Fungal Ecology 29 (2017) 103–110

Contents lists available at ScienceDirect

Fungal Ecology

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

Melanisation in the old forest lichen *Lobaria pulmonaria* reduces the efficiency of photosynthesis

Tshepiso Coral Mafole [a], Camilo Chiang [b], Knut Asbjørn Solhaug [b], Richard Peter Beckett [a, *]

[a] School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa
[b] Faculty of Environmental Sciences and Natural Resource Management, Norwegian University of Life Sciences, P.O. Box 5003, NO-1432 Ås, Norway

ARTICLE INFO

Article history:
Received 9 February 2017
Received in revised form
14 July 2017
Accepted 19 July 2017
Available online 15 August 2017

Corresponding Editor: Peter D. Crittenden

Keywords:
Lobaria pulmonaria
Melanin
Quantum yield
Non-photochemical quenching
UV-Radiation
Reflectance

ABSTRACT

The old forest lichen *Lobaria pulmonaria* synthesizes melanic pigments when exposed to ultraviolet light and high solar radiation. Here, we tested the effect of melanisation on photosynthetic efficiency. Melanisation effectively reduces high-light stress in lichen photobionts, as the photobionts of melanised thalli are healthy, based on chlorophyll contents and maximum rates of photosynthesis. However, the quantum yields of both photosynthetic CO_2 uptake and O_2 evolution were more than 40 percent lower in melanised thalli compared with control thalli. While chlorophyll fluorescence measurements suggested that melanised and pale thalli had similar apparent electron transport rates, this result was probably an artefact caused by screening reducing the light reaching the photobionts. Melanic thalli also had a higher chlorophyll *a*/*b* ratio and more xanthophyll cycle pigments, suggesting that the photosynthetic apparatus had adapted to high light. In conclusion, while protecting photobionts from high light, melanisation clearly reduced photosynthetic efficiency. Melanised thalli will be significantly disadvantaged if light levels return to lower values, more typical for those habitats in which this shade adapted lichen is most abundant.

1. Introduction

Lichens are a symbiosis between an ascomycete and one or more photosynthesizing algae or cyanobacteria, often with small amounts of basidiomycete yeasts (Spribille et al., 2016) and bacteria (Grube and Wedin, 2016). Lichens are the dominant life forms in almost 10% of the land surface of the earth (Ahmadjian, 1966), mainly in polar regions, where they form isolated turfs when there is sufficient water availability (Longton, 1988). They also inhabit the tops of mountains and occur as components of soil crust communities. These places are characterized by severe abiotic stresses such as desiccation, temperature extremes and high light intensities. Arguably, what makes lichens special, and what separates them from other eukaryotic organisms, is their ability to tolerate extreme stresses (Beckett et al., 2008). However, being highly stress tolerant has consequences, and lichens typically have very slow growth rates compared with higher plants (Honegger, 2008). Slow growth is not only a result of low resource availability (including water, which limits the time lichens can be active), but also metabolic 'costs' associated with stress tolerance mechanisms. For example, Colesie et al. (2014) compared carbon allocation in soil crust lichens in extreme Antarctic sites with more moderate sites in Germany. The Antarctic lichens allocated a high proportion of fixed carbon to low-molecular weight sugars and sugar alcohols, probably involved in desiccation and freezing tolerance. By contrast, the lichens from the much more moderate German sites had high allocation into the polysaccharide pool, indicating that they were using carbon for growth. The implication is that Antarctic lichens sacrifice growth for increased stress tolerance. However, very few studies have attempted to assess the nature and extent of costs associated with stress tolerance in lichens.

As lichens frequently grow in exposed microhabitats, they often experience high light levels, including high levels of UV. Furthermore, the amount of solar radiation, particularly at high latitudes, displays substantial annual variation. Even for lichens from lower latitudes, development of a leaf canopy causes seasonal variations in light levels. In photosynthetic tissues, excessive solar radiation can cause a variety of forms of damage, in particular as a

* Corresponding author.
E-mail address: rpbeckett@gmail.com (R.P. Beckett).

http://dx.doi.org/10.1016/j.funeco.2017.07.004
1754-5048/

consequence of the formation of reactive oxygen species (ROS) (McKenzie and Leshem, 1994). In non-photosynthetic tissues, high levels of solar radiation may cause heat stress, while UV can damage DNA (Buffoni Hall, 2002). Recently, Chowdhury et al. (2016) suggested that in *Lobaria pulmonaria* the mycobiont is likely to be the more UV-B susceptible partner, as UV-B affects overall growth more than purely photobiont responses such as chlorophyll content and the quantum yield of PSII photochemistry.

Lichens employ a diverse range of mechanisms to protect themselves from high light stress. Lichen photobionts have a well-developed xanthophyll cycle (Demmig-Adams et al., 1990). This cycle dissipates energy within the light-harvesting antenna proteins by non-photochemical quenching (NPQ), reducing the energy reaching the photosynthetic reaction centres. Photobionts undoubtedly use other mechanisms. Light stress may be worse during desiccation when carbon fixation will be inhibited, and Shevela et al. (2013) recently suggested that in dry lichens the thylakoid membranes become rearranged so that the PS II and PS I units come into contact. Excess energy can be 'spilled over' to PS I where it can, to some extent, be quenched. Both the mycobiont and the photobiont may be protected by the increased synthesis of cortical pigments. The most widespread cortical pigments are the classic lichen substances such as usnic acid, atranorin and parietin (Solhaug and Gauslaa, 2012). More unusually, in some lichens high light or UV induces the synthesis of darkly coloured melanic pigments in the upper cortex. Melanin-producing lichens come from a range of lichen orders, and grow on a variety of substrata (Gauslaa and Solhaug, 2001). Lichens with classic lichen substances or melanic compounds often coexist in open habitats, and some lichens with atranorin or usnic acid, such as *Cetraria* and *Cladonia*, additionally produce melanic compounds under very sun-exposed conditions (Solhaug and Gauslaa, 2012). While lichens appear able to effectively protect themselves against high light, very little information is available on the resulting metabolic cost. There is some evidence that synthesis of classic lichen substances may reduce the efficiency of photosynthesis. In *Xanthoria parietina*, the quantum yield of photosynthetic O_2 evolution in blue light was lower in control thalli than in parietin-free thalli, although under red light the quantum yield did not differ between control and parietin-free thalli (Solhaug and Gauslaa, 1996). However, the cost to lichens of synthesising cortical melanins is unknown.

The aim of the work presented here was to study the cost of photo-protection by melanic pigments in the lichen *L. pulmonaria*. The species has a wide distribution in Europe, Asia, North America and Africa, preferring damp habitats with high rainfall, especially in coastal areas (McCune and Geiser, 1997). *L. pulmonaria* normally grows in relatively shaded habitats. The average light level throughout the entire year for the habit where *L. pulmonaria* grows can be as low as 14 μmol m^{-2} s^{-1}, although it is ten times higher on the exposed side of tree trunks (Gauslaa and Solhaug, 2000). However, at times lichens may be exposed to higher light levels, for example following leaf fall in autumn. Lichens will receive more light when openings in forests occur, for example because of tree fall, or where local conditions such as avalanches, poor soils, or fire damage create semi-permanent clearings. While L. *pulmonaria* upregulates NPQ and xanthophyll cycle pigments in response to increased exposure to PAR and UV (MacKenzie et al., 2002), the most important adaptation is probably the synthesis of cortical melanin pigments (Solhaug et al., 2003). Here, we used a variety of techniques to compare photosynthetic performance in melanised and non-melanised thalli. We were particularly interested in studying the effect of melanisation on the efficiency of photosynthesis at lower light levels i.e. the 'quantum yield'. Results showed that while melanisation protects lichens from high PAR and UV, the cost of this adaptation is a significant reduction in carbon fixation should light levels fall to low to intermediate values.

2. Materials and methods

2.1. Lichen material

L. pulmonaria was collected from the trunks of oak trees *c.* 5 m above the ground in an old oak forest at Langangen, Norway (59° 06′ 43″ N, 9° 50′ 05″ E, 140 m above sea level) in June 2015 and April 2016. After storage at −18 °C for *c.* 4 months, half of the thalli were placed in an open site in Ås for 4 weeks to induce melanic pigments. They were sprayed with distilled water on days without rain. The other half of the thalli remained in the freezer as controls. Non-melanic (control) and melanic thalli were randomized within the two groups before start of experiments.

2.2. Gas-exchange measurements

Before all photosynthetic measurements, thalli were moistened with distilled water and acclimated at 15 °C under low light (*c.* 10 μmol m^{-2} s^{-1}) until the next day. Photosynthetic O_2 evolution was measured using an O_2 electrode chamber (model LD2, Hansatech, King's Lynn, Norfolk UK) with a red LED light source (model LH36/2R, Hansatech) at 20 °C and 5% CO_2.

Photosynthetic CO_2 uptake was measured with a portable infrared gas-analyzer (LI-6400XT, LiCor, Lincoln, NE, USA) with a LiCor 6400-24 bryophyte chamber and a LI-6400-18 RGB LED light source. In all gas-exchange measurements white light, derived from a mixture of red, green and blue LEDs was used. Before measurement, thalli were acclimated for 10 min at room temperature at 200 μmol m^{-2} s^{-1} from a LED light-source panel (Model SL-3500, Photon Systems Instruments, Brno, Czech Republic) with equal irradiances from blue, green and red light. They were then fully hydrated by spraying with distilled water and blotted with filter paper to remove superficial water. CO_2 uptake was measured at 200 μmol m^{-2} s^{-1} until stable and optimal thallus water contents were reached (assessed by typical maximal rates of photosynthesis). Unless indicated otherwise, measurements were made at a flow rate of 200 ml min^{-1} of 400 ppm CO_2 at a light level of 200 photons m^{-2} s^{-1}.

2.3. The effect of temperature and thallus water content on photosynthetic CO_2 uptake

Thalli were equilibrated for 10 min after each change in temperature before measurements were taken. Rates of photosynthesis at 15, 20, 25 and 30 °C were 2.11 ± 0.75, 1.68 ± 0.54, 1.63 ± 0.50 and 1.46 ± 0.45 μmol CO_2 m^{-2} s^{-1} (n = 5). While the optimum temperature for carbon fixation was thus 15 °C, temperature inside the IRGA cuvettes was much easier to control at 20 °C and rates were only slightly lower than those at 15 °C. Furthermore, occasional condensation occurred in the leaf chamber at 15 °C. Therefore, all subsequent carbon fixation measurements were carried out at 20 °C.

To measure the effect of thallus water content on photosynthesis, thoroughly wetted thalli (*c.* 4 cm^2) were blotted to remove excess water, weighed to obtain the maximum water content, and then put into the IRGA cuvette. After stabilization, three measurements were made at 10 s intervals, and then thalli removed from the cuvette and again weighed. This was repeated until the thalli displayed no net photosynthesis, at which point they were dried overnight at 70 °C and weighed. The relative water contents (RWC) of the thalli at each sampling interval was calculated as described by Beckett (1995).