli needed were taken from the freezer. Thalli were kept in the laboratory for 48 h at 20°C before recording their air dry mass (± 0.1 mg). At each weighing session (start and end of an experiment), five control thalli of each species were weighed air-dry. Then they were oven-dried for 24 h at 70°C before measuring their DM. The mean reduction factor in mass from the air-dry to the oven-dry state for these five controls was used to convert the air DM to the oven DM for each experimental thallus.

2.2 Growth experiments

The experiments were done at the Center for Plant Research in Controlled Environment (SKP) at the Norwegian University of Life Science (NMBU). To improve the statistical power, the first experiment, using two equal growth chambers simultaneously, was run two times using the same experimental conditions in each run. Each run used 30 new thalli of each species, 15 for each radiation treatment. Air temperature was 15°C in both growth chambers with white fluorescent tubes (Philips Master TL-D 36 W/840, Ahlsell Norge AS, Moss, Norway) providing 125 μmol m^{-2} s^{-1} photosynthetic active radiation (PAR; Table 1). PAR and temperatures were set to a level that had given fairly maximal chloro- and

Table 1 Radiation regimes in the two experiments used

	PAR µmol m^{-2} s^{-1}	UV-A W m^{-2}	UV-B W m^{-2}
First experiment[a]			
PAR	125	0	0
PAR + UV-A + UV-B[b]	125	1.0	1.0
Second experimentc[3]			
PAR + UV-A[d]	500	5.6	0
PAR + UV-A + UV-B[e]	500	7	0.4

[a] Light source: White fluorescent tubes + Q-Panel UV313. [b] using pre-burnt cellulose acetate screen. [c] LED + Q-Panel UV340. [d] using polyester screen. [e] using cling film. Spectra of UV and PAR below all screens were measured with an Optronic model 756 spectroradiometer (Optronic Laboratories, Orlando, FL, USA)

cephalolichen growth rates in a previous growth experiment (Alam et al. 2015). One of the two chambers in each run was additionally illuminated with UV-A and UV-B radiation from UV-B fluorescent tubes (Q-panel UVB 313, Largo, Gothenburg, Sweden) filtered through 0.15 mm thick cellulose di-acetate films (Rachow Kunststoff Folien GmbH, Hamburg, Germany) that had been prepared by a 24 h pre-exposure to UV radiation before start of the experiment. UV-A and UV-B radiation measured under the screens were both at 1.0 W m^{-2} (Table 1.) The daily photoperiod was 12 h; the UV radiation was on for 6 h in the middle of each photoperiod. Each thallus was kept on top of 10–12 layers of filter papers in open Petri dishes for 14 days. We sprayed all lichens with de-ionized water to keep them moistened most of the time, but in the evening they were at least partially dry before the next spraying.

Because no brown pigments were formed in the first experiment (see Section 3), we ran a second experiment for 21 days at 20°C with just *L. pulmonaria*, the most responsive species in the first experiment. Also 20°C supports high RGR in this species (Bidussi et al. 2013a). Compared to the first experiment, we now used a wider UV-range and 4 times higher PAR (500 µmol m^{-2} s^{-1}); the ratios between the various wavelength ranges (Table 1) were similar to those occurring under natural conditions, and similar to those that had successfully induced melanins under experimental conditions (Solhaug et al. 2003). Samples were illuminated with a LED panel (model SL3500 RGB, Photon systems instruments, Brno, Czech Republic) and UV-A fluorescent tubes (Q-panel UVA 340, Largo, Gothenburg, Sweden) with a UV spectrum from 300 to 350 nm similar to the natural solar radiation spectrum. UV-A and UV-B were 7 and 0.4 W m^{-2}, respectively in the PAR + UV-A + UV-B treatment (Table 1). PAR was on for 12 h; UV radiation for 8 h in the middle of each 12 h photoperiod. As before, thalli were placed on moist filter papers in Petri dishes; but with n = 10 for each treatment. Polyester foil (PET, 0.175 mm, Nordbergs Tekniska AB, Vallentuna Sweden) screened all UV-B radiation for the PAR + UV-A treatment; cling film transmitting all three wavelength ranges was used for the treatment also including UV-B. The level of UV-A and UV-B radiation beneath the polyester was 5.6 W m^{-2} and 0 W m^{-2}, respectively (Table 1). The hydration regime was slightly modified, allowing thalli to fully desiccate a short period in the evening before the next hydration at night. Thus the radiation and hydration regimes were more similar to natural field conditions than in the first experiment.

In all experiments, DM change was quantified as $RGR = (\ln (DM_{end}/DM_{start}))*1000/ \Delta t$ (mg g^{-1} day^{-1}).

2.3 Chlorophyll fluorescence measurement

We measured chlorophyll *a* fluorescence before and after all growth experiments by using a PAM 2000 fluorometer (Walz, Effeltrich, Germany). Prior to each measurement, thalli were kept hydrated in low light (10–15 µmol m^{-2} s^{-1}) at 18°C for 24 h. Thalli were further dark adapted for 15 min immediately before recording the maximum photochemical efficiency of photosystem II (Fv/Fm). Viability after each exposure was reported as mean percent of start values.

2.4 Chlorophyll and brown pigment quantification

At the end of the first replicated experiment, we randomly selected 10 thalli from each treatment and species to measure chlorophyll $a + b$ and brown pigments; for the last experiment, all thalli (n = 10) were analyzed. Each thallus was ground to fine powder with a ball mill using metal balls (Retsch model MM400, Retsch GmbH Hann, Germany). Approximately 19–22 mg DM from each sample was placed in an Eppendorf tube and, 2 ml DMSO saturated with $MgCO_3$ was added. The tubes were incubated for 30 min in an ultrasonic water bath (Ultrasonic cleaner, USC 200TH) at 70°C to improve extraction of pigments. Tubes were shaken every 10 min, and centrifuged at 15000 rpm/min for 2 min. Absorbance spectrum of the supernatant was measured by a Shimadzu UV-2101PC spectrophotometer.

The protocol for separating chlorophylls and brown pigments was as follows. First, 0.2 ml of de-ionized water was mixed with the supernatant. A solution of DMSO was made with 10% de-ionized water. First, 0.5 ml of DMSO with 10% water was pressed slowly with a standard 2 ml syringe through the Agilent Bond Elut C18 column sorbent to prepare the column for extracting brown pigments. Then 2 ml of the extract was pressed through. The brown pigments and the medullary lichen compounds passed through the column, whereas chlorophyll and carotenoids remained in the column. The 10% water added to the 100% DMSO was needed to retain all xanthophylls in the column.

Afterwards, chlorophylls and carotenoids retained in the Agilent Bond Elut C18 column, were extracted by gently

pressing 2 ml of 100% ethanol through the column. Additional 1 ml ethanol was needed to remove all chlorophylls from the column. The absorbance at 649, 665 and 750 nm of the combined 3 ml ethanol chlorophyll extracts were measured in the spectrophotometer. Chlorophylls were measured in two solutions, in DMSO before removal of brown pigments (melanin absorbance was sufficiently low at 649 and 665 nm to measure chlorophylls; see Fig. 1), and in ethanol after removal of brown pigments. Chlorophyll *a* and *b* were calculated in mg g^{-1} according to the equation from Wellburn (1994) and Lichtenthaler and Wellburn (1983) for DMSO and ethanol, respectively. The absorbance at 649, 665 and 750 nm was measured by a Shimadzu UV-2101 PC spectrophotometer. To correct for small impurities in the cuvettes, the absorbance at 750 nm (no absorbance of chlorophyll) was subtracted from the 649 and 665 nm absorbance.

The wavelength for determination of melanin was set at 450 nm. The absorbance of synthetic melanin (Sigma Aldrich, CAS 8049–97-6) increases gradually towards shorter wavelength (Fig. 1). By using 450 nm, the strong UV-absorption of the medullary carbon-based secondary lichen compounds is avoided as these do not absorb visible light. These medullary compounds strongly raise the absorbance at shorter wavelengths in the extract cleaned from chlorophylls compared to the melanin standard (Fig. 1). The absorbance was reported as: Relative brown pigments concentration = Absorbance (450 nm) / DM extracted material.

2.5 Statistical analyses

In the first experiment, two extreme outliers of *L. pulmonaria* were excluded for growth analyses (−6.1 and 34.1 mg g^{-1} d^{-1}) for the PAR-treatment. One *C. islandica* in the PAR treatment was also excluded (RGR 2× higher than the second highest value). In the second experiment two thalli experienced fragmentation during experiment, one in each treatment. These two were excluded for growth measurements.

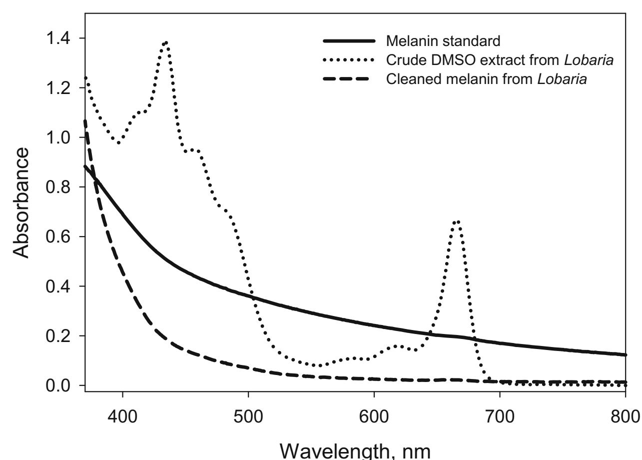

Fig. 1 Absorbance spectra of the melanin standard, a crude DMSO extract from *Lobaria pulmonaria*, and the cleaned DMSO extract after removing the chlorophylls. This extract contains melanins and colorless lichen compounds. These compounds contribute to the strong UV-absorbance in the cleaned extract

Generalized mixed linear models were run in Minitab 16 (Minitab Inc., State College, PA, USA) to test effects of treatments on measured parameters in the first growth experiment, using treatment (PAR, PAR + UV-B) as a fixed factor and experiment (1. run and 2. run) as a random factor. Lobe/branch size (DM) was included as a covariate if significant at $P < 0.05$. The requirements for species-wise analyses were met, but the factor species could not be included in a two-way analysis due to violations of the GLM requirements. For the second experiment, measured parameters (RGR, Chl *a* + *b,* Chl *a/b,* and brown pigments) were analyzed by a *t*-test for each lichen species separately. A Mann-Whitney Rank Sum Test was run for F*v*/*Fm* (normality test was not fulfilled).

3 Results

In the first experiment, the average RGR across all treatments and replications was 3.9 times higher in *L. pulmonaria* than in *C. islandica*. The treatment including UV radiation (1 W m^{-2}) significantly reduced RGR in both species (Fig. 2a-b; Table 2). For *L. pulmonaria*, RGR was 1.9 times higher during the PAR treatment (RGR = 9.20 mg g^{-1} d^{-1}) than at PAR + UV-A + UV-B; for *C. islandica,* RGR was 5.4 times higher (3.02 mg g^{-1} d^{-1}) for the treatment with just PAR. The random factor - two replicate experiments - also influenced RGR (Table 2). In *L. pulmonaria*, RGR decreased with thallus size; including individual size (58–270 mg) as a covariate significantly improved the RGR-model for *L. pulmonaria* by raising the *F*-value for treatment and thus the r^2_{adj} from 0.340 (data not shown) to 0.547 (Table 2).

The applied UV radiation in the first experiment caused a slight, but significant ($P = 0.009$) reduction in F*v*/*Fm* (% of start) in the foliose *L. pulmonaria* (Fig. 2c; Table 2). No changes in F*v*/*Fm* (% of start) were detected in the fruticose *C. islandica* (Fig. 2d; Table 2). At start, the mean F*v*/*Fm* varied just slightly between species; 0.703 ± 0.003 (±1SE) in *L. pulmonaria* and 0.722 ± 0.003 in *C. islandica*, and did not differ between thalli used for the two replicate experiments. A linear regression analysis for experiment 1 showed just weak, but significantly positive relationships between RGR and F*v*/*Fm* (given as percent of start values) for *L. pulmonaria:* (r^2_{adj} = 0.062; $P = 0.032$; $n = 58$) and *C. islandica;* (r^2_{adj} = 0.099; $P = 0.009$; $n = 59$). *Cetraria islandica* had much lower chlorophyll concentration than *L. pulmonaria*, but treatments did not significantly change chlorophyll parameters (Fig. 2e-h).

None of the treatments in the first experiment changed the brown pigmentation in any species (Fig. 2i-j). By contrast, the second experiment including *L. pulmonaria* only, gave twice

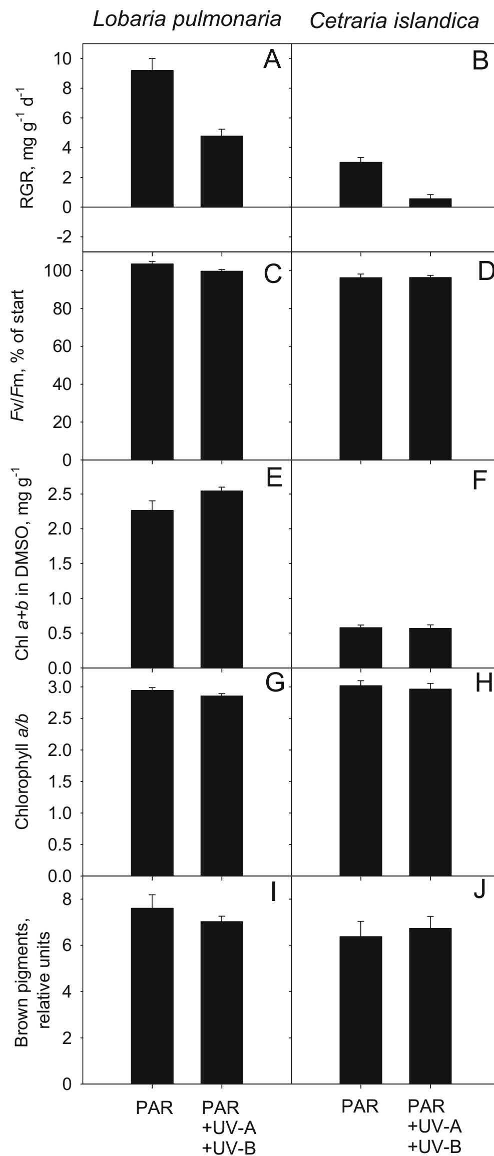

Fig. 2 Relative growth rate (RGR), *Fv/Fm* (end values given as percent of start level), chlorophyll *a* + *b*, chlorophyll *a/b*, and brown pigments in *Lobaria pulmonaria* and *Cetraria islandica* cultivated at two light treatments (Table 1) at 15°C for 14 days at 12 h photoperiod. Means ±1SE are given (n = 30 for RGR and for *Fv/Fm*; n = 10 for chlorophyll parameters and brown pigments)

as much melanins ($P < 0.001$) in the treatment with UV-B as in the one without. Here, PAR was four times higher, UV-A seven times higher, whereas UV-B was just 40% of the UV-B level given in the first experiment (Table 1). In this high-light experiment, *Fv/Fm* declined to 84.2% of start values (0.711 ± 0.002) during the PAR + UV-A treatments, whereas the addition of UV-B caused less reduced *Fv/Fm*-values (93.3% of start). Like in the first experiment (Fig. 2), UV-B did not affect chlorophylls (Table 3). However, the chlorophyll concentration (DMSO) averaged across treatments in the second experiment (PAR = 500 μmol m^{-2} s^{-1}) was just 55% of the mean total level for the first experiment

Table 2 Generalized mixed linear models for relative growth rate (RGR), *Fv/Fm* % of start, and chlorophyll *a/b* in *Lobaria pulmonaria* and *Cetraria islandica* cultivated under two light regimes (PAR, PAR + UV-B; fixed factor) in two separate experiments (random factor)

Parameter	RGR			*Fv/Fm*, % of start		
Source	df	*F*	*P*	df	*F*	*P*
Lobaria pulmonaria						
Treatment (fixed)	1	47.66	0.000	1	7.37	0.009
Experiment (random)	1	29.82	0.000	1	17.16	0.000
Lobe size (covariate)	1	31.37	0.000			
Error	54			57		
Total	57			59		
r^2_{adj}		*0.547*			*0.276*	
Cetraria islandica						
Treatment (fixed)	1	45.89	0.000	1	0.00	0.963
Experiment (random)	1	21.68	0.000	1	58.98	0.000
Error	56			57		
Total	58			59		
r^2_{adj}		*0.527*			*0.491*	

These models analyze the data summarized in Fig. 2. For RGR of *L. pulmonaria*, lobe size was a significant covariate, and was thus included in the model (RGR decreased with increased size). For chlorophyll *a* + *b*, *a/b*-ratio, and brown pigments, no models gave significant effects (data not shown)

(2.40 ± 0.08 mg g^{-1}) using just 125 μmol m^{-2} s^{-1}; the mean chlorophyll a/b-ratio in the second experiment (Table 3) was 83% of the ratio in the first low light experiment (Fig. 2). Overall growth rates in the high-light experiment (Table 3) were just 45% of those in the first optimal light experiment (Fig. 2), and there was a negative regression between *Fv/Fm* (% of start) and RGR (P = 0.007; data not shown), because those shielded from UV-B radiation had more photoinhibition, but yet higher RGR than those exposed to UV-B (Table 3). In the second experiment with just 1/3 of the replicates in the first experiment, the PAR + UV-A treatment resulted in 1.6 times higher RGR (P = 0.053; Table 3) than the PAR + UV-A + UV-B treatment.

4 Discussion

For *L. pulmonaria* in the first experiment using optimal PAR, reported growth rates in thalli shielded from UV radiation (Fig. 2), approached the highest measured RGR for lichens in earlier growth chamber studies (Alam et al. 2015; Bidussi et al. 2013a). The addition of UV radiation, which here failed to induce melanin formation, significantly reduced RGR (Fig. 2a-b). The UV-induced reduction in RGR was even stronger in the widespread *C. islandica* inhabiting open sites than in the old forest lichen *L. pulmonaria* (Fig. 2a-b).

Table 3 Relative growth rate (RGR), total chlorophyll content, chlorophyll a/b-ratio, *Fv/Fm*, and brown pigments in the second experiment with *L. pulmonaria,* run for 21 days at 20°C with two light treatments specified in Table 1

	PAR + UV-A	PAR + UV-A + UV-B	*P*-value	*t*-value
RGR, mg g^{-1} d^{-1}	3.84 ± 0.50	2.34 ± 0.52	0.053	2.088
Chl *a* + *b* in DMSO, mg g^{-1}	1.40 ± 0.06	1.21 ± 0.084	0.094	1.766
Chl *a/b*- ratio in DMSO	2.37 ± 0.08	2.38 ± 0.07	0.871	-0.164
Chl *a* + *b* in ethanol, mg g^{-1}	1.03 ± 0.07	0.89 ± 0.07	0.173	1.419
Chl *a/b*-ratio in ethanol	2.28 ± 0.05	2.27 ± 0.03	0.805	0.251
Fv/Fm, % of start[a)]	84.2 ± 2.9	93.2 ± 0.96	<0.001	60.00
Brown pigments, relative units	7.01 ± 0.62	14.44 ± 1.45	<0.001	-4.715

P- and *t*-values were computed by a *t*-test. Means ±1SE are given; *n* = 10. [a)] A Mann-Whitney Rank Sum Test was run for this parameter (normality test was not passed)

Because UV-B radiation is a more powerful in causing damage than UV-A (e.g. Rozema et al. 2002), we consider UV-B to be the main growth-reducing factor for the UV versus the PAR treatments. The strong growth-reducing UV-B effect may suggest that UV-B radiation contributes to the much lower lichen growth rates realized even under favorable field conditions (e.g. Gauslaa et al. 2006) than in the lab. The growth-reducing effect of lobe size per se in *L. pulmonaria* (Table 2) is likely related to the well-documented trade-off between growth and reproduction during aging (Gauslaa 2006; Merinero et al. 2015); the larger thalli were likely older.

In the foliose old forest lichen *L. pulmonaria*, UV-B slightly, but significantly lowered *Fv/Fm*, whereas the fruticose lichen *C. islandica* from more exposed habitats did not experienced UV-induced photoinhibition (Table 2; Fig. 2c-d). We measured *Fv/Fm* after 24 h relaxation at low light without UV-B followed by 15 min dark-adaptation. Thereby, the reported reduction in *Fv/Fm* represents long-lasting photoinhibitory damage (Gauslaa and Solhaug 1996). However, due to the weak relationship between RGR and *Fv/Fm*, the photoinhibition in the first experiment is hardly the main reason for the UV-B-induced reduction in growth. In an earlier lab-experiment (Larsson et al. 2009) with more PAR and lower growth rates than here, no adverse effects of UV-B occurred, even at similar UV-B levels. Our higher UV-B/PAR-ratio and/or more optimal light conditions for growth may have contributed to the UV-B-induced growth reductions, but also the absence of melanin formation in our first experiment may have contributed to the higher growth rates. However, the highly significant effect of the random factor experiment in the replicated first experiment suggests that there are experimental conditions like e.g. the hydration regime that are not fully controlled. To account for such variation, replication of experiment is important.

In the second experiment with much higher PAR and UV-A, the photoinhibition (Table 3) was stronger. The much lower chlorophyll content (Table 3) and lower chlorophyll *a/b*-ratio in the second compared to the first experiment likely resulted from high-PAR-induced chlorophyll degradation (Gauslaa and Solhaug 1996, 1999). PAR-induced photoinhbitory damage has likely caused the lower RGR of this experiment. The high PAR as such probably caused the lower RGR because the photoinhibition was significantly less in the full PAR + UV-A + UV-B treatment than in the one without UV-B. The absence (first experiment) and presence (second experiment) of melanin formation likely explains reported contrasts in photoinhibition between the two experiments. UV-induced melanins increased the internal shading by screening PAR for underlying photobionts (Gauslaa and Solhaug 2001; Nybakken et al. 2004), and thus causes relaxation of photoinhibition. Significant melanin formation only occurred in the second experiment (Table 3) using the UV-B/UV-A-ratio (Table 1) that has earlier been shown to induce melanins (Solhaug et al. 2003). Substantially higher PAR and UV-A, and/or faster radiation-induced desiccation, may also have contributed to the higher melanin formation. Furthermore, due to reported UV-induced increases in thickness and reduced elongation in plants (as reviewed by Robson et al. 2015), UV-B may cause a trade-off between growth and other functions also in lichens.

Realistic experimental manipulations of solar UV-B have demonstrated that damaging UV-B-associated stress is an exception for plants in most natural environments (Searles et al. 2001; Li et al. 2010). This is likely also the case for lichens (as reviewed by Solhaug and Gauslaa 2012); at least typical photobiont responses like chlorophylls and chlorophyll fluorescence parameters were not adversely affected by the UV-B treatments. In the first experiment using fairly optimal PAR levels for lichen growth according to literature (Bidussi et al. 2013a; Alam et al. 2015), the treatment with UV-B reduced growth rates in all species. In the second experiment, the UV-B-induced reduction in RGR was just marginally significant, probably because of excess PAR-induced photoinhibition and chlorophyll degradation, and too few replicates. Because UV-B did not affect pure photobiont responses, and substantially reduced growth driven by both bionts together, one may assume that the mycobiont is the more UV-B-susceptible partner in lichen symbioses.

High growth rate as such is not necessarily associated with high ecological success in lichens. For example, the common, often dominant epiphytic chlorolichen *Hypogymnia occidentalis* grows at much slower rates than the rarer cephalolichen *L. pulmonaria* across various environmental gradients (Gauslaa and Goward 2012; Bidussi et al. 2013b). Furthermore, as UV-B seems to reduce growth across contrasting species, UV-B may not necessarily change the outcome of interspecific competition in lichen-dominated communities.

5 Conclusion

UV-B reduced growth rates in studied lichens, particularly under optimal environmental conditions for growth and under conditions not supporting melanin formation. Because lichen growth was much more adversely affected than pure photobiont responses, UV-B had presumably a stronger effect on the mycobiont than on the photobiont.

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