Plant Physiology and Biochemistry 134 (2019) 123–128

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Reprint of Efficient fungal UV-screening provides a remarkably high UV-B tolerance of photosystem II in lichen photobionts☆

Peter Váczi[a], Yngvar Gauslaa[b], Knut Asbjørn Solhaug[b,*]

[a] Department of Experimental Biology, Faculty of Science, Masaryk University, Kamenice 5, 625 00, Brno, Czech Republic
[b] Faculty of Environmental Sciences and Natural Resource Management, Norwegian University of Life Sciences, P.O. Box 5003, NO-1433, Ås, Norway

ARTICLE INFO

Keywords:
Chlorophyll fluorescence
Coccomyxa
Lichen cortex
Nephroma arcticum
Trebouxia
Umbilicaria spodochroa
UV-screening

ABSTRACT

Lichen photobionts *in situ* have an extremely UV-B tolerant photosystem II efficiency (Fv/Fm). We have quantified the UV-B-screening offered by the mycobiont and the photobiont separately. The foliose lichens *Nephroma arcticum* and *Umbilicaria spodochroa* with 1: intact or 2: removed cortices were exposed to 0.7 Wm^{-2} $UV\text{-}B_{BE}$ for 4 h. Intact thalli experienced no reduction in Fv/Fm, whereas cortex removal lowered Fv/Fm in exposed photobiont layers by 22% for *U. spodochroa* and by 14% for *N. arcticum*. We also gave this UV-B dose to algal cultures of *Coccomyxa* and *Trebouxia,* the photobiont genera of *N. arcticum* and *U. spodochroa,* respectively. UV-B caused a 56% reduction in Fv/Fm for *Coccomyxa,* and as much as 98% in *Trebouxia*. The fluorescence excitation ratio (FER) technique comparing the fluorescence from UV-B— or UV-A-excitation light with blue green excitation light using a Xe-PAM fluorometer showed that these photobiont genera did not screen any UV-B or UV-A The FER technique with a Multiplex fluorometer estimated the UV-A screening of isolated algae to be 13–16%, whereas intact lichens screened 92–95% of the UV-A. In conclusion, the cortex of *N. arcticum* and *U. spodochroa* transmitted no UV-B and little UV-A to the photobiont layer beneath. Thereby, the upper lichen cortex forms an efficient fungal solar radiation screen providing a high UV-B tolerance for studied photobionts *in situ*. By contrast, isolated photobionts have no UV-B screening and thus depend on their fungal partners in nature.

1. Introduction

Lichens are long-lived associations between a mycobiont and one or more photobionts (a green alga and/or a cyanobacterium). The mycobiont provides water, minerals, and protection against high solar radiation and herbivores, whereas a main role of the photobiont is to fix CO_2. Lichens are poikilohydric organisms often inhabiting extreme environments with high solar radiation (Bjerke et al., 2002; Baniya et al., 2010). For example, the high-light adapted lichens *Rhizocarpon geographicum* and *Xanthoria elegans* survived several days exposure in the outer space (de la Torre et al., 2010). Even shade-adapted specimens of the old forest lichen *Lobaria pulmonaria* tolerates high UV-B doses due to efficient cortical UV-B screening: its cortical UV-B transmission is close to zero (Gauslaa et al., 2017). By contrast, epidermis of higher plants typically transmits 10% UV-B (Bilger et al., 2007). Exposure of an intact, but freely exposed photobiont layer in *L. pulmonaria* to UV-B increased the susceptibility of photosystem II (PSII) to UV, showing that the fungal cortex screens UV-radiation (Gauslaa et al., 2017). However, we do not know whether algal photobionts screen UV-radiation themselves.

Lichens often synthesize high amounts of secondary compounds (Molnár and Farkas, 2010; Huneck, 1999) that may screen photosynthetic active radiation (PAR) and UV radiation (Solhaug and Gauslaa, 2012). These secondary fungal compounds are often located as small crystals on the surface of the fungal hypha. Some compounds mainly occur in the upper cortex whereas others are located in the medulla (Fahselt, 1994). It is possible to non-destructively remove most of these compounds with acetone-rinsing (Solhaug and Gauslaa, 2001). However, acetone rinsing removing the majority of secondary compounds just slightly raised the UV-susceptibility of *L. pulmonaria*, probably because even a compound-deficient lichen cortex is an efficient UV-screen (Gauslaa et al., 2017).

Among green algal lichen photobionts, sporopollenin-like substances occur in the cell wall of *Coccomyxa*, but not in *Trebouxia*

DOI of original article: https://doi.org/10.1016/j.plaphy.2018.08.033

☆ This article is part of the Special Issue “Interactive effects of UV-B radiation in a complex environment” published at the journal Plant Physiology and Biochemistry 134, 2019.

* Corresponding author.
E-mail address: knut.solhaug@nmbu.no (K.A. Solhaug).

https://doi.org/10.1016/j.plaphy.2018.09.030
Received 9 April 2018; Received in revised form 23 August 2018; Accepted 24 August 2018

(Brunner and Honegger, 1985; Honegger and Brunner, 1981). Sporopollenin absorbs UV radiation, and assumingly screens damaging UV-B (Xiong et al., 1997). By using algal cultures of the lichen photobionts *Trebouxia* and *Coccomyxa*, we want to test if *Coccomyxa* having sporopollenin is more resistant against UV-B than *Trebouxia* that is deficient in this compound.

Our main objective was to quantify how much of the high UV-B tolerance of lichen photobionts (Gauslaa et al., 2017) is due to fungal cortical UV-B screening, and to which extent the algal partner itself provides additional UV-B-protection mechanisms. We aimed to test this by estimating UV screening in algal cultures using the fluorescence excitation ratio (FER) technique (Bilger et al., 1997; Cerovic et al., 2008). In addition, we compared the UV-B susceptibility of PSII efficiency in intact lichens and in cultured algal photobionts. UV-B protection may involve screening and/or by repair of UV-B damage (Pescheck et al., 2014). The FER technique can estimate the level of screening in algal cultures as well as in intact lichens, whereas the recovery of PSII efficiency during a 30 h period after UV-B exposure may also document differences in susceptibility caused by repairing mechanisms.

In this study, we will compare the UV-B susceptibility and screening in the high light-adapted lichen *Umbilicaria spodochroa* and more shade-adapted thalli of *Nephroma arcticum.* Their respective photobionts are *Trebouxia jamesii* (Hestmark et al., 2016) and *Coccomyxa* sp. (Palmqvist et al., 1994). Our final objective was to compare UV susceptibility and screening between cultures of isolated *Coccomyxa* and four *Trebouxia* species.

2. Materials and methods

2.1. Lichen material

Umbilicaria spodochroa was collected from sun-exposed rocks at Hvaler, Østfold S Norway (59°08′36″N, 10°55′21E); *Nephroma arcticum* from partly shaded N-facing rock in open spruce forests in Ski, Akershus, S Norway (59°44′22″N, 10°58′34″). We selected these two species because they were sufficiently large and robust to allow freely exposed photobiont layers of the respective photobiont genera to be prepared. Both were collected in typical habitats of the two respective species. Furthermore, we wanted to use one lichen with and one without a sporopollenin-producing photobiont. Many thalli of both species were collected in May, air-dried, and stored at −18 °C. We completed all experiments within less than one month after collection. Before the UV exposure, lichens were moistened and placed at approx. 30 µmol m^{-2} s^{-1} light for one day to recover from possible photoinhibition prior to collection in the field (Solhaug 2018). For direct exposure of the photobiont layer, a portion of approximately 1 cm^2 upper cortex was carefully scraped off each specimen of *U. spodochroa*, whereas approx. 1 cm^2 of the lower cortex including the medulla was removed from *N. arcticum* thalli. A highly fragile structure did not allow removal of the upper cortex in the latter species. We carefully scraped off the upper cortex from healthy uniform lichen thalli using a scalpel under a preparation microscope. The following categories, (1) intact thalli, (2) scraped thalli with exposed photobiont layers, as well as (3) cultured photobionts were exposed to UV irradiation before Fv/Fm were measured by chlorophyll fluorescence imaging tools.

2.2. Photobiont cultivation

Stock cultures of the lichen photobionts *Trebouxia erici* (UTEX 911), *T. assymmetrica* (SAG 48.88), *T. jamesii* (SAG 2103) and *Coccomyxa* sp. (P. Váczi lab. collection) were maintained in an axenic culture on Bold's Basal Medium (BBM-agar, prepared according to Ahmadjian, 1993) in Petri dishes. The photobionts were cultivated at 20 °C using 16/8 h light/dark photoperiod with irradiance of 30 µmol m^{-2} s $^{-1}$ (Vaczi and Bartak, 2006). Prior to the experiments, algal cultures were transferred into a liquid BBM medium (Ahmadjian, 1993), suspended by gentle stirring and distributed evenly (10 mg FW/disk) on nitrocellulose membrane discs, pore size of 0.45 µm by vacuum filtration. Membrane disks with photobiont culture were maintained for 24 h at the cultivation conditions specified above before start of experiment.

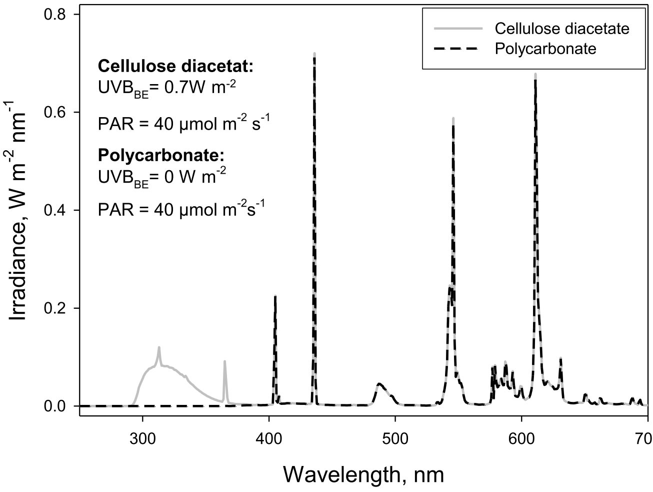

Fig. 1. Irradiance spectra of Philips TL 18W/827 white fluorescent tubes combined with Philips TL 20W/12 RS broadband UVB fluorescent tubes screened with cellulose diacetate (grey line) or both cellulose diacetate and polycarbonate (dashed line).

2.3. UV exposure

The membrane discs with photobionts were placed on Petri dishes with agar (BBM with 1% agar) covered with UV transmitting cling film to prevent drying during exposure. We exposed discs with pure photobionts as well as intact lichen thalli and prepared photobiont layers, placed on agar (BBM with 1% agar) in Petri dishes, to 0.7 W m^{-2} biologically effective UV-B$_{BE}$ radiation from UV-B broadband fluorescent tubes, Philips TL 20W/12 RS for 4 h. This UV-B treatment represents a common, high UV-B level during a sunny summer day in Norway (Solhaug et al., 2003). Cellulose diacetate film (Jürgen Rachow, Hamburg, Germany, 0.10 mm, 50% cut-off at 295 nm) screened the radiation below 295 nm. During both UV exposure and subsequent recovery, the samples were exposed to PAR of 40 µmol m^{-2} s^{-1} from Philips TL 18W/827 fluorescent tubes. The spectral irradiance distributions were measured with an Optronic model 756 spectroradiometer (Optronic Laboratories, Orlando, Florida, USA) (Fig. 1), and the biologically effective UVB$_{BE}$ was estimated with the green weighting function normalised to 1 at 300 nm (Green et al., 1974). Control samples had a cover of polycarbonate sheets that screened all UV radiation.

Three 4 h UV-exposure experiments were done. In the first experiment, we compared the four different photobiont species. In the second experiment, we simultaneously exposed (1) intact thalli, (2) thalli with scraped cortex of *U. spodochroa,* and (3) its cultivated photobiont *Trebouxia jamesii* to UV. Likewise, the third experiment simultaneously used (1) intact thalli, (2) thalli with scraped cortex of *N. arcticum,* (3) and its cultivated photobiont genus *Coccomyxa.*

2.4. Photosystem II efficiency

Maximal photosystem II efficiency (Fv/Fm, Van Kooten and Snell, 1990) was measured after 15 min dark adaptation using Handy FluorCam imaging chlorophyll fluorometer (Photon System Instruments, Brno, Czech Republic; all exciting light λ_{max} = 620 nm). During the measuring protocol, the samples were exposed to low measuring light (1 µmol m^{-2} s^{-1}) to determine basal fluorescence signal Fo and subsequently to strong saturating pulse (3000 µmol m^{-2} s^{-1}) to

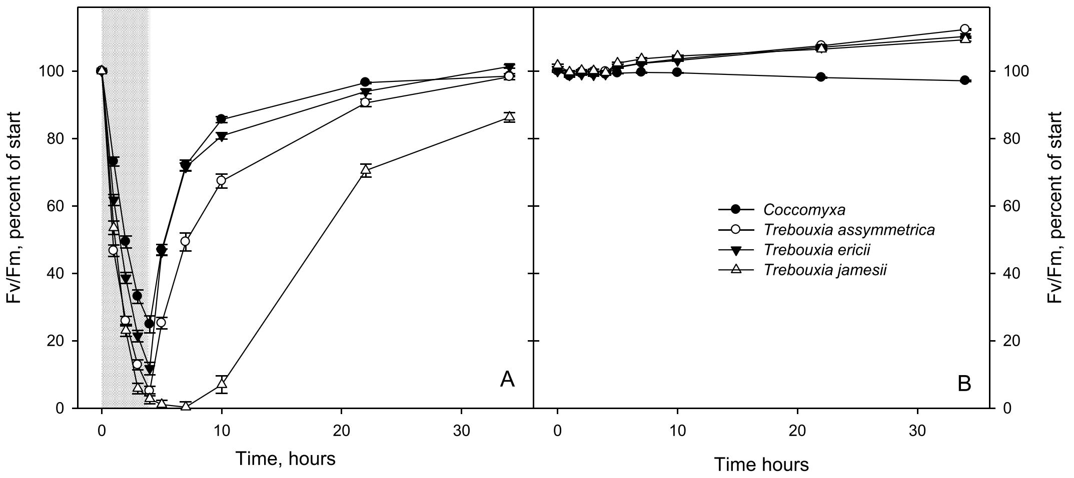

Fig. 2. Maximum photosystem II efficiency (Fv/Fm) as percent of start values for algal cultures exposed for 4 h to (A) 0.7 W m^{-2} UV-B$_{BE}$ (grey area) + PAR (40 μmol photons m^{-2} s^{-1}) or (B) 0 W m^{-2} UV-B$_{BE}$ + PAR (40 μmol photons m^{-2} s^{-1}). The subsequent recovery 36 h after both UV and control treatments occurred at a PAR level of 40 μmol photons m^{-2} s^{-1}. Error bars show SE and n = 10.

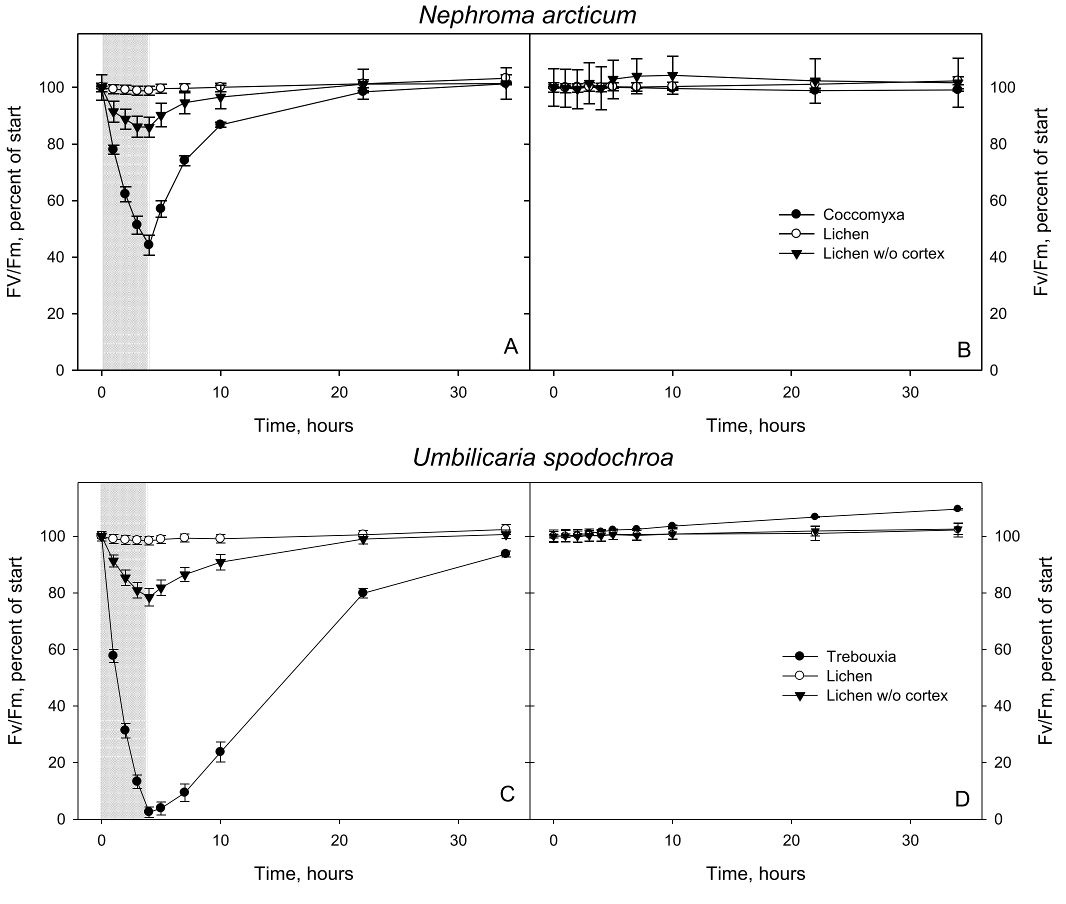

Fig. 3. Maximum photosystem II efficiency (Fv/Fm) as percent of start values for symbiotic photobionts in intact thalli, in exposed photobiont layer, and in asymbiotic algal cultures for *Nephroma arcticum* and *Umbilicaria spodochroa* exposed to exposed for 4 h to (A) 0.7 W m^{-2} UV-B$_{BE}$ (grey area) + PAR (40 μmol photons m^{-2} s^{-1}) or (B) 0 W m^{-2} UV-B$_{BE}$ + PAR (40 μmol photons m^{-2} s^{-1}). The subsequent recovery 30 h after both UV and control treatments occurred at a PAR level of 40 μmol photons m^{-2} s^{-1}. Error bars show SE and n = 10.

determine maximal fluorescence Fm. Fv/Fm was calculated using the formula: (Fm-Fo)/Fm.

Measurements were done before the UV exposure, during the UV-B exposure (after 1, 2, 3, 4 h) and during the recovery period (after 1, 3, 6, 18, 30 h).

2.5. Fluorescence excitation ratio method (FER)

UV screening was measured with the fluorescence excitation ratio (FER) technique using the fluorometers Multiplex 3 (FORCE A, Orsay, France) and Xe-PAM fluorometer (Walz, Effeltrich, Germany). Both instruments excite chlorophyll fluorescence at two wavelengths: one UV-wavelength absorbed by cells or cell walls before the beam reaches

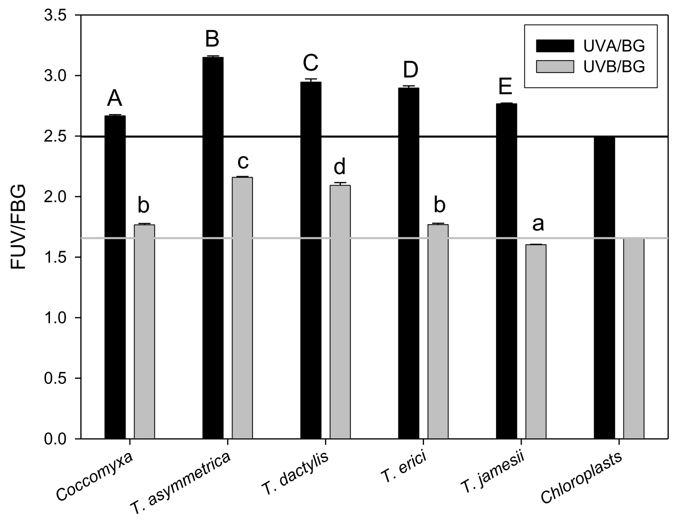

Fig. 4. The ratio between UV-A (black columns) or UV-B (grey columns) and blue-green (BG) excited chlorophyll fluorescence for various lichen photobiont cultures and isolated chloroplast from measured with a Xe-PAM fluorometer. Values above the respective horizontal lines indicate that UV screening is less than for chloroplasts used as a non-UV screening control. Bars marked with the same letter are not significantly different. Error bars show SE (n = 5).

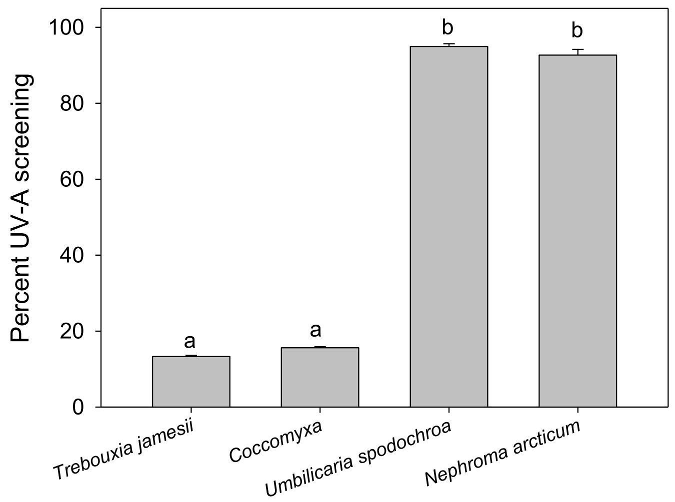

Fig. 5. Percent UV-A screening estimated with the Multiplex fluorometer for intact thalli of *Umbilicaria spodochroa* and *Nephroma arcticum* and cultures of their photobionts *Trebouxia jamesii* and *Coccomyxa*, respectively. Bars marked with the same letter are not significantly different. The error bars show SE (n = 5).

the chloroplasts, and one blue-green or red wavelength penetrating freely into the chloroplasts. By comparing the ratio between the fluorescence excited by these two wavelengths, we can estimate screening above the chloroplast level.

We used the fluorometer Multiplex 3 (Cerovic et al., 2008) to estimate UV-A screening in lichens and in photobionts. During measurement, we used moist thalli and photobionts on nitrocellulose membrane discs. We measured far-red chlorophyll fluorescence excited with UV-A (λ_{max} = 368 nm, 18 nm half bandwidth) ($F_{UV\text{-}A}$) and with red light (λ_{max} = 630 nm, 22 nm half bandwidth) (F_R). Signals in darkness without any specimen in front of the instrument was subtracted from all measurements. Furthermore, the values were normalised with signals from isolated *Vicia faba* chloroplasts assumed to have no screening. Percent UV-A screening was estimated with the formula: 100 *(1-($F_{UV\text{-}A}/F_R$)).

UV-A and UV-B screening were also measured in lichen photobionts with the Xe-PAM fluorometer using the method of Bilger et al. (1997). Photobiont cells were concentrated on filter paper by dripping a photobiont suspension on the paper. The paper covered with photobionts was attached to the sample holder in the fluorometer with double-sided tape. The setup of the instrument with filters, sample holder and light source followed Burchard et al. (2000) and Pescheck et al. (2010). The fluorescence signals induced by UV-A (λ_{max} = 366 nm, 32 nm half bandwidth), UV-B (λ_{max} = 314 nm, 18 nm half bandwidth) and broadband blue-green (λ = 420–550 nm) were normalised with fluorescence signals from blue plastic foil (fluorescence standard, Walz); the signal from a non-fluorescing green foil was subtracted from all measurements. The signals from photobionts were compared with the signal from isolated chloroplasts assumed to have very low screening. Because the normalised F_{UV}/F_{BG} ratios were slightly higher for lichen photobionts than for isolated chloroplasts, we presented the results as F_{UV}/F_{BG} ratios for both photobionts and chloroplasts. Calculation of percent screening based on zero screening for chloroplasts would have resulted in negative values probably caused by slight screening in the chloroplasts.

2.6. Statistical analysis

We used factorial analysis of variance and Fisher's LSD test (Dell Statistica ver.13, 2016) to determine the significant differences between ($P > 0.05$) measured parameters.

3. Results

3.1. UV-B effects on photosystem II efficiency in algal cultures

Mean Fv/Fm at start (time = 0) differed among all ($P < 0.001$; one-way ANOVA) algal cultures. It was highest in *Coccomyxa* (0.738 ± 0.001), lower in *T. erici* (0.651 ± 0.002), but lowest in *T. assymetrica* (0.580 ± 0.001) and *T. jamesii* (0.590 ± 0.002). During the UV-B_{BE} exposure to 0.7 W m^{-2} for 4 h, all algal cultures rapidly experienced strong reductions in maximum Fv/Fm. UV-B reduced Fv/Fm by 75% in *Coccomyxa* and by 88, 95 and 97% in *T. ericii, T. assymmetrica* and *T. jamensii*, respectively. *Coccomyxa, T. ericii,* and *T. assymmetrica* recovered almost 100% during 30 h under 40 μmol photons m^{-2} s^{-1}, whereas the recovery of *T. ericii* was 86% (Fig. 2A). Control algal cultures not exposed to UV-B had quite constant Fv/Fm during the experiment (Fig. 2B).

3.2. UV-B effects on photobionts in intact lichens, exposed photobiont layers, and in asymbiotic photobiont cultures

When the three categories of the *N. arcticum* photobiont were exposed to 4 h UV-B, Fv/Fm was reduced by 56% in the *Coccomyxa* culture (start Fv/Fm = 0.709 ± 0.003), by 14% in scraped thalli (start Fv/Fm = 0.559 ± 0.025), whereas UV-B had no effect on photobionts in intact thalli (start Fv/Fm = 0.677 ± 0.010). Nevertheless, all samples recovered completely during 30 h under 40 μmol m^{-2} s^{-1} (Fig. 3A).

Likewise, UV-B exposure of the three photobiont categories of *U. spodochroa* resulted in 98% reduction of Fv/Fm in cultured *T. jamesii* (start Fv/Fm = 0.609 ± 0.004), 22% reduction in the scraped thalli (start Fv/Fm = 0.656 ± 0.011) and no reduction in the photobiont of the intact thalli (start Fv/Fm = 0.661 ± 0.011). Fv/Fm for both intact and scraped thalli completely recovered within 30 h at 40 μmol m^{-2} s^{-1}, whereas cultured *T. jamesii* recovered to 94% of the original Fv/Fm (Fig. 3C). By contrast, Fv/Fm did not change in any categories of *N. arcticum* and *U. spodochroa* photobionts during the experiment excluding UV-B (Fig. 3B and D).

3.3. Estimation of UV-A and UV-B screening using the Xe-PAM fluorometer

The ratio between UV induced fluorescence and blue-green induced fluorescence (FUV/FBG) was similar or slightly higher for algal cultures compared to isolated chloroplasts (Fig. 4). We assumed that chloroplasts had very low UV-A and UV-B screening. Similar or higher FUV/FBG ratios in photobionts than in chloroplasts were consistent with low or no screening for all species both in the UV-A and UV-B spectral range.

3.4. Estimation of UV-A screening using the multiplex fluorometer

UV-A screening in pure *T. jamesii* and *Coccomyxa* cultures was 13.3 and 15.6%, respectively, whereas intact thalli of *U. spodochroa* and *N. articum* had UV-A screening of 95.0 and 92.7%, respectively (Fig. 5).

4. Discussion

Cortical screening by the mycobiont efficiently protects PSII against harmful UV-B radiation in photobionts in *N. arcticum* and *U. spodochroa* (Fig. 2), as previously shown for the old forest lichen *Lobaria pulmonaria* (Gauslaa et al., 2017). Yet, even exposed photobiont layer were substantially more UV-B-resistant than pure photobiont cultures (Fig. 3), presumably because algal cells in a photobiont layer are surrounded by medullary hyphae with UV-B screening compounds. Another explanation may be that algae within the lichen has acclimated to higher UV than algae cultured for a long time in a lab. Additional, but low UV screening in pure algal photobionts was confirmed by the two FER techniques.

With respect to the two FER techniques, the portable Multiplex instrument measures UV-A screening only, whereas the Xe-PAM fluorometer assesses UV-A and UV-B screening independently. However, UV-A screening may also indicate UV-B screening because many UV-A-absorbing compounds absorb even more in the UV-B range. For algal cultures, the Multiplex instrument showed less than 15% screening assuming no UV-screening in the reference chloroplasts, whereas Xe-PAM indicated that the algal cultures had similar or even less screening than chloroplasts. Together, these measurements show that pure cultures of studied lichen photobionts hardly screen any UV. By contrast, the Multiplex instrument showed 85% UV-A screening for intact lichens. This is likely an underestimation of the UV-B screening because the cortical secondary compound usnic acid in *N. arcticum* absorbs much more UV-B than UV-A (McEvoy et al., 2007). Furthermore, if cortical transmission of *N. arcticum* and *U. spodochroa* is similar to unpigmented shade-adapted thalli of the old forest lichen *Lobaria pulmonaria* (Gauslaa et al., 2017), their cortical UV-B screening should be close to 100%.

The FER data did not support the hypothesis that the sporopollenin present in *Coccomyxa* screens UV (Figs. 4 and 5). Nevertheless, *Coccomyxa* seems to be more resistant against UV-B than *Trebouxia*, but the explanation is likely rather efficient repair of UV damage than efficient screening. In the macroalgae *Ulva clathrate,* UV-B resistance with no screening is based on fast repair rate of PSII, whereas UV-B resistance in *Rhizoclonium riparium* found in the same sites is based on UV-B screening (Pescheck et al., 2014).

Free-living micro-algae have a wide range of susceptibility to UV-B, and this variation is partly due to variable contents of sporopollenin (Xiong et al., 1997). Yet, the reduction in Fv/Fm after UV-B exposure in the most resistant free-living species (Xiong et al., 1997) is much greater than in the algae of intact lichens (Fig. 3).

Photosystem II is one of the targets for UV-B damage (Vass, 2012) and is easy to measure with chlorophyll fluorescence. Another important effect of UV-B is DNA damage. When DNA absorbs UV-B, it is often damaged by the production of cyclobutane pyrimidine dimers and 6-4 photoproducts; see e.g. Jansen et al. (1998). Such damage may also occur in the fungal partner of the lichen. Therefore, our results do not necessarily show that intact lichens are highly UV-B resistant because measurement of PSII efficiency will only assess damage in the algal partner. Significantly reduced growth rates of lichens exposed to natural levels of UV-B compared to lichens shielded from UV-B show that UV-B negatively affects lichens despite the fact that the algal photobiont is well protected (Chowdhury et al., 2017).

In conclusion, natural levels of UV-B do not reduce PSII efficiency in photobionts of intact *N. arcticum* and *U. spodochroa* because the lichen cortex in both species efficiently screens UV. By contrast, their photobionts *Coccomyxa* and *Trebouxia* are highly susceptible to UV-B because they lack UV-screening. Therefore, *Coccomyxa* and *Trebouxia* totally depend on their fungal partner to survive UV-B under natural conditions.

Author contributions

PV and KAS designed the study. PV and KAS performed the experimental studies. All results and data were analyzed and interpreted by PV, YG and KAS. PV, YG and KAS wrote the manuscript.

Acknowledgements

We thank Professor Wolfgang Bilger at Christian-Albrechts University Kiel for the use of his Xe-PAM instrument and for useful discussions during measurements with this instrument.

Support of the Financial Mechanisms of EEA/Norway (Norwegian funds) travel grant No. 4/003/09/1 and the Masaryk University Specific Research grant (MUNI/A/0967/2009) are acknowledged.

References

Ahmadjian, V., 1993. The Lichen Symbiosis. John Wiley & Sons, New York.

Baniya, C.B., Solhøy, T., Gauslaa, Y., Palmer, M.W., 2010. The elevation gradient of lichen species richness in Nepal. Lichenol 42, 83–96. https://doi.org/10.1017/s0024282909008627.

Bilger, W., Rolland, M., Nybakken, L., 2007. UV screening in higher plants induced by low temperature in the absence of UV-B radiation. Photochem. Photobiol. Sci. 6, 190–195.

Bilger, W., Veit, M., Schreiber, L., Schreiber, U., 1997. Measurement of leaf epidermal transmittance of UV radiation by chlorophyll fluorescence. Physiol. Plantarum 101, 754–763.

Bjerke, J.W., Lerfall, K., Elvebakk, A., 2002. Effects of ultraviolet radiation and PAR on the content of usnic and divaricatic acids in two arctic-alpine lichens. Photochem. Photobiol. Sci. 1, 678–685.

Brunner, U., Honegger, R., 1985. Chemical and ultrastructural studies on the distribution of sporopolleninlike bio-polymers in 6 genera of lichen phycobionts. Canadian Journal of Botany-Revue Canadienne De Botanique 63, 2221–2230. https://doi.org/10.1139/b85-315.

Burchard, P., Bilger, W., Weissenbäck, G., 2000. Contribution of hydroxycinnamates ans flavonoids to epidermal shielding of UV-A and UV-B radiation in developing rye primary leaves as assessed by ultraviolet-induced chlorophyll fluorescence measurements. Plant Cell Environ. 23, 1373–1380.

Cerovic, Z.G., Moise, N., Agati, G., Latouche, G., Ben Ghozlen, N., Meyer, S., 2008. New portable optical sensors for the assessment of winegrape phenolic maturity based on berry fluorescence. J. Food Compos. Anal. 21, 650–654.

Chowdhury, D.P., Solhaug, K.A., Gauslaa, Y., 2017. Ultraviolet radiation reduces lichen growth rates. Symbiosis 73, 27–34. https://doi.org/10.1007/s13199-016-0468-x.

de la Torre, R., Sancho, L.G., Horneck, G., de los Rios, A., Wierzchos, J., Olsson-Francis, K., Cockell, C.S., Rettberg, P., Berger, T., de Vera JPP, Ott, S., Frias, J.M., Melendi, P.G., Lucas, M.M., Reina, M., Pintado, A., Demets, R., 2010. Survival of lichens and bacteria exposed to outer space conditions - results of the Lithopanspermia experiments. Icarus 208, 735–748.

Fahselt, D., 1994. Secondary biochemistry of lichens. Symbiosis 16, 117–165.

Gauslaa, Y., Alam, M.A., Lucas, P.-L., Chowdhury, D.P., Solhaug, K.A., 2017. Fungal tissue per se is stronger as a UV-B screen than secondary fungal extrolites in *Lobaria pulmonaria*. Fungal Ecology 26, 109–113. https://doi.org/10.1016/j.funeco.2017.01.005.

Green, A.E.S., Sawada, T., Shettle, E.P., 1974. The middle ultraviolet reaching the ground. Photochem. Photobiol. 19, 251–259.

Hestmark, G., Lutzoni, F., Miadlikowska, J., 2016. Photobiont associations in co-occurring umbilicate lichens with contrasting modes of reproduction in coastal Norway. Lichenol 48, 545–557. https://doi.org/10.1017/s0024282916000232.

Honegger, R., Brunner, U., 1981. Sporopollenin in the cell-walls of *Coccomyxa* and *Myrmecia* phycobionts of various lichens - an ultrastructural and chemical investigation. Canadian Journal of Botany-Revue Canadienne De Botanique 59, 2713–2734. https://doi.org/10.1139/b81-322.

Huneck, S., 1999. The significance of lichens and their metabolites. Naturwissenschaften 86, 559–570.

Jansen, M.A.K., Gaba, V., Greenberg, B.M., 1998. Higher plants and UV-B radiation: balancing damage, repair and acclimation. Trends Plant Sci. 3, 131–135.

McEvoy, M., Solhaug, K.A., Gauslaa, Y., 2007. Solar radiation screening in usnic acid-containing cortices of the lichen *Nephroma arcticum*. Symbiosis 43, 143–150.

Molnar, K., Farkas, E., 2010. Current results on biological activities of lichen secondary metabolites: a review. Zeitschrift fur Naturforschung Section C-A Journal of Biosciences 65, 157–173.

Palmqvist, K., Samuelsson, G., Badger, M.R., 1994. Photobiont-related differences in carbon acquisition among green-algal lichens. Planta 195, 70–79.

Pescheck, F., Bischof, K., Bilger, W., 2010. Screening of ultraviolet-A and ultraviolet-B radiation in marine green macroalgae (Chlorophyta). J. Phycol. 46, 444–455.

Pescheck, F., Lohbeck, K.T., Roleda, M.Y., Bilger, W., 2014. UVB-induced DNA and photosystem II damage in two intertidal green macroalgae: distinct survival strategies in UV-screening and non-screening Chlorophyta. J. Photochem. Photobiol. B Biol. 132, 85–93. https://doi.org/10.1016/j.jphotobiol.2014.02.006.

Solhaug, K.A., 2018. Low-light recovery effects on assessment of photoinhibition with chlorophyll fluorescence in lichens. Lichenol 50, 139–145.

Solhaug, K.A., Gauslaa, Y., 2001. Acetone rinsing - a method for testing ecological and physiological roles of secondary compounds in living lichens. Symbiosis 30, 301–315.

Solhaug, K.A., Gauslaa, Y., 2012. Secondary lichen compounds as protection against excess solar radiation and herbivores. Prog. Bot. 73, 283–304.

Solhaug, K.A., Gauslaa, Y., Nybakken, L., Bilger, W., 2003. UV-induction of sunscreening pigments in lichens. New Phytol. 158, 91–100.

Váczi, P., Barták, M., 2006. Photosynthesis of lichen symbiotic alga *Trebouxia erici* as affected by irradiance and osmotic stress. Biol. Plantarum 50, 257–264.

Van Kooten, O., Snell, J., 1990. The use of chlorophyll fluorescence nomenclature in plant stress physiology. Photosynth. Res. 25, 147–150.

Vass, I., 2012. Molecular mechanisms of photodamage in the photosystem II complex. Biochim. Biophys. Acta Bioenerg. 1817, 209–217. https://doi.org/10.1016/j.bbabio.2011.04.014.

Xiong, F.S., Komenda, J., Kopecky, J., Nedbal, L., 1997. Strategies of ultraviolet-B protection in microscopic algae. Physiol. Plantarum 100, 378–388.