



## **Preface**

This thesis is the final part of my master degree in General Ecology at the department of Ecology and Natural Resource Management (INA), Norwegian University of Life Sciences (NMBU). The one year project now has come to an end. It has been great learning experience for me.

I would like to express my sincere thanks to my two supervisors Professor Knut Asbjørn Solhaug and Professor Yngvar Gauslaa, Norwegian University of Life sciences for their guidance and cooperation during the whole process. Both of you were always available whenever I need to discuss about any problem.

I would like to thank my family, specially my husband for all the support and inspiration.

This Master thesis is submitted to the Department of Ecology and Natural Resource Management, Norwegian University of Life Sciences.

Best Regards  
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Ås, Norway.  
July, 2015

## Abstract

This study investigates relative growth rates and brown pigments synthesis under the influence of UV-B radiation in short term growth chamber experiments. One chlorolichen, *Cetraria islandica*, and the two cephalolichens *Lobaria pulmonaria* and *Peltigera aphthosa* were cultivated for two-weeks at two treatments, PAR and PAR+UV-B. PAR level was  $125 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  for 12h photoperiod and UV-B level was  $1 \text{ Wm}^{-2}$  for 6 h in the middle of the photoperiod. All three lichen species responded significantly to applied treatments. General Linear Model was performed with growth parameters (RGR,  $\text{RT}_{\text{A}}\text{GR}$ ) as responses and treatment as factor. UV-B had adverse impact on lichens by decreasing both the RGR and  $\text{RT}_{\text{A}}\text{GR}$ . By contrast, PAR treatment (no UV-B) supported high RGR and  $\text{RT}_{\text{A}}\text{GR}$  for all three lichens. However, *P. aphthosa* was more affected by UV-B radiation than *C. islandica* and *L. pulmonaria*. *Peltigera aphthosa* had negative RGR and  $\text{RT}_{\text{A}}\text{GR}$  due to photoinhibition. Chl *a* and *b* were measured in two solutions, in DMSO and in ethanol. There were no significant differences in Chl *a+b*, Chl *a/b* and brown pigments synthesis between treatments.

A second growth chamber experiment was run with only *L. pulmonaria* for 3-weeks at two treatments, PAR+UV-B+UV-A and PAR+UVA, to investigate the role of high intensity UVA and PAR in brown pigments synthesis. In the PAR+UV-B+UV-A treatment, the level of UV-A and UV-B were  $7$  and  $0.4 \text{ Wm}^{-2}$ , respectively, and PAR was  $500 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . In the PAR+UV-A treatment, polyester had been placed to screen UV-B, the level of UV-A beneath the polyester was  $5.6 \text{ Wm}^{-2}$  and the PAR level was same. Photoperiod was 12 h; UV radiation for 8 h in the middle of the daily 12h photoperiod for both treatments. Chl *a+b* and brown pigment synthesis significantly differed between the treatments. Brown pigment synthesis was twice as high at PAR+UV-B+UV-A than at PAR+UVA. The higher brown pigments synthesis in this second experiment suggests that higher intensity of UV-A and/or PAR boost the synthesis of brown pigments in the lichens.

**Keywords:** *Cetraria islandica*, *Lobaria pulmonaria*, *Peltigera aphthosa*, Relative growth rate, Relative thallus area growth rate, Chlorophyll, UV-B, UV-A, PAR, Brown pigments.

## Abbreviations

A	Area
Chl <i>a+b</i>	Chlorophyll <i>a+b</i>
Chl <i>a/b</i>	Chlorophyll <i>a/b</i>
DM	Dry mass
$F_v/F_m$	Maximal quantum yield of PSII
RGR	Relative growth rate
RT <sub>A</sub> GR	Relative thallus area growth rate
STM	Specific thallus mass

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## 1. Introduction

Lichens are stable, self-supporting symbiotic poikilohydric associations of a fungus species (mycobiont) and one or more photosynthetic partners (photobiont) where the photobiont produces food and the mycobiont provides shelter. The main body of lichen, referred to as a thallus, is formed by the fungal partner, constituting 50 - 90 % of total thallus biomass (Honegger 2012). The shape of a lichen thallus varies from leaf-like to filamentous to powder like. Depending on their shape, lichens are mostly grouped into three growth forms – foliose (leafy), fruticose (shrubby) or crustose (crusty) (Perez-Llano 1944). The estimation of global total known lichen species ranges approximately between 13,500 to 20, 000 (Feuerer & Hawksworth 2007), comprising 60 families and 400 genera (Perez-Llano 1944). Lichens have significant roles in nature. Unlike other photosynthetic organisms, photobionts (algae/cyanobacteria) take up atmospheric CO<sub>2</sub> to produce carbohydrates through photosynthesis. Lichen-dominated ecosystems occur in many places on Earth and cover approximately 8% of terrestrial ecosystem (Honegger 2012; Larson 1987). Lichens are capable to survive in extreme environments with high, as well as low temperature, strong solar exposures even under scarcity of water and low nutrient availability (Nash III 1996) . They are capable to survive even in space (Sancho et al. 2007). They can colonize various substrates, on and within rocks, on the surface of soils, living leaves, tree trunk and branches or on various man-made construction such as brick, plastic, glass, metal, leather, etc. However, lichens are mostly dominant in higher latitude and altitude, especially Arctic and alpine areas are covered by terricolous and saxicolous lichens (Bjerke et al. 2002). High altitude lichen mostly experience variable environmental conditions, in terms of high visible light and UV-B light.

Solar radiation on the earth surface reach with variable wavelengths where the wavelength of ultraviolet radiation (100-400 nm) is shorter than the visible light (400-700 nm). Radiation with shorter wavelengths have higher energy than longer wavelengths. The shortest and most energy-rich UV-C (100-280 nm) radiation cannot transmit through the atmosphere as it is mostly absorbed by ozone (O<sub>3</sub>). UV-B radiation (280-315 nm) is partly absorbed in the atmosphere, and partly reaches the earth surface. UV-A (315-400 nm) radiation mostly passes through the ozone layer (Madronich et al. 1998). UV-B exposure has been reported to increase significantly in both high and low latitudes due to the greenhouse gas (e.g. chlorofluorocarbon) inducing stratospheric ozone layer depletion (Aphalo et al. 2012). However, the ozone layer

depletion is more prominent near the Northern and Southern poles. The study about the adverse impact of UV-B on biosphere has started during the 1980's period when the stratospheric Ozone layer depletion was discovered (Solomon 1999). UV-B has strong impact on the biosphere. In general, high UV-B doses impact DNA, proteins, membranes, and may reduce photosynthesis with consequent impact on plant growth. It can generate reactive oxygen species which may harm cellular processes (Buffoni Hall et al. 2003). Plants have protective mechanism to avoid harmful UV-B radiation. They acquire UV-B absorbing phenolic compounds in the epidermal tissue, which act as sunscreen and prevent UV-B radiation to penetrate (Jenkins 2009).

The investigation about the effects of increased UV-B radiation has begun during the last three decades. However, most studies have been performed for higher plants and bryophytes (Caldwell et al. 1998). The effect of UV-B on lichens is less studied, although major lichen habitats are located on exposed higher latitude and altitude. Due to higher latitudinal gradient of ozone layer, the biological impact on treeless Arctic lichen will be more prominent compare to low latitude vegetation. Thus we need to investigate closely to observe the sustainability and vulnerability of lichens, when they are exposed to UV-B radiation.

Generally, lichens are known as slow growing and long-lived organism in nature, providing less opportunity to study their growth parameters in response to different environmental factors under laboratory condition in short time period. However, recent studies (Alam et al. 2015; Bidussi et al. 2013) have already showed that lichens grow fast in growth chambers within few weeks. Besides growth parameters, the synthesis of lichen compounds (Solhaug & Gauslaa 2004; Solhaug et al. 2003) can also be investigated within short time periods. Most of the growth chamber experiment were performed to assess the lichens growth in relation to hydration and temperature regime or light use efficiency. The aim of this study is to investigate how UV-B radiation may affect lichen growth under controlled growth chamber condition. Some growth chamber and field experiments studies showed that UV-B radiation enhanced photosystem II efficiency (Sonesson et al. 1995) and had no adverse effect on lichen's relative growth rate (Larsson et al. 2009). Contrast result are also documented, where altered UV-B radiation slightly reduced or did not affect photosystem II efficiency (Bjerke et al. 2005; Solhaug et al. 2003). The direct impact of UV-B radiation on lichens growth is poorly studied so far. However, many studies have focused on the synthesis of lichen compounds (parietin, usnic acid) in response to UV-B radiation, specifically parietin (Solhaug et al. 2003) and usnic acid

(Bjerke et al. 2002; Hall et al. 2002; Larsson et al. 2009; McEvoy et al. 2006; Solhaug et al. 2003; Solhaug & Gauslaa 2012), whereas few have studied regulating factors of melanin synthesis (Solhaug et al. 2003).

Lichens are often protected from UV-B radiation by producing various UV-B-screening secondary compounds such as parietin, usnic acid and melanins (Gauslaa & Solhaug 2001; McEvoy et al. 2006; Solhaug & Gauslaa 1996; Solhaug & Gauslaa 2012). These pigments are produced by the fungal partner and form a layer above the algal partner. Overlaying UV-B-absorbing lichen compounds thus protect chlorophylls from the exposure of UV-B radiation. However, these pigment formations as a response of UV-B irradiance vary seasonally (Gauslaa & McEvoy 2005) and differs considerably from lichen to lichen (Bjerke et al. 2002). Melanic compounds are one of the widely known UV-B radiation screening compounds which are present in a wide variety of organisms in various kingdoms (Riley 1997) including humans (Brenner & Hearing 2008; Routaboul et al. 1999). In lichens, the brownish and amorphous melanic compounds are produced in the upper cortex and they may screen high radiation (Gauslaa & Solhaug 2001; Nybakken et al. 2004). Melanic lichens are easy to recognize by the naked eye in the open habitat for their dark brown cortical melanic compounds. I want to investigate how UV-B radiation may influence lichens growth and which factors actually take part in the synthesis of brown pigments. Thus, three common melanin producing lichens, one chlorolichen *Cetraria islandica* and the two, cephalolichens *Lobaria pulmonaria* and *Peltigera aphthosa* were selected for this study.

Melanic pigments complicate measurements of chlorophylls because the recommended extraction method for lichens (Palmqvist & Sundberg 2002) extract both chlorophylls and melanins (Meeßen et al. 2013) with partly overlapping absorbances. Extraction of chlorophyll without melanins may be avoided by using acetone or ethanol in which melanins are not dissolved (Meeßen et al. 2013). However, DMSO is a better solvent for chlorophyll extraction, because grinding is often not needed and there is less loss of solvent due to much lower vapor pressure than for acetone. As the studied lichen species can form melanic pigments, there is a need to develop rapid methods that can extract chlorophylls and melanic compounds with DMSO and separate and measure them separately.

The major objectives of this study are:

- a. To study the growth of lichens as relative growth rate (RGR) and relative thallus area growth rate ( $RT_{\Delta}GR$ ) under UV-B radiation and photosynthetic active radiation.
- b. To investigate UV-B radiation impact on lichen growth.
- c. To develop methods that quantify chlorophyll *a*, *b* and brown material separately.
- d. To assess whether UV-B has any impact on chlorophyll *a*, *b* and brown pigments.

## 2. Material and Methods

### 2.1 Lichen material

The three selected lichen species were: the chlorolichen *Cetraria islandica* (L.) Ach and the two, cephalolichens *Lobaria pulmonaria* (L.) Hoffm. and *Peltigera aphthosa* (L.) Wild (Fig. 1). I collected *C. islandica* and *P. aphthosa* from an open and mixed (birch and pine) forest, located near Sørmarka Konferansehotell, Ski, Akershus (59° 48' 54"N, 10° 54' 34"E, 170 m above from sea level); *L. pulmonaria* from an old open broadleaved oak forest, located in Langangen, Porsgrunn, Telemark (59° 06 43 N, 9° 50 05 E, 140 m above from sea level).

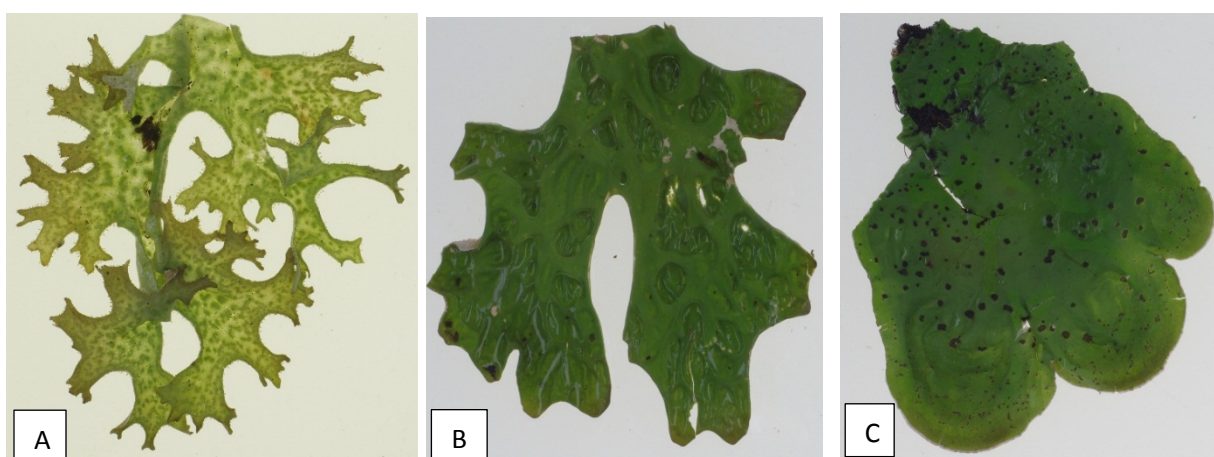


Fig. 1: A- *Cetraria islandica*, B- *Lobaria pulmonaria*, C- *Peltigera aphthosa*. The photos show typical experimental thalli of the three species, taken on glass plates with light from both below and above.

Photos courtesy: Dipa Paul Chowdhury

### 2.2 Growth Chamber Experiment

The growth chamber experiments have been performed at the Center for Plant Research in Controlled Environment (SKP) at the Norwegian University of Life Science (NMBU) during September to November 2014. All collected thalli were first cleaned to remove attached moss, debris. Then they were air dried and stored in the freezer (-18°C) until (1 months) experiments. In total, 180 young and healthy thalli without reproductive organs, 60 thalli for each species, were randomly selected for the experiment. Not more than 2-3 entire branches (hereafter called thalli) of approximately 10-18 cm<sup>2</sup> were taken from each thallus. All thalli were air dried in the

laboratory for 48 h at 20° C before weighing their air dry mass ( $\pm 0.1$  mg). In addition, 10 control thalli of each species were weighted at the beginning and at the end of the growth chamber experiment. Then, these control thalli were oven-dried for 24 h at 70° C before I recorded their oven-dried mass (DM). The reduction factor in dry mass from the air-dried to the oven-dried state was used to calculate the DM for all experimental thalli before and after the experiment. Afterwards, all thalli were sprayed by the de-ionized water until they were fully hydrated. Each hydrated thallus was then photographed, and I measured their thallus area (A) by applying ImageJ (1.48v) software before and after the growth chamber experiment. The area of *C. islandica* was not measured due to its complex three-dimensional thallus structure.

A second growth experiment was run with only *L. pulmonaria*. In total, I used 20 young and healthy thalli for this experiment and 5 thalli as control.

### 2.2.1 Experimental condition

The growth experiment was performed in a growth chamber at constant air temperature of 15° C. I used two separate growth chambers to establish two different growth regimes. One chamber was illuminated with white fluorescent tubes (Philips Master TL-D 36W/840, Ahlsell Norge AS, Moss, Norway) as a light source of photosynthetic active photon flux (PAR) density of 125  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . The other chamber was illuminated with the same PAR, but with added ultraviolet radiation density of 1  $\text{Wm}^{-2}$  (Q-panel UV 313, Largo, Gothenburg, Sweden). The UV radiation was filtered through 0.15 mm thick cellulose di-acetate film (Rachow Kunststoff Folien GmbH, Hamburg, Germany). The daily photoperiod was 12 h for PAR; the UV-B light was on for 6 h in the middle of the photoperiod for all experimental days. In total 180 thalli were cultivated in both growth chambers (90 in each) for 14 days. All thalli from all species were placed on top of 10-12 layers with filter papers in one large open plastic box. The samples were sprayed with de-ionized water three times a day. Thereby, all thalli remained moistened most of the time.

The second growth experiment was run in one small room without windows at room temperature (approximately 20°C). Samples were illuminated with a LED panel (model SL3500 RGB, Photon systems instruments, Brno, Czech Republic) and UV-A tubes (Q-panel UV 340, Largo, Gothenburg, Sweden). The level of UV-A and UV-B were 7 and 0.4  $\text{Wm}^{-2}$  respectively. PAR was 500  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  for 12 h; UV radiation for 8 h in the middle of the daily 12h

photoperiod. Two small boxes containing 2 small petri-dishes, each with 5 thalli, were placed under the light sources. I placed polyester (standard overhead foil) that screens all UV-B radiation above one box; the other box had normal cling film that transmits all three wavelength ranges (Fig. 2). The level of UVB and UVA radiation beneath the polyester were  $0 \text{ Wm}^{-2}$  and  $5.6 \text{ Wm}^{-2}$ . There were 2 cm distance between the box and screens to allow free air circulation. Samples were hydrated by spraying once a day for an experimental period of 21 days. Moistening of the thalli was done once each evening, just after the 12h photoperiod. Therefore, all thalli remain desiccated during the day time.

Spectra of both UV313 and UV340 tubes were measured with the Optronic OL756 spectroradiometer (Optronic Laboratories, Orlando, FL USA) (Fig. 3).

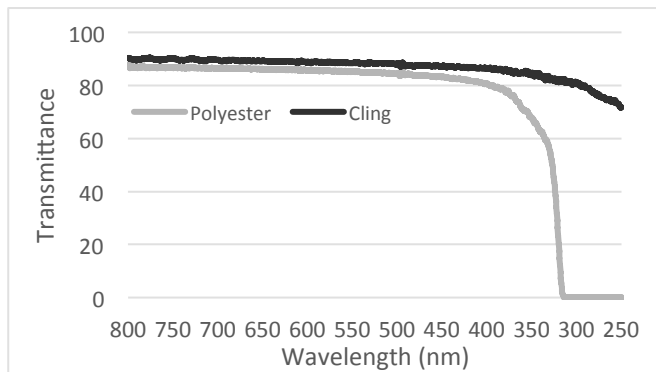


Fig. 2: Transmittance spectra of the two screens, polyester (overhead foil) and cling film.

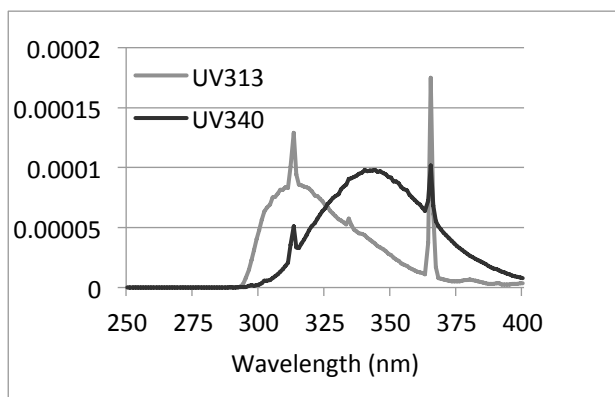


Fig. 3: Spectra of the UV313 and the UV340 tubes used in the experiments.

### 2.2.2 Growth rate measurement

I estimated relative growth rates by measuring their changes in DM and A before and after the experiment. DM change was quantified as  $RGR = (\ln(DM_{end}/DM_{start})) * 1000 / \Delta t \text{ (mg g}^{-1}\text{day}^{-1}\text{)}$

and relative thallus area growth was quantified as  $RT_{\Delta}GR = (\ln (A_{end}/A_{start})) * 100 / \Delta t$  ( $\text{mm}^2 \text{cm}^{-2} \text{day}^{-1}$ ) for the two foliose species only.

### **2.3 Chlorophyll fluorescence measurement**

Chlorophyll *a* fluorescence for all thalli was measured before and after the growth chamber experiment by using a PAM 2000 fluorometer (Walz, Effeltrich, Germany). Prior to each measurement occasions, all thalli were kept hydrated in low light ( $10\text{-}15 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) at  $18^{\circ}\text{C}$  for 24 h to recover from photoinhibition. Thalli were further dark adapted for 15 min immediately before I recorded the maximum photochemical efficiency of photosystem II ( $F_v/F_m$ ).

### **2.4 Chlorophyll and brown pigments analyses**

I quantified chlorophylls and brown pigments (melanic compounds) in the 10 randomly selected thalli from each treatment of 1<sup>st</sup> experiment. For 2<sup>nd</sup> experiment, chlorophylls and brown pigments were measured for all the thalli.

#### **2.4.1 Chlorophyll and brown pigments extraction**

First, 10 thalli from each treatment and from each of three species from 1<sup>st</sup> experiment were randomly selected to measure chlorophyll *a+b* and brown pigments from 1<sup>st</sup> experiment. Each thallus was ground to fine powder with a ball mill using small metal ball (Retsch model MM400, Retsch GmbH Hann, Germany). Approximately 19-22 mg of dry lichen from each ground sample was placed in an Eppendorf tube. Then, 2 ml DMSO with  $\text{MgCO}_3$  was added to the Eppendorf tube. The tubes were then incubated for 30 min in an ultrasonic water bath (Ultrasonic cleaner, USC 200TH) at  $70^{\circ}\text{C}$  to improve extraction of pigments with DMSO. The tubes were shaken every 10 min. The tubes with the well-mixed solution were centrifuged at 15000 rpm/min for 2 min. I measured the absorbance spectrum of the supernatant by a spectrophotometer (Shimadzu UV-210IPC).

For separating brown pigments, various procedures were performed. First, 0.2 ml of de-ionized water was mixed with the supernatant into the cuvettes. A solution of DMSO was made with 10% de-ionized water added. Half ml of this 10% water-mixed DMSO was passed through the Agilent Bond Elut C18 column sorbent to prepare the column for extracting brown pigments.



Then, the water mixed supernatant was passed through the column gently by using a syringe. However, the brown pigments were passed through the column. Chlorophyll and carotenoids were held back in the column. The extract was taken into the cuvettes and the absorbance measured. It was observed that by using 100% DMSO, xanthophylls were not completely retained in the column. Therefore, 10% de-ionized water was used to completely retain all carotenoids in the column.

Now, chlorophylls and carotenoids, which remained 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 take out all chlorophylls from the column. The absorbance at 649, 665 and 750 nm of the combined 3 ml ethanol chlorophyll extracts were then measured in the spectrophotometer. Each Agilent Bond Elut C18 column was used for only 5 samples before it was replaced by a new column.

Similarly, I extracted chlorophylls and brown pigments for all the thalli from 2<sup>nd</sup> experiment.

#### **2.4.2 Measurement of chlorophyll**

Chlorophyll content was measured in two solutions, in DMSO before removal of brown pigments and in ethanol after removal of brown pigments. Chlorophyll *a* and *b* was calculated in mg g<sup>-1</sup> 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, respectively. To correct for small impurities etc in the cuvettes the absorbance at 750 nm was subtracted from the 649 and 665 nm absorbances because chlorophyll does not absorb at 750 nm. These absorbance values were then multiplied by their respective solution volume and then, divided by their respective dry weights.

##### **DMSO**

$$\text{Chl } a = 12.19*(A_{665} - A_{750}) - 3.45*(A_{649} - A_{750})$$

$$\text{Chl } b = 21.99*(A_{649} - A_{750}) - 5.32*(A_{665} - A_{750})$$

##### **Ethanol**

$$\text{Chl } a = 13.95*(A_{665} - A_{750}) - 6.88*(A_{649} - A_{750})$$

$$\text{Chl } b = 24.96*(A_{649} - A_{750}) - 7.32A*(A_{665} - A_{750})$$

### 2.4.3 Measurement of brown pigments

Brown pigments were measured as relative absorbance at 450 nm. The wavelength for determination of melanin was chosen to be 450 nm because the absorbance of synthetic melanin (Fig. 4) is increasing gradually towards shorter wavelength. The absorption of other secondary lichen compounds are avoided as these do not absorb in the visible light. The absorbance at 450 nm was measured and the value divided by the weight of extracted material.

Relative brown pigments concentration = Absorbance (450 nm) / DM

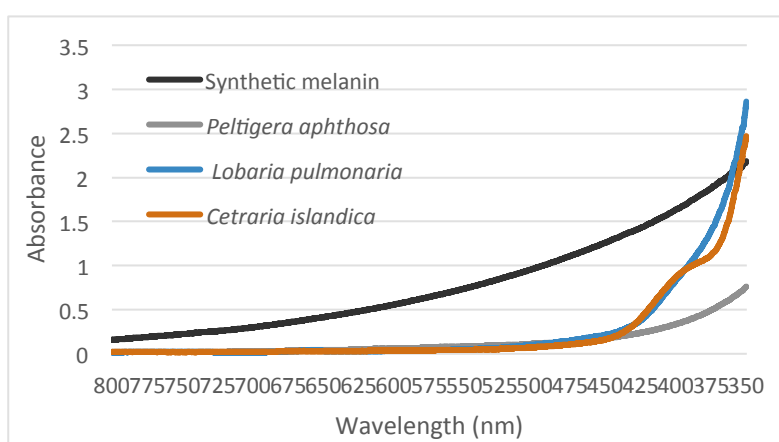


Fig.4: Absorbance spectrum of *Cetraria islandica*, *Lobaria pulmonaria*, *Peltigera aphthosa* and synthetic melanin.

### 2.5 Statistical analyses

All statistical analyses were run in Minitab 16 (Minitab Inc., State College, PA, USA). General Linear Models (GLM) and Non-parametric test (Kruskal-Wallis test), depending on whether the data satisfied the GLM requirements or not, was used to observe the difference between treatments on different parameter among three lichens. In GLM, growth parameters (RGR,  $RT_{AGR}$ ),  $\Delta STM$ , Chl  $a+b$ , Chl  $a/b$  and  $Fv/Fm$  were used as responses and treatments as model. In the non-parametric test (Kruskal-Wallis test), parameters were used as responses and treatments as factor.

### 3. Results

#### 3.1 Experiment 1

##### 3.1.1 Effects of UV-B on Relative Growth Rate (RGR)

Relative growth rate was much higher without UV-B than with UV-B ( $P < 0.001$ ; Fig. 5 and Tab. 2). Relative growth rate (RGR) for the PAR+UV-B treatment, *L. pulmonaria* showed highest growth rate ( $4.86 \pm 0.48 \text{ mg g}^{-1} \text{ day}^{-1}$ ) (mean  $\pm$  1 SE;  $n = 30$ ), *C. islandica* showed just slightly positive growth rate ( $0.32 \pm 0.30 \text{ mg g}^{-1} \text{ day}^{-1}$ ), significantly lower than in *L. pulmonaria*, whereas *P. aphthosa* had negative growth rate ( $-3.21 \pm 0.50 \text{ mg g}^{-1} \text{ day}^{-1}$ ). The RGRs without UV-B (the PAR treatment) for *L. pulmonaria*, *C. islandica* and *P. aphthosa* were  $9.60 \pm 1.25 \text{ mg g}^{-1} \text{ day}^{-1}$ ,  $3.06 \pm 0.46 \text{ mg g}^{-1} \text{ day}^{-1}$  and  $2.23 \pm 0.45 \text{ mg g}^{-1} \text{ day}^{-1}$ , respectively. UV-B significantly reduced the growth of all three lichen species (Fig. 5 and Tab. 2). Thereby, all three lichen species showed higher growth rate in absence of UV-B compared to the treatment with PAR + UV-B (Fig. 5). However, the impact of UV-B on RGR varied depending on lichen species. Among three lichen species, *L. pulmonaria* showed the highest tolerance of UV-B and *P. aphthosa* the lowest tolerance with negative growth rates.

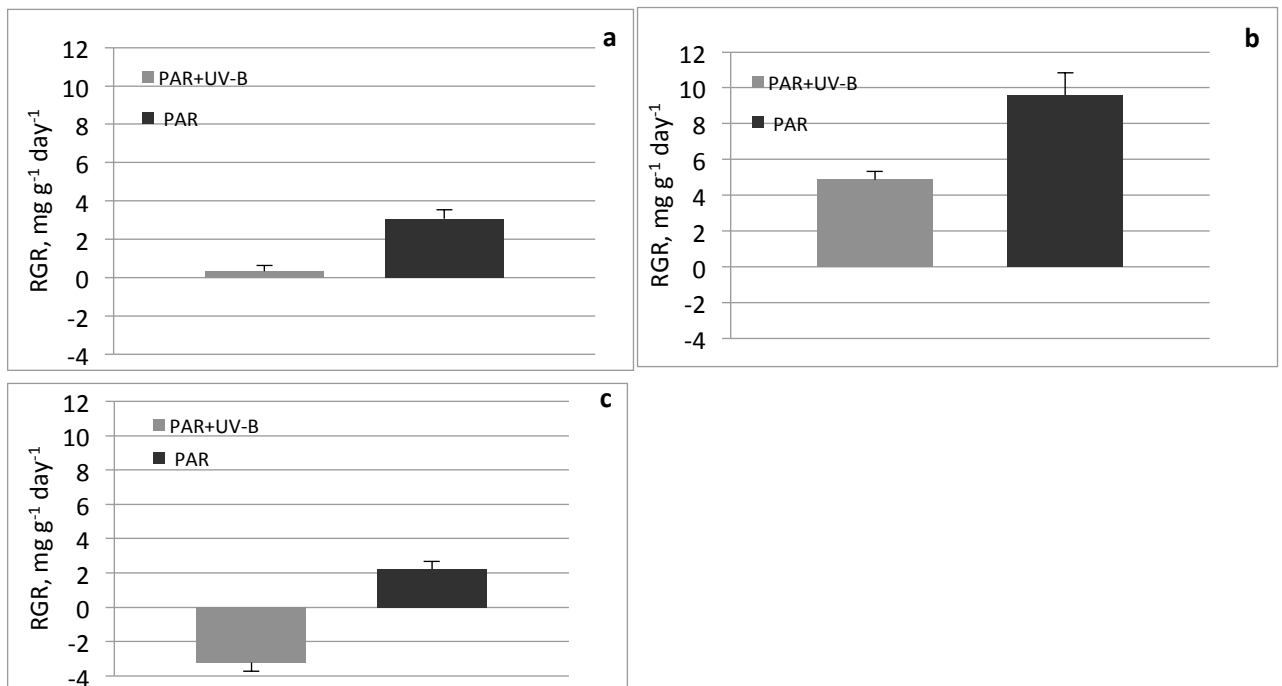


Fig. 5: Relative growth rates (RGR) in *Cetraria islandica* (a), *Lobaria pulmonaria* (b) and *Peltigera aphthosa* (c), exposed to visible light only (PAR) or visible light combined with UV-B (PAR+UV-B) for 2 weeks. The error bars show  $\pm$  1 SE;  $n=30$ .

### 3.1.2 Effects of UV-B on Relative Thallus Area Growth Rate (RT<sub>A</sub>GR)

The RT<sub>A</sub>GR for *L. pulmonaria* and *P. aphthosa* was  $0.02 \pm 0.06 \text{ mm}^2 \text{ cm}^{-2} \text{ day}^{-1}$  and  $-0.29 \pm 0.05 \text{ mm}^2 \text{ cm}^{-2} \text{ day}^{-1}$ , respectively (mean  $\pm$  1SE, n=30) for the PAR+UV-B treatment. Without UV-B exposure (the PAR treatment), the RT<sub>A</sub>GR was  $0.39 \pm 0.06 \text{ mm}^2 \text{ cm}^{-2} \text{ day}^{-1}$  for *L. pulmonaria* and just  $0.01 \pm 0.06 \text{ mm}^2 \text{ cm}^{-2} \text{ day}^{-1}$  for *P. aphthosa*. Thereby, UV-B had adversely affected thallus area growth in *L. pulmonaria* and *P. aphthosa* (Tab. 2). The RT<sub>A</sub>GR of *L. pulmonaria* without UV-B was fifteen times higher than in those exposed to UV-B. However, *P. aphthosa* showed negative area growth under UV-B treatment (Fig 6). Moreover, *P. aphthosa* had less area growth than *L. pulmonaria* for the PAR treatment.

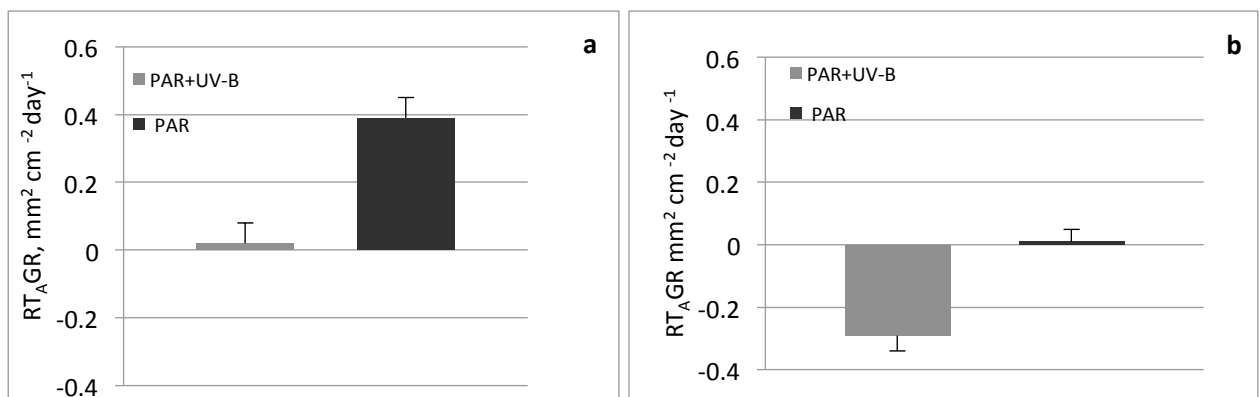


Fig. 6: Relative thallus area growth rate (RT<sub>A</sub>GR) in *Lobaria pulmonaria* (a) and *Peltigera aphthosa* (b), exposed to visible light only (PAR) or visible light combined with UV-B (PAR+UV-B) for 2 weeks. The error bars show  $\pm$  1 SE; n=30.

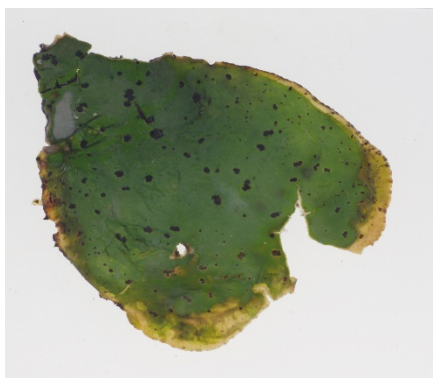


Fig. 7: Wet thalli of *Peltigera aphthosa* from the treatment PAR+UV-B, showing damage around the corner of the thallus.

Thus, *P. aphthosa* had much less tolerance of UV-B radiation than *L. pulmonaria*. In addition, some damage is visible around the corner of *P. aphthosa* for few thallus under UV-B treatment

(Fig. 7).  $RT_{AGR}$  of *C. islandica* could not be computed because its three-dimensional thallus structure prevented a reliable estimate of A.

### 3.1.3 Effects of UV-B on the changes in Specific Thallus Mass ( $\Delta STM$ )

Changes in STM ( $\Delta STM$ ) over the two weeks' cultivation experiment for the two treatments (PAR+UV-B, PAR) followed similar patterns as RGR and  $RT_{AGR}$ . Both *L. pulmonaria* and *P. aphthosa* showed higher  $\Delta STM$  for the PAR treatment in comparison to the PAR+UV-B treatment (Fig 8). The average  $\Delta STM$  of *L. pulmonaria* for the PAR treatment was  $8.60 \pm 1.47$   $mg\ cm^{-2}$  (mean  $\pm$  1 SE; n=30), which decreased to  $6.89 \pm 0.92$  in thalli exposed to PAR+UV-B. In *P. aphthosa*, the average  $\Delta STM$  values for PAR and PAR+UV-B were substantially lower with just  $3.02 \pm 0.65$   $mg\ cm^{-2}$  and  $-0.29 \pm 0.49$   $mg\ cm^{-2}$ , respectively. Noticeably, *P. aphthosa* showed stronger decrease and negative  $\Delta STM$  for the PAR+UV-B treatment, as the biomass growth and area growth for *P. aphthosa* showed negative values as well (Fig 5 and 6). Also *L. pulmonaria* had decreased, but still positive  $\Delta STM$  under the UV-B exposure. Treatments significantly differed for *P. aphthosa* whereas *L. pulmonaria* did not show any significant difference between the treatments (Table 2).

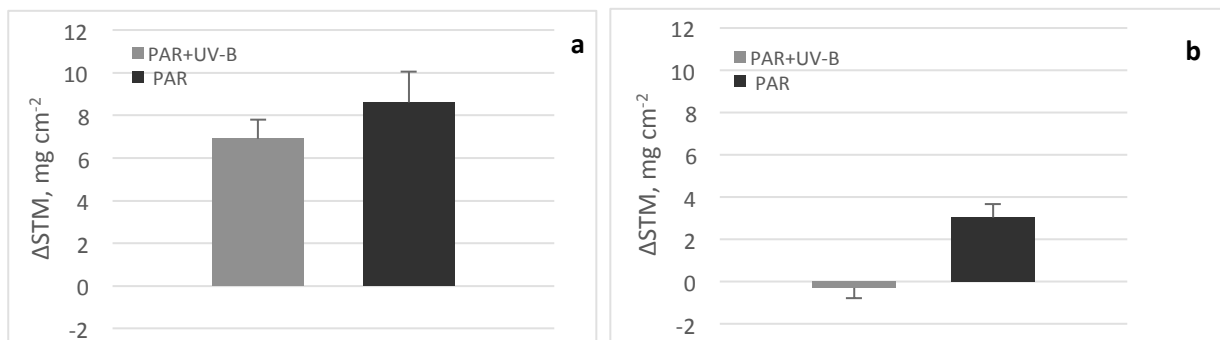


Fig. 8: Changes in STM ( $\Delta STM$ ) for *Lobaria pulmonaria* (a) and *Peltigera aphthosa* (b), exposed to visible light only (PAR) or visible light combined with UV-B (PAR+UV-B) for 2 weeks. The error bars show  $\pm$  SE and n=30.

### 3.1.4 Chlorophyll fluorescence

The average  $F_v/F_m$  for *C. islandica*, *L. pulmonaria* and *P. aphthosa* showed larger variation at the end of both treatments (UV-B+PAR, PAR) than before, evidenced by the higher standard errors at the end (Table 2). There were no differences in  $F_v/F_m$  between the treatments for *C. islandica* and *L. pulmonaria* after treatment (Table 1 and 2). By contrast, *P. aphthosa* had significantly lower  $F_v/F_m$  values after both treatments than *C. islandica* and *L. pulmonaria*. Furthermore, *P. aphthosa* had lower  $F_v/F_m$  with UV-B ( $0.489 \pm 0.022$ ) (mean  $\pm$  1 SE; n = 30) than without UV-B ( $0.609 \pm 0.021$ ) at the end of cultivation (Table 1 and 2).

Table 1: Maximal photosystem II efficiency ( $F_v/F_m$ ) of *Cetraria islandica*, *Lobaria pulmonaria* and *Peltigera aphthosa* at the start and end of two treatments, UV-B+PAR and PAR. The values show average values  $\pm$  1 SE; n=30.

Treatments	<i>Cetraria islandica</i>		<i>Lobaria pulmonaria</i>		<i>Peltigera aphthosa</i>	
	Start	End	Start	End	Start	End
UV-B+PAR	0.731 $\pm$ 0.003	0.705 $\pm$ 0.008	0.707 $\pm$ 0.003	0.704 $\pm$ 0.006	0.723 $\pm$ 0.002	0.489 $\pm$ 0.022
PAR	0.711 $\pm$ 0.005	0.684 $\pm$ 0.012	0.695 $\pm$ 0.005	0.718 $\pm$ 0.005	0.707 $\pm$ 0.006	0.609 $\pm$ 0.021

Table 2: One way ANOVA of RGR and  $F_v/F_m$  in *Cetraria islandica*, *Peltigera aphthosa* and *Lobaria pulmonaria* and of  $RT_{AGR}$  and  $\Delta STM$  for *L. pulmonaria* and *P. aphthosa* cultivated for 14 days at two treatments (UV-B+PAR, PAR). A non-parametric test (Kruskal-Wallis test) was run for RGR and  $\Delta STM$  for *L. pulmonaria* and of  $F_v/F_m$  for *P. aphthosa*. In these cases,  $r^2_{adj}$  were not computed.

Parameter	<i>d.f</i>	RGR		$RT_{AGR}$		$F_v/F_m$		$\Delta STM$	
Source									
<b><i>Cetraria islandica</i></b>		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>p</i>
Treatment	1	24.9	0.000	n/a		1.89	0.175	n/a	
Error	58								
Total	59								
$r^2_{adj}$		28.83%				1.48%			
<b><i>Lobaria pulmonaria</i></b>		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>p</i>
Treatment	1		0.000	17.2	0.000	2.78	0.101		0.000
Error	58								
Total	59								
$r^2_{adj}$				21.54%		2.93%			
<b><i>Peltigera aphthosa</i></b>		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>p</i>
Treatment	1	64.05	0.000	20.85	0.000		0.000	16.2	0.000
Error	58								
Total	59								
$r^2_{adj}$		51.66%		25.17%				20.48%	

### 3.1.5 Chlorophylls

#### Chlorophyll *a+b*

Chlorophyll *a+b* varied among lichen species ( $P < 0.001$ ; GLM data not shown), but neither between treatments nor the two methods used for extraction (DMSO and ethanol) (Fig.9 and Tab. 3), except for Chl *a+b* of *L. pulmonaria*, measured in ethanol ( $P < 0.05$ , Tab. 3).

*C. islandica* had the lowest Chl *a+b* concentration and *L. pulmonaria* the highest for both treatments (PAR+UV-B and PAR). In *C. islandica*, the average Chl *a+b* concentrations (DMSO) for PAR+UV-B and PAR were  $0.569 \pm 0.049 \text{ mg g}^{-1}$  (mean  $\pm$  SE,  $n=10$ ) and  $0.578 \pm 0.037 \text{ mg g}^{-1}$  respectively. By contrast, *L. pulmonaria* had much higher average Chl *a+b* concentration (DMSO;  $2.543 \pm 0.053 \text{ mg g}^{-1}$ ) for the PAR+UV-B treatment. *P. aphthosa* had also fairly high Chl *a+b* concentrations (DMSO);  $2.126 \pm 0.090 \text{ mg g}^{-1}$  and  $2.020 \pm 0.095 \text{ mg g}^{-1}$  for the PAR+UV-B and PAR treatment, respectively.

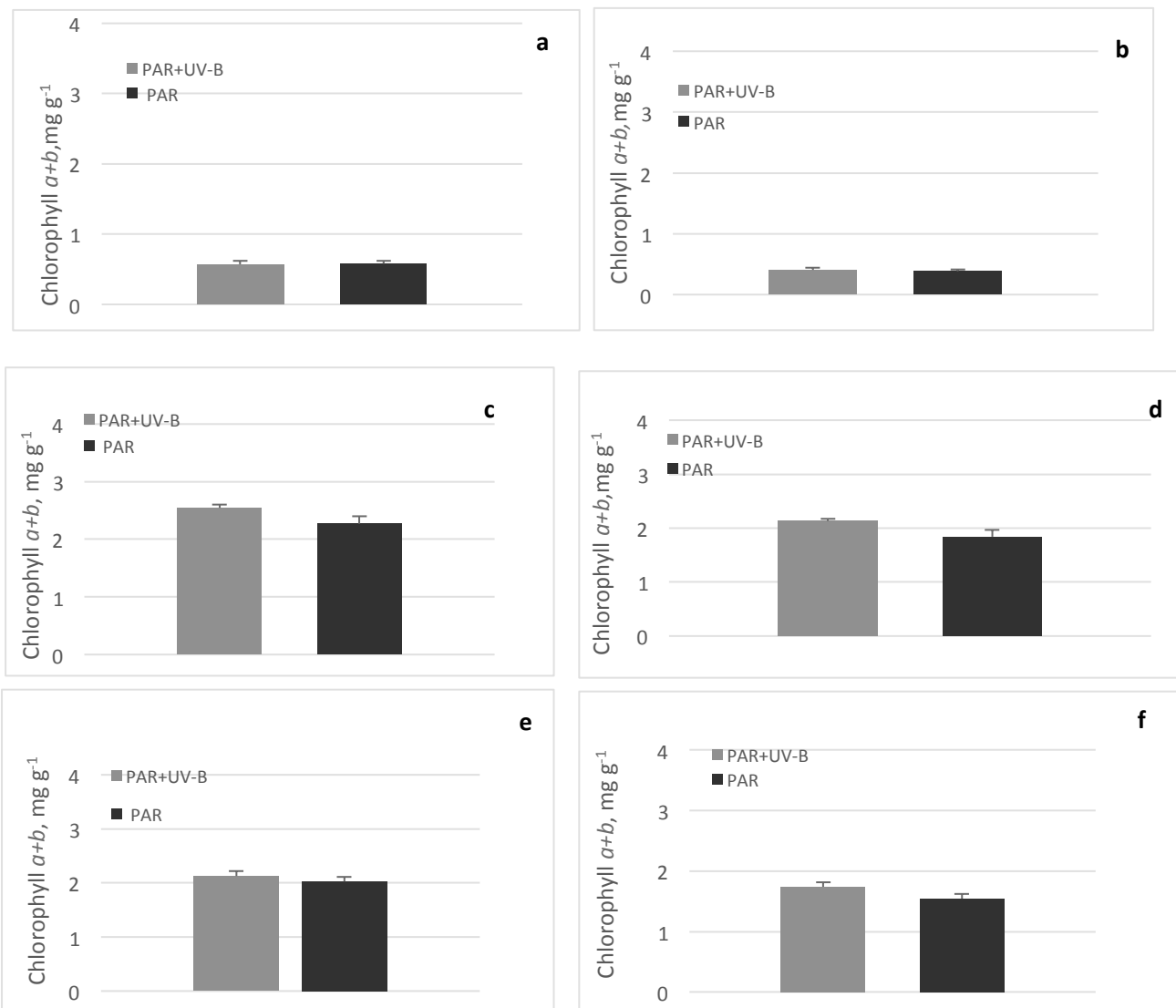


Fig. 9: Chlorophyll *a+b* (in mg g<sup>-1</sup>) in *Cetraria islandica* (a,b), *Lobaria pulmonaria* (c,d) and *Peltigera aphthosa* (e,f) after a two-weeks exposure to two treatments; PAR and PAR+UV-B, measured in DMSO (a, c, e) and in ethanol (b, d, f). The error bars show ± 1 SE; n=10.



## Chlorophyll *a/b*

The chlorophyll *a/b* ratio significantly varied among species ( $P < 0.001$ ; GLM data not shown) but not between the treatments (Fig. 10 and Tab. 3). The average Chl *a/b* ratio for all three lichen species was slightly higher for the PAR treatment than the PAR+UV-B in both measurements (DMSO and ethanol); except Chl *a/b* ratio (ethanol) of *L. pulmonaria*. Treatments were significantly differed only for the Chl *a/b* ratio of *P. aphthosa*, measured in DMSO.

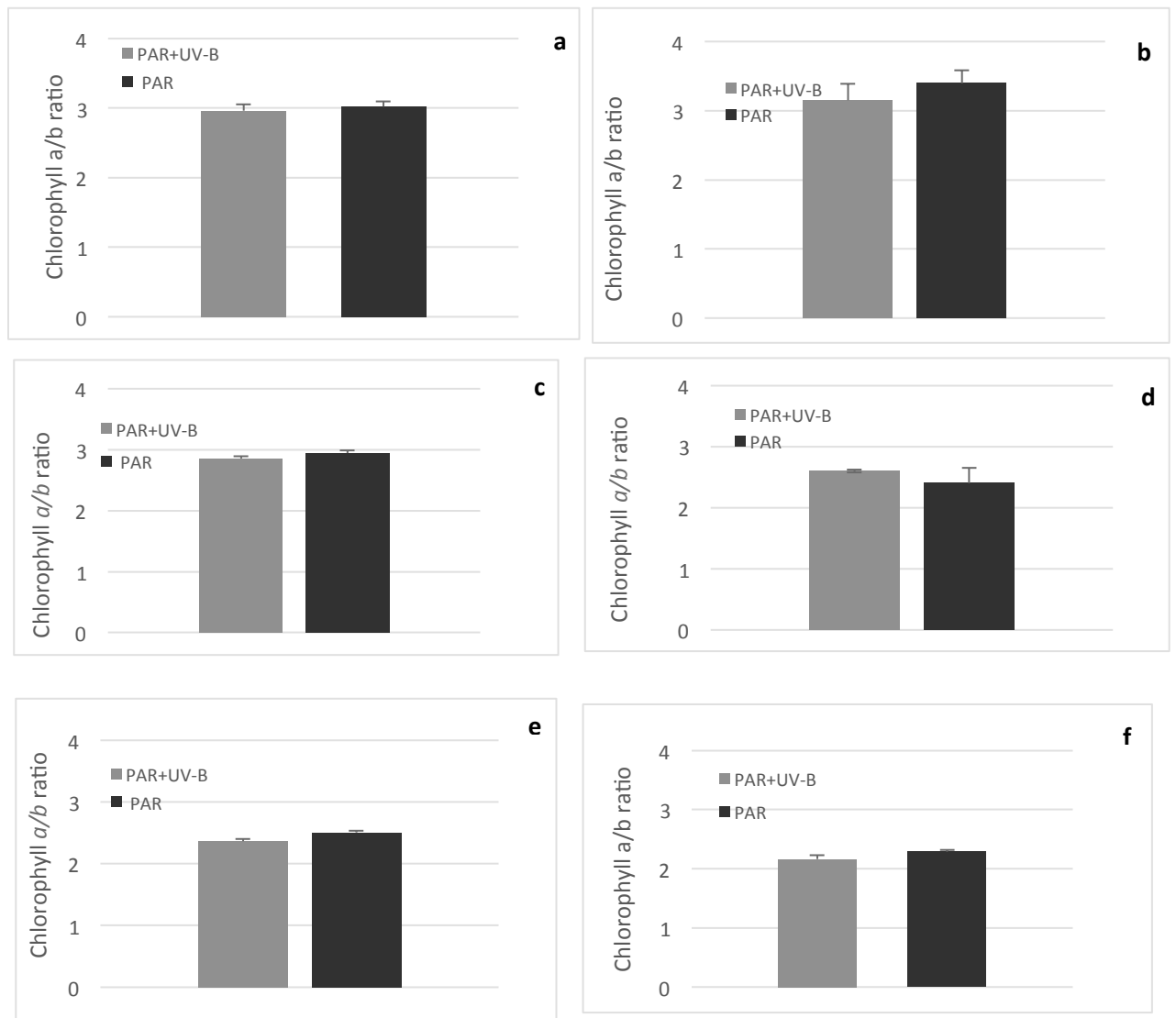


Fig. 10: Chlorophyll *a/b* in *Cetraria islandica* (a,b), *Lobaria pulmonaria* (c,d) and *Peltigera aphthosa* (e,f) after a two-weeks exposure to two treatments; PAR and PAR+UV-B, measured in DMSO (a. c. e) and in ethanol (b. d. f). The error bars show  $\pm 1$  SE:  $n=10$ .

### 3.1.6 Brown pigments synthesis

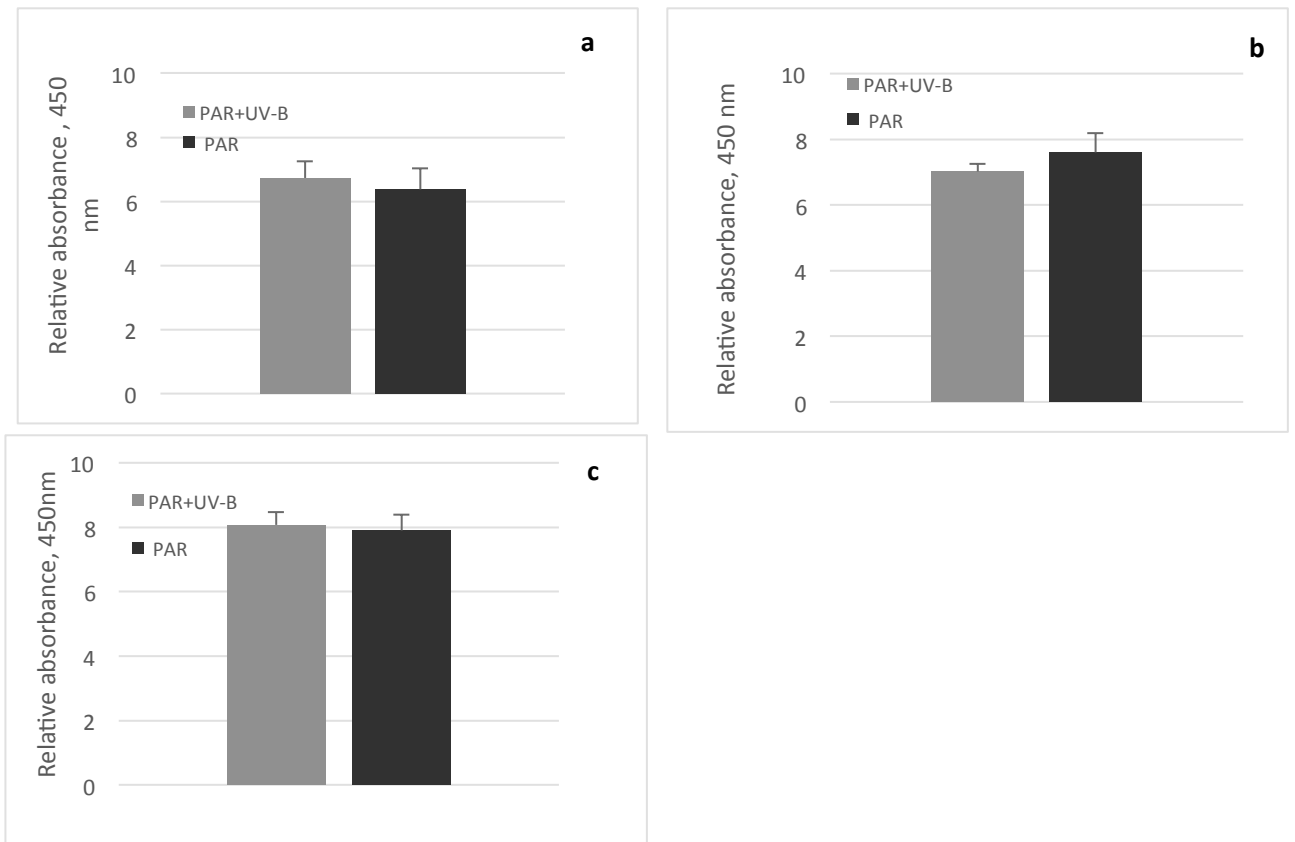


Fig. 11: Brown pigments (measured as relative absorbance at 450 nm) in *Cetraria islandica* (a), *Lobaria pulmonaria* (b) and *Peltigera aphthosa* (c), after a two-weeks exposure to two treatments; PAR and PAR+UV-B. The error bars show  $\pm 1$  SE; n=10.

Brown pigments absorbance or synthesis was slightly (but not significantly) higher with UV-B than the PAR treatment alone for *Cetraria islandica* and *Peltigera aphthosa* (Fig. 11 and Tab. 3). However, *L. pulmonaria* had more brown pigments absorbance for the PAR treatment compared to PAR+UV-B. For *L. pulmonaria*, the brown pigments absorption with PAR was  $7.60 \pm 0.58$  (mean  $\pm$  SE, n=10) and slightly less with PAR +UV-B treatment ( $7.01 \pm 0.24$ ). For *C. islandica*, the average brown pigments absorption was  $6.72 \pm 0.52$  and  $6.37 \pm 0.66$  for the PAR+UV-B and PAR treatments, respectively. *P. aphthosa* had the highest brown pigment absorption with UV-B treatment ( $8.07 \pm 0.39$ ) than without UV-B ( $7.90 \pm 0.49$ ).

Table 3: One way ANOVA of chlorophyll *a+b*, chlorophyll *a/b* and brown pigments in *Cetraria islandica*, *Lobaria pulmonaria* and *Peltigera aphthosa* for two treatments (UV-B+PAR, PAR). A non-parametric test (Kruskal-Wallis) was run for Chlorophyll *a+b* (DMSO), Chlorophyll *a+b* (ethanol), Chlorophyll *a/b* (ethanol) for *C. islandica* and of Chlorophyll *a/b* (ethanol) for *L. pulmonaria*. In these cases,  $r^2_{adj}$  were not computed.

Parameter Source	<i>d.f</i>	DMSO				Ethanol				Brown pigments	
		Chl <i>a+b</i>		Chl <i>a/b</i>		Chl <i>a+b</i>		Chl <i>a/b</i>		<i>F</i>	<i>p</i>
<b><i>Cetraria islandica</i></b>											
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
Treatment	1		0.940	1.47	0.230		0.143		0.525	0.18	0.680
Error	18										
Total	19										
$r^2_{adj}$				0.79%						0.00%	
<b><i>Lobaria pulmonaria</i></b>											
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
Treatment	1	3.62	0.073	2.44	0.135	4.9	0.040		0.880	0.85	0.368
Error	18										
Total	19										
$r^2_{adj}$		12.13%		7.06%		17.02%				0.00%	
<b><i>Peltigera aphthosa</i></b>											
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
Treatment	1	0.65	0.432	7.93	0.011	3.23	0.089	2.61	0.124	0.07	0.795
Error	18										
Total	19										
$r^2_{adj}$		0.00%		26.73%		10.52%		7.81%		0.00%	

## 3.2 Experiment 2

### 3.2.1 Effects of absence of UV-B and addition of UV-A on Relative growth rate (RGR) and Relative thallus area growth rate (RT<sub>A</sub>GR)

A second growth chamber experiment with just *L. pulmonaria* was run with two experimental treatments, one with visible light and UV-A (PAR+UV-A) or another one combined with UV-B (PAR+UV-B+UV-A). RGR and RT<sub>A</sub>GR of *L. pulmonaria* were not significantly differed (Tab. 4). RGR and RT<sub>A</sub>GR of *L. pulmonaria* tended to decrease biomass and area growth with UV-B exposure (the PAR+UV-B+UV-A treatment) than without UV-B (the PAR +UV-A treatment) (Fig. 12). The average RGR was  $1.61 \pm 0.39 \text{ mg g}^{-1} \text{ day}^{-1}$  (mean  $\pm$  SE, n=10) with UV-B and  $2.51 \pm 0.85 \text{ mg g}^{-1} \text{ day}^{-1}$  without UV-B. The average RT<sub>A</sub>GR was less with UV-B ( $0.09 \pm 0.05 \text{ mm}^2 \text{ cm}^{-2} \text{ day}^{-1}$ ); without UV-B it was higher ( $0.16 \pm 0.05 \text{ mm}^2 \text{ cm}^{-2} \text{ day}^{-1}$ ). Therefore, UV-B seemed to have influence on both RGR and RT<sub>A</sub>GR, although few samples showed that effect (Tab. 4).

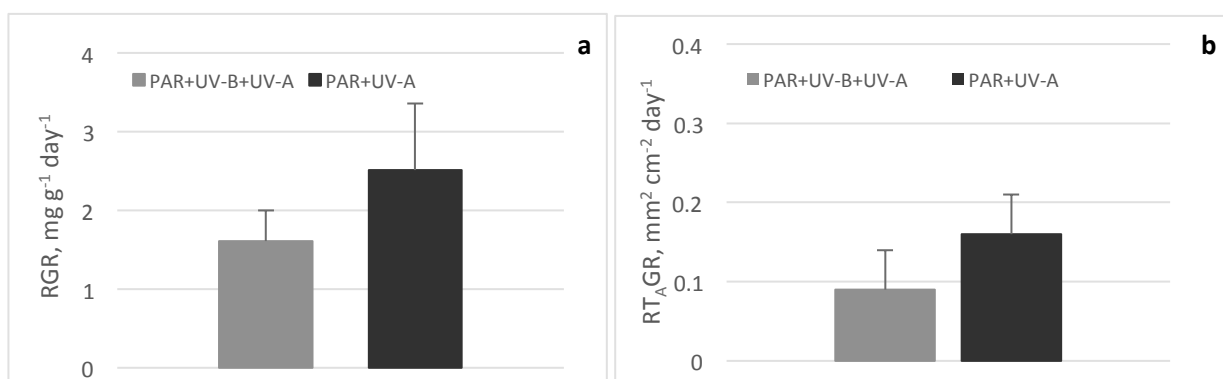


Fig. 12: Relative growth rate (RGR) (a) and relative thallus area growth rate (RT<sub>A</sub>GR) (b) in *Lobaria pulmonaria*, cultivated under visible light and UV-A (PAR+UV-A) or visible light combined with UV-B and UV-A (PAR+UV-B +UV-A) for 3 weeks. The error bars show  $\pm 1$  SE; n=10.

Table 4: One way ANOVA of RGR, RT<sub>A</sub>GR, Chlorophyll *a+b* (DMSO), Chlorophyll *a+b* (Ethanol), Chlorophyll *a/b* (DMSO), Chlorophyll *a/b* (Ethanol) and brown pigments in *Lobaria pulmonaria*, cultivated for 3 weeks under two treatments (PAR+UV-B+UV-A, PAR+UV-A).

Parameter Source	df	RGR		RT <sub>A</sub> GR		Chl <i>a+b</i> (DMSO)		Chl <i>a+b</i> (Ethanol)		Chl <i>a/b</i> (DMSO)		Chl <i>a/b</i> (Ethanol)		Brown pigments	
		F	P	F	P	F	P	F	p	F	p	F	p	F	p
<i>Lobaria pulmonaria</i>															
Treatment	1	0.94	0.345	1.0	0.330	19.21	0.000	16.91	0.001	0.03	0.871	0.06	0.805	22.23	0.000
Error	18														
Total	19														
<i>r</i> <sup>2</sup> adj		0.00%		0.02%		48.94%		45.58%		0.00%		0.00%		52.77%	

### 3.2.2 Chlorophylls

The treatments highly significantly influenced the chlorophyll  $a+b$  concentrations for both chlorophyll methods (DMSO and ethanol). However, the chlorophyll  $a/b$  ratio showed no difference between the treatments (Fig. 13 and Tab. 4). Total chlorophyll content was higher for the treatment PAR+UV-B+UV-A than the PAR+UV-A treatment. Thalli exposed to PAR+UV-A were visible more bleached than thalli exposed to UV-B in addition (Fig. 15). Chlorophyll  $a/b$  was similar for both treatments and for both measurements (Fig. 13).

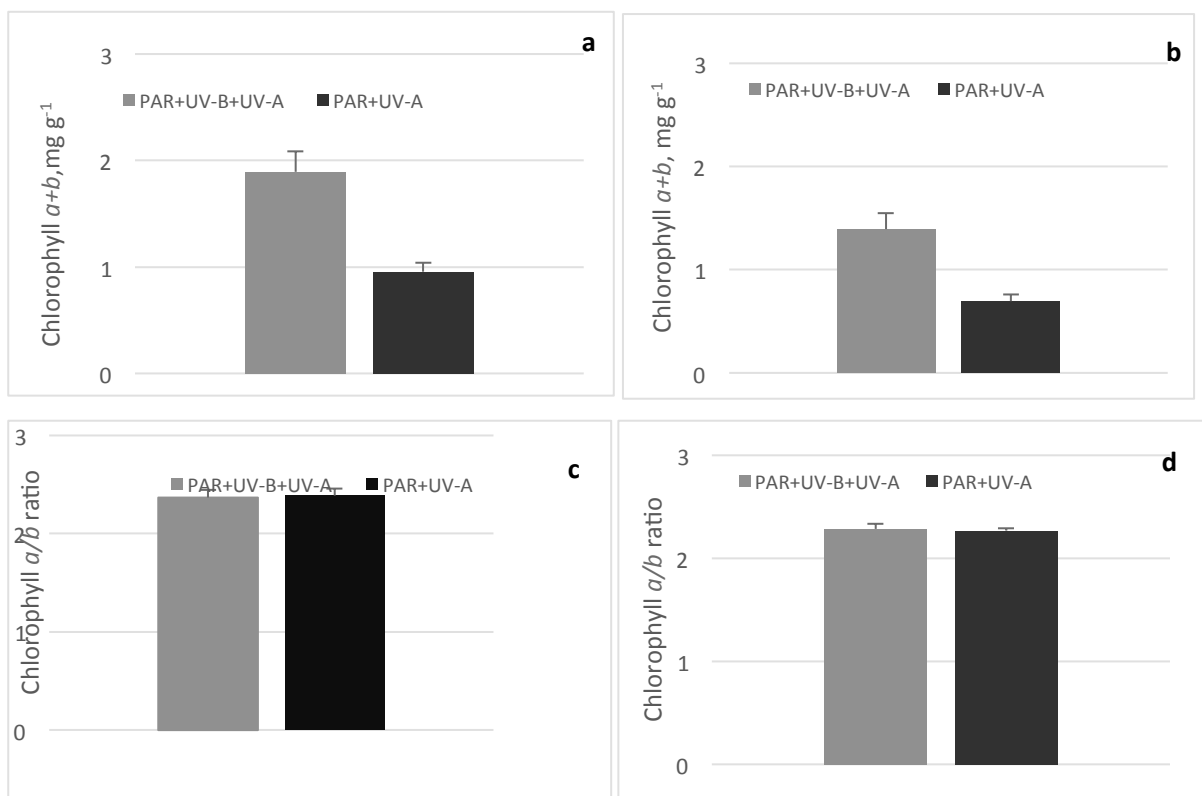


Fig. 13: Chlorophyll  $a+b$  (a,b) and Chlorophyll  $a/b$  (c,d) in *Lobaria pulmonaria* ; after a three-weeks exposure to two treatments; PAR+UV-B+UV-A and PAR+UV-A, measured in DMSO (a, c,) and in Ethanol (b, d). The error bars show  $\pm 1$  SE; n=10.

### 3.2.3 Brown pigments

Brown pigments synthesis highly significantly differed between the treatments (Tab. 4). The relative absorbance at 450 nm was two times more for the treatment PAR+UV-B+UV-A than the PAR+UV-A treatment (Fig. 14). The average relative absorbance at 450 nm was  $14.44 \pm 1.44$  (mean  $\pm$ SE, n=10) with UV-B and  $7.01 \pm 0.62$  without UV-B. This higher brown pigment synthesis probably melanin and color difference is also visible between the treatments (Fig. 15).

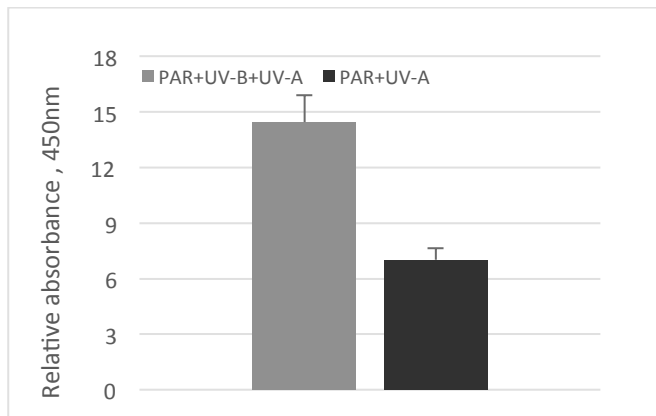


Fig. 14: Brown pigments (measured as relative absorbance at 450 nm) in *Lobaria pulmonaria*, after a three-weeks exposure to two treatments; PAR+UV-B+UV-A and PAR+UV-A. The error bars show  $\pm 1$  SE; n=10.

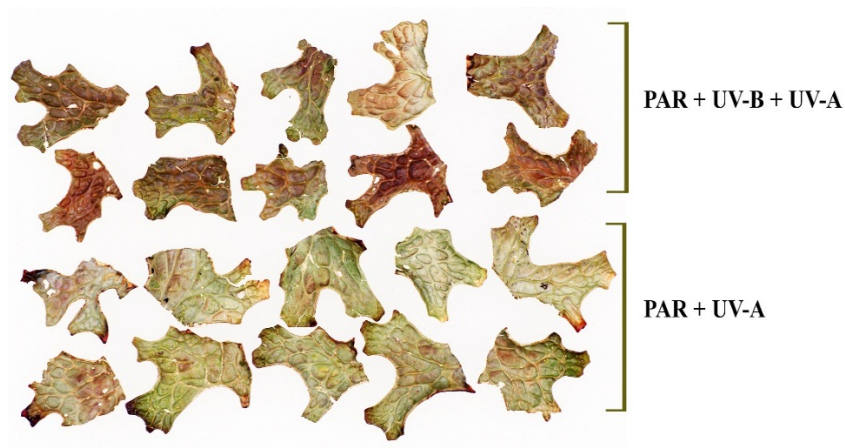


Fig. 15: Photographs of dry thalli of *Lobaria pulmonaria*, cultivated for three-weeks long under two treatments; PAR+UV-B+UV-A and PAR+UV-A.

## 4. Discussion

### 4.1 Impact of UV-B on lichen growth

Assessing lichen growth in growth chambers (Alam et al. 2015; Bidussi et al. 2013; Larsson et al. 2009) or in the field (Gauslaa & Goward 2012; Larsson et al. 2012) is a powerful tool to investigate how different factors may influence lichen growth. Several studies have shown that short time period growth chamber experiments may provide significant growth rates (e.g. RGR and  $RT_{AGR}$ ) (Bidussi et al. 2013; Larsson et al. 2009). This study showed that UV-B influenced growth in chamber experiment. Lichen growth depends on many environmental factors such as surrounding temperature, water availability, light source, nutrient supply. As lichens are poikilohydric (Heber et al. 2006), they show faster and higher growth rate in most laboratory experiment when they are kept moistened most of the time under favorable temperature (Alam et al. 2015; Bidussi et al. 2013). Here, all studied lichen species showed increased biomass gain and area growth (area of *C. islandica* was not measured) during 14 days cultivation in the absence of UV-B radiation. By contrast, all studied lichen species with UV-B radiation showed significant decrease of biomass gain and area growth.

Higher plants often reduce height and leaf area growth when exposed to UV-B radiation (Jansen et al. 1998) and synthesis of UV screening flavonoids is increased (Paul & Gwynn-Jones 2003). This study is the first as far as I know in which effects of UV-B on lichen growth is measured. Under the experimental conditions in the growth chambers, a clear negative effect on lichen growth was shown. However, this study (experiment 1) has the same limitation as several growth chamber experiments with UV-B effects on higher plants because low levels of UV-A and PAR were used in combination with relatively high UV-B levels (Tab. 5) (Paul & Gwynn-Jones 2003). Low levels of PAR and UV-A may limit the ability of the lichens to repair UV-B damage (Jansen et al. 1998). Further experiments under natural irradiance levels are needed to confirm the ecological relevance of UV-B for lichen growth.

In the first experiment, the impact of the two treatments (with UV-B and without UV-B) on total chlorophyll concentration (Chl *a+b*) for three studied lichen species was indifferent for both solution (DMSO and ethanol). However, the varied impact of UV-B on three lichen species may be related by their chlorophylls concentration. The total chlorophyll concentration (Chl *a+b*) was highest for *L. pulmonaria* and lowest for *C. islandica* (Fig.9). Chl *a+b* concentration for *P. aphthosa* was at an intermediate level. Nevertheless, *P. aphthosa* had negative growth

rate. For this species, chlorophyll fluorescence ( $F_v/F_m$ ) may have a role for their negative growth. The measurement of  $F_v/F_m$  is used as an indicator of the maximal photosystem II activity in plants (Maxwell & Johnson 2000) and in lichens (Nayaka et al. 2009). The  $F_v/F_m$  of *P. apthosa* significantly differed between the treatments ( $P < 0.001$ ; Tab. 1). The normal  $F_v/F_m$  values for the cephalolichen, *P. apthosa* ranges between 0.6 to 0.76 (Jensen & Kricke 2002). The average  $F_v/F_m$  of *P. apthosa* was  $0.489 \pm 0.022$  with UV-B at the end of cultivation (Tab. 1) which is much lower than their normal average values. The lower  $F_v/F_m$  of *P. apthosa* under UV-B radiation shows that this lichen was more photoinhibited than the other two species, which may have caused their negative RGR. Biomass gain depends on area expansion as well because lichens are capable to absorb more light by expanding their area, which will subsequently facilitate other resource accumulation (water, nutrients etc.) in the thallus (Dahlman & Palmqvist 2003). The negative area growth of *P. apthosa* followed the negative biomass growth under UV-B treatment (Fig. 5c and Fig. 6c).

In the second experiment, Chl  $a+b$  concentration significantly differed between treatments (PAR+UV-B+UV-A and PAR+UV-A). In addition, brown pigment synthesis also significantly differed between treatments. Chl  $a+b$  and brown pigment were higher for the treatment PAR+UV-B+UV-A than PAR+UV-A. The brown pigments probably protected the chlorophylls from the excessive light exposure (high visible light intensity used in the 2<sup>nd</sup> experiment).

#### **4.2 Brown pigments (melanic compounds) synthesis and role of UV-A and PAR**

Generally, lichens protect their photobionts from solar radiation by synthesizing screening pigments (Solhaug et al. 2003), such as parietin, usnic acid (Bjerke et al. 2002; McEvoy et al. 2006) and melanin (Gauslaa & Solhaug 2001); which are deposited on the outer surface of fungal hyphae. Brown pigment extraction and quantification is rarely studied in lichens. Therefore, I have tried to extract and quantify brown pigments (melanin) in this study. Brown pigments synthesis of all three studied species in the first experiment did not significantly differ between treatments (PAR and UV-B+PAR). For *C. islandica* and *P. apthosa*, brown pigment synthesis tend to be slightly higher with UV-B than without UV-B. The highest brown pigments synthesis occurred in *P. apthosa* with UV-B (Fig. 11). Noticeably, there was no color change for brown pigments synthesis (dark brown color of melanin) in any of the experimented thallus



of three lichens. Earlier experiments on melanin synthesis were done in the field under natural sunlight both in short (Solhaug et al. 2003) and long periods (Gauslaa & Solhaug 2001), and in every occasion, lichens synthesized melanin, which is visible as a dark brown colour. The first experiment was performed in the growth chamber with low visible light ( $125 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) and UV radiation density was  $1 \text{ Wm}^{-2}$ . I used Q-panel UV 313 (Fig.3) as UV radiation source which gives less intensity of UV-A with UV-B radiation. However, lichens receive high visible light and high intensity of UV-A radiation with UV-B in the field experiment because of natural sunlight (Tab. 5). Having noticed that, the second growth chamber experiment for only *L. pulmonaria* was performed with Q-panel UV 340 (Fig. 3), which gives a UV spectrum similar to solar radiation with much more UV-A than the Q-panel UV 313 tubes (Fig. 3). In addition, I used high visible light ( $500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ).

It is difficult to simulate full solar radiation under lab conditions. In the 2<sup>nd</sup> experiment the ratio between UV-B, UV-A and PAR was quite close to solar radiation by using UV340 tubes, combined with a high intensity LED lamp (Tab. 5). In the first experiment, the used UV-B level was close to maximum UV-B level in Norway during midsummer, whereas the level of UV-A and PAR were 15-25 times lower than in solar radiation. Very high UV-B combined with low UV-A and PAR may explain less growth for UV-B exposed thalli in 1<sup>st</sup> experiment, because UV-A and blue light is required for repair of DNA damage (Jansen et al. 1998).

After 3 weeks cultivation, *L. pulmonaria* showed twice the amount of brown pigments synthesis with PAR+UV-B+UV-A than with PAR+UV-A (Fig. 14). The level of brown pigment synthesis was similar in the first experiment (both treatments, PAR and PAR+UV-B) and second experiment (the treatment PAR+UV-A). Therefore, it is not only UV-B alone, but also higher intensity of UV-A and/or PAR that is required to synthesize brown pigments in the lichen. However, the reason behind this behavior is not investigated in this study. Further studies should be done to separate the effects of UV-A and PAR by using filters that screen both UV-B and UV-A. Preliminary results from a recent field experiments with filters that screen all UV or UV-B only indicate that UV-A is partly responsible for brown pigment synthesis (Solhaug K.A., personal communication).

It seems to be a principal difference between induction of melanin synthesis in the lichens in this study compared with UV induction of parietin in *Xanthoria parietina*. In *X. parietina* UV will

induce parietin synthesis even in darkness (Solhaug and Gauslaa 2004), whereas no melanin was induced in experiment 1 with low visible light (Fig. 11).

Table 5: Comparison of the amount of UV-B, UV-A and PAR in full solar radiation in Southern Norway with the amount of UV-B, UV-A and PAR used in the 1<sup>st</sup> and 2<sup>nd</sup> experiments. (UV-B and UV-A estimated with UV calculator from

[http://cprm.acom.ucar.edu/Models/TUV/Interactive\\_TUV/](http://cprm.acom.ucar.edu/Models/TUV/Interactive_TUV/))

	<b>UV-B</b>	<b>UV-A</b>	<b>PAR</b>
<b>Solar radiation</b>	1.5 Wm <sup>-2</sup>	48 Wm <sup>-2</sup>	2000 μmol photons m <sup>-2</sup> s <sup>-1</sup>
<b>1<sup>st</sup> experiment</b>	1.0 Wm <sup>-2</sup>	1 Wm <sup>-2</sup>	125 μmol photons m <sup>-2</sup> s <sup>-1</sup>
<b>2<sup>nd</sup> experiment</b>	0.4 Wm <sup>-2</sup>	7 Wm <sup>-2</sup>	500 μmol photons m <sup>-2</sup> s <sup>-1</sup>

#### 4.3 Separation of brown pigments from chlorophyll by use of C18 column

Melanin may be extracted with DMSO or with NaOH solution (Meeßen et al. 2013). However, DMSO also extracts chlorophylls (Palmqvist & Sundberg 2002). Therefore, melanin was separated from chlorophyll by using a C18 column (Agilent Bond Elut C18 column used in this study). After separation the amount of brown pigments and chlorophyll could be measured individually. This may not be possible in the first crude DMSO extract due to overlapping spectra. However, the brown pigment level in this experiment was so low that the chlorophyll measured in the crude extract was the same as in the purified extract. However, in high melanin containing lichens as *Bryoria* chlorophyll determination in crude DMSO extracts may be impossible (Solhaug K. A. personal communication). Separation of melanin and chlorophyll with the C18 column may be a new useful method for chlorophyll measurements in lichens with high melanin content. Separating melanin from chlorophyll with C18 column will be faster and cheaper than analyzing chlorophyll with HPLC as suggested by Palmqvist and Sundberg (2002)

## **5. Conclusions**

Despite the slow growing nature of lichens in the field, their fast growing response in short term laboratory experiments has proven its usefulness in lichen physiological studies. All three studied lichen species showed significant increase of RGR and  $RT_A$ GR in absence of UV-B and decrease under the influence of UV-B radiation. Lichens are in general protective against UV-B radiation by producing different lichen compounds including synthesis of brown pigments. Besides UV-B, considerable high amount of UV-A and/or high PAR seem to be necessary for brown pigments synthesis. The underneath reason is not investigated in this study.

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## 7. Appendix

### Experiment 1.

**Appendix 1.** Table shows the values of RGR,  $RT_{AGR}$ ,  $\Delta STM$ ,  $F_v/F_m$  with treatments in *Cetraria islandica*.

Treatment	Repl	RGR	$RT_{AGR}$	$\Delta STM$	$F_v/F_m$
UV B+PAR	1	-0.91	-	-	0.71
UV B+PAR	2	1.11	-	-	0.62
UV B+PAR	3	1.12	-	-	0.70
UV B+PAR	4	-4.01	-	-	0.63
UV B+PAR	5	-3.46	-	-	0.67
UV B+PAR	6	0.55	-	-	0.71
UV B+PAR	7	1.96	-	-	0.70
UV B+PAR	8	0.21	-	-	0.63
UV B+PAR	9	0.35	-	-	0.70
UV B+PAR	10	0.82	-	-	0.68
UV B+PAR	11	-0.33	-	-	0.70
UV B+PAR	12	-1.02	-	-	0.68
UV B+PAR	13	-0.98	-	-	0.65
UV B+PAR	14	-1.30	-	-	0.72
UV B+PAR	15	-0.97	-	-	0.67
UV B+PAR	16	1.35	-	-	0.78
UV B+PAR	17	-1.27	-	-	0.72
UV B+PAR	18	0.86	-	-	0.73
UV B+PAR	19	1.14	-	-	0.69
UV B+PAR	20	0.67	-	-	0.73
UV B+PAR	21	0.99	-	-	0.73
UV B+PAR	22	1.69	-	-	0.70
UV B+PAR	23	-1.80	-	-	0.76
UV B+PAR	24	3.35	-	-	0.74
UV B+PAR	25	0.77	-	-	0.61
UV B+PAR	26	0.90	-	-	0.75
UV B+PAR	27	2.40	-	-	0.77
UV B+PAR	28	1.12	-	-	0.78
UV B+PAR	29	1.74	-	-	0.72
UV B+PAR	30	2.73	-	-	0.77
PAR	1	2.13	-	-	0.64
PAR	2	0.00	-	-	0.68
PAR	3	5.75	-	-	0.64
PAR	4	2.52	-	-	0.58
PAR	5	2.58	-	-	0.56
PAR	6	0.94	-	-	0.64
PAR	7	0.41	-	-	0.48

PAR	8	1.21	-	-	0.64
PAR	9	1.17	-	-	0.64
PAR	10	1.40	-	-	0.69
PAR	11	0.85	-	-	0.64
PAR	12	1.22	-	-	0.67
PAR	13	0.28	-	-	0.71
PAR	14	-0.40	-	-	0.66
PAR	15	-0.18	-	-	0.59
PAR	16	4.78	-	-	0.75
PAR	17	4.27	-	-	0.73
PAR	18	4.86	-	-	0.74
PAR	19	4.74	-	-	0.74
PAR	20	4.34	-	-	0.75
PAR	21	5.43	-	-	0.72
PAR	22	5.36	-	-	0.75
PAR	23	3.04	-	-	0.78
PAR	24	3.18	-	-	0.72
PAR	25	3.94	-	-	0.73
PAR	26	3.60	-	-	0.77
PAR	27	5.42	-	-	0.72
PAR	28	3.47	-	-	0.73
PAR	29	4.15	-	-	0.72
PAR	30	11.62	-	-	0.72



**Appendix 2.** Table shows the values of RGR,  $RT_{AGR}$ ,  $\Delta STM$ ,  $F_v/F_m$  with treatments in *Lobaria pulmonaria*

Treatment	Repl	RGR	$RT_{AGR}$	$\Delta STM$	$F_v/F_m$
UV B+PAR	1	8.49	0.44	5.86	0.71
UV B+PAR	2	11.19	0.53	8.58	0.72
UV B+PAR	3	6.42	0.40	3.38	0.71
UV B+PAR	4	7.10	0.22	7.11	0.67
UV B+PAR	5	9.51	0.34	8.98	0.72
UV B+PAR	6	3.72	0.19	2.65	0.67
UV B+PAR	7	6.03	0.45	2.13	0.69
UV B+PAR	8	5.36	0.07	6.77	0.69
UV B+PAR	9	2.41	-0.04	4.02	0.71
UV B+PAR	10	6.23	-0.23	12.75	0.60
UV B+PAR	11	4.84	0.08	5.76	0.71
UV B+PAR	12	5.44	0.21	4.79	0.68
UV B+PAR	13	6.62	-0.02	9.96	0.70
UV B+PAR	14	2.58	0.15	1.47	0.69
UV B+PAR	15	5.57	0.16	5.77	0.68
UV B+PAR	16	2.69	0.13	1.96	0.72
UV B+PAR	17	2.99	-0.94	18.87	0.74
UV B+PAR	18	2.57	-0.94	18.25	0.75
UV B+PAR	19	1.56	-0.24	5.76	0.72
UV B+PAR	20	1.27	0.00	1.74	0.72
UV B+PAR	21	1.69	-0.18	4.96	0.70
UV B+PAR	22	8.11	-0.46	19.41	0.77
UV B+PAR	23	6.54	0.30	5.14	0.75
UV B+PAR	24	5.06	0.00	7.36	0.71
UV B+PAR	25	4.73	-0.38	12.67	0.73
UV B+PAR	26	3.92	0.11	4.03	0.72
UV B+PAR	27	2.04	0.12	1.20	0.66
UV B+PAR	28	0.93	-0.10	2.81	0.68
UV B+PAR	29	2.37	-0.06	4.21	0.69
UV B+PAR	30	7.80	0.20	8.42	0.75
PAR	1	13.09	0.96	4.95	0.71
PAR	2	34.38	1.14	38.01	0.73
PAR	3	18.42	0.95	13.29	0.73
PAR	4	19.61	1.05	13.56	0.71
PAR	5	3.98	0.04	5.08	0.65
PAR	6	15.40	0.76	11.48	0.71
PAR	7	-5.85	0.68	-16.23	0.73
PAR	8	9.75	0.58	5.75	0.70
PAR	9	11.15	0.51	8.91	0.71
PAR	10	5.54	0.05	7.26	0.67

PAR	11	8.40	0.32	7.52	0.74
PAR	12	3.75	-0.01	5.52	0.68
PAR	13	9.39	0.36	8.49	0.73
PAR	14	9.22	0.02	13.42	0.69
PAR	15	10.04	0.62	5.49	0.73
PAR	16	4.28	0.05	5.51	0.72
PAR	17	10.38	0.27	11.39	0.75
PAR	18	6.24	-0.12	10.92	0.76
PAR	19	6.74	0.33	4.92	0.69
PAR	20	10.33	0.66	5.31	0.73
PAR	21	4.99	-0.04	7.80	0.67
PAR	22	7.09	0.24	6.72	0.72
PAR	23	12.65	0.53	10.82	0.75
PAR	24	11.55	0.06	16.50	0.76
PAR	25	7.62	0.19	8.38	0.73
PAR	26	2.47	0.20	0.68	0.72
PAR	27	7.13	0.19	7.55	0.75
PAR	28	7.81	0.34	6.44	0.72
PAR	29	7.15	0.37	4.95	0.72
PAR	30	15.33	0.36	17.84	0.76

**Appendix 3.** Table shows the values of RGR,  $RT_{AGR}$ ,  $\Delta STM$ ,  $F_v/F_m$  with treatments in *Peltigera aphthosa*.

Treatment	Repl	RGR	$RT_{AGR}$	$\Delta STM$	$F_v/F_m$
UV B+PAR	1	0.56	0.07	-0.20	0.54
UV B+PAR	2	-0.44	0.07	-1.61	0.52
UV B+PAR	3	1.39	-0.19	4.73	0.61
UV B+PAR	4	-0.63	0.10	-2.28	0.37
UV B+PAR	5	0.17	-0.01	0.45	0.43
UV B+PAR	6	-2.06	-0.11	-1.32	0.31
UV B+PAR	7	-6.61	-0.66	0.01	0.31
UV B+PAR	8	0.00	-0.30	4.33	0.65
UV B+PAR	9	-1.63	0.11	-3.72	0.58
UV B+PAR	10	-2.26	-0.01	-3.00	0.44
UV B+PAR	11	-5.67	-0.84	3.83	0.38
UV B+PAR	12	-5.54	-0.73	2.47	0.32
UV B+PAR	13	-4.43	-0.44	-0.02	0.52
UV B+PAR	14	-4.48	-0.60	2.20	0.35
UV B+PAR	15	-1.25	-0.40	3.89	0.37
UV B+PAR	16	-3.77	-0.28	-1.34	0.48
UV B+PAR	17	-7.17	-0.47	-3.39	0.29
UV B+PAR	18	-4.14	-0.19	-3.08	0.49
UV B+PAR	19	-8.80	-0.70	-2.52	0.42
UV B+PAR	20	-6.19	-0.30	-4.41	0.39
UV B+PAR	21	-3.45	-0.34	-0.05	0.61
UV B+PAR	22	-4.41	-0.68	3.40	0.63
UV B+PAR	23	-7.06	-0.64	-0.92	0.45
UV B+PAR	24	-4.79	-0.27	-2.92	0.46
UV B+PAR	25	-5.25	-0.31	-2.96	0.64
UV B+PAR	26	-5.59	-0.44	-1.70	0.49
UV B+PAR	27	-2.41	-0.30	0.84	0.63
UV B+PAR	28	0.98	0.27	-2.42	0.69
UV B+PAR	29	-0.36	-0.31	3.92	0.65
UV B+PAR	30	-1.18	-0.04	-1.09	0.68
PAR	1	5.38	0.34	2.85	0.56
PAR	2	5.44	0.31	3.35	0.70
PAR	3	4.96	0.07	6.11	0.53
PAR	4	3.82	0.23	2.09	0.42
PAR	5	2.71	0.50	-3.18	0.61
PAR	6	0.99	-0.24	4.89	0.54
PAR	7	1.42	0.35	-2.91	0.51
PAR	8	0.49	-0.06	1.56	0.45
PAR	9	0.92	0.19	-1.40	0.44
PAR	10	-0.39	0.22	-3.50	0.60

PAR	11	1.52	-0.10	3.60	0.31
PAR	12	-2.52	-0.43	2.55	0.60
PAR	13	-4.25	-0.43	0.08	0.48
PAR	14	0.04	0.15	-2.05	0.45
PAR	15	1.01	0.15	-0.67	0.67
PAR	16	3.44	0.00	4.98	0.70
PAR	17	2.45	-0.18	6.17	0.70
PAR	18	0.92	-0.14	3.22	0.69
PAR	19	3.57	-0.33	10.14	0.66
PAR	20	0.27	-0.08	1.54	0.70
PAR	21	2.75	0.10	2.44	0.62
PAR	22	-0.12	-0.28	3.86	0.70
PAR	23	1.01	0.03	0.94	0.71
PAR	24	4.07	0.14	3.74	0.69
PAR	25	4.82	0.21	3.85	0.71
PAR	26	2.55	-0.19	6.50	0.67
PAR	27	7.17	0.05	9.77	0.74
PAR	28	2.91	-0.25	7.86	0.67
PAR	29	5.39	0.07	6.72	0.75
PAR	30	4.07	0.02	5.55	0.72

**Appendix 4.** Table shows the values of Chl *a*, Chl *b* (measured in DMSO and in ethanol) and brown pigments synthesis with treatment in *Cetraria islandica*.

Treatment	Repl	DMSO		Ethanol		Brown pigments
		Chl <i>a</i>	Chl <i>b</i>	Chl <i>a</i>	Chl <i>b</i>	
UV-B+PAR	1	0.44	0.14	0.25	0.12	5.25
UV-B+PAR	2	0.24	0.11	0.20	0.07	9.27
UV-B+PAR	3	0.45	0.15	0.32	0.10	5.79
UV-B+PAR	4	0.49	0.16	0.37	0.11	6.25
UV-B+PAR	5	0.67	0.23	0.46	0.18	8.27
UV-B+PAR	6	0.47	0.16	0.29	0.14	7.27
UV-B+PAR	7	0.34	0.10	0.28	0.06	5.38
UV-B+PAR	8	0.41	0.14	0.31	0.09	8.88
UV-B+PAR	9	0.30	0.09	0.23	0.06	4.40
UV-B+PAR	10	0.47	0.15	0.34	0.09	6.54
PAR	1	0.35	0.12	0.19	0.09	5.71
PAR	2	0.48	0.17	0.34	0.10	5.77
PAR	3	0.34	0.12	0.19	0.06	7.10
PAR	4	0.57	0.18	0.39	0.12	7.07
PAR	5	0.43	0.15	0.25	0.08	7.38
PAR	6	0.46	0.14	0.32	0.10	3.37
PAR	7	0.57	0.22	0.44	0.12	11.21
PAR	8	0.37	0.11	0.27	0.07	6.18
PAR	9	0.39	0.12	0.30	0.07	4.67
PAR	10	0.39	0.12	0.28	0.07	5.31

**Appendix 5.** Table shows the values of Chl *a*, Chl *b* (measured in DMSO and in ethanol) and brown pigments synthesis with treatment in *Lobaria pulmonaria*.

Treatment	Repl	DMSO		Ethanol		Brown pigments
		Chl <i>a</i>	Chl <i>b</i>	Chl <i>a</i>	Chl <i>b</i>	
UV-B+PAR	1	1.90	0.64	1.61	0.62	5.16
UV-B+PAR	2	1.86	0.63	1.53	0.58	7.50
UV-B+PAR	3	1.71	0.59	1.36	0.53	7.74
UV-B+PAR	4	2.03	0.70	1.60	0.62	6.91
UV-B+PAR	5	1.95	0.67	1.50	0.59	7.40
UV-B+PAR	6	1.67	0.57	1.45	0.55	7.51
UV-B+PAR	7	2.00	0.71	1.66	0.65	7.08
UV-B+PAR	8	1.84	0.64	1.47	0.55	6.33
UV-B+PAR	9	1.91	0.70	1.56	0.62	7.45
UV-B+PAR	10	1.96	0.76	1.65	0.61	7.11
PAR	1	1.82	0.62	1.57	0.59	5.74
PAR	2	2.28	0.85	1.88	0.78	6.84
PAR	3	1.64	0.58	0.29	1.45	6.91
PAR	4	1.55	0.50	1.25	0.44	9.09
PAR	5	1.51	0.53	1.17	0.46	7.68
PAR	6	1.15	0.37	0.94	0.33	4.72
PAR	7	1.55	0.50	1.21	0.42	11.29
PAR	8	1.89	0.64	1.46	0.57	7.79
PAR	9	1.95	0.65	1.47	0.58	7.02
PAR	10	1.54	0.53	1.11	0.44	8.94

**Appendix 6.** Table shows the values of Chl *a*, Chl *b* (measured in DMSO and in ethanol) and brown pigments synthesis with treatment in *Peltigera aphthosa*.

Treatment	Repl	DMSO		Ethanol		Brown pigments
		Chl <i>a</i>	Chl <i>b</i>	Chl <i>a</i>	Chl <i>b</i>	
UV-B+PAR	1	1.66	0.73	1.40	0.67	8.03
UV-B+PAR	2	1.27	0.55	1.01	0.47	8.49
UV-B+PAR	3	1.41	0.61	1.07	0.49	8.58
UV-B+PAR	4	1.88	0.82	1.39	0.70	6.68
UV-B+PAR	5	1.43	0.64	1.01	0.52	7.27
UV-B+PAR	6	1.37	0.59	1.06	0.59	8.33
UV-B+PAR	7	1.51	0.66	1.28	0.57	11.11
UV-B+PAR	8	1.29	0.53	1.05	0.46	7.03
UV-B+PAR	9	1.73	0.67	1.43	0.60	7.17
UV-B+PAR	10	1.39	0.53	1.18	0.46	8.00
PAR	1	1.35	0.56	1.04	0.47	6.33
PAR	2	1.28	0.52	0.94	0.42	7.10
PAR	3	1.58	0.59	1.21	0.48	9.31
PAR	4	1.73	0.67	1.32	0.56	6.96
PAR	5	1.92	0.73	1.40	0.59	9.95
PAR	6	1.41	0.59	1.10	0.49	5.70
PAR	7	1.22	0.50	0.90	0.42	7.46
PAR	8	1.24	0.50	0.90	0.40	9.26
PAR	9	1.34	0.53	0.98	0.43	7.06
PAR	10	1.38	0.56	0.97	0.43	9.89

## Experiment 2.

**Appendix 7.** Table shows the values of RGR, RT<sub>A</sub>GR, Chl *a*, Chl *b* (measured in DMSO and in ethanol) and brown pigments with treatments in *Lobaria pulmonaria*.

Treatments	Repl	RGR	RT <sub>A</sub> GR	DMSO		Ethanol		Brown pigments
				Chl <i>a</i>	Chl <i>b</i>	Chl <i>a</i>	Chl <i>b</i>	
PAR+UV-B+UV-A	1	0.83	-0.16	1.32	0.53	1.06	0.45	10.29
PAR+UV-B+UV-A	2	1.48	0.07	1.48	0.57	1.14	0.46	11.14
PAR+UV-B+UV-A	3	1.41	0.19	2.01	0.77	1.48	0.59	16.86
PAR+UV-B+UV-A	4	3.61	0.40	1.45	0.59	1.04	0.44	18.33
PAR+UV-B+UV-A	5	0.35	-0.11	1.47	0.61	1.04	0.44	17.10
PAR+UV-B+UV-A	6	2.90	0.12	0.94	0.38	0.71	0.32	8.50
PAR+UV-B+UV-A	7	-0.50	-0.04	0.81	0.36	0.58	0.27	7.57
PAR+UV-B+UV-A	8	2.44	0.13	1.97	0.79	1.43	0.63	16.31
PAR+UV-B+UV-A	9	1.31	0.04	1.35	0.62	0.92	0.42	20.16
PAR+UV-B+UV-A	10	2.30	0.27	0.57	0.33	0.33	0.17	18.15
PAR+UV-A	1	-0.47	0.00	0.75	0.34	0.57	0.26	8.51
PAR+UV-A	2	2.93	0.22	0.46	0.22	0.34	0.16	9.58
PAR+UV-A	3	8.80	0.12	0.66	0.26	0.49	0.20	4.26
PAR+UV-A	4	2.08	0.21	0.67	0.27	0.49	0.21	6.83
PAR+UV-A	5	-0.89	0.34	0.36	0.18	0.24	0.10	7.65
PAR+UV-A	6	1.98	0.13	0.85	0.34	0.63	0.27	7.92
PAR+UV-A	7	1.73	0.20	0.70	0.31	0.49	0.23	8.39
PAR+UV-A	8	3.49	0.42	0.61	0.23	0.44	0.19	3.14
PAR+UV-A	9	1.69	-0.03	0.57	0.23	0.38	0.17	6.86
PAR+UV-A	10	3.81	0.00	1.09	0.42	0.74	0.33	6.97





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