Acta Physiologiae Plantarum (2022) 44:123
https://doi.org/10.1007/s11738-022-03455-x

ORIGINAL ARTICLE

Unpigmented lichen substances protect lichens against photoinhibition of photosystem II in both the hydrated and desiccated states

Nqobile Truelove Ndhlovu[1] · **Farida Minibayeva**[2] · **Richard Peter Beckett**[1,3]

Received: 20 November 2021 / Revised: 22 July 2022 / Accepted: 3 September 2022

Abstract
Lichen secondary metabolites have been suggested to play a great diversity of roles in lichen biology by acting as UV screens, antimicrobials, herbivore deterrents or allelopathic compounds. However, evidence is also beginning to accumulate that in addition to these roles, even faintly pigmented or unpigmented lichen substances can reduce photoinhibition caused by high levels of photosynthetically active radiation (PAR). Here we used an acetone rinsing technique to remove lichen substances from four common epiphytic Afromontane macro lichens, *Parmelia perlata*, *Ramalina celastri*, *Usnea dasaea*, and *Heterodermia leucomela*. Results showed that lichen substances can increase the tolerance of lichens to the photoinhibition of PSII of their photobionts when hydrated, apparently by increasing reflectance. However, in the field, lichens may suffer more photoinhibition when fully or partially desiccated. Here we show for the first time that, except for the atranorin-containing *Heterodermia*, lichen substances can also protect desiccated lichens. Interestingly, removal of substances has no effect on reflectance when lichens are dry, suggesting that lichen substances protect photobionts in other ways.

Keywords Secondary metabolites · Light stress · Desiccation · Photoinhibition · Chlorophyll fluorescence

Introduction

Lichenization involved the development of thalli that neither symbiont could form on their own, enabling lichens to grow in habitats that were previously unavailable to their free-living symbionts. However, in sunny or well-lit areas the photobionts need to tolerate high light intensities, which can cause a temporary or longer-lasting reduction of photosynthesis called photoinhibition (Beckett et al. 2021). It has been well established that in field situations photoinhibition is a regular occurrence. For example, Gauslaa and Solhaug (2000) and Jairus et al. (2009) demonstrated that sustained reductions in photosynthesis can occur in tree-inhabiting lichens suddenly exposed to high light e.g., as a result of the felling of surrounding trees. There is a lack of consensus as to the precise mechanism of photoinhibition. However, the majority of workers consider that when photosynthetic organisms absorb more light energy than they can use in photosynthesis, the excess energy activates oxygen producing reactive oxygen species (ROS) (Gururani et al. 2015; Pospíšil 2016; Roach and Krieger-Liszkay 2019; Zavafer and Mancilla 2021). In general, poikilohydric organisms are likely to be particularly sensitive to high light stress. Although not specifically tested for lichens, in drying poikilohydric higher plants phosphorylation can continue after carbon fixation has stopped, and the energy absorbed will increase ROS production (e.g. Georgieva et al. 2007; for review see Challabathula et al. 2018). Furthermore, even though lichens may rapidly dry out when exposed to high light, they can nevertheless be photo-inhibited even when

Communicated by K. Jan Strzałka.

✉ Richard Peter Beckett
rpbeckett@gmail.com

Nqobile Truelove Ndhlovu
nqoohndhlovu@gmail.com

Farida Minibayeva
minibayeva@kibb.knc.ru

[1] School of Life Sciences, University of KwaZulu-Natal, Private Bag X01, Scottsville 3209, South Africa

[2] Kazan Institute of Biochemistry and Biophysics, Federal Research Center, Kazan Scientific Center of RAS", PO Box 30, Kazan 420111, Russia

[3] Open Lab 'Biomarker', Kazan (Volga Region) Federal University, Kremlevskaya str. 18, 420008 Kazan, Russia

Published online: 19 September 2022

desiccated (Kershaw and MacFarlane 1980; Mafole et al. 2019). Therefore, lichen photobionts require protection from photoinhibition whether hydrated or desiccated.

Lichens tolerate high light stress using three broad sets of mechanisms (Beckett et al. 2021). First, ROS formation can be reduced by the production in the upper cortex of pigments that screen the photobionts against excessive light, or by using non-photochemical quenching (NPQ) to dissipate the excess energy absorbed radiationlessly as heat. Second, once formed ROS, can be scavenged. Finally, lichens can repair ROS-induced damage. To some extent, the fungal upper cortex protects the photobiont against the effects of high light. A moist cortex can transmit approximately 90% of visible light in rainforest lichens, 45% in lichens from brighter sites (Dietz et al. 2000), but only 10% in lichens from extremely exposed sites (Büdel and Lange 1994). Transmittance is much lower in the upper cortex of desiccated than hydrated thalli (Ertl 1951). The fungal hyphae in the upper cortex themselves are partly responsible for screening the photobionts from excessive light. However, the presence of secondary compounds, which occur as hydrophobic crystals on the hyphal cell walls, can greatly reduce transmittance. Lichens are known to synthesize a great diversity of secondary compounds, and more than 1050 have been described (Goga et al. 2020; Huneck and Yoshimura 1996; Molnar and Farkas 2010). Some compounds are intensely pigmented, and directly absorb PAR. For example, melanins are brown pigments that have been shown to increase the tolerance of lichen photobionts to photoinhibition (Mafole et al. 2019). In addition, Phinney et al. (2019) reported that susceptibility to photoinhibition in the lichen *Letharia vulpina* (that contains mainly the bright yellow vulpinic acid) is much higher following extraction of lichen acids using the acetone rinsing technique of Solhaug et al. (2010). However, the great majority of more "classic" secondary compounds absorb very poorly in the visible region (Huneck and Yoshimura 1996), and intuitively seem unlikely to play any role in photoprotection. As they absorb well in the UV range, they are considered to protect lichens from the harmful effects of UV radiation (Solhaug and Gauslaa 2012). Furthermore, they have been suggested to play important roles in lichen biology by acting as antimicrobials, herbivore deterrents or allelopathic compounds (Molnár and Farkas 2010). Nevertheless, there is some evidence that even colorless secondary metabolites can provide photoprotection from high levels of PAR. For example, Legaz et al. (1986) found higher concentrations of usnic acid and atranorin in the thalli of *Evernia prunastri* during the brighter summer months than in winter. Furthermore, extraction of the colorless secondary metabolite atranorin from *Physcia aipolia* significantly increased photoinhibition caused by high light (Solhaug et al. 2010). This increased sensitivity to light appeared to be because the reflectance of the thallus was greatly reduced, and the visible appearance of the lichen changed from pale grey to green when hydrated. Differences in the reflectance of dry thalli with and without lichen substances were much smaller. Irrespective of the mechanism, preliminary data suggest that even unpigmented lichen substances can provide significant photoprotection for lichen photobionts.

Apart from the study with *Physcia* described above, there is little information available on whether unpigmented lichen substances can protect lichens from high PAR. Therefore, the first aim of the work presented here was to use the acetone rinsing method to test the ability of unpigmented lichen substances to reduce photoinhibition in four common South African Afromontane macro lichens. Three of these lichens contain the faintly yellow lichen substance usnic acid, while the fourth contains the unpigmented atranorin. Secondly, we also tested the effectiveness of lichen substances at preventing photoinhibition in desiccated lichens, which has not previously been tested in any species. Photoinhibition was assessed by measuring the maximal (or optimal) quantum yield (F_V/F_M) using chlorophyll fluorescence (Ottander and Oquist 1991; Huner et al. 1993). Results presented here show that secondary metabolites can protect lichen photobionts from high PAR, even if they are only faintly pigmented or unpigmented, and furthermore photoprotection occurs whether thalli are wet or dry.

Materials and methods

Lichen material

The lichens used were first *Parmelia perlata* (Huds.) Ach., *Ramalina celastri* (Sprengel) Krog and Swinscow, and *Usnea dasaea* Stirt., all reported to contain mainly usnic acid (Payal and Sharma, 2016; Fazio et al. 2007; Bannister et al. 2020) and *Heterodermia leucomela* (L.) Poelt, reported to contain almost exclusively atranorin (Culberson, 1966). The identity of the main lichen substances present in the lichens was confirmed by high performance liquid chromatography as described by Pawlik-Skowrońska and Bačkor (2011), and comparison with standards of usnic acid and atranorin from Sigma-Aldrich. Lichens were collected dry from an Afromontane forest at Fort Nottingham, KwaZulu Natal, South Africa and most were growing on a small tree, *Leucosidea sericea* Eckl. and Zeyh. Lichens were collected growing close to each under similar environmental conditions. The photobionts of these lichens have been reported to belong to the Chlorophycean genus *Trebouxia* (Rambold et al. 1998). Lichens were stored dry for up to 1 month at − 24 °C until the start of the experiments.

Acetone rinsing

Lichen substances were removed using the "acetone rinsing" technique of Solhaug et al. (2003). In all cases, lichens were initially left overnight over silica gel to ensure they were completely dry. They were then gently shaken in 100% acetone for 10 min. Acetone was then discarded, and the process repeated three times. After acetone rinsing, the thalli were left at room temperature overnight to allow residual acetone to evaporate. Subsamples were rehydrated overnight, and photobiont vitality checked by measuring F_V/F_M as described below. Rinsing four times had no effect on F_V/F_M in *Parmelia* or *Heterodermia*, but slightly reduced values in *Usnea* and *Ramalina*. The extraction was repeated for the latter two species, and results showed that reducing rinsing from four to two times prevented damage.

Reflectance spectra measurements

Out of the four species tested, only the thalli of *Parmelia* were sufficiently large to accurately measure reflectance spectra with the equipment available. Reflectance spectra (300–800 nm) were measured at random positions of the thalli with a spectrometer (model SD2000, Ocean Optics) connected to an output port sphere with a 400 µm thick fiber. Measurements were done by directly placing the upper cortex of each lichen thalli (factorial combination of desiccated, hydrated, with and without secondary metabolites). The thalli were then illuminated by a halogen lamp (model DH2000, Ocean Optics) through a 600 µm optical fiber connected to the input port of the integrating sphere (ISP-50-REFL, OceanOptics).

Effective quantum yield of PSII (F_V/F_M)

A Hansatech FMS2 (Hansatech instruments, King's Lynn, England) was used to measure F_V/F_M, defined as:

$$F_V/F_M = (F_M - F_O)/F_M,$$

where, F_M is the maximum fluorescence (reaction centres closed) and F_O is the minimum fluorescence (reaction centres opened). All thalli were dark adapted for 10 min before measurement, and measurements carried out using standard Hansatech leaf clips.

Photoinhibition

For each species 40 samples were used, 10 for each treatment combination of hydrated and desiccated with and without secondary metabolites. All thalli were initially in a desiccated state. To expose hydrated thalli to high light, thalli were acetone rinsed if required, the acetone allowed to evaporate overnight, all (rinsed and unrinsed) thalli placed on wet filter paper at 30 µmol m^{-2} s^{-1} overnight and an initial F_V/F_M measurement taken. Thalli were then exposed to high light. To expose desiccated thalli to high light, thalli were acetone rinsed if required, the acetone allowed to evaporate for 24 h, all thalli (rinsed and unrinsed) hydrated overnight as above, an initial F_V/F_M measurement taken, and then allowed to air dry overnight. They were then exposed to the photoinhibitory light, and immediately rehydrated by placing them on wet filter paper. Lichens were photoinhibited using a LED panel (Model SL-3500, Photon System Instruments, Brno, Czech Republic) that provides cool white light. The exposure to light needed to reduce F_V/F_M down to c. 0.2–0.3 for untreated lichens was determined in preliminary experiments. Species differed in their sensitivity, and much longer exposures were needed for dry compared with wet material; Table 1 indicates the exposure times and intensities used. Lichens with and without lichen substances received the same exposure times and intensities. Initial measurements of F_V/F_M were taken at the start of the experiment as indicated above, immediately after the exposure to high light and again at intervals for up to 24 h. During recovery, lichens were exposed to normal laboratory light (c. 5 mol m^{-2} s^{-1}) as recommended by Solhaug (2018). All species were highly desiccation tolerant, and when not photoinhibited were found to recover from desiccation within minutes of rehydration (data not shown). However, some of the recovery that occurred in photoinhibited dry lichens during the first 30 min of rehydration may represent recovery from desiccation stress.

Table 1 Times of exposure and light intensities used to photoinhibit lichens to F_V/F_M values of c. 0.2–0.3

	Times of exposure to a photoinhibitory light	
	Wet state	Dry state
Parmelia sulcata	900 µmol m^{-2} s^{-1} for 4 h	900 µmol m^{-2} s^{-1} for 10.5 h
Ramalina celastri	600 µmol m^{-2} s^{-1} for 4.5 h	1200 µmol m^{-2} s^{-1} for 24 h
Usnea dasaea	400 µmol m^{-2} s^{-1} for 5 h	900 µmol m^{-2} s^{-1} for 27 h
Heterodermia leucomela	600 µmol m^{-2} s^{-1} for 5 h	1350 µmol m^{-2} s^{-1} for 24 h

Statistics

All data from the photoinhibition experiments were subjected to a two-way analysis of variance (ANOVA) in Microsoft Excel.

Results

Reflectance spectra of *Parmelia perlata*

Extracting lichen substances from *Parmelia* greatly reduced reflectance compared to control thalli when the lichen was in the rehydrated state (Fig. 1). In desiccated thalli reflectance was generally higher, but removing secondary lichen substances had little effect on reflectance.

Photoinhibition of wet and dry thalli with or without lichen substances

As discussed in Materials and Methods, despite all being collected from the same place, and occupying superficially similar habitats (small twigs at the periphery of the canopy), species differed in their sensitivity to photoinhibition (Table 1). Furthermore, much longer exposures were needed to reduce F_V/F_M down to c. 0.2–0.3 for dry compared with wet material (Table 1). After exposure to the photoinhibitory light, thalli with lichen substances removed usually displayed lower values of F_V/F_M compared with thalli with lichen substances still present, and often displayed reduced rates of recovery back to initial values (Fig. 2). For *Ramalina* and *Parmelia*, removal of substances had similar effects on

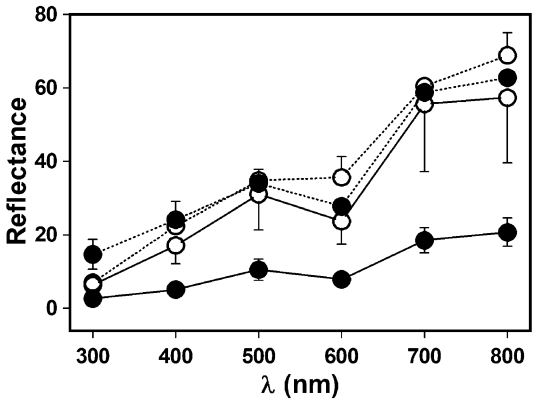

Fig. 1 The effect of acetone rinsing on reflectance in hydrated and desiccated thalli of *Parmelia perlata*. Dashed lines indicate desiccated thalli, while solid lines indicate hydrated thalli. Open symbols are thalli containing lichen substances, while closed symbols indicate thalli that had been acetone rinsed to remove lichen substances. Error bars indicate the standard error, $n=6$; overlapping error bars have been removed

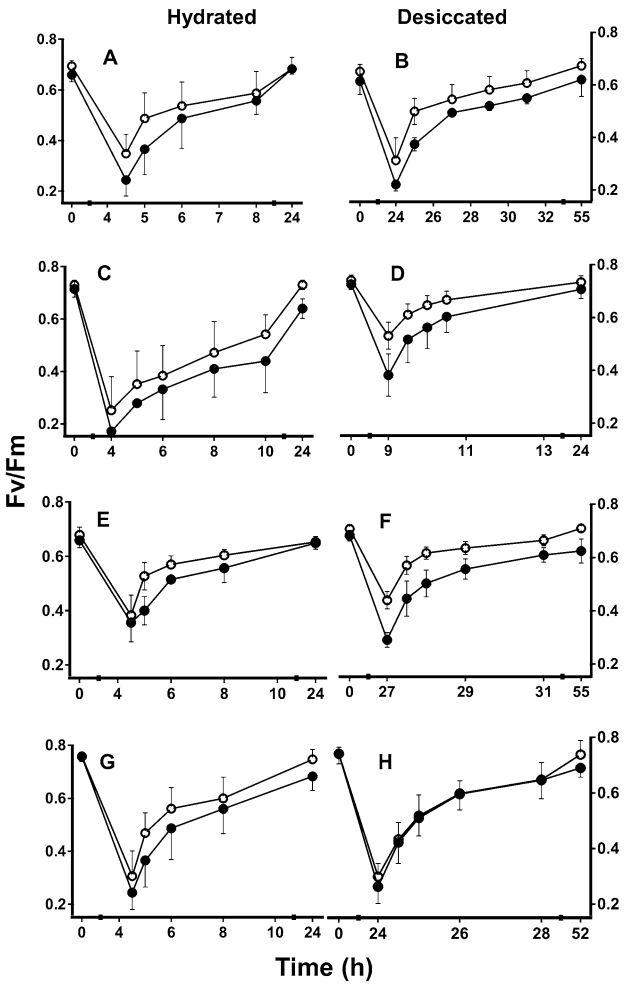

Fig. 2 The recovery from a photoinhibition in untreated (open symbols) and thalli with lichen substances removed using acetone rinsing (closed symbols) in hydrated (**A**, **C**, **E**, **G**) and desiccated (**B**, **D**, **F**, **H**) thalli of *Ramalina celastri* (**A**, **B**), *Parmelia perlata* (**C**, **D**), *Usnea dasaea* (**E**, **F**) and *Heterodermia leucomela* (**G**, **H**). Error bars indicate the standard error, $n=10$; overlapping error bars have been removed

the susceptibility of hydrated and desiccated thalli to photoinhibition; photoinhibition reduced F_V/F_M to lower levels than control thalli, and F_V/F_M took longer to recover (Fig. 2A–D). In *Usnea* removal of lichen substances had more effect in dry material. In hydrated material removal of lichen substances slightly reduced F_V/F_M after photoinhibition, and reduced the rate of recovery (Fig. 2E). In desiccated material, removal had more effect on F_V/F_M after photoinhibition, and slowed down recovery, particularly during the early stages. By contrast, in *Heterodermia* the removal of lichen substances slightly increased the susceptibility of hydrated thalli to photoinhibition but had almost no effect on the susceptibility of desiccated thalli (Fig. 2G, H). Two-way ANOVA indicated that removing lichen substance significantly ($P<0.05$) affected the recovery of both hydrated and

desiccated lichens from photoinhibition, the only exception being desiccated material of *Heterodermia*, where there was no significant effect of lichen substance removal ($P > 0.05$).

Discussion

The results presented here clearly show that even in lichens possessing only faintly pigmented or unpigmented lichen substances, photoinhibition is higher when the lichen substances are removed (Fig. 2). Unfortunately, our equipment for measuring reflectance required a disk of c. 1 cm in diameter, and we were therefore only able to test *Parmelia*. In this species, lichen substance removal greatly reduced the reflectance of wet thalli (Fig. 1). Assuming lichen substance removal affected the reflectance of the other species in the same way, it seems likely that in hydrated thalli reduced reflectance is responsible for the greater photoinhibition in thalli with lichen substances removed. However, except for the atranorin-containing *Heterodermia*, lichen substances can also protect desiccated lichens, even though in *Parmelia*, the one species we were able to test, removal of substances has no effect on reflectance when lichens are dry (Fig. 1). This suggests that there are other reasons why lichen substances can photoprotect photobionts in dry lichens.

Effect of the removal of lichen substances on thallus reflectance in *Parmelia*

Removal of usnic acid greatly decreases the reflectance of visible light in hydrated thalli of *Parmelia*, particularly at longer wavelengths (Fig. 1). Desiccated thalli have much higher reflectances than wet thalli, and by contrast, lichen substance removal has little effect on reflectance. Results obtained here are very similar to those reported following atranorin removal in *Physcia* by Solhaug et al. (2010). The precise reasons for the decrease in reflectance that occurs following lichen substance removal remain unclear. Probably, at least part of the reason for the effect is that crystals of lichen substances directly reflect light. However, lichen substance removal does not affect the reflectance of desiccated lichens. Therefore, Solhaug et al. (2010) suggested that as they are hydrophobic, the lichen substances stop water from entering the intercellular hyphal spaces in the cortex, and in hydrated thalli it is actually the air-filled cavities that reflect light.

Effect of removal of lichen substances on susceptibility of lichens to high light induced reductions in F_V/F_M

To varying degrees, usnic acid removal significantly increases the sensitivity of F_V/F_M to inhibition following a photoinhibitory light stress in photobionts of *Parmelia*, *Ramalina* and *Usnea* in both the hydrated and desiccated states (Fig. 2A–F). By contrast, in *Heterodermia*, atranorin appears to increase tolerance to reductions in F_V/F_M only when the lichen was hydrated, and has no effect when the lichen is desiccated (Fig. 2G, H). The main reason that lichen substances increase tolerance to photoinhibition in the hydrated state is probably because they considerably increase reflectance (Fig. 1). In an analogous way, the leaves of many higher plants from bright environments protect themselves from photoinhibition by a high reflectance as a result of a coat of hairs or wax or even salt crystals (Robinson et al. 1993). However, it is more difficult to explain why lichen substances can improve tolerance to photoinhibition in dry lichens, while apparently not increasing thallus reflectance (Fig. 1). Various explanations are possible. First, while lichen substances may not increase reflectance, they may reduce transmission, although this does not appear likely given that they are only faintly pigmented or unpigmented. Neither atranorin or usnic acid absorb in the PAR region of the electromagnetic spectrum (Fazio et al. 2009; Medina and Avalos-Chacon 2015). Possibly, the lichen substances can scatter light to some extent, although this does not appear to result in an increase in reflectance. Second, as discussed in the Introduction, lichen substances can have very high antioxidant activity (Kosanić et al. 2011; Fernández-Moriano et al. 2016). Although not proven for lichen photobionts, it seems likely that as for bryophytes (Heber et al. 2006), even when desiccated, light shining on chloroplasts may generate harmful radicals. However, it is currently unknown whether lichen substances can scavenge ROS produced by photobiont chloroplasts. Regardless of the mechanism, it seems clear that usnic acid can protect lichen photobionts from photoinhibition even when thalli are desiccated.

It is not clear why unlike usnic acid, atranorin does not protect desiccated *Heterodermia* (Fig. 2G, H). In all species studied the photobiont belongs to the same genus, *Trebouxia*, so photobiont differences seem unlikely. Possibly atranorin is less effective in decreasing transmittance, or is less effective as an antioxidant. However, both atranorin and usnic acid have been reported to possess antioxidant properties (Sepahvand et al. 2021; White et al. 2014).

Conclusion

As discussed in the Introduction, lichen substances play many roles in lichen biology. It now seems clear that in addition to their better elucidated roles in UV screening and acting as antimicrobials or grazing deterrents, they can also protect the photobionts from high PAR. While Solhaug et al. (2010) reported that unpigmented substances can photoprotect hydrated thalli of *Physcia*, it appears that such protection

may be a rather general phenomenon. Furthermore, we show here for the first time that lichen substances can even protect photobionts from high light when a lichen is desiccated. Many lichens spend a great proportion of their lives desiccated, even in temperate climates (Leisner et al. 1997). Although it requires longer to photoinhibit photobionts when lichens are desiccated, arguably photoprotection is equally or even more important when they are desiccated as when they are hydrated. However, more work is needed to understand how lichen substances such as usnic acid can improve photoprotection in desiccated lichens, and why some lichen substances are more effective than others.

Author contribution statement Ms. NN carried out all the experiments. All authors contributed equally to the planning of the experiments, and the writing of the manuscript.

Acknowledgements Richard Beckett thanks the University of KwaZulu Natal Research Fund, and also acknowledges that the work presented here was supported by the Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030). Farida Minibayeva thanks the Russian Science Foundation (Grant № 18-14-00198, reflection experiments) and the Russian government assignment of FRC Kazan Scientific Center of RAS for financial support. The authors thank Knut Solhaug for assistance with using high performance liquid chromatography to identify lichen substances.

Declarations

Conflict of interest None.

References

Bannister J, Harrold P, Blanchon D (2020) Additional lichen records from New Zealand 51 .*Usnea Dasaea* Stirt. Australas Lichenol 86:114–117

Beckett RP, Minibayeva FV, Solhaug KA, Roach T (2021) Photoprotection in lichens: adaptations of photobionts to high light. Lichenologist 53:21–33. https://doi.org/10.1017/S0024282920000535

Büdel B, Lange OL (1994) The role of cortical and epinecral layers in the lichen genus *Peltula*. Cryptogam Bot 4:262–269

Challabathula D, Zhang QW, Bartels D (2018) Protection of photosynthesis in desiccation-tolerant resurrection plants. J Plant Physiol 227:84–92. https://doi.org/10.1016/j.jplph.2018.05.002

Culberson WL (1966) Chemistry and taxonomy of the lichen genera *Heterodermia* and *Anaptychia* in the Carolinas. Bryologist 69:472–487

Dietz S, Büdel B, Lange OL, Bilger W (2000) Transmittance of light through the cortex of lichens from contrasting habitats. Bibliotheca Lichenologica 75:171–182

Ertl L (1951) Über die Lichtverhältnisse in Laubflechten. Planta 39:245–270. https://doi.org/10.1007/BF01909397

Fazio AT, Adler MT, Bertoni MD, Sepúlveda CS (2007) Lichen secondary metabolites from the cultured lichen mycobionts of *Teloschistes chrysophthalmus* and *Ramalina celastri* and their antiviral activities. Z Naturforschung C 62:543–549. https://doi.org/10.1515/znc-2007-7-813

Fazio AT, Bertoni MD, Adler MT, Ruiz LB, Rosso ML, Muggia L, Hager A, Stocker-Wörgötter E, Maier MS (2009) Culture studies on the mycobiont isolated from *Parmotrema reticulatum* (Taylor) Choisy: metabolite production under different conditions. Mycol Prog 8:359–365. https://doi.org/10.1007/s11557-009-0609-1

Fernández-Moriano C, Gomez-Serranillos MP, Crespo A (2016) Antioxidant potential of lichen species and their secondary metabolites. A systematic review. Pharm Biol 54:1–17. https://doi.org/10.3109/13880209.2014.1003354

Gauslaa Y, Solhaug KA (2000) High-light-intensity damage to the foliose lichen *Lobaria pulmonaria* within a natural forest: the applicability of chlorophyll fluorescence methods. Lichenologist 32:271–289. https://doi.org/10.1006/lich.1999.0265

Georgieva K, Szigeti Z, Sarvari E, Gaspar L, Maslenkova L, Peeva V, Peli E, Tuba Z (2007) Photosynthetic activity of homoiochlorophyllous desiccation tolerant plant Haberlea rhodopensis during dehydration and rehydration. Planta 225:955–964. https://doi.org/10.1007/s00425-006-0396-8

Goga M, Elečko J, Marcinčinová M, Ručová D, Bačkorová M, Bačkor M (2020) Lichen metabolites: an overview of some secondary metabolites and their biological potential. In: Merillon JM, Ramawat K (eds) Co-evolution of secondary metabolites. Springer, Cham

Gururani MA, Venkatesh J, Tran LSP (2015) Regulation of photosynthesis during abiotic stress-induced photoinhibition. Mol Plant 8:1304–1320. https://doi.org/10.1016/j.molp.2015.05.005

Heber U, Bilger W, Shuvalov VA (2006) Thermal energy dissipation in reaction centres and in the antenna of photosystem II protects desiccated poikilohydric mosses against photooxidation. J Exp Bot 57:2993–3006. https://doi.org/10.1093/jxb/erl058

Huneck S, Yoshimura I (1996) Identification of lichen substances. Springer, Berlin. https://doi.org/10.1007/978-3-642-85243-5_2

Huner NPA, Öquist G, Hurry VM, Krol M, Falk S, Griffith M (1993) Photosynthesis, photoinhibition and low temperature acclimation in cold tolerant plants. Photosyn Res 37:19–39. https://doi.org/10.1007/BF02185436

Jairus K, Lõhmus A, Lõhmus P (2009) Lichen acclimatization on retention trees: a conservation physiology lesson. J App Ecol 46:930–936. https://doi.org/10.1093/10.1111/j.1365-2664.2009.01672.x

Kershaw KA, MacFarlane JD (1980) Physiological-environmental interactions in lichens X. Light as an ecological factor. New Phyt 84:687–702. https://doi.org/10.1111/j.1469-8137.1980.tb04781.x

Kosanić M, Ranković B, Vukojević J (2011) Antioxidant properties of some lichen species. J Food Sci Tech 48:584–590. https://doi.org/10.1007/s13197-010-0174-2

Legaz ME, Avalos A, Detorres M, Escribano MI, Gonzalez A, Martinfalquina A, Pérezurria E, Vicente C (1986) Annual variations in arginine metabolism and phenolic content of *Evernia prunastri*. Env Exp Bot 26:385–396. https://doi.org/10.1016/0098-8472(86)90027-4

Leisner JMR, Green TGA, Lange OL (1997) Photobiont activity of a temperate crustose lichen: long-term chlorophyll fluorescence and CO_2 exchange measurements in the field. Symbiosis 23:165–182

Mafole TC, Solhaug KA, Minibayeva FV, Beckett RP (2019) Tolerance to photoinhibition within lichen species is higher in melanised thalli. Photosynthetica 57:96–102. https://doi.org/10.32615/ps.2019.008

Medina MG, Avalos-Chacon R (2015) Physiological performance of a foliose microlichen *Umbilicaria antarctica* as affected by supplemental UV-B treatment. Czech Pol Rep 5:222–229. https://doi.org/10.5817/CPR2015-2-19

Molnar K, Farkas E (2010) Current results on biological activities of lichen secondary metabolites: a review. Z Natur C 65:157–173

Ottander C, Öqvist G (1991) Recovery of photosynthesis in winter-stressed scots pine. Plant Cell Environ 14:345–349. https://doi.org/10.1111/j.1365-3040.1991.tb01511.x

Pawlik-Skowrońska B, Bačkor M (2011) Zn/Pb-tolerant lichens with higher content of secondary metabolites produce less phytochelatins than specimens living in unpolluted habitats. Environ Exp Bot 72:64–70. https://doi.org/10.1016/j.envexpbot.2010.07.002

Payal P, Sharma MC (2016) GC–MS analysis and biological activities of medicinally important lichen: *Parmelia perlata*. Int J Pharm Phytochem Res 8:1975–1985

Phinney NH, Gauslaa Y, Solhaug KA (2019) Why chartreuse? The pigment vulpinic acid screens blue light in the lichen *Letharia vulpina*. Planta 249:709–718. https://doi.org/10.1007/s00425-018-3034-3

Pospíšil P (2016) Production of reactive oxygen species by photosystem II as a response to light and temperature stress. Front Plant Sci 7:1950. https://doi.org/10.3389/fpls.2016.01950

Rambold G, Friedl T, Beck A (1998) Photobionts in lichens: possible indicators of phylogenetic relationships? Bryologist 101:392–397

Roach T, Krieger-Liszkay A (2019) Photosynthetic regulatory mechanisms for efficiency and prevention of photo-oxidative stress. Ann Plant Rev 2:1–34. https://doi.org/10.1002/9781119312994.apr0666

Robinson SA, Lovelock CE, Osmond CB (1993) Wax as a mechanism for protection against photoinhibition—a study of *Cotyledon orbiculata*. Bot Acta 106:307–312. https://doi.org/10.1111/j.1438-8677.1993.tb00753.x

Sepahvand A, Studzinska-Sroka E, Ramak P, Karimian V (2021) *Usnea* sp.: antimicrobial potential, bioactive compounds, ethnopharmacological uses and other pharmacological properties; a review article. J Ethnopharm 268:113656. https://doi.org/10.1016/j.jep.2020.113656

Solhaug KA (2018) Low-light recovery effects on assessment of photoinhibition with chlorophyll fluorescence in lichens. Lichenologist 50:139–145. https://doi.org/10.1017/S0024282917000640

Solhaug KA, Gauslaa Y (2012) Secondary lichen compounds as protection against excess solar radiation and herbivores. Prog Bot 73:283–304. https://doi.org/10.1007/978-3-642-22746-2_11

Solhaug KA, Gauslaa Y, Nybakken L, Bilger W (2003) UV-induction of sun-screening pigments in lichens. New Phytol 158:91–100. https://doi.org/10.1046/j.1469-8137.2003.00708.x

Solhaug KA, Larsson P, Gauslaa Y (2010) Light screening in lichen cortices can be quantified by chlorophyll fluorescence techniques for both reflecting and absorbing pigments. Planta 231:1003–1011. https://doi.org/10.1007/s00425-010-1103-3

White PAS, Oliveira RCM, Oliveira AP, Serafini MR, Araújo AAS, Gelain DP, Moreira JCF, Almeida JRGS, Quintans JSS, Quintans-Junior LJ, Santos MRV (2014) Antioxidant activity and mechanisms of action of natural compounds isolated from lichens: a systematic review. Molecules 19:14496–14527. https://doi.org/10.3390/molecules190914496

Zavafer A, Mancilla C (2021) Concepts of photochemical damage of photosystem II and the role of excessive excitation. J Photochem Photobiol C Photochem Rev 47:100421. https://doi.org/10.1016/j.jphotochemrev.2021.100421