Blue light screening reduce blue light photosynthetic efficiency of cyanolichens compared with chlorolichens

LI XIE

Norwegian University of Life Sciences
Department of Ecology and natural resource management
Master Thesis 60 credits 2008

Abstract

Cyanolichens have phycobiliproteins that mainly absorb light in the green part of the spectrum. Thereby, phycobiliproteins enhance the utilization of light transmitted through a canopy. The combination of phycobiliproteins and chl *a* may thus improve photosynthesis in shaded forest sites.

We compared the chlorolichens *Lobaria pulmonaria* and *Peltigera leucophlebia* with the cyanolichens *Lobaria hallii* and *Peltigera praetextata* by measuring light response curves for photosynthetic CO_2 uptake, O_2 evolution, as well as photosystem II efficiency in blue, green and red light, respectively. Maximal photosynthetic CO_2 uptake was slightly higher for both cyanolichens than for the chlorolichens in green light. In red light there was no difference in maximal CO_2 uptake, whereas both cyanolichens had substantially lower photosynthesis in blue light. The same trend occurred for photosynthetic O_2 evolution. Apparent electron transport rate (ETR) did not differ between red and green light in any of the species. For the cyanolichens, ETR showed no sign of light saturation in blue light, indicating that little blue light absorbed is used in photosynthesis.

Reflectance spectra showed that green light was less reflected in the cyanolichens, which may partly explain the slightly higher cyanobacterial photosynthetic CO_2 uptake in green light. At the same time, the reflectance patterns in the blue region cannot explain the reduced photosynthesis in cyanolichens in blue light. Transmittance of light through the combined cortex and photobiont layer indicated that also the blue light was efficiently absorbed. Screening was estimated indirectly by comparing chlorophyll fluorescence ratios between chlorophyll fluorescence excited with blue and red light. Much lower blue/red ratios occurred in the cyanolichens *L. hallii* and *P. praetextata* than in the chlorolichens *L. pulmonaria* and *P. leucophlebia*, indicating that screening of blue light in the cyanolichens inhibited blue light from reaching the photosynthetic apparatus.

Cyanobacteria may contain the UV and blue light absorbing compound

scytonemin. HPLC analyses showed that *L. hallii* contained some scytonemin that partly may explain the blue light screening, whereas *P. praetextata* contained no scytonemin, Therefore, the mechanism for low cyanolichen photosynthesis in blue light remains unknown.

Keywords: *Lobaria pulmonaria*, *Lobaria hallii*, *Peltigera leucophlebia*, *Peltigera praetextata*, chlorolichen, cyanolichen, blue light screening, scytonemin

Abbreviations

ETR: Electron transport rate

F_m: Maximal fluorescence in dark-adapted samples

F_o: Minimum fluorescence in dark-adapted samples

F_v: Variable fluorescence ($F_v=F_m-F_o$)

F_m': maximal fluorescence in illuminated samples

F_t: Minimum fluorescence in illuminated samples

F_v/F_m: Maximal quantum yield of PSII

$(F_m'- F_t)/ F_m'$: Effective quantum yield of PSII

PAR: Photosynthetically active radiation

PSII: photosystem II

QY: Quantum yield

Preface

This thesis marks the end of my master studies in the Department of Ecology and Natural Resource Management (INA), and two wonderful years as a student at the Norwegian University of Life Sciences (UMB). It has been a great journey and I want to thank everybody sharing it with me.

I owed my deepest appreciation to my main-supervisor, Professor Knut Asbjørn Solhaug for great supervision during the process of lab work and writing. My co-supervisor, Professor Yngvar Gauslaa, deserves a great deal of thanks for all the help and supports.

Moreover, further accolade shall be given to my parents and my friends, especially Jiemeng Zhou who supported me a lot in the life aspect.

Today I finished my thesis and I will continue to challenge myself in future with what I learned. This is not the end but only the start.

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

Norwegian University of Life Sciences, Ås, Norway

December 2011

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1 Introduction

Lichens, which are symbiotic associations between a mycobiont (heterotrophic fungus) and one or more photobionts (green algae and/or cyanobacteria autotrophic host), they are dominant life forms in about 8% of the land surface of the earth (Nash, 2008). Their ability to tolerate the extreme stress makes them occur in some of the most extreme environments on Earth. It is well known that the strong irradiation will cause the photoinhibition in plants (Aro et al., 1993). Until recently, the research on photoprotection in plant is mostly on the UV light, because it directly damages membranes and DNA of plants. The ability of plant pigments as flavonoids to absorb in the UV and/or photosynthetically active regions of the spectrum and thereby act as internal light filter have been discussed for quite a time.

Lichens are poikilohydric organisms that tolerate desiccation for long periods (e.g. Kranner et al. 2008). More than 800 different secondary compounds have been identified (Huneck, 2001). And various secondary compounds that may screen excess radiation (Solhaug et al., 2003, Huneck, 1999, Gauslaa and Solhaug, 1996, Ingólfsdóttir, 2002, Solhaug and Gauslaa, 2012)

Photosynthesis is the process by which autotrophic organisms convert light energy to chemical energy in the form of glucose (Spoehr and McGee, 1924). Life on earth is directly or indirectly dependent on photosynthesis. Chlorophylls absorb mainly red and blue light, whereas green light is more reflected from and transmitted through the leaves giving plants their green color (Lawlor, 2001). The cyanobacteria in cyanolichens have chlorophyll *a* and no chlorophyll *b* since they do not have chlorophyll *b* containing light harvesting complexes. Instead they have phycobilisomes as antenna complexes. The phycobilisomes contain the phycobiliproteins allophycocyanin, phycocyanins and phycoerythrin that mainly absorb light in the green part of the spectrum (Blankenship 2002). The phycobiliproteins are accessory pigments to chlorophyll in the photosystems . Thereby, phycobiliproteins may enhance the utilization of green light transmitted through a canopy. The combination of

phycobiliproteins and chlorophyll *a* may thus improve photosynthesis in shaded forest sites with relatively more green light.

The rate of a physiological activity plotted against wavelength of light is called action spectrum. It shows which wavelength of light is most effectively used in a specific chemical reaction (Taiz and Zeiger, 2010). Most plants have the regular action spectrum of photosynthesis (Fig.1). When viewing an action spectrum, it will be seen that the rate of photosynthesis is higher at the blue and red light wavelengths.

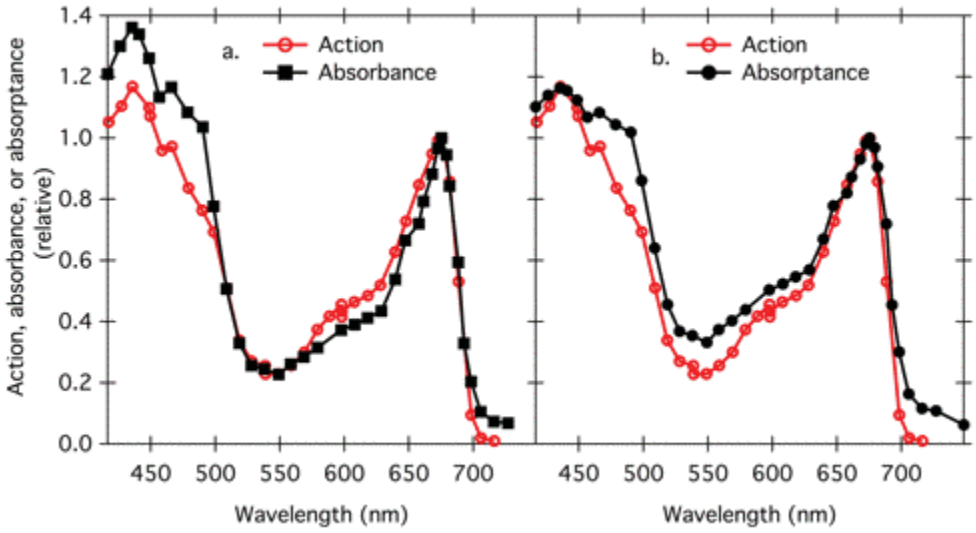

Figure 1 Action spectrum of photosynthesis in green algae *Ulva* plotted with (a) thallus absorbance and (b) thallus absorptance (Haxo and Blinks, 1950).

The efficiency of photosynthesis can be shown by quantum yield (QY). QY is defined by number of photochemical products divided by total number of photons absorbed (Taiz and Zeiger, 2010). Quantum yield can be calculated for CO_2 uptake, O_2 evolution and electron transport rate (ETR) for the linear part of the light response curve in low light. Apparent QY is calculated based on the number of photons that the sample is illuminated with, while the absolute QY is based on the number of photons absorbed by the photosynthetic apparatus. If a sample contain screening pigments, the apparent QY of CO_2 uptake or O_2 evolution will decrease. This reduction can be used to estimate screening (Solhaug and Gauslaa, 1996). The QY of ETR is the same as the PSII effeciency. ETR often correlates well with CO_2

uptake (Maxwell and Johnson, 2000). However, measurement of F_v/F_m is not directly affected by screening pigments and apparent ETR is increased with screening pigments because less light reaches the photosynthetic apparatus resulting increas yiled of PSII.(Solhaug et al., 2010)

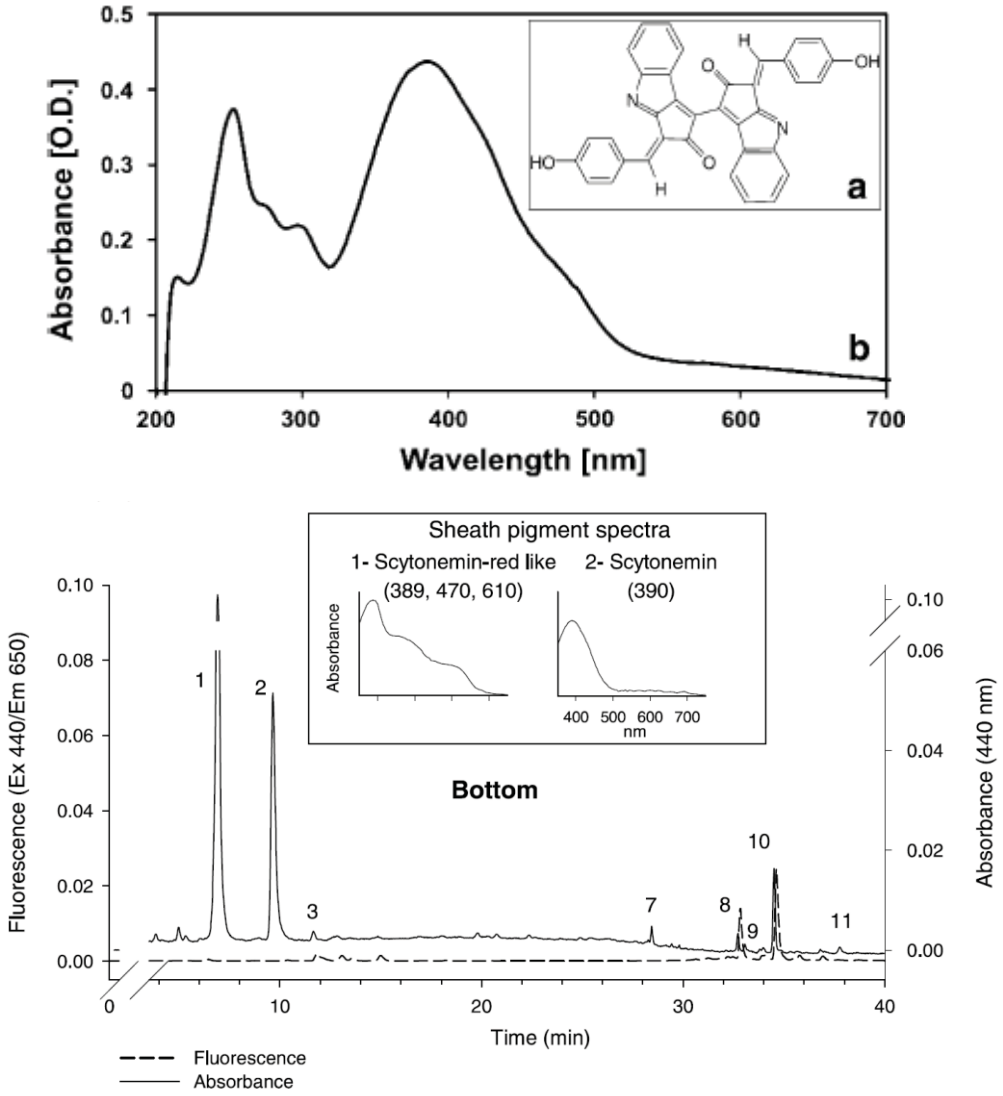

Figure 2 Upper panel: Structure of scytonemin (a) and its absorption spectrum showing a maximum at 386 nm (b) (Rastogi et al., 2010). Lower panel: HPLC chromatogram from a microbial mat sample from Markham Ice Shelf with spectrum of the scytonemin peak and the scytonemin-red like peak (Vincent et al. 2004).

In this study we focus on scytonemin, a pigment synthesized by many strains of cyanobacteria, and also found in many cyanolichens, where it acts as a bacterial sunscreen with a broad absorption from 325-425 nm and a separate maxima at 250 nm (Proteau et al., 1993), and its biosynthesis triggered by

exposure to UV light (Bandaranayake, 1998). It also has absorption in blue region of the light spectrum (Fig.2).

Problem statement

In this work spectral dependency of photosynthesis is compared in cyanolichens and green algal lichens. Most cyanolichens live in the shaded areas with higher proportion of green light, so we suppose that cyanolichens with phycobiliproteins in their cyanobacteria may improve light absorption and photosynthetic efficiency in shaded sites.

Hypotheses

Hypothesis 1. Cyanolichens utilize green light more efficient than chlorolichens

This hypothesis was tested with various methods. However, the surprising result was that blue light was much less efficiently utilized than green and red light. This may be due to less efficient use of blue light in cyanobacteria photosynthesis or it may be due to blue light screening. Several cyanobacteria and cyanolichens contain the pigment scytonemin (Balskus et al., 2011, Büdel, 1999). Scytonemin is localized in extracellular polysaccharide sheath of cyanobacteria (e.g. Sinha & Häder 2008). Scytonemin has been extensively studied in relation to UV screening (Büdel et al., 1997). However, the absorbance spectrum of scytonemin also extends into blue light (Fig.2). Therefore, it is a potential blue light screening compound.

Hypothesis 2. Reduced efficiency of blue light in photosynthesis is caused by blue light screening by scytonemin.

Scytonemin can not be extracted from intact dry thalli with the acetone-rinsing method (Solhaug & Gauslaa 1996). This hypothesis was therefore tested by measuring the amount of scytonemin with destructively extraction in two cyanolichens to estimate if the concentration was high enough to be responsible for reduced blue light photosynthesis.

2 Materials and methods

2.1 Lichen materials

Lichen genus *Lobaria*

Lobaria pulmonaria and *Lobaria hallii* were collected on April 2010 from upper Fraser site under open canopy condition in British Columbia (54°05'52″ N, 121°52'41″ W, 610m a.s.l, Canada). *Lobaria pulmonaria* is a widespread foliose lichen, distributed over parts of Europe, Asia and Africa. It is characterized by two photobionts, the green alga *Dictyochloropsis reticulata* and the nitrogen-fixing cyanobacterium *Nostoc sp (Tschermak-Woess, 1995)*. *Lobaria hallii* is infrequent over conifers in open to somewhat shady forests in humid regions throughout, except possibly absent in hyper maritime localities. (Goward, 1994). *L. hallii* only contains the cyanobacterium *Nostoc* sp.

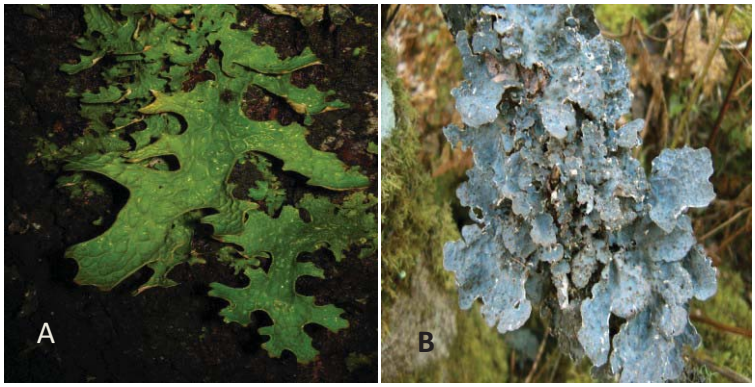

Figure 3 A is *Lobaria pulmonaria* (green algae *Dictyochloropsis reticulata*) and B is *Lobaria hallii* (cyanobacterium *Nostoc*) (Lichenflora)

Lichen genus *Peltigera*

Genus *Peltigera* was collected on November 2011 from a shady deciduous forest in Vinterbro (59 44′45″ N, 10°45′41″ E, 45m a.s.l, Norway). *Peltigera leucophlebia* is found in North America, Asia, and Europe. This and other species in the genus contain a green algae in the genus *Coccomyxa* and also cyanobacteria in the genus *Nostoc* assymbionts (Walewski, 2007). *Peltigera praetextata* was recently associated with mosses occur on rocks and trees that help to keep them damp and hence metabolically active (Darbishire, 1926). They contain cyanobacterium *Nostoc* as photobiont.

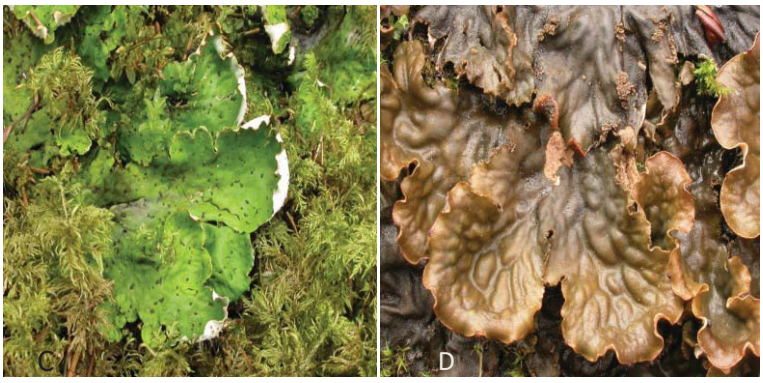

Figure 4 C is *Peltigera leucophlebia* (green algae *Coccomyxa*) and D is *Peltigera praetextata* (cyanobacterium *Nostoc*) (Lichenflora)

2.2 Chlorophyll fluorescence measurement and CO_2 gas exchange

Chlorophyll fluorescence was measured with a portable, modulated fluorometer (PAM-2000, Walz, Effeltrich, Germany), operated by a computer and connected to the cuvette of the gas-exchange system (a CIRAS-1 Portable Photosynthesis System (PP-systems, UK) using a PLC (N) cuvette in which the heat-absorbing glass had been exchanged with plain glass to allow measurement of chlorophyll fluorescence). Ten thalli were prepared for each species with the area around 10 cm^2. Prior to experimental use, thalli were sprayed with water and left to reactivate for 12 hours in the laboratory at 18°C and low irradiance at 30 μmol photons $m^{-2}s^{-1}$, from a LED panel with equal irradiance from blue, green and red light from a high intensity LED light source (Model SL-3500, Photon System Instruments, Brno, Czech Republic) with separately regulated blue, green and red LEDs (see Fig. 5). During the experiment, each thallus was measured under 3 colors (red, green and blue) with 7 levels of irradiance from low to high (0, 20, 50, 100, 200, 400, 600 μmol photons $m^{-2}\,s^{-1}$) which were measured by separate LI-COR quantum sensor model LI-190 (Lincoln, Nebraska，USA）connected to a LI-COR LI-250 meter. Measurements at each irradiance value were carried out after 10 minutes under each of the irradiance when the thallus had reached a stable CO_2 uptake, chlorophyll fluorescence was also taken at that time to get the

effective quantum yield value of PSII (Fv′/Fm′). Apparent electron transport rate (ETR) is calculated by ETR=0.5 × PAR ×effective quantum yield value of PSII

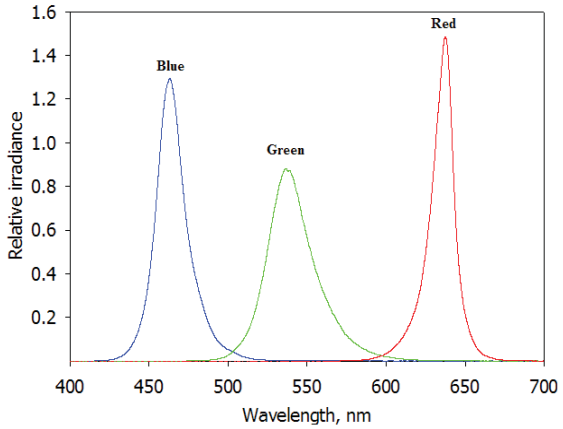

Figure 5 SL3500 LED light source

2.3 O_2 evolution

O_2 evolution was measured with CB1-D2 Manual Oxygen Electrode Control Units and LD2 Electrode Chamber （Hansatech King's Lynn, Norolk, UK） at 18°C (Solhaug and Gauslaa, 1996). A modified electrolyte (one part saturated KCl solution, one part 0.4 M borate buffer at pH 9.0 and two parts 1.0 M sodium bicarbonate solution previously adjusted to pH 9.0 by the addition of $NaHCO_3$) was prepared for electrode disk (Delieu and Walker, 1981).

The lichen thalli with the area 10 cm^2 were placed in the cuvette and O_2 evolution was measured with increasing photon flux rates (0, 20, 50, 100, 200, 400, 600 μmol photons m^{-2} s^{-1}) under different color using LED light source (Model sl-3500). Stable O_2 evolution values under each of irradiance were recorded after 1 min.

2.4 Reflectance and transmittance

In order to get the light absorbance of those thalli, reflectance spectra of upper cortex and transmittance spectra of upper cortex and photobiont layer were measured. Lichen thalli were moistened before measurement of

reflectance spectra. Visible spectra (400-1000 nm) were measured with an Ocean Optics SD 2000 spectrometer (Ocean Optics, Dunedin, Fla., USA) connected to an integrating sphere (ISP-50-REFL OceanOptics) with 400 µm fiber. A halogen light (DH2000 OceanOptics) was connected to the integrating sphere through a 600 µm fiber illuminating the sample at the sphere port. Reference reflectance spectra were recorded with a reflectance standard (WS-2, Ocean Optics). Thereafter, every thallus was placed under the integrating sphere.

Before transmittance measurement each thallus was striped lower cortex and medulla layer. The upper cortex of a small, smooth and soredia-free portion of an air-dry thallus was fixed to double-sided Scotch tape under a dissecting microscope. The lower cortex and medulla were removed by scraping until the lower part of the green photobiont layer was exposed. Then the sample of cortex and photobiont layer, size 2 mm, was removed carefully from the double-side tape with a scalpel (Gauslaa and Solhaug, 2001).

To measure the transmittance spectra, each prepared sample was placed into holder (transparent plastic wrap) to keep it smooth. Then the sample was placed on the integrating sphere against the 400µm fiber that connects to Ocean Optics SD2000 spectrometer. Visible spectra (400-1000 nm) from a DH2000 (Ocean Optics) halogen light source was applied through a 600 µmol thick optical fiber to the upper side of the cortex. Before the sample measurement an empty transparent preservative film was measured to make a standard spectrum.

2.5 Fluorescence Excitation Ratio method:

To measure screening percentage, the instrument "*Multiplex*" (FORCE-A, France) was used. In principle the relative screening of UV-A, blue, green and red light can be estimated. The instrument *Multiplex* can measure the fluorescence exited by blue green and red light (Fig. 6). By comparing these fluorescence values with a none-screening reference, percentage screening can be calculated.

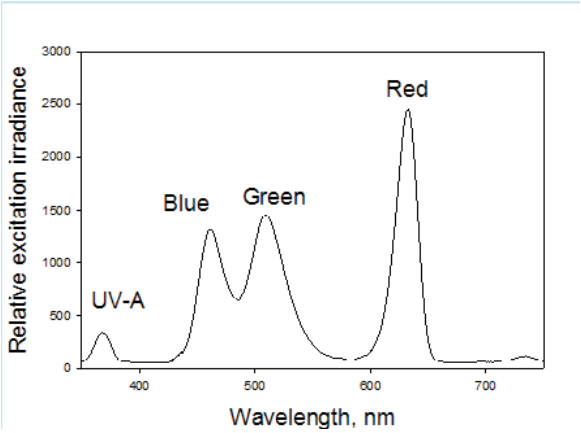

Figure 6 Excitation wavelengths of the “Multiplex” instrument

FR_B (far red fluorescence excited with blue light), FR_G (far red fluorescence excited with green light) and FR_R (far red fluorescence excited with red light) were first calibrated against the lower side of green leaf (*Myosotis silvaticum*) without epidermis as a non-screening standard instead of the blue standard from the producer. Then five thalli of each lichen species were measured for FR_B, FR_G and FR_R.

Calculations:

First all lichen fluorescence values were normalized against the unscreened leaf without epidermis.

Normalized blue light excited fluorescence:

Evaporation 1: $FR\_B_{norm} = FR\_B_{lichen}/(FR\_B_0/FR\_R_0)$

Normalized green light excited fluorescence:

Evaporation 2: $FR\_G_{norm} = FR\_G_{lichen}/(FR\_G_0/FR\_R_0)$

FR_B_0, FR_G_0 and FR_R_0 are the far red fluorescence excited from the unscreened leaf with blue, green or red light respectively.

After having calculated the normalized fluorescence values percent screening could be calculated with following formulas.

Evaporation 3: Percent green light screening=100 × (1-(FR_G/ FR_R))

Evaporation 4: Percent Blue light screening=100 × (1-(FR_B/ FR_R))

The principle of the method is shown in Figure 4.

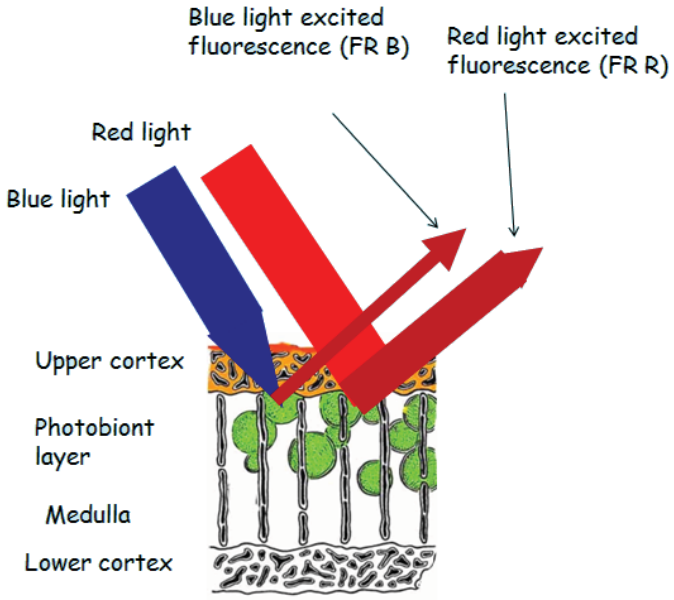

Figure 7 Principle of fluorescence excitation ratio method.

2.6 Scytonemin analysis

2.6.1 Compounds separation

HPLC is a chromatographic technique that can separate a mixture of compounds and is used in biochemistry and analytical chemistry to identify, quantify and purify the individual components of the mixture. During this experiment lichen extracts were analyzed on a 1200 Series HPLC including a 1040-M diode-array detector and a fraction collector G1364C (Agilent Technologies, Waldbronn, Germany). Separation was achieved on an ODS Hypersil 4.6×250 mm column.

Dried lichen thalli around 30 mg were homogenized for pigment extraction with a ceramic mortar with acetone. The mortar and pestle was washed with additional acetone. The combined extracts were centrifuged at 4000 g for 5 min. The supernatant was evaporated to dryness and redissolved in 1 ml acetone. All steps were done under dim light. Sample volumes of 20 µl were injected into the HPLC. Scytonemin and chlorophyll *a* were eluted by a binary gradient system of degassed solvents and monitored at 385 nm. Absorption spectra were recorded between 350-600 nm on the HPLC separated peaks.

Solvent A was distilled water, whereas solvent B consisted of 75% acetonitrile, 15% methanol and 10% tetrahydrofuran (Büdel et al., 1997). The run started with 70% B. with 11 min, solvent B increase to 100% for 15 min. at the end of the run, solvent B was reduced to 85% within 1 min, and the column flushed with 15% B for 5 min before the next run started. The identification of compounds was based on retention times and spectra of the peaks (Figs. 8 and 14) compared with retension times and spectra in published data (Fig. 2) (Rastogi et al., 2010, Vincent et al., 2004).

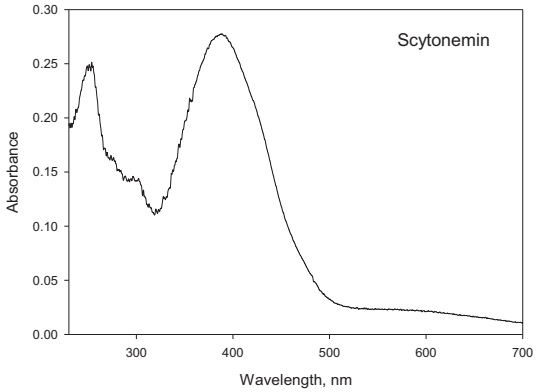

Figure 8 The spectra of scytonemin standard which was measured by UVPC. the standard scytonemin was extracted by HPLC from lichen species *L. hallii*.

2.6.2 Preparation of scytonemin standard

We first separated scytonemin that had been extracted from one thallus with acetone as described above. 50 µl of solution was injected into the HPLC in each run, and the scytonemin from 5 runs was collected with the fraction collector. Then the absorbance of this pooled scytonemin solution was measured on a Shimadzu UV2001 PC spectrophotometer (see spectrum in Fig. 8) and its concentration was calculated using the extinction coefficient 112.6 $Lg^{-1}cm^{-1}$ at 384 nm (Garcia-Pichel et al., 1992). This scytonemin solution with known concentration was then again injected into the HPLC as a standard.

Since we then know both the content and the area of the peak of scytonemin, we can calculate the scytonemin content per unit peak area and calculate scytonemin content of each thallus. In this measured, screening is calculated

with the equation:

Evaporation 5: Percent screening =100 x (1-10^{-Abs})

Where Abs is the absorbance of an evenly distribute layer of scytonemin as in the lichen thallus.

2.7 Statistics

In the experiment, ANOVA in Minitab (versions 1.5, Minitab, Inc., Pennsylvania, USA) was used to calculate the significance of differences in photosynthesis in both two genera and photobionts under the maximum irradiance of blue, green and red light. Three-way ANOVA was used to test the CO_2 uptake, O_2 evolution and quantum yield of CO_2 uptake, O_2 evolution. Two-way ANOVA was used to test the F_v/F_m. One- way ANOVA followed by Tukeys pairwise comparison test was used to compare difference in effective quantum yield of PSII between different irradiances of blue light for the cyanolichens. For discussion of results, $P<0.01$ was used as the limit for significance if not anything else is stated.

3 Results

3.1 Effective quantum yield of PSII and Electron transport rate

The chlorolichens (*Lobaria pulmonaria* and *Peltigera leuocophlebia*) had much higher maximal quantum yield of PSII (F_v/F_m) compared to the cyanolichens (*Lobaria hallii* and *Peltigera praetextata*) (Tabs. 1 and 2). The *Peltigera* species had slightly higher, but clearly significant higher F_v/F_m than the *Lobaria* species, and the difference between the cyanolichens and the chlorolichens was slightly higher for *Lobaria* than for *Peltigera* shown by the significant genus x photobiont interaction (Tab. 2)

Table 1 Maximal quantum yield of PSII (F_v/F_m) of different species. Each value is the mean of 10 measurements ± 1SE.

	L. hallii	*L. pulmonaria*	*P. praetextata*	*P. leucophlebia*
F_v/F_m	0.55±0.041	0.71±0.033	0.61±0.026	0.72±0.014

Table 2 Two-way ANOVA of F_v/F_m (significance yield $P<0.01$). Calculation is based on the result of 10 thalli measurements for each species.

variables	*df*	*F*	*P*
Genus	1.	45.180	0.000
Photobiont	1	738.320	0.000
Genus×Photobiont	1	29.53	0.000
Error	116		
Total	119		

All species had decreasing effective PSII yield under red and green light with increasing irradiance (Fig. 9). Under blue light, effective PSII yield of both two cyanolichens first rapidly increased in low light before it slightly decreased at the highest irradiances. For *L. hallii* the increase was highly significant, whereas the increase was not significant for *P. praetextata* (Tab. 3).

For chlorolichens the yield immediately started to decline also in blue light.

The response of electron transport rate (ETR) to different PAR is shown for in Fig. 10. Apparent electron transport rate was similar and reach the light saturation at about 400 µmol m^{-2} s^{-1} irradiance for red and green light in both

chlorolichens and cyanolichens. However, in blue light the cyanolichens, ETR showed no sign of light saturation whereas chlorolichens showed normal light saturation. Comparison of ETR for the two chlorolichens in blue light, *L. pulmonaria* had lower maximum value than *P. leuocophlebia.* For both *L. hallii* and *P. praetextata* ETR increased linearly with blue light up to the maximum irradiance of 600 µmol m^{-2} s^{-1}.

Table 3 Effective quantum yields of PSII at various irradiances for *L. hallii* and *P. praetextata*. The values are average values of 10 measurements ± standard error (SE). Values with different letters are significant different at 5% level (Tukeys pairwise comparison test).

Irradiance	*L. hallii*	*P. pratextata*
0	0.55±0.03^{c}	0.61±0.03^{ab}
20	0.65±0.02^{a}	0.65±0.05^{a}
50	0.64±0.03^{a}	0.65±0.04^{a}
100	0.63±0.03^{a}	0.64±0.03^{ab}
200	0.06±0.02^{ab}	0.63±0.03^{ab}
400	0.58±0.03^{bc}	0.60±0.02^{bc}
600	0.55±0.03^{c}	0.56±0.02^{c}

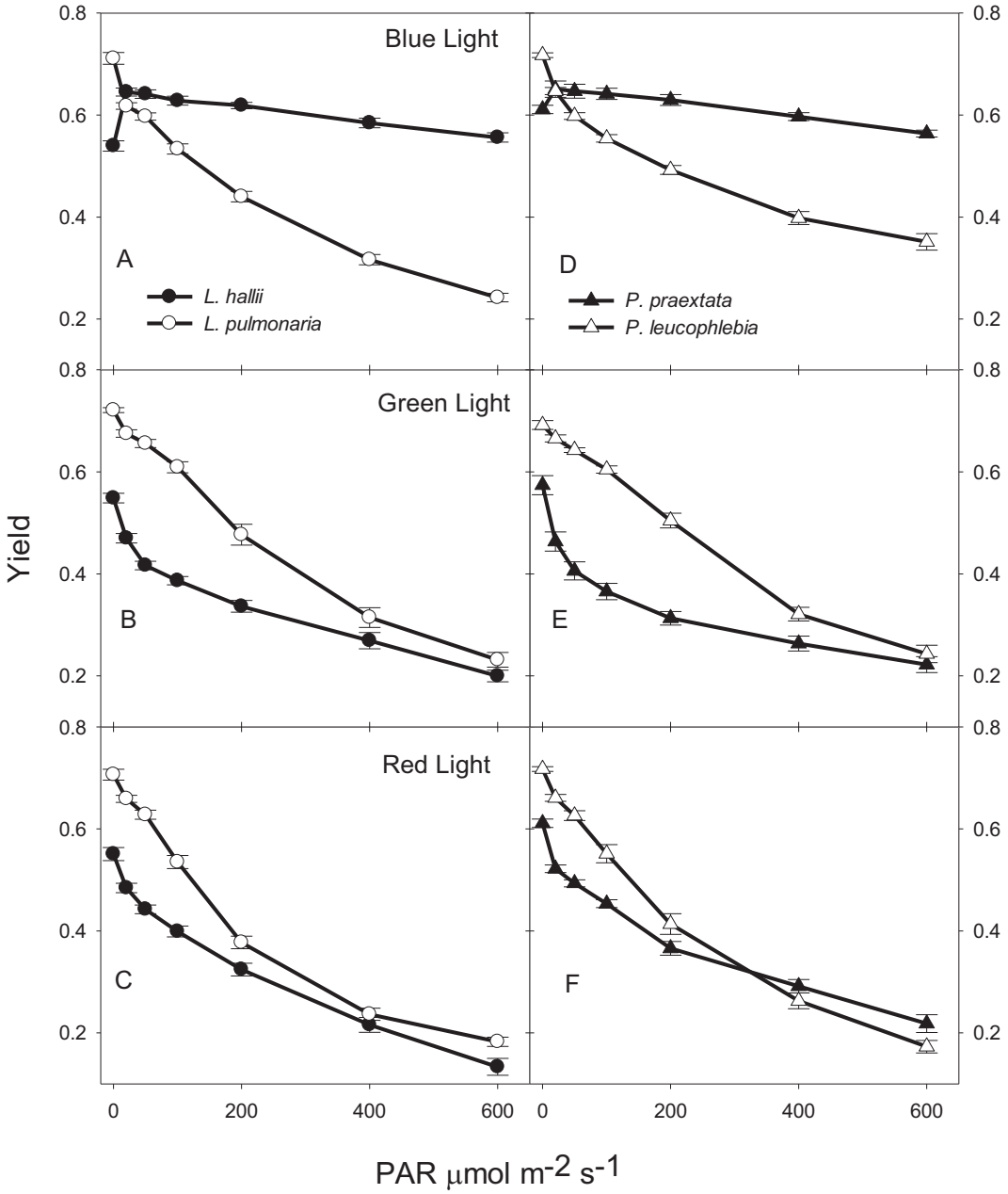

Figure 9 Light response curves of yield value for *L. pulmonaria* and *L. hallii* (A, B and C) and for *P. leucophlebia and P. praetextata* (D, E and F) in blue (A and D), green (B and E) red (C and F) light. Each curve is the mean of light response curves for 10 thalli and the error bars show SE.

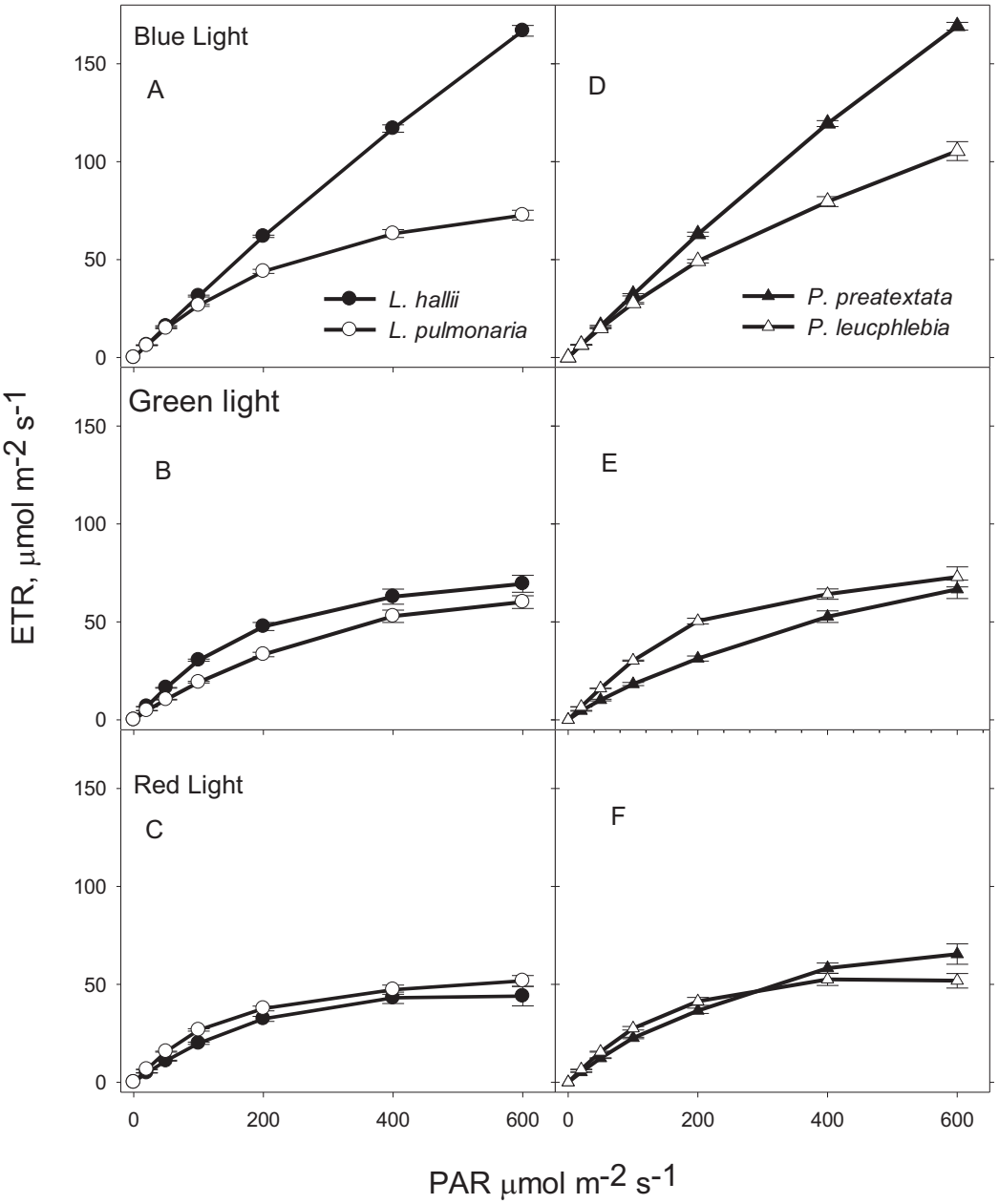

Figure 10 Light response curves of apparent ETR for *Lobaria pulmonaria* and *L. hallii* (A, B and C) and for P*eltigera leucophlebia and P. praetextata* (D, E and F) in blue (A and D), green (B and E) red (C and F) light. Each curve is the mean of light response curves for 10 thalli and the error bars show SE.

3.2 Photosynthesis

To track the real photosynthesis of the two lichen genus, we present the CO_2 uptake and O_2 evolution in Figure 11 and Figure 12. For genus *Lobaria*, the cyanolichen *L.hallii* had lower dark respiration. But for genus *Peltigera*, the chlorolichen *P. leuocophlebia* had lower dark respiration. The cyanolichens had higher photosynthesis measured as both CO_2 uptake and O_2 evolution under red and green light (Figs. 11 and 12). Whereas in both two genera, the cyanolichens had substantially lower photosynthesis in blue light. This phenomenon is also shown by the highly significant interaction (P=0.000) between color and photobiont (Table. 6).

Apparent quantum yields of CO_2 uptake and O2 evolution for the cyanolichens *L. hallii and P. praetextata* were much lower in blue light than in red light (Tabs. 4, 5 and 7). Apparent quantum yields were also lower for the chlorolichens in blue light compared with red light but the difference was smaller. The interaction of color and photobiont type is shown by the significant color x photobiont interaction for O_2 evolution (Tab. 7)

The reduction in the blue/red ratio of quantum yields for cyanolichens compared with chlorolichens was less for CO_2 uptake than for O_2 evolution (Tabs. 4 and 5). Combined with somewhat higher variation in the estimation of QY for CO_2 uptake than for O_2 evolution, no significant interaction between photobiont and color was achieved for the QY of CO_2 uptake (Tab. 7).

Assuming that absorbed light have about the same efficiency in photosynthetic CO_2 uptake regardless of color, the ratio of apparent QY between blue and red light and between green and red light can be used as a measure of the screening of blue and green light relative to blue light. By this method it is estimated that only 48 and 39 % blue light is transmitted for the cyanolichens *L. hallii* and *P. praetextata* and respectively, whereas 59 and 65 % is transmitted for chlorolichens *L. pulmonaria* and *P. leucophlaebia* respectively. For green light, 74 and 91% is transmitted for cyanolichen *L. hallii* and *P. praetextat,* whereas 57 and 95 % is transmitted for chlorolichens

L. pulmonaria and *P. leucophlaebia* (Tab. 4). By using the ratio of quantum yield under blue and red light and green and red light measured by O_2 evolution method it is estimated that only 36 and 15 % blue light is transmitted for cyanolichens *L. hallii* and *P. praetextata* respectively, whereas 71 and 52 % is transmitted for chlorolichens *L. pulmonaria* and *P. leucophlaebia* (Tab. 5).

Table 4 Apparent quantum yields of CO_2 uptake. The rates in quantum yields in blue light compared with red light and Green light compared with red light is shown. Each value is the mean of 10 measurements ±1SE.

color	*L.halli*	*L.pulmonaria*	*P. praetextata*	*P. leucophlebia*
blue	0.012±0.003	0.020±0.008	0.021±0.006	0.035±0.011
green	0.019±0.007	0.019±0.007	0.049±0.012	0.052±0.008
red	0.025±0.005	0.033±0.008	0.054±0.009	0.054±0.011
Blue/Red	0.484±0.049	0.590±0.091	0.394±0.041	0.652±0.051
Green/Red	0.743±0.083	0.575±0.067	0.918±0.074	0.9560.085

Table 5 Apparent quantum yields of O_2 evolution. The rates in quantum yields in blue light compared with red light and Green light compared with red light is shown. Each value is the mean of 10 measurements ±1SE.

color	*L. halli*	*L. pulmonaria*	*P. praetextata*	*P. leucophlebia*
blue	0.020±0.004	0.039±0.005	0.006±0.010	0.018±0.005
green	0.043±0.006	0.042±0.005	0.027±0.003	0.026±0.003
red	0.056±0.002	0.055±0.007	0.043±0.005	0.035±0.005
Blue/Red	0.36±0.060	0.71±0.018	0.15±0.114	0.52±0.050
Green/Red	0.66±0.064	0.81±0.064	0.63±0.050	0.74±0.042

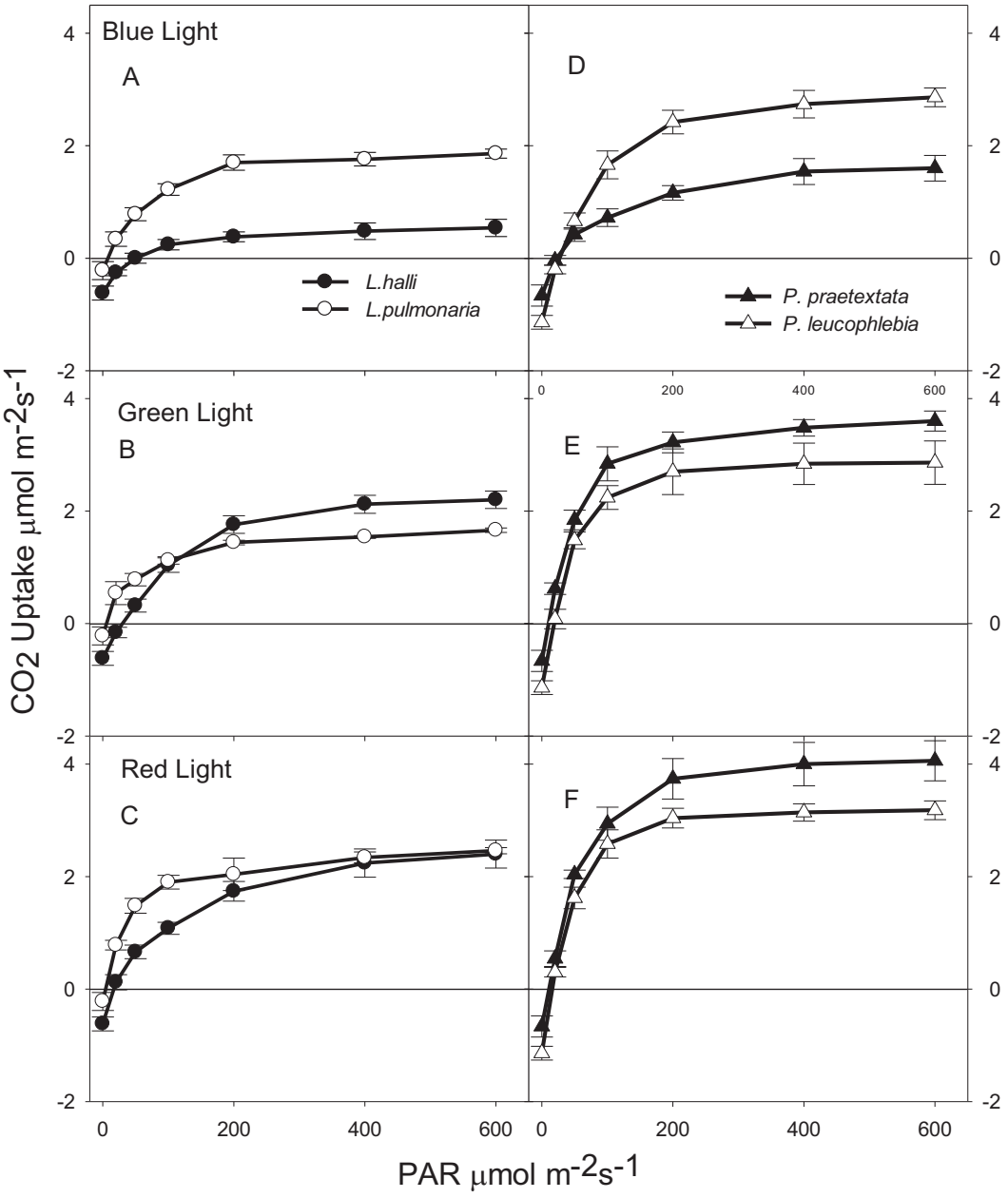

Figure 11 Light response curves for CO_2 uptake for *Lobaria pulmonaria* and *L. hallii* (A, B and C) and for P*eltigera leucophlebia and P. praetextata* (D, E and F) in blue (A and D), green (B and E) red (C and F) light. Before measurement all thalli were acclimated in hydrated condition for 24h at 30 µmol photons m^{-2} s^{-1}. Each curve is the mean of light response curves for 10 thalli. The error bars show SE.

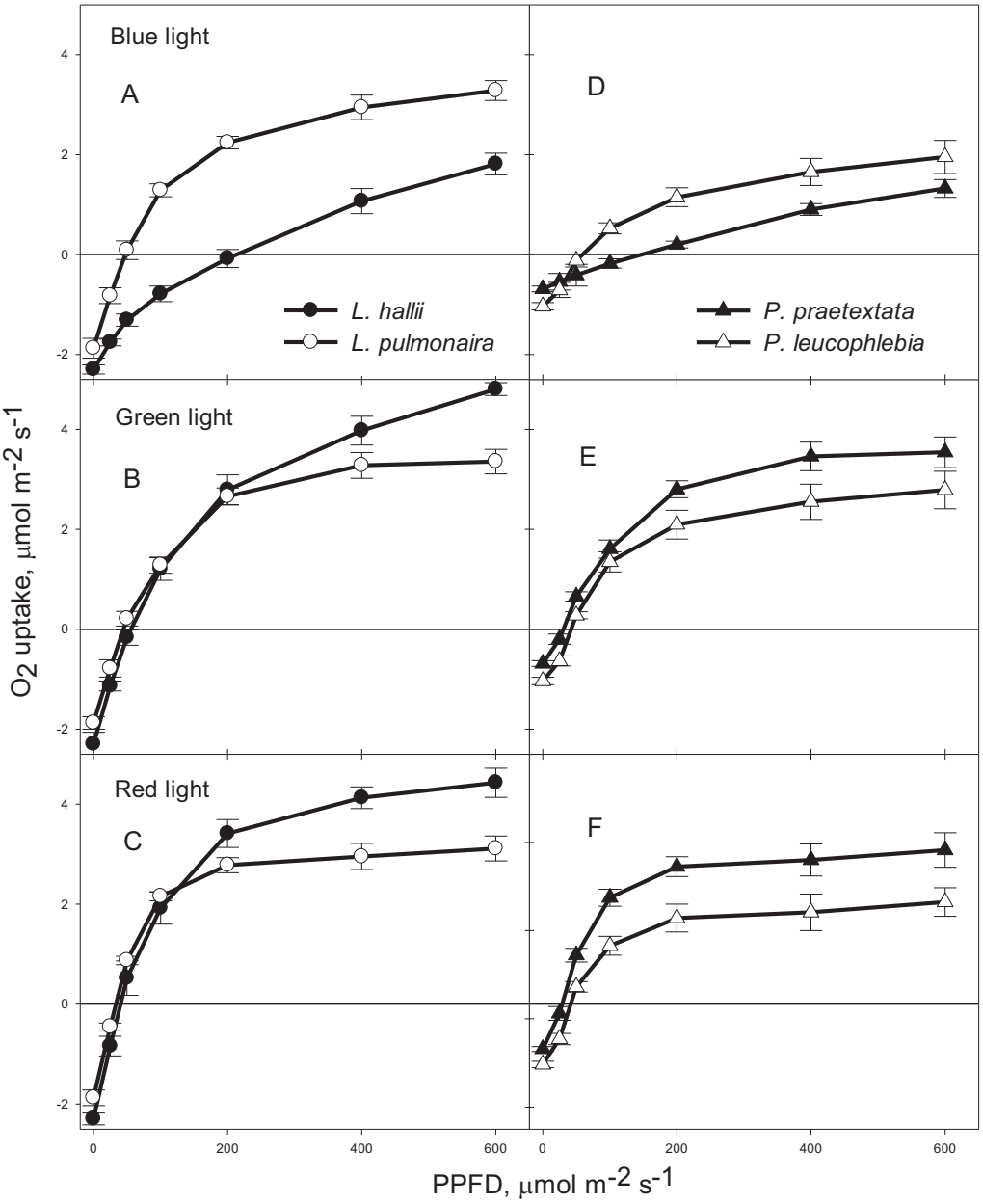

Figure 12 Light response curves for photosynthetic O_2 evolution for thalli genus *Lobaria* and *Peltigera*. Before measurement all thalli were acclimated in hydrated condition for 24h at 30 µmol photons m^{-2} s^{-1}. Each curve is the mean of light response curves for 10 thalli. The error bars show SE.

Table 6 Three-way ANOVA of CO_2 uptake and O_2 evolution at 600 µmol photons $m^{-2}s^{-1}$ (significance yield *P*<0.01). Calculation is based on the result of 10 thalli measurements for each species.

variables	df	*F*		*P*	
		CO2 uptake	O2 evolution	CO2 uptake	O2 evolution
Genus	1	238.23	6.58	0.000	*0.000*
Photobiont	1	0.33	7.97	0.569	*0.007*
Color	2	38.96	58.55	0.000	*0.000*
Genus×Photobiont	1	2.42	0.17	0.126	*0.681*
Genus×Color	2	0.43	1.24	0.653	*0.300*
Photobiont×Color	2	25.22	34.45	0.000	*0.000*
Genus×Photobiont×Color	2	1.08	2.13	0.349	*0.129*
Error	48				
Total	59				

Table 7 Three-way ANOVA of quantum yield of CO_2 uptake and O_2 evolution (significance yield *P*<0.01). Calculation is based on the result of 10 thalli measurements for each species.

variables	df	*F*		*P*	
		QY of CO_2	QY of O_2	QY of CO_2	QY of O_2
Genus	1	113.51	72.79	0.000	0.000
Photobiont	1	6.65	9.36	0.013	0.004
Color	2	27.78	68.25	0.000	0.000
Genus×Photobiont	1	0.01	0.02	0.920	0.878
Genus×Color	2	6.85	0.67	0.002	0.516
Photobiont×Color	2	1.62	11.25	0.208	0.000
Genus×Photobiont×Color	2	0.96	0.19	0.392	0.828
Error	48				
Total	59				

3.3 Reflectance and transmission

Both cyanolichens *L. hallii* and *P. praetextata* had much lower reflection and transmission of green light probably due to phycobiliproteins (Fig. 13). However, in the same genus class, there was no difference in reflection and transmission in blue light. Also transmittance of light through the combined cortex and photobiont layer indicated that the blue light was efficiently absorbed by the cyanolichens.

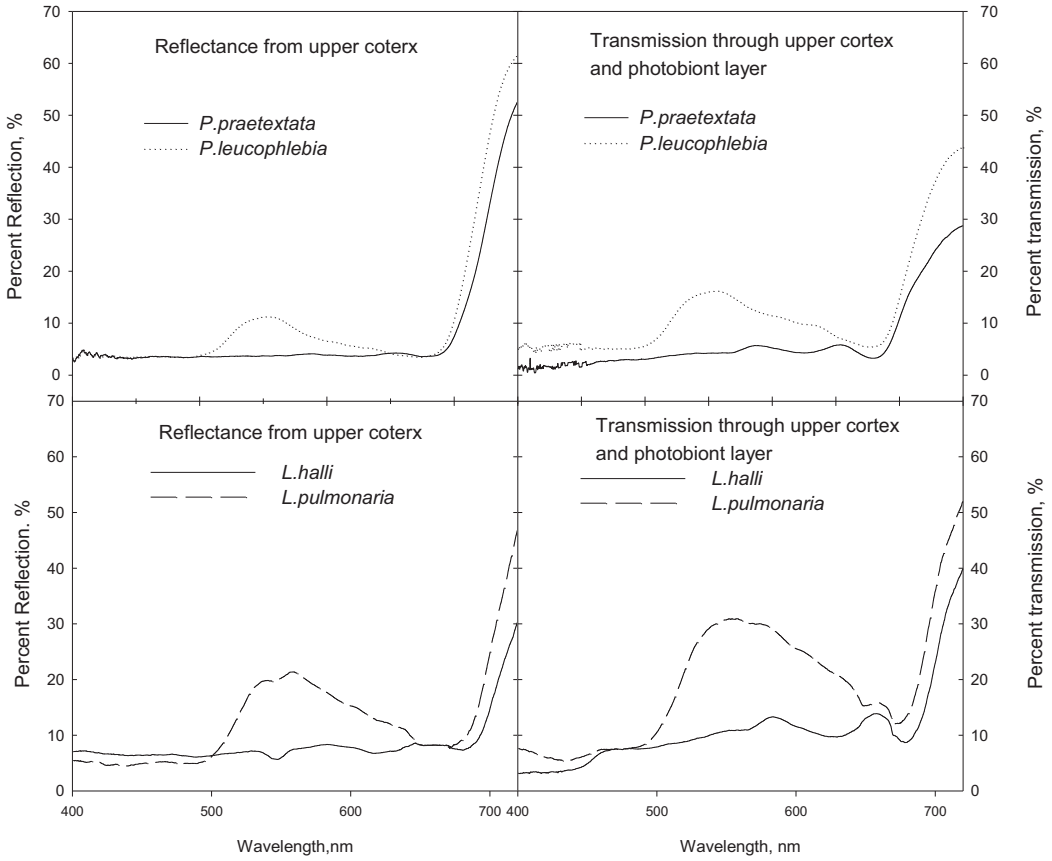

Figure 13 Reflectance from upper cortex and transmission through upper cortex and photobiont from thalli of *L. hallii, L. pulmonaria, P. praetextata* and *P. leucophlaebia*. Each curve is the average of five measurements.

3.4 Blue light screening estimated with fluorescence excitation ratio

Using the fluorescence excitation ratio method it was estimated that the cyanolichen *L. hallii* screened around 87% blue light, and *P. praerextata* also had 83% blue light screening while the chlorolichen *L. pulmonaria* and *P. leucophlebia* had blue light screening of 32% and 9% (Tab. 8).

Table 8 The percent of blue light screening is the average value of 10 measurements ± standard error. All thalli were hydrated for 24h at 30 µmol photons m^{-2} s^{-1}.

Lichen species	Blue light screening, %	Green light screening, %
L. hallii	87.48 ± 2.54	59.67 ± 1.50
L. pulmonaria	28.22 ± 1.41	16.59 ± 1.52
P. preatextata	83.39 ± 0.65	47.49 ± 2.03
P. leucophlebia	9.49 ± 0.27	13.69 ± 0.15

3.5 HPLC analysis and content calculation

The HPLC results (Figs. 14 and 15) show that only *L. hallii* have absorbance peaks of the screening pigments scytonemin and red scytonemin, but *P. praetextata* did not. Some unidentified carotenoids can also be seen for both cyanolichens in the HPLC chromatogram (Figs. 14 and 15). Peak C in the *L. hallii* chromatogram was not identified (Fig. 14C). The concentration of scytonemin in *L. hallii* was 0.45 mg/g (Tab. 9).

Table 9 Content of scytonemin in *L. hallii* and *P. praetextata* measured by HPLC. Each thallus weighed around 30 mg and had an area of about 4 cm^2.

species	Scytonemin content, mg					Average concentration	
	sample 1	sample 2	sample 3	sample 4	sample 5	average, mg/g	average, mg/cm^2
L. hallii	0.010	0.022	0.026	0.020	0.037	0.45 ± 0.077	0.0057 ± 0.0012
P. praetextata	0.000	0.000	0.000	0.000	0.000	0.000	0.000

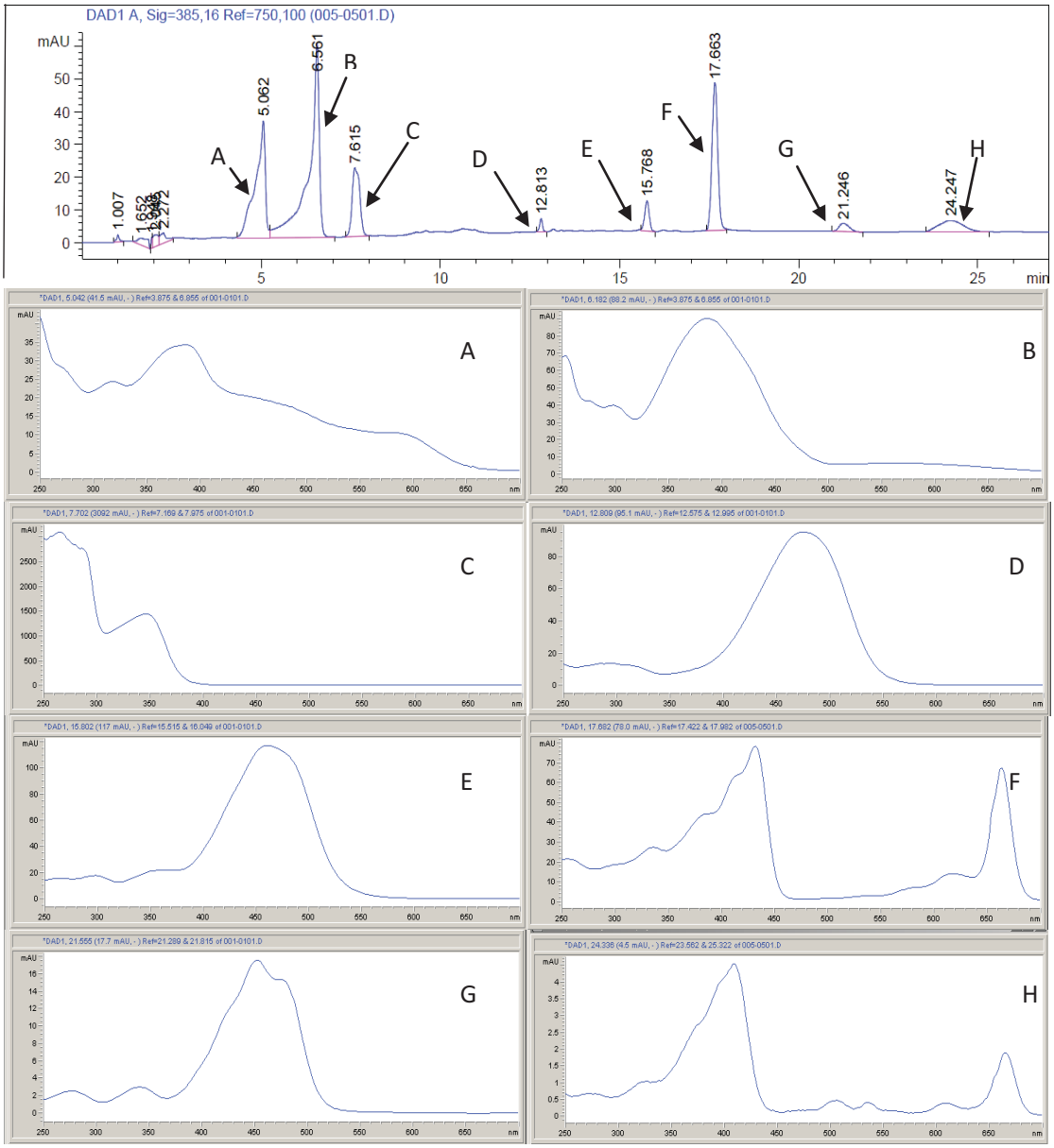

Figure 14 HPLC chromatogram of *L. hallii with spectra for the individual peaks recorded wih the HPLC photodiodearray detector.* A is red scytonemin (Mueller et al., 2005). B is scytonemin. C is some unknown substance. D, E and G are carotenoids. F and H are Chlorophyll *a.*

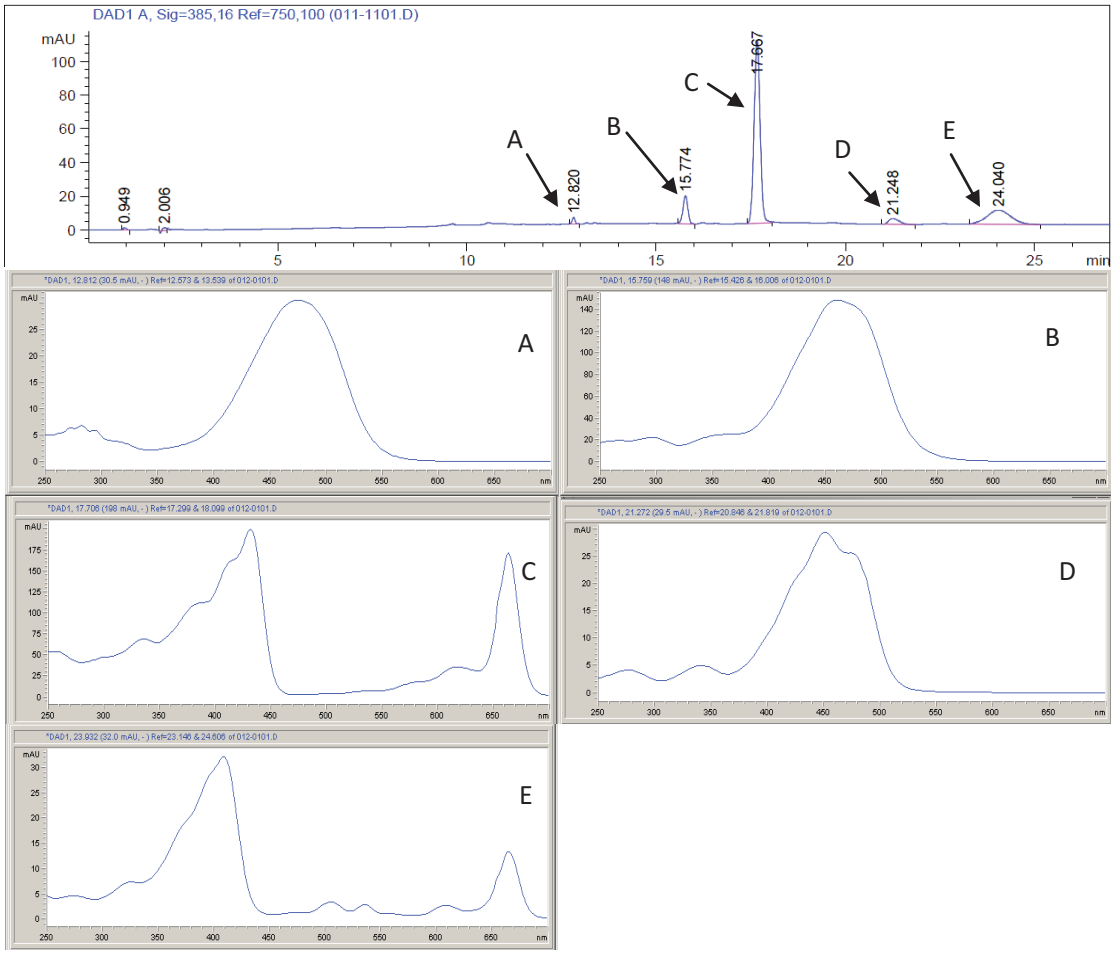

Figure 15 HPLC chromatogram of *P. praetextata with spectra for the individual peaks record wih the HPLC photodiodearray detector.* A, B and D are carotenoids. C and E are chlorophyll *a*.

4 Discussion

The cyanolichens *L. hallii* and *P. praetextata* had much lower photosynthetic CO_2 uptake and photosynthetic O_2 evolution in blue light than the chlorolichens *L. pulmonaria* and *P. leucophlebia* (Figs. 11 and 12). To our knowledge it is the first time that this is shown.

4.1 Lower photosynthesis of cyanolichens in blue light

The reduced photosynthesis in blue light may be caused by either screening of light before it reaches the photosynthetic apparatus or reduced efficiency of blue light in cyanolichen photosynthetic apparatus. Screening may be caused by either increased reflection or increased absorptance by screening pigments (Solhaug et al., 2010). There were no difference in absorptance of blue light between cyanolichens and chlorolichens since a very low fraction of blue light was either reflected (Fig. 12) and (Gauslaa, 1984) or transmitted through the cortex plus photobiont layer (Fig. 12). Therefore, most blue light must be absorbed either by screening pigments or by the photobiont layer. The cortex of the cyanolichens contain almost no colored lichen acids (Krog et al., 1994). However, cyanobacteria contain the UV and blue light absorbing pigment in their sheath. We tested the second hypothesis by using HPLC analysis to calculate the content of scytonemin in these two cyanolichens. The result of HPLC shows that there was low concentration of only 0.045% scytonemin in *L. hallii* and even no content in *P. praetextata* (Tab. 7) while *Peltula* species may contain up to 8% scytonemin on a dry weight basis (Büdel 1997). If it is assumed that scytonemin is evenly distributed as one layer above the photosynthetic active cells using the extinction coefficient of 112.6 $Lg^{-1}cm^{-1}$ at 384 nm (Garcia-Pichel et al., 1992) it can be calculated that the screening at 385 nm is 77% using equation 5, whereas the screening in blue light where scytonemin has approximately half the absorbance (Fig. 13) will be 50%. However, in a real cyanolichen thallus scytonemin will be located

all around the cyanobacterial cells with much less content per area cell. Assuming that scytonemin will be distributed on a four times higher cyanobacterial surface area (spherical cyanobacterial cells) giving an absorbance of 0.075 and the screening caused by scytonemin may be estimated with equation 5 to be around 15%. Therefore, the main cause reduced blue light photosynthesis cannot be scytonemin.

It was tried to prepare isolated thallus fragments for direct measurement of cortical transmittance as it has been done by e.g (Dietz et al., 2000) and (McEvoy et al., 2007). However, this was not successful because the cortex was very fragile making it impossible to prepare large enough fragments for measurement. Indirect measurement of screening with the fluorescence excitation ratio method shows that *L. hallii* and *P. praetxtata* screen 87 and 83% blue light respectively relative to red light (Table 4). Both cyanolichen also showed some green light screening estimated with this method.

Lower apparent quantum yield of O_2 evolution and CO_2 uptake in blue light shown by the blue/red ratio of the quantum yields (Tabs. 4 and 5) also indicates that there is screening of blue light in the same way as the blue light absorbing pigment parietin reduce the quantum yield of O_2 evolution in *Xanthoria parietina (Gauslaa and Solhaug, 1996)*

The apparent quantum yield of PSII (F_v'/F_m') is almost equal at low irradiances under blue light, whereas ETR is not saturated at high irradiances (Fig. 9). This indicates that there is a screening, indicating that blue light does not reach the photosynthetic apparatus. However, some blue light must reach the photosynthetic apparatus because there is some CO_2 uptake and O_2 evolution also in blue light although it is much lower than in red and green light.

One aspect that complicates chlorophyll fluorescence measurements in cyanobacteria is that some of the fluorescence contributing to F_0 comes from phycocyanin (Campbell et al., 1998). This effect will increase the F_0 fluorescense resulting in decreased F_v/F_m values in cyanobacteria compared

with higher plants or green algae explaining why cyanolichens have lower F_v/F_m values than chlorolichen (Fig. 9).

In chlorolichens, the lower photosynthesis in green light compared to the blue and red light is due to chlorophyll in chlorolichen absorbs fewer green light photons than blue and red light photons (Figs. 11 and 12). Cyanolichen have higher photosynthesis than chlorolichen in green light (Figs. 11B, E and 12B, E). Instead of chloroplasts cyanobacteria in cyanolichens have an elaborate and highly organized system of internal membranes which function in photosynthesis. Cyanobacteria contain the accessory phycobiliprotein pigments phycoerythrin and phycocyanin that are located in phycobilisomes as antenna pigments (Blankenship, 2002). These pigments absorb green light much more than chlorophyll and they have a low absorbance in blue light (Fig. 16). Comparison of spectrum of the blue LED light used for photosynthesis in this study with the phycocyanin and phycoerythrin spectrum (Fig. 5) shows only partly overlap and therefore low absorbance. Therefore, reduced blue light absorbance by phycocyanin and phycoerythrin may contribute to reduced blue light photosynthesis. However, as discussed above, blue light is well absorbed in the two cyanolichens in this study, so this can only partly be an explanation for the low blue light photosynthesis.

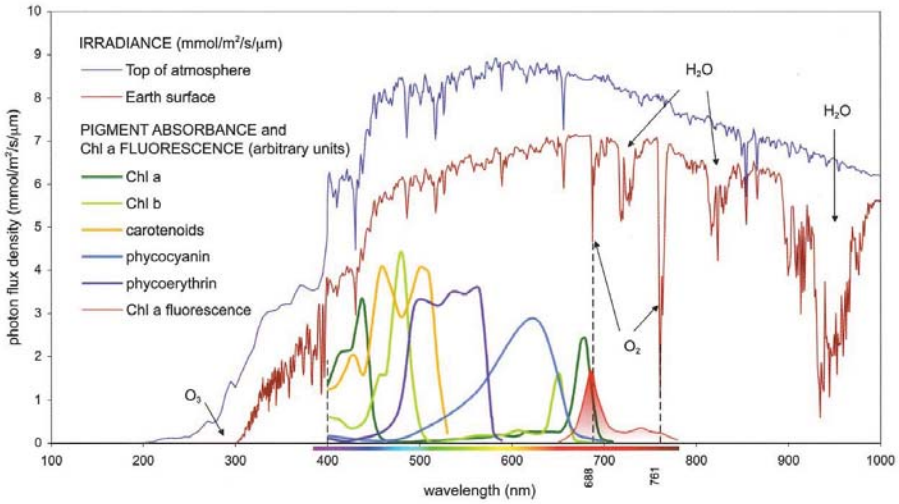

Figure 16. Irradiance and absorbance of various photosynthetic pigments. Cover page of (Papageorgiou and Govindjee, 2004)

4.2 Increased quantum yield of PSII in low blue light

The effective quantum yields of PSII increases in low blue light for both cyanolichens in low blue irradiance (Tab. 3). A similar result has not been shown before for cyanolichens or cyanobacteria in general. The only similar result known for me is in diatoms for which (Schreiber, 1998) showed that the effective quantum yield of PSII increased in low light. However, he did not test different light spectral qualities. In addition, diatoms contain chlorophyll *a* and *c* (Sugahara et al., 1971) while cyanolichens only contain chlorophyll *a*. This phenomenon needs further study.

5 Conclusion

In this study we confirm our first hypothesis that green light is more efficiently used in cyanolichens than in chlorolichens. To the second hypothesis, we have found that cyanolichens have lower photosynthesis under blue light. The lower photosynthesis was caused by blue light screening. But the content of the blue light absorbing compound scytonemin was too low to explain the screening. Therefore, the cause of blue light screening in the two cyanolichens in this study is still unknown. It needs further research.

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7 Appendix

Appendix 1 Yield value of PSII for *Lobaria pulmonaria*

Irradiance / Sample	Red light, μmol m^{-2} s^{-1}						
	0	20	50	100	200	400	600
1	0.738	0.679	0.632	0.508	0.351	0.220	0.193
2	0.736	0.676	0.636	0.538	0.376	0.216	0.156
3	0.729	0.662	0.646	0.563	0.402	0.227	0.179
4	0.732	0.643	0.621	0.557	0.371	0.268	0.199
5	0.723	0.690	0.670	0.601	0.457	0.317	0.240
6	0.742	0.631	0.582	0.466	0.304	0.175	0.131
7	0.684	0.649	0.631	0.547	0.392	0.248	0.198
8	0.623	0.623	0.582	0.479	0.371	0.214	0.166
9	0.705	0.659	0.627	0.553	0.369	0.237	0.180
10	0.699	0.677	0.650	0.538	0.379	0.243	0.183
AVG	0.711	0.659	0.628	0.535	0.377	0.237	0.183
SD	0.036	0.022	0.028	0.040	0.039	0.038	0.029
Irradiance / Sample	Green Light, μmol m^{-2} s^{-1}						
	0	20	50	100	200	400	600
1	0.738	0.665	0.644	0.559	0.356	0.249	0.168
2	0.736	0.683	0.681	0.633	0.517	0.328	0.261
3	0.729	0.681	0.660	0.652	0.491	0.325	0.235
4	0.732	0.701	0.682	0.625	0.498	0.317	0.221
5	0.723	0.698	0.684	0.649	0.570	0.414	0.290
6	0.742	0.667	0.650	0.586	0.431	0.219	0.169
7	0.684	0.675	0.652	0.608	0.479	0.319	0.218
8	0.623	0.688	0.658	0.632	0.556	0.403	0.310
9	0.705	0.674	0.648	0.590	0.432	0.291	0.223
10	0.699	0.619	0.598	0.557	0.437	0.279	0.220
AVG	0.711	0.675	0.656	0.609	0.477	0.314	0.232
SD	0.036	0.023	0.025	0.035	0.065	0.061	0.046
Irradiance / Sample	Blue Light, μmol m^{-2} s^{-1}						
	0	20	50	100	200	400	600
1	0.738	0.647	0.627	0.574	0.473	0.336	0.235
2	0.736	0.624	0.626	0.558	0.427	0.305	0.222
3	0.729	0.614	0.601	0.550	0.456	0.332	0.251
4	0.732	0.626	0.601	0.556	0.457	0.337	0.273
5	0.723	0.618	0.590	0.515	0.396	0.293	0.223
6	0.742	0.619	0.594	0.551	0.488	0.367	0.291
7	0.684	0.597	0.576	0.495	0.405	0.269	0.201
8	0.623	0.575	0.557	0.471	0.396	0.274	0.230
9	0.705	0.643	0.616	0.534	0.443	0.311	0.242
10	0.699	0.615	0.584	0.532	0.458	0.341	0.254
AVG	0.711	0.618	0.597	0.534	0.440	0.317	0.242
SD	0.036	0.021	0.022	0.032	0.033	0.032	0.026

Appendix 2 Yield value of PSII for *Lobaria hallii*

Irradiance / Sample	Red light, μmol m^{-2} s^{-1}						
	0	20	50	100	200	400	600
1	0.581	0.509	0.452	0.405	0.307	0.210	0.110
2	0.500	0.497	0.439	0.387	0.294	0.192	0.132
3	0.556	0.502	0.452	0.419	0.359	0.249	0.181
4	0.574	0.527	0.474	0.438	0.372	0.294	0.220
5	0.565	0.452	0.395	0.342	0.252	0.172	0.083
6	0.528	0.477	0.422	0.372	0.316	0.154	0.075
7	0.506	0.489	0.477	0.443	0.366	0.263	0.172
8	0.568	0.423	0.405	0.355	0.287	0.175	0.104
9	0.578	0.485	0.447	0.411	0.349	0.253	0.183
10	0.559	0.481	0.459	0.416	0.338	0.195	0.074
AVG	0.552	0.484	0.442	0.399	0.324	0.216	0.133
SD	0.030	0.029	0.027	0.034	0.039	0.046	0.052
Irradiance / Sample	Green Light, μmol m^{-2} s^{-1}						
	0	20	50	100	200	400	600
1	0.581	0.492	0.452	0.403	0.365	0.310	0.220
2	0.500	0.435	0.360	0.331	0.285	0.201	0.157
3	0.556	0.481	0.413	0.399	0.361	0.322	0.216
4	0.574	0.497	0.433	0.417	0.382	0.300	0.226
5	0.565	0.479	0.439	0.394	0.368	0.292	0.240
6	0.528	0.471	0.408	0.380	0.286	0.185	0.127
7	0.506	0.415	0.414	0.400	0.334	0.264	0.202
8	0.568	0.457	0.428	0.396	0.342	0.309	0.214
9	0.578	0.491	0.394	0.366	0.309	0.224	0.206
10	0.559	0.502	0.401	0.360	0.304	0.238	0.196
AVG	0.552	0.472	0.414	0.385	0.334	0.265	0.200
SD	0.030	0.028	0.026	0.026	0.036	0.050	0.034
Irradiance / Sample	Blue Light, μmol m^{-2} s^{-1}						
	0	20	50	100	200	400	600
1	0.581	0.627	0.611	0.613	0.595	0.560	0.510
2	0.500	0.610	0.642	0.624	0.610	0.542	0.564
3	0.556	0.674	0.683	0.664	0.640	0.624	0.579
4	0.574	0.623	0.605	0.594	0.598	0.557	0.517
5	0.565	0.670	0.669	0.664	0.645	0.630	0.602
6	0.528	0.639	0.616	0.609	0.604	0.594	0.563
7	0.506	0.653	0.652	0.639	0.625	0.586	0.564
8	0.568	0.651	0.647	0.595	0.624	0.580	0.536
9	0.578	0.681	0.664	0.663	0.644	0.609	0.577
10	0.559	0.623	0.622	0.617	0.602	0.562	0.548
AVG	0.552	0.645	0.641	0.628	0.619	0.584	0.556
SD	0.030	0.025	0.027	0.028	0.019	0.030	0.029

Appendix 3 Yield value of PSII for *Peltigera leucophlebia*

Irradiance / Sample	Red light, μmol m^{-2} s^{-1}						
	0	20	50	100	200	400	600
1	0.738	0.687	0.662	0.594	0.426	0.318	0.194
2	0.724	0.659	0.627	0.543	0.409	0.227	0.190
3	0.717	0.671	0.620	0.512	0.321	0.226	0.154
4	0.711	0.664	0.642	0.600	0.465	0.325	0.213
5	0.723	0.667	0.629	0.596	0.413	0.283	0.202
6	0.700	0.683	0.648	0.636	0.546	0.316	0.222
7	0.688	0.611	0.570	0.494	0.386	0.217	0.138
8	0.727	0.644	0.645	0.542	0.435	0.258	0.169
9	0.720	0.664	0.640	0.544	0.403	0.274	0.153
10	0.726	0.657	0.576	0.452	0.330	0.184	0.094
AVG	0.717	0.661	0.626	0.551	0.413	0.263	0.173
SD	0.014	0.021	0.030	0.056	0.064	0.049	0.039
Irradiance / Sample	Green Light, μmol m^{-2} s^{-1}						
	0	20	50	100	200	400	600
1	0.738	0.675	0.647	0.606	0.486	0.332	0.255
2	0.724	0.644	0.631	0.566	0.470	0.302	0.261
3	0.717	0.637	0.638	0.605	0.477	0.332	0.244
4	0.711	0.645	0.620	0.602	0.520	0.382	0.289
5	0.723	0.648	0.640	0.607	0.521	0.363	0.274
6	0.700	0.672	0.659	0.618	0.554	0.364	0.293
7	0.688	0.663	0.625	0.577	0.467	0.256	0.220
8	0.727	0.703	0.663	0.648	0.558	0.308	0.108
9	0.720	0.689	0.648	0.617	0.564	0.309	0.271
10	0.726	0.679	0.656	0.601	0.428	0.262	0.217
AVG	0.717	0.666	0.643	0.605	0.505	0.321	0.243
SD	0.014	0.022	0.015	0.022	0.046	0.042	0.054
Irradiance / Sample	Blue Light, μmol m^{-2} s^{-1}						
	0	20	50	100	200	400	600
1	0.738	0.672	0.626	0.566	0.527	0.429	0.389
2	0.724	0.647	0.579	0.522	0.440	0.298	0.330
3	0.717	0.664	0.603	0.535	0.483	0.404	0.330
4	0.711	0.660	0.608	0.587	0.506	0.412	0.412
5	0.723	0.648	0.614	0.550	0.473	0.371	0.366
6	0.700	0.651	0.611	0.568	0.509	0.437	0.395
7	0.688	0.597	0.556	0.516	0.463	0.411	0.317
8	0.727	0.663	0.610	0.573	0.516	0.429	0.390
9	0.720	0.642	0.597	0.563	0.518	0.399	0.346
10	0.726	0.627	0.577	0.563	0.489	0.392	0.239
AVG	0.717	0.647	0.598	0.554	0.492	0.398	0.351
SD	0.014	0.022	0.021	0.023	0.028	0.040	0.051

Appendix 4 Yield value of PSII for *Peltigera praetextata*

Irradiance / Sample	Red light, μmol m^{-2} s^{-1}						
	0	20	50	100	200	400	600
1	0.612	0.521	0.5	0.462	0.419	0.348	0.257
2	0.638	0.522	0.449	0.429	0.334	0.266	0.163
3	0.587	0.541	0.505	0.42	0.295	0.238	0.16
4	0.591	0.556	0.527	0.479	0.338	0.268	0.138
5	0.598	0.546	0.515	0.5	0.401	0.317	0.232
6	0.604	0.493	0.471	0.446	0.41	0.314	0.285
7	0.639	0.482	0.478	0.449	0.382	0.311	0.253
8	0.64	0.534	0.498	0.454	0.407	0.345	0.295
9	0.635	0.514	0.491	0.462	0.331	0.273	0.209
10	0.566	0.508	0.498	0.432	0.34	0.234	0.19
AVG	0.611	0.522	0.493	0.453	0.366	0.291	0.218
SD	0.026	0.023	0.022	0.024	0.043	0.041	0.055
Irradiance / Sample	Green Light, μmol m-2 s-1						
	0	20	50	100	200	400	600
1	0.612	0.432	0.387	0.388	0.366	0.323	0.269
2	0.638	0.386	0.285	0.239	0.219	0.207	0.131
3	0.587	0.437	0.39	0.379	0.32	0.22	0.225
4	0.591	0.43	0.4	0.337	0.348	0.323	0.27
5	0.598	0.376	0.348	0.356	0.315	0.305	0.268
6	0.604	0.512	0.437	0.359	0.346	0.265	0.268
7	0.639	0.521	0.446	0.399	0.335	0.289	0.213
8	0.64	0.549	0.453	0.373	0.293	0.197	0.154
9	0.635	0.503	0.464	0.404	0.308	0.25	0.225
10	0.566	0.49	0.448	0.42	0.281	0.255	0.198
AVG	0.611	0.464	0.406	0.365	0.313	0.263	0.222
SD	0.026	0.059	0.056	0.051	0.042	0.046	0.0498
Irradiance / Sample	Blue Light, μmol m-2 s-1						
	0	20	50	100	200	400	600
1	0.612	0.685	0.67	0.665	0.635	0.599	0.572
2	0.638	0.695	0.677	0.664	0.628	0.582	0.539
3	0.587	0.698	0.696	0.681	0.672	0.643	0.574
4	0.591	0.703	0.687	0.681	0.667	0.618	0.585
5	0.598	0.703	0.687	0.658	0.662	0.611	0.588
6	0.604	0.604	0.634	0.63	0.618	0.581	0.533
7	0.639	0.573	0.569	0.588	0.573	0.568	0.536
8	0.64	0.621	0.627	0.646	0.641	0.6	0.583
9	0.635	0.615	0.619	0.613	0.618	0.602	0.559
10	0.566	0.611	0.602	0.591	0.581	0.566	0.569
AVG	0.611	0.651	0.647	0.642	0.629	0.597	0.564
SD	0.026	0.05	0.042	0.034	0.034	0.024	0.02

Appendix 5 CO_2 Uptake of genus *Lobaria*

Irradiance / Sample		Red light, μmol m^{-2} s^{-1}						
		0	20	50	100	200	400	600
Lobaria hallii	1	-0.8	0.1	0.7	0.8	2	2.7	2.9
	2	-0.9	-0.4	0.2	1.1	1.8	2.2	2.3
	3	-0.2	0.3	0.7	0.9	1.1	1.3	1.5
	4	-0.7	0.3	0.9	1.2	1.7	2.6	2.8
	5	-0.5	0.3	0.8	1.4	2.1	2.4	2.5
	AVG	-0.620	0.120	0.660	1.080	1.740	2.240	2.400
	SD	0.277	0.303	0.270	0.239	0.391	0.559	0.557
Lobaria pulmonaria	1	0.3	0.9	1.5	1.9	2.3	2.4	2.5
	2	-0.5	0.7	1.8	1.8	2.2	2.3	2.4
	3	-0.6	0.5	1	1.5	0.9	2	2.3
	4	-0.1	0.8	1.5	2.1	2.3	2.4	2.5
	5	-0.2	1	1.6	2.2	2.5	2.6	2.6
	AVG	-0.220	0.780	1.480	1.900	2.040	2.340	2.460
	SD	0.356	0.192	0.295	0.274	0.647	0.219	0.114
Irradiance / Sample		Green Light, μmol m^{-2} s^{-1}						
		0	20	50	100	200	400	600
Lobaria hallii	1	-0.8	0.1	0.4	1.4	1.7	2.2	2.4
	2	-0.9	-0.3	0	0.8	2.1	2.2	2.3
	3	-0.2	-0.4	0.1	0.7	1.2	1.5	1.6
	4	-0.7	-0.2	0.6	1.2	1.8	2.3	2.3
	5	-0.5	0	0.5	1.1	2	2.4	2.5
	AVG	-0.620	-0.160	0.320	1.040	1.760	2.120	2.220
	SD	0.277	0.207	0.259	0.288	0.351	0.356	0.356
Lobaria pulmonaria	1	0.3	1.3	0.8	1.1	1.5	1.5	1.6
	2	-0.5	0.2	0.8	1.2	1.3	1.6	1.8
	3	-0.6	0.2	0.4	0.9	1.4	1.5	1.6
	4	-0.1	0.6	1.1	1.3	1.5	1.5	1.6
	5	-0.2	0.4	0.8	1.1	1.5	1.6	1.7
	AVG	-0.220	0.540	0.780	1.120	1.440	1.540	1.660
	SD	0.356	0.456	0.249	0.148	0.089	0.055	0.089
Irradiance / Sample		Blue Light, μmol m^{-2} s^{-1}						
		0	20	50	100	200	400	600
Lobaria hallii	1	-0.8	-0.2	0	0.2	0.3	0.2	0.2
	2	-0.9	-0.3	-0.2	0	0.3	0.6	0.6
	3	-0.2	-0.3	0.2	0.5	0.7	0.9	1
	4	-0.7	-0.4	-0.2	0.1	0.2	0.1	0.2
	5	-0.5	-0.1	0.2	0.4	0.4	0.6	0.8
	AVG	-0.620	-0.260	0.000	0.240	0.380	0.480	0.560
	SD	0.277	0.114	0.200	0.207	0.192	0.327	0.358
Lobaria pulmonaria	1	0.3	0.5	0.6	1	1.3	1.5	1.6
	2	-0.5	0.2	0.7	1.2	1.8	1.8	1.9
	3	-0.6	-0.1	0.5	1	1.5	1.6	1.9
	4	-0.1	0.5	1	1.5	2.1	2.2	2.1
	5	-0.2	0.6	1.1	1.4	1.8	1.7	1.8
	AVG	-0.220	0.340	0.780	1.220	1.700	1.760	1.860
	SD	0.356	0.288	0.259	0.228	0.308	0.270	0.182

Appendix 6 CO_2 Uptake of genus *Peltigera*

Irradiance / Sample		Red light, μmol m^{-2} s^{-1}						
		0	20	50	100	200	400	600
Pertigera praetextata	1	-1.4	0.0	2.0	2.2	2.8	3.1	3.1
	2	-0.6	0.6	2.1	3.9	5.0	5.4	5.3
	3	-0.5	0.8	2.3	3.2	3.8	4.1	4.1
	4	-0.4	0.6	1.9	2.5	3.7	3.8	4.0
	5	-0.5	0.7	1.9	2.9	3.4	3.6	3.8
	AVG	-0.664	0.540	2.040	2.940	3.740	4.000	4.060
	SD	0.420	0.313	0.167	0.658	0.805	0.863	0.796
Pertigera leucophlebia	1	-1.5	0.5	2.1	2.7	2.8	2.8	2.7
	2	-1.1	0.2	1.6	2.4	3.1	3.3	3.3
	3	-1	0.5	2	3.5	3.7	3.6	3.7
	4	-1.3	0.1	1.2	2.1	2.8	2.8	3
	5	-0.8	0.2	1.2	2.2	2.8	3.2	3.2
	AVG	-1.140	0.300	1.620	2.580	3.040	3.140	3.180
	SD	0.270	0.187	0.427	0.563	0.391	0.344	0.370
Irradiance / Sample		Green Light, μmol m^{-2} s^{-1}						
		0	20	50	100	200	400	600
Pertigera praetextata	1	-1.4	0.3	1.9	2.6	2.9	2.9	2.9
	2	-0.6	0.9	2.2	3.3	3.5	3.6	3.8
	3	-0.5	0.7	1.8	1.8	2.7	3.6	3.9
	4	-0.4	0.5	2.1	3.5	3.7	3.7	3.7
	5	-0.5	0.7	1.2	3.0	3.3	3.6	3.7
	AVG	-0.664	0.620	1.840	2.840	3.220	3.480	3.600
	SD	0.420	0.228	0.391	0.673	0.415	0.327	0.400
Pertigera leucophlebia	1	-1.5	-0.5	1.1	2.8	4.2	4.2	4.3
	2	-1.1	0.5	1.9	2	2.1	2.3	2.3
	3	-1	0.2	1.5	2.7	2.8	2.9	2.9
	4	-1.3	0.3	1.7	1.8	1.9	2.1	2.1
	5	-0.8	-0.1	1.2	1.9	2.5	2.7	2.7
	AVG	-1.140	0.080	1.480	2.240	2.700	2.840	2.860
	SD	0.270	0.390	0.335	0.472	0.908	0.823	0.865
Irradiance / Sample		Blue Light, μmol m^{-2} s^{-1}						
		0	20	50	100	200	400	600
Pertigera praetextata	1	-1.4	-0.1	0.2	0.3	0.7	1.0	1.3
	2	-0.6	-0.1	0.2	0.5	1.4	2.4	2.5
	3	-0.5	-0.3	0.6	0.9	1.2	1.4	1.4
	4	-0.4	0.1	0.8	1.2	1.4	1.5	1.5
	5	-0.5	0.2	0.3	0.7	1.1	1.4	1.3
	AVG	-0.664	-0.040	0.420	0.720	1.160	1.540	1.600
	SD	0.420	0.195	0.268	0.349	0.288	0.518	0.510
Pertigera leucophlebia	1	-1.5	-0.3	1.2	2.6	3.1	3.6	3.4
	2	-1.1	-0.2	0.7	1.7	2.6	2.8	3
	3	-1	-0.3	0.4	1.2	1.9	2.1	2.4
	4	-1.3	-0.3	0.5	1.4	2.4	2.6	2.8
	5	-0.8	0.1	0.5	1.4	2.1	2.6	2.7
	AVG	-1.140	-0.200	0.660	1.660	2.420	2.740	2.860
	SD	0.270	0.173	0.321	0.555	0.466	0.546	0.371

Appendix 7 O_2 evolution of genus *Lobaria*

Sample	Irradiance	Red light, μmol m^{-2} s^{-1}						
		0	20	50	100	200	400	600
Lobaria hallii	1	-1.97	-1.31	-0.22	1.09	3.94	3.94	4.60
	2	-2.49	-0.23	1.58	2.49	3.39	3.84	3.84
	3	-2.60	-0.65	0.87	2.60	3.90	4.98	5.42
	4	-2.40	-1.20	-0.24	1.20	2.40	3.84	3.84
	5	-2.63	-0.81	0.61	2.22	3.44	4.04	4.45
	AVG	-2.42	-0.84	0.52	1.92	3.41	4.13	4.43
	SD	0.27	0.44	0.77	0.72	0.62	0.48	0.65
Lobaria pulmonaria	1	-1.28	-0.21	1.07	1.92	2.35	2.56	2.56
	2	-1.97	-0.59	0.79	1.97	2.56	2.36	2.56
	3	-1.70	-0.43	0.64	2.34	2.98	3.19	3.19
	4	-1.92	-0.43	1.07	2.35	3.21	3.85	3.85
	5	-2.21	-0.60	0.80	2.21	2.81	2.81	3.41
	AVG	-1.82	-0.45	0.87	2.16	2.78	2.95	3.11
	SD	0.35	0.16	0.19	0.20	0.34	0.59	0.56
Sample	Irradiance	Green Light, μmol m^{-2} s^{-1}						
		0	20	50	100	200	400	600
Lobaria hallii	1	-2.85	-1.31	-0.44	1.31	2.19	3.72	4.38
	2	-1.81	-0.90	0.23	1.81	3.39	3.39	4.97
	3	-1.52	-1.08	0.00	1.30	3.47	4.77	4.98
	4	-1.92	-0.96	0.00	1.20	2.88	4.56	5.04
	5	-2.43	-1.41	-0.61	0.40	2.02	3.44	4.65
	AVG	-2.10	-1.14	-0.16	1.21	2.79	3.98	4.81
	SD	0.53	0.22	0.35	0.51	0.67	0.65	0.28
Lobaria pulmonaria	1	-1.92	-0.21	0.64	1.07	2.35	2.56	2.77
	2	-2.36	-1.18	-0.20	1.18	2.17	2.76	2.96
	3	-1.91	-0.85	0.00	0.85	2.98	3.61	3.19
	4	-2.14	-1.07	0.21	1.71	2.99	3.85	3.85
	5	-1.61	-0.60	0.40	1.61	2.81	3.61	4.02
	AVG	-1.99	-0.78	0.21	1.28	2.66	3.28	3.36
	SD	0.28	0.39	0.33	0.36	0.38	0.58	0.55
Sample	Irradiance	Blue Light, μmol m^{-2} s^{-1}						
		0	20	50	100	200	400	600
Lobaria hallii	1	-2.41	-1.97	-1.53	-1.31	-0.44	0.44	1.31
	2	-2.49	-1.58	-1.13	-0.45	0.45	1.81	2.03
	3	-2.17	-1.73	-1.30	-0.87	-0.43	0.65	1.30
	4	-2.16	-1.68	-0.96	-0.48	0.24	1.44	2.40
	5	-2.63	-1.82	-1.62	-0.81	-0.20	1.01	2.02
	AVG	-2.37	-1.76	-1.31	-0.78	-0.08	1.07	1.81
	SD	0.20	0.15	0.27	0.35	0.40	0.56	0.49
Lobaria pulmonaria	1	-1.28	-0.43	0.43	1.07	1.92	2.35	2.99
	2	-2.36	-1.38	-0.59	0.99	1.97	2.36	2.76
	3	-1.49	-0.64	0.21	1.28	2.34	3.19	3.61
	4	-2.14	-0.86	0.00	1.71	2.57	3.42	3.85
	5	-1.81	-0.80	0.40	1.41	2.41	3.41	3.21
	AVG	-1.82	-0.82	0.09	1.29	2.24	2.95	3.28
	SD	0.45	0.35	0.42	0.29	0.28	0.55	0.45

Appendix 8 O_2 evolution of genus *Peltigera*

Sample	Irradiance	Red light, μmol m^{-2} s^{-1}						
		0	20	50	100	200	400	600
Pertigera praetextata	1	-0.64	0.42	1.49	2.90	4.32	4.95	5.24
	2	-0.73	0.07	1.32	2.72	3.67	3.89	4.11
	3	-0.46	-0.17	1.37	2.80	3.54	3.60	4.11
	4	-0.89	-0.44	0.83	1.84	2.67	2.22	2.39
	5	-0.67	0.31	1.71	3.12	3.36	3.24	3.24
	AVG	-0.68	0.04	1.34	2.67	3.51	3.58	3.82
	SD	0.16	0.35	0.32	0.49	0.59	0.99	1.07
Pertigera leucophlebia	1	-0.82	-0.22	0.99	1.87	2.42	2.86	2.91
	2	-1.35	-0.76	0.60	1.84	2.33	2.55	2.55
	3	-1.05	-0.39	1.16	2.31	2.97	3.41	3.58
	4	-0.96	-0.36	0.54	1.38	2.11	1.56	2.41
	5	-1.07	-0.90	0.68	1.75	3.00	3.28	3.17
	AVG	-1.05	-0.53	0.79	1.83	2.56	2.73	2.92
	SD	0.20	0.29	0.27	0.33	0.40	0.74	0.47
Sample	Irradiance	Green Light, μmol m^{-2} s^{-1}						
		0	20	50	100	200	400	600
Pertigera praetextata	1	-0.64	-0.35	0.35	1.27	2.76	4.17	4.60
	2	-0.73	-0.29	0.73	2.28	3.16	3.82	3.74
	3	-0.46	0.17	0.86	1.88	3.02	3.82	3.88
	4	-0.89	0.06	0.44	1.06	2.00	2.22	2.34
	5	-0.67	-0.49	0.61	1.71	2.87	3.12	3.36
	AVG	-0.68	-0.18	0.60	1.64	2.76	3.43	3.58
	SD	0.16	0.28	0.21	0.49	0.45	0.78	0.83
Pertigera leucophlebia	1	-0.82	-0.33	0.38	1.37	1.98	2.31	2.75
	2	-1.35	-0.92	0.11	0.98	2.00	2.55	3.03
	3	-1.05	-0.66	0.39	1.82	2.70	3.08	3.47
	4	-0.96	-0.72	0.24	1.62	2.29	2.59	2.53
	5	-1.07	-0.34	0.51	1.75	2.77	3.68	3.79
	AVG	-1.05	-0.59	0.33	1.51	2.35	2.84	3.11
	SD	0.20	0.26	0.15	0.34	0.37	0.55	0.52
Sample	Irradiance	Blue Light, μmol m^{-2} s^{-1}						
		0	20	50	100	200	400	600
Pertigera praetextata	1	-0.64	-1.13	-0.99	0.00	0.07	0.50	0.85
	2	-0.73	-0.37	-0.29	0.00	0.44	1.17	1.91
	3	-0.46	-0.91	-0.46	-0.17	0.17	0.74	1.31
	4	-0.89	-1.28	-1.61	-0.33	0.00	0.89	1.17
	5	-0.67	-0.31	-0.18	0.00	0.37	1.28	1.77
	AVG	-0.68	-0.80	-0.71	-0.10	0.21	0.92	1.40
	SD	0.16	0.44	0.59	0.15	0.19	0.32	0.44
Pertigera leucophlebia	1	-0.82	-0.38	-0.05	0.71	1.21	1.81	1.98
	2	-1.35	-1.30	-0.54	0.16	0.87	1.57	1.84
	3	-1.05	-0.44	0.39	0.83	1.76	2.20	2.70
	4	-0.96	-0.60	0.00	0.66	1.14	1.81	2.17
	5	-1.07	-1.02	-0.23	0.57	1.47	2.15	2.60
	AVG	-1.05	-0.75	-0.09	0.59	1.29	1.91	2.26
	SD	0.20	0.40	0.34	0.25	0.34	0.26	0.38