

Chapter 20

Lichen Photosynthesis. Scaling from the Cellular to the Organism Level

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Summary

Photosynthesis in lichens is intimately linked to the photosynthetic capacities of the photobiont, *i.e.* autotrophic algae and cyanobacteria, that form the lichen association together with a fungal partner. Lichen photosynthesis in nature is also affected by a complex mixture of internal and external factors.

Intrathalline localization of photobiont cells, structure of photobiont layer, functional photobiont-mycobiont interlink, and physico-chemical properties of the fungal part of thallus are considered important internal characteristics affecting photosynthesis and utilization of photosynthetic products in lichens. In this chapter, a brief introduction into the anatomy and morphology is provided from a view point of function. Special attention is given to cellular

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structure of photobionts, and especially to the chloroplast of unicellular alga *Trebouxia*, the most abundant symbiotic alga in lichen association. Since lichens are typical poikilohydric organism with no active control of their hydration status, the photosynthetic responses of lichens to full, partial and severely limited water supply are described. In addition the protective mechanisms activated during thallus desiccation are discussed. Several aspects of lichen photosynthesis including light-response curves, photoinhibition, activation of photoprotective mechanisms and reactive oxygen species-induced changes in the amount and activity of antioxidative substances are reviewed. Lichens can photosynthesize over a wide temperature range, including subzero temperature. The photobiont also exhibits response depending on nitrogen availability and exposure to heavy metals.

I. Introduction

Photosynthesis is a key metabolic function of lichens. Therefore a understanding of mechanisms and limitations of photosynthesis in this symbiotic organisms is desirable. Since many lichens are considered extremophilic organisms, that exhibit great tolerance against a variety of stress factors, lichen photosynthesis in response to several of these stress factors has been investigated in detail. Defining and characterizing the range of conditions that lichen can tolerate continuous to be an active area of research. The photosynthetic machinery of lichens responds to these challenges with a number of biophysical

and biochemical protective mechanisms. Although some of these protective mechanisms, may have well-characterized homologous counterparts in plants, many mechanisms in lichens are not yet fully understood. This lack of knowledge has given generations of lichenologist, plant physiologist and ecophysiological the motivation to study lichen photosynthesis under natural and controlled conditions. In recent years, molecular, proteomic and metabolomic approaches are increasingly employed to study lichen photosynthesis. In addition miniaturized and automated measuring devices are now available that enable detailed long-term studies of photosynthesis in the field. This chapter gives an overview of our current knowledge of lichen photosynthesis, with an emphasis on mechanisms that have been revealed under stress conditions.

Abbreviations: APX – Ascorbate peroxidase; CA – Carbonic anhydrase; CAT – Catalase; CCM – Carbon concentrating mechanism; DEPS – Deepoxidation state of xanthophyll-cycle pigments; DW – Dry weight; Fo – Background chlorophyll fluorescence; F_m – Maximal fluorescence yield; F_v – Variable chlorophyll fluorescence; F_v/F_m – Potential quantum yield of photochemical processes in photosystem II; FW – Fresh weight; GSH – Reduced glutathione; GSSG – Oxidised glutathione; NPQ – Non-photochemical quenching; PAR – Photosynthetically active radiation; Pn – Net photosynthesis; Pn_{max} – Maximum rate of net photosynthesis; PSII – Photosystem II; φ_{PSII} – Effective quantum yield of photochemical processes in photosystem II; qE – Energy dependent quenching; qI – Photoinhibitory quenching; qT – State 1-state 2 transition quenching; ROS – Reactive oxygen species; RuBisCo – Ribulose bis phosphate carboxylase oxygenase; RWC – Relative water contents; SOD – Superoxid dismutase; UV-A – Ultraviolet radion (B); UV-B – Ultraviolet radion (B); WP – Water potential

II. Lichen Anatomy and Morphology

A. Symbionts and Thallus Forms

Liches are symbiotic organisms consisting of at least two partners: A photobiont and mycobiont that form the structures of a lichenized association, *i.e.* the lichen thallus. The mycobiont, *i.e.* fungal partner (in most cases Ascomycetes) forms the majority of the lichen thallus biomass. The mycobiont is with only a few exceptions, responsible for thallus morphology and growth form. The photobiont, *i.e.* photosynthesizing partner, is an alga or cyanobacterium. The relation



Fig. 20.1. *Placopsis contortuplicata* is a lichen that has two photosynthesizing partners located in a thallus. In the central orange part (cephalodium), there is a nitrogen-fixing cyanobacterium (*Nostoc* sp.). Marginal white thal- lus parts have an algal photobiont (Photo M. Barták).

between these two partners is considered a mutualistic symbiosis.

While bipartite lichens consist of one mycobiont and one photobiont species, tripartite lichens have two photobionts in a single mycobiont. Tripartite lichens have typically an alga (see Chap. 17) and cyanobacteria (see Chap. 14) in association with the mycobiont. Areas where these two photobionts are located have anatomical differences in the majority of cases – see e.g. *Placopsis contortuplicata* (Fig. 20.1).

Regarding thallus morphology, lichens are divided into three main and several additional morphological groups. In this chapter, only the main ones are treated: crustose lichens, foliose lichens, and fruticose lichens (for more details see e.g. Büdel and Scheidegger 1996).

Crustose lichens are tightly attached to the substrate (upper surface of stones and rocks) with their lower surface (see Fig. 20.2). A typical feature that distinguishes crustose lichens from the foliose and fruticose lichens is that crustose lichens cannot be removed from the surface without a lot of force and destruction of the thallus structure.

Foliose lichens possess generally a flat leaf-like thallus, which is attached to the surface only partially by special structures: rhizines, cilia, tomentum or umbilicus (see Fig. 20.3). Foliose lichens exhibit considerable difference in coloration and surface structure between the upper and lower thal- lus surface. The thallus of foliose lichens is often divided into lobes that show various degree of branching. The size of the lobes can vary from several millimeters to more than 10 cm. Among foliose lichens, two sub- groups can be distinguished: laciniate and umbilicate. While laciniate thalli typically exhibit numerous lobes arranged radially or forming overlapping structures, umbilicate lichens create circular thalli consisting of one unbranched lobe.

Lichens with fruticose thallus morphology typically form three-dimensional branching structures. These structures may be arranged upward (shrub-like structures) or form hanging branched structures oriented downward from a basal holdfast attached to the substrate (typically tree branches or stems). Fruticose lichens form a cluster of free-standing, branching tubes, which are usually round in cross section (see Fig. 20.4). Some fruticose lichens, however, may have flattened branches such as e.g. *Ramalina* sp.

B. Intrathalline Location of Photobionts and Their Photosynthetic Properties

More than 40 algal and cyanobacterial genera have been described that participate as photobionts in lichen association (Ahmadjian 1993). The most frequent algal photobionts include species of the *Trebouxia*, *Trentepohlia*, *Coccomyxa*, and *Dictyochloropsis* clades. In cyanobacterial lichens, *Nostoc*, *Scytonema*, *Stigonema*, *Gloeocapsa*, and *Calothrix* are the most common genera. Recently, thanks to advanced molecular biology-based taxonomic approaches, a majority of species previously assigned to the genus *Trebouxia* have been reclassified as *Asterochloris* (Peksa and Škaloud 2010). In this chapter, however, the term *Trebouxia* is used even for those newly reclassified algal

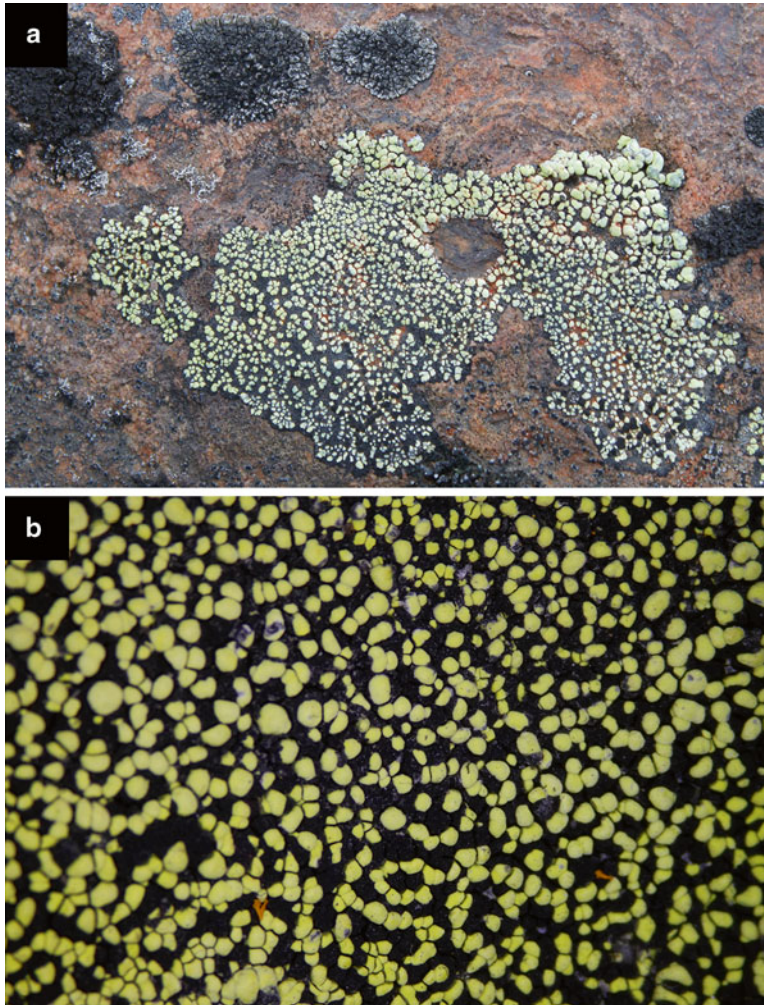


Fig. 20.2. *Rhizocarpon geographicus* is a typical lichen with crustose morphology showing black and yellow thalli parts (lower panel). Crustose lichens are tightly attached to stone or rock surfaces (upper panel) (Photos M. Barták).

species. For majority of lichens, symbiotic algae and/or cyanobacteria are located beneath the upper cortex layer that is formed by densely packed fungal hyphae (see Figs. 20.4 and 20.5).

Since more than 50 % of all known lichens possess the unicellular alga *Trebouxia* in their thalli, anatomical and physiological properties are briefly overviewed here. More than 40 species of *Trebouxia* isolated from lichen thalli are currently described. *Trebouxia* species are defined using shape of chloroplast (see Fig. 20.6), as well as pyrenoid (see Chap. 7) and pyrenoglobuli configuration as critical features (Peksa and

Škaloud 2011). The pyrenoid is a large central part of the chloroplast, rich in proteins and lipids. It contains the photosynthetic enzyme RuBisCo and is the place of starch synthesis. Within a pyrenoid, there are several pyrenoglobuli that are lipid-rich storage inclusions (Ahmadjian 2001). The pyrenoids contained in *Trebouxia* cells also possess a carbon-concentrating mechanism that can enhance photosynthetic performance (Smith and Griffiths 1998).

Carbon concentrating mechanism (CCM) is a term that refers to the ability of pyrenoid-containing algal chloroplasts, as well as carboxysome-containing cyanobacteria to



Fig. 20.3. Foliose thalli of *Umbilicaria antarctica*, a macrolichen, are abundant in maritime Antarctica. In the dry state, the *upper side* of the thallus exhibits a pale *brown color* (left), while the *lower side* of the thallus is *black* (right). There is only one point that connects the thallus to a surface (typically rock); this umbilicus is located in the *center* of the thallus (right) (Photo M. Barták).

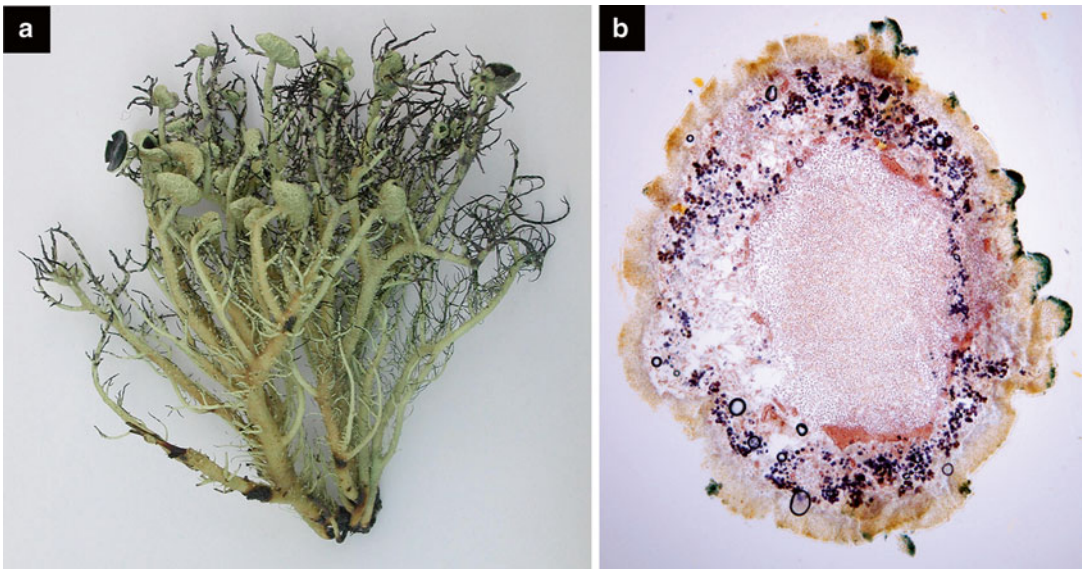


Fig. 20.4. Cluster of fruticose thalli (left) and cross section of the middle part of a single thallus of *Usnea aurantiaco-atra* (right) showing typical anatomical structures. From the thallus periphery to the thallus center, the outer cortex (brown), medulla with symbiotic algae (individual dark points) forming round-shaped layer, and a cell-free internal space (light red) can be distinguished (Photos M. Barták (left), J. Rotkovská (right)).

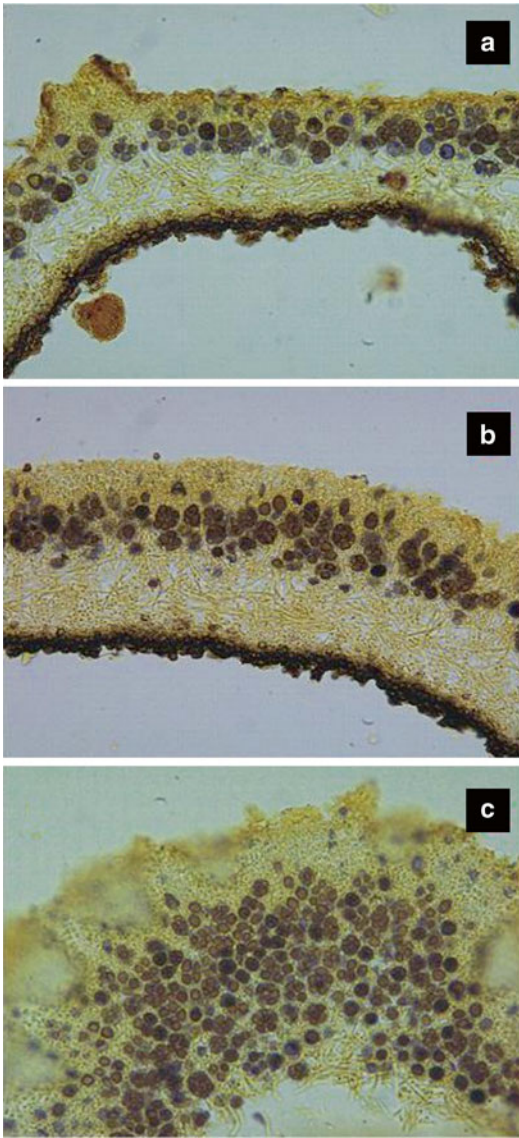


Fig. 20.5. Cross sections of the foliose lichen *Lasallia pustulata* that show the marginal (a), intermediate (b), and central thallus part, with hemispherical outgrowth called pustulus (c). The upper cortex contains an algal layer with different thickness in panels a, b & c. The medulla, and lower cortex with rhizines can be seen from top to the bottom at individual panels (a, b & c) (Photos J. Dubová).

locally increase the amount of inorganic carbon that can so be used to efficiently perform carbon fixation. Lichens use CCM during favourable conditions, when the thallus is

hydrated and light is sufficiently available. Studies made on isolated photobionts (e.g. Palmqvist and Badger 1996) indicate a key role of carbonic anhydrase (CA) in such lichens. It has been shown for *Trebouxia* sp. and *Coccomyxa* sp. that CA is located both intra- and extracellularly. Species-specific differences in total CA content have been determined by Palmqvist and coworkers (1997).

Polyols (*i.e.* sugar alcohols) are photosynthetic products of symbiotic algae. In lichens, polyols (mainly ribitol, arabitol, mannitol) have several physiological roles (see below). Natural levels of polyols in lichens vary within 1.4–8.8 mg/g dry weight (ribitol) and 0.4–29.0 mg/g (mannitol) between species and physiological state – for review see Hájek et al. (2009). The polyols can make up between 2 and 10 % of the thallus dry weight (Palmqvist 2000). The stored polyols are utilized for supplying the mycobiont with carbon, as energy-storage compound within algae, and as a cryoprotectant. In agreement with these functions is that the highest content of polyols is typically found in late summer (Sundberg et al. 1999).

In the majority of lichens, ribitol is produced in algal cells and then transported to fungal part of a lichen, where it is transformed to mannitol and can then be utilized for metabolic processes. When *Trebouxia* is in lichen association it produces ribitol, but when cultured axenically at laboratory conditions, sucrose is the main photosynthate. This observation may indicate that sucrose is the preferred carbon storage component in autotrophic conditions, while ribitol is produced to exchange carbon with the fungal partner. Interestingly *Trebouxia*, when isolated from a lichen thallus and cultivated on agar, can grow both autotrophically and heterotrophically (Archibald 1977) suggesting that some carbon may also be imported from the fungal partner by the alga. In contrast to lichens in association with alga, cyanolichens utilize glucose as the main transport compound to the mycobiont.

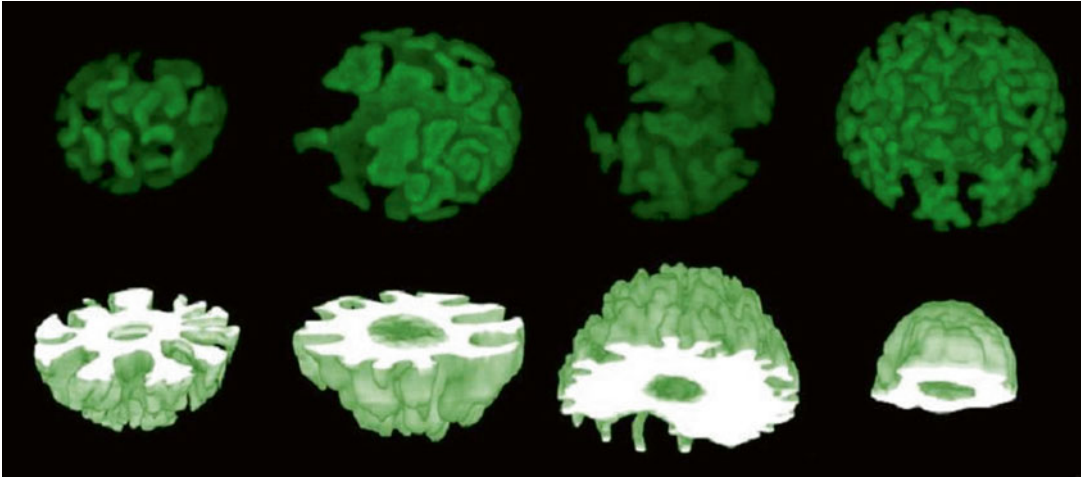


Fig. 20.6. Chloroplast shapes of *Trebouxia/Asterichloris* species. Upper row from left to right: *A. glomerata*, *A. glomerata*, *A. irregularis*, *Aastrochloris* sp. Lower row from left to right: cross sections of *Asterochloris* sp. (Provided by P. Škaloud, Charles University, Prague).

III. Dependence of Photosynthesis on Physical Factors

A. Hydration-Dependent Photosynthesis

Due to their poikilohydric nature, lichens can respond to the changes in the thallus water content rapidly. While dehydrated lichens are virtually metabolically inactive, increases of the thallus water content leads to gradual re-activation of physiological processes (including photosynthesis and respiration) – Palmqvist and Sundberg 2000. The majority of lichens exhibit dry thalli that are more contracted and possess a different spatial organization compared to their wet state. Extensive packing of thalli layers decreases the volume occupied by the lichen in the dry state. On a cell level, symbiotic algae and cyanobacteria as well as the fungal hyphae are a lot reduced in volume. The cells of the algal photobionts are far from the round shape observed in wet lichens, but are typically collapsed into star-shape structures and densely packed in-between mycobiont cells (de los Rios et al. 1999).

Desiccation also has consequences for the ultrastructure in severely desiccated cells of

photobionts. For example the pyrenoglobuli may be re-distributed in a pyrenoid and pyrenoglobuli-free pyrenoid matrix areas may be observed in pyrenoid cross section (Ascaso et al. 1988). Moreover, pyrenoglobuli density may be altered according to severity and rapidity of drying. Due to cytorhisis, *i.e.* loss of intracellular water resulting in cell collapse (protoplast, however remains attached to cell wall), the cell wall of photobionts is much less resistant to desiccation-induced changes than the thick cell wall of mycobionts (Scheidegger et al. 1995).

During rehydration, both photo- and mycobiont cells undergo volumetric changes accompanied with gradual re-activation of physiological processes including photosynthesis (see e.g. Sancho and Kappen 1989). When rewetted from the dry state, lichen thalli show an almost linear increase in photosynthesis and respiration with time of hydration (Green et al. 2011). Duration of rehydration and the rate of restoration of photosynthetic processes are, however, species-specific and also dependent on the ecophysiology of the lichen in the preceding period (*i.e.* number of hydration/dehydration cycles, prevailing temperature, the length of

period spent dehydrated etc.). At optimum hydration, *i.e.* when the full cellular turgor of photosynthesizing symbiont is reached, the lichens exhibit maximum photosynthetic rate. The optimum water content in a lichen thallus varies amongst species. When the content is above the optimum, surface or extracellular water represents a physical barrier for CO₂ transfer from the air to a photobiont. Over hydration therefore leads to the limitation of CO₂ supply and inhibits the rate of photosynthesis. This phenomenon is called hypersupersaturation effect on photosynthesis (Lange and Green 1996) and was observed in several lichen species such as *e.g.* *Ramalina maciformis* Lange (1980), *Xanthoria calcicola* and *Lecanora muralis* Lange (2002), and *Teloschistes capensis* (Lange et al. 2006).

It is well established that, lichens can perform close-to-maximum or even maximum photosynthesis at hydration levels below full thallus hydration. Several studies that applied gasometric measurements of lichen photosynthesis reported such high photosynthesis rates in partially dehydrated thalli (see below). However, such photosynthetic studies expressed water status of thalli as relative water content (RWC, %, Eq. 20.1).

$$\text{RWC} = \frac{(\text{FWa} - \text{DW}) * 100}{(\text{FWw} - \text{DW})} \quad (20.1)$$

where FWa is fresh weight of actually hydrated lichen thallus, FWw is fresh weight of fully hydrated lichen thallus, and DW is dry weight of fully dehydrated thallus. In majority of lichens, maximum photosynthetic rates are reached within the RWC range of 60–90 %. However, species-specific differences in ability to hold water in their thalli (per unit of dry weight) exist. Therefore gasometric measurements of photosynthesis are difficult to compare between lichen species, especially when these measurements were obtained in the field.

In contrast to gasometric measurements, the hydration-response curves of photosynthesis measured by chlorophyll fluorescence can be related directly to water potential

(WP). In this approach, WP of lichen thallus is measured directly in a chamber of a chilled-mirror dewpoint meter. Immediately after the WP is evaluated, the lichen sample is exposed to light and effective quantum yield of photosystem II (ϕ_{PSII}) is measured using a saturation pulse method. In this way, simultaneous measurements of water status and photosynthetic activity can be performed on gradually desiccating lichens (see *e.g.* Jupa et al. 2012). Using the approach, photosynthesis under water stress can be compared between poikilohydrous lichens (Fig. 20.7) and homoihydrous higher plants.

While higher plants can hardly perform positive photosynthesis below -2.5 MPa, the majority of lichens do not show any significant decrease within the range of WP -10.0 to 0 MPa (M. Barták, unpublished data). With further dehydration of the lichen thalli, photosynthesis, assessed by effective quantum yield of PSII (ϕ_{PSII}), declines in a curvilinear manner towards critical WP at which $\phi_{\text{PSII}} = 0$. For the majority of lichens, critical WP is about -30.0 MPa (*e.g.* *Xanthoria elegans* – Barták et al. 2005), however, some species of the genus *Umbilicaria* have critical WP of as low as -40.0 MPa (Fig. 20.7). The gradual inhibition of photosynthetic processes with ongoing thallus dehydration leads to the activation of protective mechanisms that involves mainly interconversion of xanthophyll-cycle pigments (Calatayud et al. 1997). Such changes are manifested in an dehydration-dependent increase of non-photochemical quenching (NPQ). The rate of NPQ increase is higher when the WP decreases from 0 to -15 MPa than at more pronounced thalli dehydration within the WP range of -30 to -15 MPa (*Lasallia pustulata*, Moudrá 2009).

Dehydration-induced inhibition of photosynthetic processes may be altered by the rapidity of desiccation and light conditions during desiccation. Generally, fast dehydration leads to more pronounced stress to PSII. Therefore, after fast dehydration, a slower and incomplete recovery is observed. This behavior is also observed in the isolated photobiont *Trebouxia erici* (Gasulla et al. 2009).

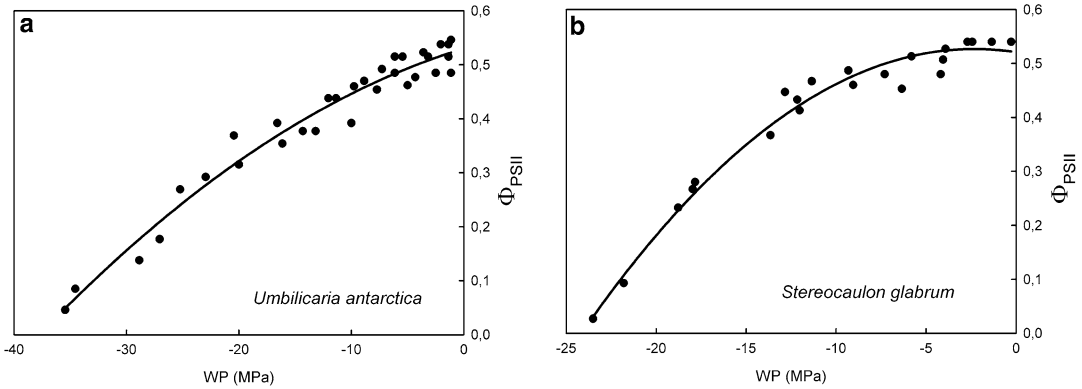


Fig. 20.7. Effective quantum yield of PSII (Φ_{PSII}) in response to dehydration. With gradual dehydration of the lichen thallus, no substantial decrease of Φ_{PSII} is between a water potential (WP) of 0 (full hydration) to -12 MPa. Further thalli dehydration (WP below -12 MPa) leads to a rapid decrease of Φ_{PSII} . The critical WP, at which full inhibition of Φ_{PSII} occurs is typically about -25 MPa (see *Stereocaulon glabrum* – right). However, several species may exhibit higher tolerance of Φ_{PSII} against thallus dehydration (see *Umbilicaria antarctica* – left).

Structural and functional properties of the lichen thallus may affect dehydration-induced inhibition of photosynthesis as well. Kosugi et al. (2009) showed that an association of the photobionts with the mycobionts in lichen thalli increases tolerance to photo-inhibition under drying conditions, compared to isolated photobiont.

A detailed analysis of non-photochemical quenching components (qE , qT , qI) reveals that they differ between lichen thalli passing fast and slow dehydration. Apart from well-known protective mechanisms activated during dehydration of lichen thalli, *i.e.* light-dependent xanthophyll cycle pigments conversion, and state 1–2 transition, there is another effective quencher in the photosynthetic apparatus of PSII of desiccating lichens. It is not related to zeaxanthin formation and independent on light. According to Heber et al. (2006), it may involve conformational changes in pigment-protein complexes in PSII. In this way, highly-effective dissipation centers are formed in which excitation energy is trapped (Heber et al. 2010). Recently, the presence of a strong quencher located in the PSII core and/or PSII antennas has been reported from *Parmelia sulcata* desiccating on light (Veerman et al. 2007), however its chemical structure is not known.

B. Light-Dependent Photosynthesis

1. Light Response Curves

Optimally-hydrated, lichens respond to light in a similar manner to higher plants and light response curves of photosynthesis can be determined in both systems and differences evaluated. At low light intensities of photosynthetically active radiation (PAR), lichens exhibit linear increase in net photosynthetic rate (P_n) with increasing light followed by a curvilinear P_n -PAR relationship until constant photosynthesis ($P_{n_{\text{max}}}$) in saturating light is reached.

The great majority of lichens is adapted to low light and thus reach typical light-saturated photosynthesis in the PAR range of $100\text{--}400 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Demmig-Adams et al. 1990a). However, species from sun-exposed habitats exist that exhibit light-saturated photosynthesis at $1,100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Green et al. 1997). In general, lichens have much lower maximum (light-saturated) photosynthetic rates expressed per area or dry weight unit of thallus than higher plant leaves. This is because, photobionts (alga, cyanobacterium or both) constitute only a small proportion to the lichen thalli, while the bulk is made up of the

fungal partner. Typically, maximum rate of net photosynthesis (Pn_{max}) does not exceed $6 \mu\text{mol} (\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$ as shown e.g. for cyanobacterial lichen *Collema tenax* (Lange et al. 1998), algal species *Xanthoria elegans* (Barták et al. 2005), and *Lecanora muralis* (Lange and Green 2008). For *Lecanora muralis* a Pn_{max} as high as 7.5 was reported for optimally hydrated thalli when measured under controlled laboratory conditions. Many lichens, however, have much lower Pn_{max} ranging $1.0\text{--}2.0 \mu\text{mol} (\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$ as shown for *Lobaria pulmonaria* and *Platismatia glauca* (Sundberg et al. 1997).

2. Photoinhibition

Photoinhibition of photosynthesis frequently occurs in hydrated lichens exposed to high light intensities. Such high-exposure to lichen can occur in wet open habitats such as rocks and light-exposed clear cuts or forest edges. It is well established that the sensitivity to photoinhibition is higher in the lichens possessing cyanobacterial photobionts than those having algal partners (Demmig-Adams et al. 1990b). When photoinhibited, cyanobacterial lichens do not show fast return of photosynthetic parameters to pre-photoinhibition parameters during dark recovery. This in contrast to lichens with algal photobiont, which exhibit recovery to initial values typically within tens of minutes.

When the photosynthetic apparatus of the lichen photobionts is exposed to high light doses, reactive oxygen species (ROS) are formed that are destructive to the photosynthetic machinery (see Chap. 11), especially photosystem II. ROS may cause damage or destruction of pigment-protein complexes and inhibition of photochemical processes. To avoid or reduce the negative consequences of ROS formation, lichen photobionts activate several photoprotective mechanisms that are similar to higher plants.

a. Zeaxanthin Formation

Several laboratory-based studies with high light-treated lichens demonstrated

zeaxanthin formation in algal lichens (e.g. Vráblíková et al. 2005). Zeaxanthin is involved in effective thermal energy dissipation from overenergized photosystem II of algal photobionts (Demmig-Adams et al. 1990a), i.e. high light-induced conversion of violaxanthin to zeaxanthin (DEPS value). The recovery to pre-photoinhibition violaxanthin to zeaxanthin ratios is relatively fast ranging from 3 to 10 h (Barták et al. 2008). While the zeaxanthin formation is well established in algal photobionts, cyanobacterial photobionts do not possess such mechanism.

b. Involvement of Glutathione

Glutathione is low-weight thiol that has the function of an antioxidant and plays a role in the photoprotection of lichens. Phytochelatin, another antioxidant is synthesized from glutathione. The amount of intrathalial glutathione ranges typically in lichens between 1.2 and $3.3 \mu\text{mol/g}$ dry weight. Glutathione levels respond not only to actual light conditions and functioning of photosynthetic apparatus of algal and fungal symbionts during light-induced oxidative stress, but also possess seasonal variation as well as spatial variation within the thallus (Kranner and Grill 1996).

The amount of glutathione increases during lichen dehydration (Kranner and Birtić 2005; Tretiach et al. 2012), while both increases and decreases can be induced by heavy metals and toxic compounds (Mrak et al. 2010).

If exposed to strong, brief high light treatment, reduced glutathione (GSH) is converted to oxidized glutathione (GSSG) and the amount of total glutathione (GSht) decreases in lichens as algal cells are increasingly photoinhibited. Severe photoinhibition leads to degradation of GSH (down to 12–30 % of GSht before light treatment Štěpíková et al. 2007) to glutamyl-cystein. Glutamyl-cystein in turn can be used to synthesize reduced glutathione during recovery from high light treatment. An increase of glutathione content by long-term high light treatment is reported for higher plants (Burritt and MacKenzie 2003), but has never been demonstrated for lichens.

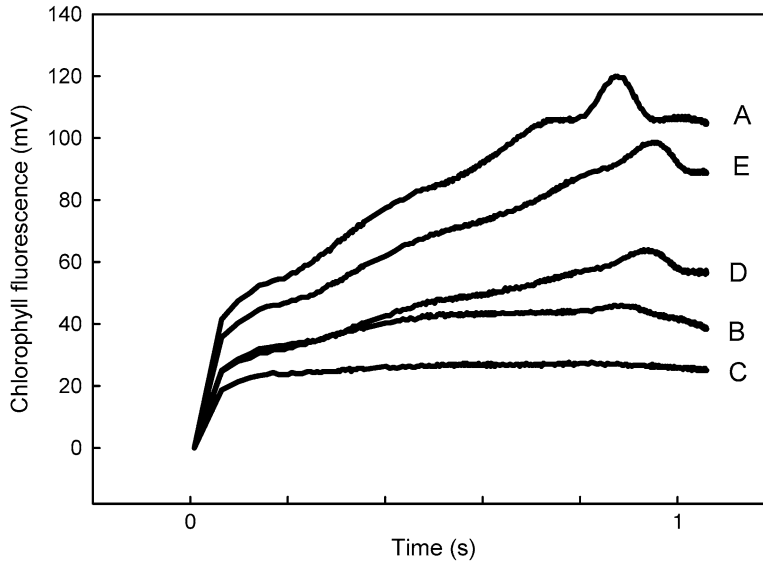


Fig. 20.8. Photoinhibition and recovery in *Usnea antarctica* assessed by fast chlorophyll fluorescence induction curves (OJIP). The fluorescence kinetics are caused by a *short light* pulse applied to a *dark-adapted* lichen thallus. Photoinhibition is characterized by a decrease in chlorophyll fluorescence yield. Partial inhibition (*B*) and full inhibition (*C*) can be compared to the uninhibited control (*A*). After photoinhibition treatment, a 30 or 60 min dark recovery period leads to partial (*D*) and almost complete (*E*) restoration of PSII fluorescence characteristics. This fast recovery indicates high resistance of PSII in *U. antarctica* to short-term high light treatment.

The extent of photoinhibition and recovery can be sensitively monitored in a lichen thallus by several chlorophyll fluorescence techniques and the derived chlorophyll fluorescence parameters. In chloroplasts, photoinhibition can lead to a gradual inactivation and photodestruction of chlorophyll molecules. This leads to a decrease in chlorophyll fluorescence (variable fluorescence – F_v) and efficiency of absorbed energy transfer through PSII and PSI. Figures 20.8 and 20.9 show photoinhibition-induced changes in the shape of fast and slow chlorophyll fluorescence induction kinetics in wet lichen thalli exposed to high doses of photosynthetically active radiation.

c. Antioxidative Enzymes

In symbiotic lichen algae, antioxidative enzymes are involved in cell protection when ROS are formed (del Hoyo et al. 2011). Several studies reported altered levels of

catalase (CAT), superoxide dismutase (SOD) and ascorbate peroxidase (APX) during dehydration stress (e.g. Mayaba and Beckett 2001; Gasulla et al. 2009), heavy metal-induced stress (Weissman et al. 2006), and exposure to SO_2 (Kong et al. 1999). It appears very likely that CAT, SOD and APX would be also involved in protecting lichens against ROS formed during high-light stress in hydrated lichens, but experimental evidence is lacking.

3. Effects of UV-B Radiation

Lichens are generally resistant against high doses of UV radiation, especially when dehydrated and thus physiologically inactive. Some algal lichens, however, show limited resistance (Unal and Uyanikgil 2011). Experiments conducted so far led to the conclusion that UV enhancement typically lead to an increase of UV-absorbing pigments and compounds such as phenolics

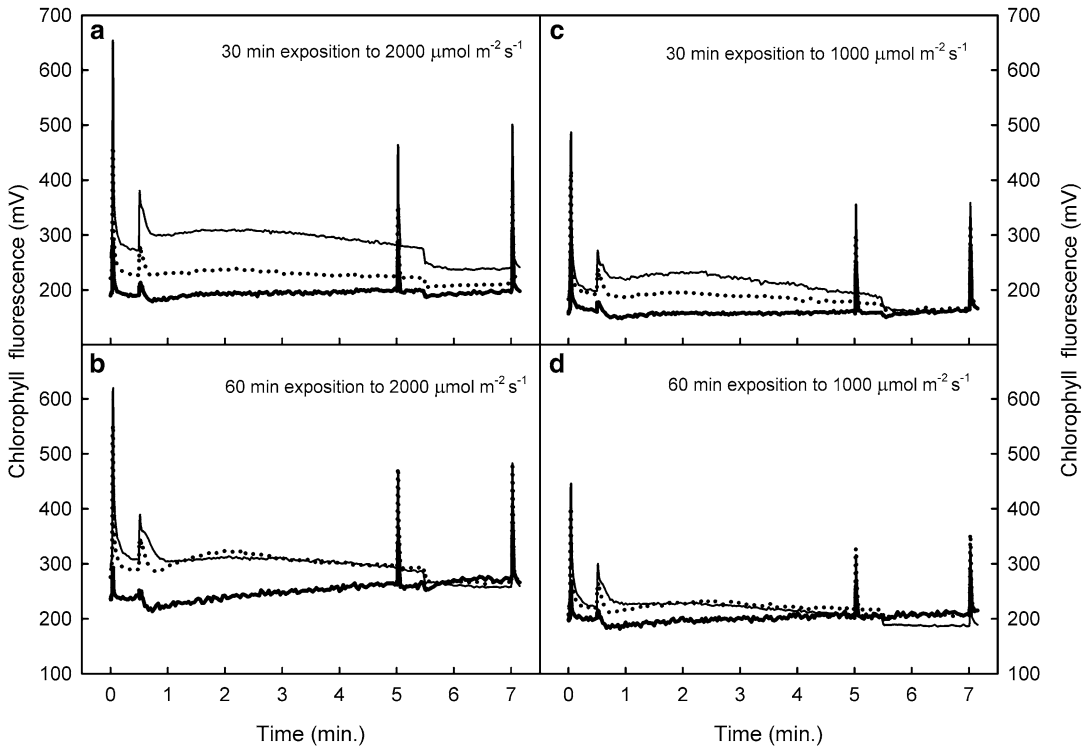


Fig. 20.9. Slow chlorophyll fluorescence kinetics and maximum fluorescence induced by saturation light pulses applied to lichens in *dark-adapted* and *light-adapted* states. Untreated *Usnea antarctica* (solid line) is exposed to a 30/60 min high light treatment (solid bold line) and consequent dark recovery (dashed line). The recovery in the *dark* is faster when thalli are exposed for longer periods (60 min) indicating the involvement of photoprotective and regulatory process in chloroplasts (Reprinted from Barták et al. 2012 with the permission of the Masaryk University Press).

(Buffoni Hall et al. 2002), usnic acid (Bjerke et al. 2002), rhizocarpic acid (Rubio et al. 2002), parietin (Solhaug and Gauslaa 2004). However, these protective substances cannot fully prevent DNA damage and cell death (Unal and Uyanikgil 2011). UV screening compounds are typically located in the upper cortex of lichen thallus and thus reduce the amount of harmful radiation reaching the algal layer.

Photosynthesis in most lichens appears not to be negatively effected by UV radiation. For example, Lud et al. (2001) reported no change of fluorometric parameters in *Turgidosculum complicatulum* exposed to extra UV-B radiation in Antarctica. Lichens from shade habitats, however, may exhibit some limited sensitivity of supplemental UV and resulting species-specific effects on photosynthetic rate and biomass production (Larsson et al. 2009). For a majority of lichens, exposure to

increased quantities of UV-B radiation leads to increased synthesis of photoprotective, UV-absorbing compounds, such as phenolics (Buffoni Hall et al. 2002) parietin, melanic compounds (Solhaug and Gauslaa 2004; Solhaug et al. 2003) and usnic acid (McEvoy et al. 2006). In lichen thalli – in the upper cortex layer, in particular – these compounds protect photosynthetic pigments located in a photobiont cells in underlying layer.

C. Temperature-Dependence of Photosynthesis

Similarly to higher plants, the rate of photosynthesis and production in lichens is also temperature-dependent including aquatic lichens (Davis et al. 2002). Therefore, temperature response curves can be measured and interspecific differences in temperature

tolerance can be evaluated. Due to poikilohydric character of the lichen thallus, however, an increase in thallus temperature is accompanied with gradual loss of water. In natural conditions, temperature-induced change in photosynthetic rate can therefore not be distinguished from dehydration-dependent changes. Several studies evaluated photosynthetic temperature-response curves in lichens that were maintained optimally hydrated in laboratory studies. For many lichens, the temperature optimum for photosynthesis varies between 10 and 22 °C. Individual temperature optima depend on lichen adaptation/acclimation to particular growing habitats (tropical, mountainous, polar etc.), as well as other physical environmental factors including light intensity. If a lichen is exposed to a lower than optimum light level, a decrease in optimum temperature for photosynthesis is observed (Reiter et al. 2008). Many lichens are capable of maintaining high photosynthetic rates (of about 80 % of maximum photosynthesis) within a temperature range of 5–20 °C (Hájek et al. 2001). The critical maximum temperature for lichen photosynthesis is about 40 °C for both tropical and high-mountain lichen species (Lange et al. 2004; Hájek et al. 2001). However this parameter (maximum temperature) has mostly theoretical value, as such high temperature will usually also cause rapid dessication of the lichen in most environments. At 40 °C, wet and fully photosynthetically active thalli can hardly be found in the field.

The only exception are tropical lichens. At temperature above optimum, absolute rate of lichen respiration increases as well as proportion of respiration to photosynthesis. This is attributed mainly to the respiration of mycobiont which forms substantial part of overall thallus CO₂ release. Short-term high temperature stress in hydrated lichens leads to the loss of effectivity of photosynthetic processes in photosystem II and, if the capacity of protective mechanisms is low, to the injury of PSII structure in photosynthesizing symbiont (e.g. destruction of the Mn-cluster – Oukarroum et al. 2012), as

well as partial disintegration of thylakoid membrane structure.

Contrastingly to higher plants, majority of lichens can perform photosynthesis at temperature far below zero even under snow cover (Kappen 1993, Pannowitz et al. 2006). This capability is facilitated by the presence of molecules in lichen thallus, such as e.g. polyols that prevent freezing. The critical temperature for lichen photosynthesis can drop to values about –20 °C as has been demonstrated by gasometric (Kappen et al. 1996) and fluorometric studies (Barták et al. 2007) in lichens from polar and alpine regions. In many field and laboratory studies, photosynthetic parameters, such as e.g. F_v/F_m and ϕ_{PSII} are used as indicators of cryoresistance of lichens and their ability to survive long-term periods at freezing temperature (in dry state) without significant damage to photosynthetic apparatus. However, freezing experiments with lichen species from cold habitats showed that wet thalli may perform substantial photosynthesis at below zero temperature (e.g. *Usnea antarctica* – see Fig. 20.10). During rapid cooling, both F_v/F_m and ϕ_{PSII} of lichen thallus decline, in a S-curve type manner. While F_v/F_m is more or less constant within the range of 20 to –10 °C, ϕ_{PSII} tends to decline more rapidly indicating gradual temperature-dependent inhibition of Photosystem II.

The algal photobionts are remarkable resilient to freezing. Even when shock-frozen in liquid nitrogen, lichen symbiotic algae of the genus *Trebouxia* survive to a certain extent (typically 30 % of algal population, Hájek et al. 2012) and are able to restore their photosynthetic performance during subsequent cultivation.

IV. Important Chemical Factors Affecting Photosynthesis

A. Availability of Nutrients

The typical lichen habitat only provides poor access to nutrients. This limitation also effects photosynthetic performance. Consequently,

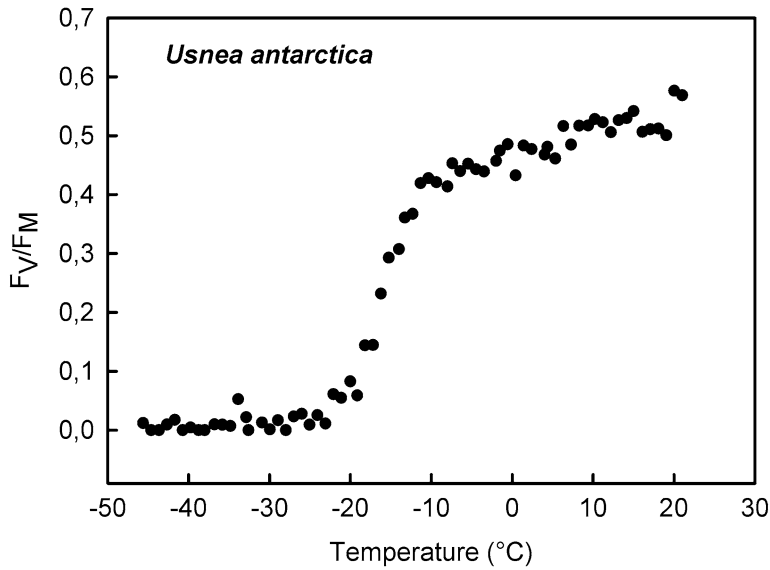


Fig. 20.10. Temperature-dependent decrease of potential quantum yield of PSII (F_v/F_m – potential yield of PSII photosynthesis) recorded during linear cooling of *Usnea antarctica* thallus from +20 to –30 °C (cooling rate 0.5 °C/min). Photochemical processes in PSII show slight decrease in the temperature range (–12 to +20 °C), dramatic decrease within the range of –12 to –20 °C. The critical point for photosynthesis (assessed by chlorophyll fluorescence) is about –22 °C.

lichens can increase photosynthetic rate when nutrients – nitrogen in particular – are available (see e.g. Davis et al. 2000). A study by Palmqvist et al. (1998) demonstrated that lichens with higher nitrogen content exhibit higher gross photosynthetic rate, but lower photosynthetic nitrogen use efficiency. It is well established that when nitrogen is available to a lichen, larger amount of nitrogen is invested in both, the algal and fungal partner. The nitrogen partitioning between the algal and fungal partner is likely species specific (Palmqvist et al. 2002). Nitrogen fertilization experiments show that additional nitrogen is used to synthesize proteins, chlorophyll and ribitol, the photosynthetic product of algal photobiont that can be supplied to the fungal partner (Dahlman et al. 2003).

B. Heavy Metals

With few exceptions of heavy metal-tolerant species (e.g. *Xanthoria parietina*), lichens are considered good indicators of toxic environmental pollution (Beckett and Brown

1983). It is well established that lichen abundance and distribution decreases with heavy presence of heavy metals. This susceptibility is a result of lichens' disposition to accumulate heavy metals. Heavy metal accumulation is thought to lead to ROS formation, and consequently to lipid peroxidation and damages to proteins and nucleic acids.

On the level of the photobiont, a decrease in the integrity of chlorophyll molecules that can in extreme cases also lead to a severe decrease in of chlorophyll content, that limits and inhibits photosynthetic processes (Bačkor and Fahsett 2008; Bačkor and Loppi 2009). Interestingly evidence from laboratory studies suggest that heavy metal-sensitive and – tolerant *Trebouxia* strains exist.

While there is amply evidence from laboratory experiments, field studies that investigate the effect of heavy metals on photosynthesis are less frequent. Garty et al. (2002) reported a decrease of capacity of photosynthetic processes in PSII (F_v/F_m) in epilithic fruticose lichen *Ramalina maciformis* exposed to a mixture of heavy metal ions

from industrial pollution. The decrease in photosynthetic performance is attributed to the degradation of chlorophylls, leading to a decrease in variable chlorophyll fluorescence. Therefore, heavy metal exposure in lichens may be assessed as an decrease in minimum (F_0) fluorescence, variable chlorophyll fluorescence (F_v) and chlorophyll fluorescence parameters derived from those signals (Kupper et al. 1998).

The extent of heavy metal-induced inhibition of photosynthetic processes differs among lichen species. Several species, especially those grown near copper mines, exhibit increased tolerance to heavy metals. Such organisms have increased content of phytochelatin, glutathione (Bačkor et al. 2009) compared to similar organisms living away from pollution sources.

V. Lichen Photosynthesis in the Field

A. Overview of Typical Environments

Over last several decades, numerous short- and long-term studies have been conducted that involved measurements of lichen photosynthesis in the field. In addition to the pioneering studies of O. Lange and his co-workers, who started photosynthesis measurements of lichens in semi-desert ecosystems (Lange et al. 1970), a wide range ecosystems and biotopes has been investigated (see below). Initially these studies have utilized infra-red gas (CO_2) analysers, that were later complemented by a variety of chlorophyll fluorescence measurements and environmental sensors.

Detailed field studies can assess the diurnal courses of lichen photosynthesis simultaneously with microclimatological and environmental parameters. Such approaches enables the identification of the key factors that limit photosynthesis in particular location (see e.g. Lange 2002), as well as primary productivity of lichens (e.g. Green and Lange 1991; Uchida et al. 2006).

In tropical rain forests, net photosynthetic rate of algal and cyanobacterial lichens is

generally high, but also show season-dependent variation (Lange et al. 2004). During moist period (*i.e.* overcast days), light is limiting, while during the periods with high photosynthetically active radiation, thalli dehydration is limiting lichen photosynthesis.

Water limitation is the key factor that determines lichen photosynthesis in deserts and semideserts, where lichens are components of soil crusts. It has been shown for a variety of lichen species from the Negev desert (Lange et al. 1977; Palmer and Friedmann 1990) and Namib desert (Lange et al. 1994, 2006) that aerial moisture is a crucial factor for photosynthesis and the survival of crust-forming lichens. Nocturnal fog and/or early morning dew hydrates lichen so that they are able to perform positive net photosynthesis for a short period of time after sunrise.

Several long-term studies on lichen photosynthesis in mountainous ecosystems have been conducted (Reiter and Turk 2000; Reiter et al. 2008). These studies addressed interaction of water availability and temperature and their effect on photosynthetic performance of *Xanthoria elegans*, *Umbilicaria cylindrica*, and *Brodoa atrofusca*, respectively. Continuous field measurements of net photosynthesis and respiration revealed that in the particular alpine ecosystem, the species were photosynthetically active only 16–25 % of the observation time. The most limiting factor for photosynthesis was dehydration of thalli. Interspecific comparison showed that *X. elegans* is the most photosynthetically active among the three species.

Similar long-term studies were carried out in polar regions, particularly in Antarctica. Using automation the effective quantum yield (Φ_{PSII}) of lichen photosynthesis was continuously evaluated. Measurements can be recorded over weeks (see Fig. 20.11) and even several seasons (Schroeter et al. 2011). These measurements clearly show the limitation of lichen photosynthesis by thallus dehydration, freezing of thalli and unavailability of light during the polar night.

While continuous long-term field measurements of lichen photosynthesis provide



Fig. 20.11. Field installation of a fluorometer for long-term measurements of effective quantum yield of photosynthetic processes in *Xanthoria elegans* (James Ross Island, Antarctica) (Photo M. Barták).

unparalleled insights in the ecophysiology of lichens, field studies that acquire data in week or month intervals can also be very informative. Such studies can reveal general trends in acclimation of lichen photosynthesis and provide additional data that require direct interactions with the sample (e.g. quantification of photosynthetic pigments) (Piccotto and Tretiach 2010).

B. Special Environments

In addition to ecosystems on Earth, lichen photosynthesis has been also investigated in extraterrestrial space. Here lichen photosynthesis may serve as a marker for surviving conditions experienced in space (Gomez et al. 2012). Within the last decade, several preflight tests have performed to test the resistance of lichens to freezing temperature, vacuum and UV radiation using chlorophyll fluorescence characteristics in

Rhizocarpon geographicum and *Xanthoria elegans*.

Short-term exposure of lichens to extra-terrestrial (orbital) conditions was evaluated in the year 2005 within the FOTON_M2 spacecraft mission using the BIOPAN facility. Based on these studies, a long-term (18 months) lichen exposure experiment was performed within the EXPOSE-E experiment onboard the International Space Station (ISS) in 2008/2009. These experiments showed that the experimental lichen species survive open space conditions in a dry state and restore their photosynthetic activity with only minor signs of damage when rewetted under laboratory conditions (de la Torre et al. 2010).

VI. Methods for Assessing Lichen Photosynthesis

A wide array of methods is available to study photosynthesis in lichens and their isolated photobionts. Assessment of photosynthetic CO_2 fixation by measuring gas exchange is frequently employed in the field and laboratory. Due to the small dimensions of lichen thalli, necessity of maintaining a constant hydration state, impossibility of deattaching crustose lichen thalli from stone surfaces, these gasometric measurements typically require specifically-modified measuring chambers (e.g. clap-cuvette – Lange et al. 1986, 2007). In spite of these difficulties, many studies demonstrate the acquisition of gas exchange measurements to characterize lichen photosynthesis.

Measurements of chlorophyll fluorescence parameters are also widely used in lichen photosynthesis studies. Fluorometers are relatively robust and cost-effective instruments. Apart of generally well-established indicators of potential (F_v/F_m) and actual (ϕ_{PSII}) effectivity of PSII processes, there are several parameters used in the evaluation of stress in lichen photosynthesis, such as e.g. non-photochemical quenching (NPQ, q_N) and its components related to the involvement of energy-dependent (qE), state one-state two

transition (qT) and photoinhibitory (qI) processes in PSII. Such approach enables to determine negative effects of particular stress factors in chloroplast of a lichen photobiont. Almost all chlorophyll fluorescence measuring techniques are used in lichens to address specific aspects of lichen photosynthesis. Fast chlorophyll fluorescence transients (OJIPs) were exploited to characterize absorbed light energy transformation in PSII and photochemical photosynthetic machinery. At OJIP curves measured on symbiotic lichen alga *Trebouxia*, Ilík et al. (2006) reported unusual dip that differs from evidence from higher plant. It was attributed to fast reoxidation of electron carriers in chloroplastic thylakoid membranes caused by activation of ferredoxinNADP + oxidoreductase or Mehlerperoxidase. Oukarroum et al. (2012) used OJIPs to evaluate heat stress effect in PSII of optimally hydrated thallus of *Parmelia tiliacea*. The study focused on high temperature-induced occurrence of K and M peaks at the OJIP transient. K and M values are indicative for heat stress effects in PSII. Recently, visualization techniques are increasingly used in many fluorometric studies (e.g. Barták et al. 2000) enabling to distinguish intrathalline differences in lichen photosynthetic performance (see Fig. 20.12). Tens of chlorophyll fluorescence parameters are available to evaluate the heterogeneity of photosynthesis caused over lichen thalli by patterned distribution of photosynthesizing photobionts (Jensen et Siebke 1997) photoinhibitory (Barták et al. 2006), temperature and osmotic stress (Hájek et al. 2006). Recently, development of automated and even robotic fluorometers that are used in many applications in higher plants is very promising and future application chlorophyll fluorescence imaging in ecophysiological studies of lichen photosynthesis might be expected.

Oxymetric methods in lichen photosynthesis research have only been employed recently and only to a limited extent. In principle, the method is based on a Clark-type oxygen electrode housed in a chamber that interfaces with the lichen. Typically

these instruments are used to assess photosynthetic O₂ evolution in response to light, temperature and hydration status of lichens (Charles 2011; Aubert et al. 2007). Due to the small chamber volumes of these instruments a constant hydration state of lichen thallus can be maintained for longer periods than during CO₂ exchange measurements.

Another application of oxymetric methods is the measurement of oxygen exchange in algal/cyanobacterial cultures of photobionts isolated from lichen thalli and cultivated in liquid media. However the isolated photobionts cultivated in cultures differ in their photosynthetic performance from their natural behaviour of their lichenized forms. Studies that contrast the performance of mycobiont and photobiont collective with the isolated photobiont can be used to characterize features that arise from interactions within lichens.

Carbon isotopes are also used in the evaluation of lichen photosynthesis. Stable isotope (¹³C) discrimination is typically used to distinguish between photosynthesis and respiration and also between the presence or absence of carbon concentration mechanisms (Máguas et al. 1993). Radioactive carbon isotope (¹⁴C) is used for identification of photosynthetic products of lichen photobionts and secondary metabolites such as sugars, polyols and lichen acids (Eisenreich et al. 2011). Recently, the approach has been used as an effective tool to assess lichen metabolomics in *Cladonia portentosa* (Freitag et al. 2012).

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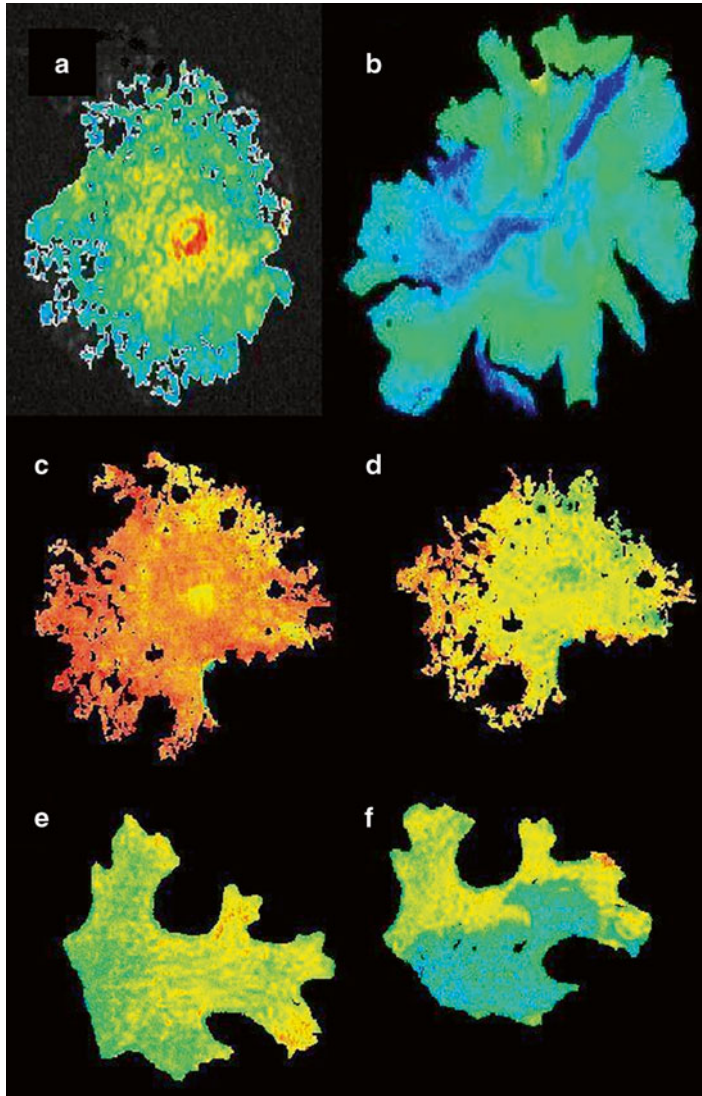


Fig. 20.12. Chlorophyll fluorescence imaging visualizes several physiological and photosynthetic processes in lichens. **(a)** Image of the chlorophyll fluorescence signal (intensity indicated by color) reveals higher amount of chlorophyll (*red*) and less developed *dark pigmentation* (*yellow*) of the central (young and growing) thallus part of *Lasallia pustulata*: Older and more *dark-pigmented* marginal parts show low chlorophyll fluorescence (*green* and *blue*). **(b)** Intrathalline heterogeneity of effective quantum yield of photosynthetic processes (Φ_{PSII}) in *Umbilicaria antarctica*. The *blue* line across the thallus area indicates inhibited photosynthesis due to mechanical stress caused by repeated dehydration-dependent movements. In response to desiccation, the thallus bends along by the *blue* line. **(c, d)** Distribution of photoinhibition (assessed by F_v/F_m) in a fully-hydrated *Lasallia pustulata* thallus induced by high light treatment. The reduction of F_v/F_m from pre-photoinhibitory state ($F_v/F_m=0.648$) to high light state ($F_v/F_m=0.530$) is indicated by color shift from orange (see **c**) to *yellow* and *green* (see **d**). **(e, f)** Photoinhibition caused by high light ($650 \mu\text{mol m}^{-2} \text{s}^{-1}$ for 2 d) in the dry thallus of foliose lichen *Lobaria pulmonaria*. Maximum photosynthetic activity (Φ_{PSII}) is observed close to the tips of thallus lobes in the untreated control (*red* and *yellow* in panel **e**). In the desiccated state, a part of thallus bends and so provides a shield against incident radiation. As a consequence, one part of thallus exhibits very limited photoinhibition (indicated in *yellow* in panel **f**) while the rest of thallus (unshielded by overlapped part) is severely photoinhibited (*blue*).

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