Oecologia (1996) 108:412–418

Knut A. Solhaug · Yngvar Gauslaa

Parietin, a photoprotective secondary product of the lichen *Xanthoria parietina*

Received: 21 December 1995 / Accepted: 4 May 1996

Abstract Secondary lichen products can be extracted from air-dry thalli of *Xanthoria parietina, Xanthoparmelia conspersa* and *Parmelina tiliacea* with 100% acetone without affecting either short- or long-term viability. In *Xanthoria parietina* damage by acetone started to occur as water content reached the critical lower limit for photosystem II (PSII) activity. Extraction of the blue-light absorbing cortical pigment parietin increased the susceptibility of both air-dry and hydrated thalli to high light. Damage by high light levels caused a permanent reduction in F_v/F_m, quantum yield for photosynthetic O_2 production and photosynthetic capacity measured after a 2-day recovery period at low light levels (20 μmol photons m^{-2} s^{-1}). Parietin therefore protects the photosynthetic apparatus of *Xanthoria parietina* against damage by high light levels. Extraction of UV-absorbing pigments from *Xanthoparmelia conspersa* and *Parmelina tiliacea* did not increase photoinhibition after 24 h exposure to high light.

Key words Acetone-rinsing · Chlorophyll fluorescence · Cortical pigments · Light screening · Photosynthesis

Introduction

Various kinds of photoprotection have been clearly demonstrated for several lichens (Demmig-Adams et al. 1990). One possible photoprotective strategy would be to increase nonphotochemical quenching, as shown in hydrated and air-dry thalli of *Anaptychia ciliaris* (Valladares et al. 1995). Another strategy would be to reduce the light level for the photobiont by increasing the reflectance or absorbance of light in cortical layers. The upper surface reflectance was higher (Gauslaa 1984) and the light transmission lower (Ertl 1951) for dry than for hydrated thalli. A lichen thallus growing in full sunlight developed an upper cortex twice as thick as that of a shade specimen (Tobler 1925), and light transmittance through the upper cortex was reduced in thalli adapted to high light levels (Ertl 1951). Only 8–11% of incident ambient light intensity was found to reach the photobiont layer in high-light adapted *Peltula* (Büdel and Lange 1994). However, photoprotective mechanisms do not necessarily provide adequate protection in lichens exposed to high light, and even air-dry thalli of some forest lichens have been shown to be highly susceptible to excess light (Gauslaa and Solhaug 1996).

Most fungal partners of lichens produce secondary products which may contribute as much as 30% of the thallus dry weight (Huneck 1973). Production of secondary products has mainly, but not exclusively, been reported in mycobionts that establish a successful symbiosis with a photobiont in a lichen thallus (Ahmadjian 1993). Various significant roles of secondary products in the lichen symbiosis have been suggested (reviewed by Rundel 1978; Fahselt 1994). Coloured secondary products deposited mainly in the cortex may protect the photobiont against excessive light (Rundel 1978; Lawrey 1986; Fahsehlt 1994), and thallus concentrations of usnic acid (Rundel 1969) and parietin (Hill and Woolhouse 1966) are correlated with the light intensity in the habitat. Parietin is a widespread, orange antraquinone found in lichen genera such as *Caloplaca, Xanthoria* and *Teloschistes* that form conspicuous and characteristic communities in various open and sun-exposed habitats. Since parietin efficiently absorbs blue light and is situated mainly in the upper cortex, parietin may help to protect the photosynthetic apparatus of the photobiont against strong light. However, in the absence of conclusive evidence this role is conjectural, and Kappen (1994), questioning whether the lichen is a mutualistic system, concluded that there is little evidence for any benefit to the photobiont in the lichen symbiosis.

Preliminary experiments indicated that air-dry lichen thalli tolerated a complete extraction of secondary products by repeated soakings in 100% acetone. One objective of this study was therefore to study short- and long-

K. A. Solhaug (✉) · Y. Gauslaa
Department of Biology and Nature Conservation,
Agricultural University of Norway, P.O. Box 5014,
N-1432 Ås, Norway

term effects of acetone rinsing of air-dry thalli in order to develop an experimental design that allows testing of current hypotheses on the ecological significance of secondary products in lichens. The second objective was to test the hypothesis that parietin provides photoprotection in *Xanthoria parietina* (L.) Th. Fr., by studying the effects of removal of this blue-light absorbing cortical pigment on the photobiont's susceptibility to high light levels. The final objective was to compare the effects of removal of the light-absorbing cortical pigment in *X. parietina* with the removal of the UV-absorbing cortical pigments usnic acid and atranorin in *Xanthoparmelia conspersa* (Ach.) Hale and *Parmelina tiliacea* (Hoffm.) Hale, respectively.

Materials and methods

Plant material

Xanthoria parietina and *Parmelina tiliacea* thalli were collected from the south side of sun-exposed stems of avenue trees of *Populus tremula* L. and *Fraxinus excelsior* L. respectively, *Xanthoparmelia conspersa* thalli were collected on a southwest-facing rock. All species were sampled at Ås (59°30′N, 10°47′E, 100 m altitude), south-eastern Norway, in an open agricultural landscape, a few days before start of the experiments.

Isolation of pure parietin

About 10 g of air-dry *X. parietina* thalli was extracted for 30 min with 100 ml 100% acetone at room temperature. During partial evaporation in air yellow needle-shaped crystals precipitated in the acetone extract. These crystals were recrystallized from acetone. The crystals had a sharp melting point at 213°C, indicating that they contained relatively pure parietin. The melting point of parietin is reported to be 206–209°C (Culberson 1969; Steiner and Hauschild 1970). Then 10 mg crystals were dissolved in 250 ml acetone and the molar extinction coeffisient (ε) at 434 nm (λmax) was measured as 12,133. This extinction coeffisient was later used for estimation of parietin content.

Acetone treatment

Lichen thalli were air-dried at 20°C, 30–50% relative humidity (RH) and low light levels for 24 h, then rinsed four times repeatedly with 100% acetone at room temperature for 5 min each time. The four acetone extracts were combined and diluted to 25 ml before measurement of absorbance at 434 nm. Thalli were thereafter left for another 24 h to be sure that residual of acetone had evaporated completely.

A separate experiment tested the effect of thallus water content immediately before acetone rinsing on thallus viability. A total of 32 similar *X. parietina* thalli were weighed in the air-dry state before being wetted. Excess water was removed with blotting paper, and samples were allowed to air-dry at 20°C and 30% RH. During drying thalli were successively selected, reweighed and acetone-rinsed after periods of 0–3 h. Five similarily treated control thalli were used to find the air-dry/oven(105°C) dry weight ratio, and this ratio was used to calculate water content in the acetone-rinsed thalli. Another set of 20 *Xanthoria parietina* thalli were treated similarly, but they were repeatedly weighed and their chlorophyll *a* fluorescence measured during the drying cycle in low light (<5 μmol photons m^{-2} s^{-1}).

The bryophytes *Polytrichum commune* Hedw. and *Hypnum cupressiforme* Hedw. were air-dried at room-temperature for 3 days, then soaked in 100% acetone for 30 min. After the acetone had evaporated completely the samples were sprayed with water and left for 24 h in low light at room temperature before measurement of maximum quantum yield of photosystem II (F_v/F_m).

Light exposure of lichens

Thalli were moistened by spraying them with distilled water, and preconditioned for 48 h at 20 μmol photons m^{-2} s^{-1} at 16 C and 70% RH. The thalli were then continuously exposed to 1000, 250 and 20 μmol photons m^{-2} s^{-1} for 24 h by placing the thalli in specific distances from a Philips HPI 400 W mercury lamp. About 25% of the energy in the photosynthetically active region (400–700 nm) is in the parietin-absorbing range (400–475 nm), a similar fraction to sunlight. A plexiglass tray with a 10-cm layer of water inserted between the lamp and the lichens minimized heat radiation. Photon fluence rates were measured with a Licor quantum sensor. The thalli were frequently sprayed with distilled water to avoid drying, epecially those subjected to the strongest light. After 24 h all thalli were transferred to 20 μmol photons m^{-2} s^{-1} for 6 days. Chlorophyll fluorescence was repeatedly measured during light exposure and during the 6-day recovery period.

Dry thalli were exposed to 1000 μmol photons m^{-2} s^{-1} for exposure durations varying from 48 to 192 h. After each light treatment five thalli were hydrated and placed under 20 μmol photons m^{-2} s^{-1} for a 48-h recovery period before measurement of chlorophyll fluorescence and O_2 evolution.

Photosynthetic O_2 evolution

Oxygen evolution was measured with a leaf-disc electrode (Model LD2, Hansatech King's Lynn, Norfolk, UK) at 20°C and 5% CO_2. The lichen thalli were placed in the cuvette and O_2 evolution was measured with increasing photon fluence rates in the range 0–1090 μmol photons m^{-2} s^{-1} using light-emitting diodes (LH36 U, Hansatech). Apparent quantum yields were calculated from the linear portions of the light response curves.

Apparent quantum yields in blue light were determined using a blue Kodak filter (λmax=430 nm; Fig. 1) in combinations of neutral density filters. A halogen lamp (Hansatech LS2) was the light source.

Chlorophyll *a* fluorescence

Chlorophyll *a* fluorescence induction curves were recorded with a portable fluorometer [plant efficiency analyser (PEA), Hansatech]. The lichen thalli were dark-adapted for 15 min before measurement except in the experiment with varying water content when measurements were made in low light (<5 μmol photons m^{-2} s^{-1}). Fluorescence induction curves of 5 s duration were recorded during illumination with light of 1500 μmol photons m^{-2} s^{-1} from light emitting diodes. F_v/F_m was estimated by the instrument, and photoinhibition defined as a reduction in F_v/F_m compared with low-light control thalli. Fluorescence was measured three times at different positions on each thallus, and the mean value was used as one observation.

Synthesis of new parietin

Acetone-rinsed thalli of *X. parietina* without parietin were attached to the northern side of their original *Populus tremula* stems with epoxy resin (Araldite) (Richardson 1967) on 12 October 1994. The amount of parietin in these thalli was repeatedly determined until 30 May 1995 by harvesting five new thalli each time. Transplanted control thalli that had not been acetone-rinsed were treated in the same way.

Results

Absorbance spectra of secondary lichen products

The orange acetone-extract from *X. parietina* had maximal absorbance at 434 nm and low absorbance in the UV region of the spectrum, while the faintly yellowish acetone-extracts from *Xanthoparmelia conspersa* and the colourless extract of *P. tiliacea* had maximal absorbances around 330 nm in the UV region (Fig. 1). The 100% acetone did not extract any chlorophyll from the lichen thalli, since there was no trace of increased absorbance around 660 nm.

Effects of acetone rinsing on long-term viability

Air-dry thalli of *X. parietina* became grey after the parietin was removed, contrasting with the original orange colour (Fig. 2). Chlorophyll dominates the colour of hydrated parietin-free as well as normal thalli.

A preliminary experiment in late spring, when ten acetone-rinsed thalli were transplanted back to their original *P. tremula* trees situated in an open farmland, showed that the thalli were able to resynthesize parietin and that the lobes grew about 2–3 mm in the first year. The five north-facing transplants remained vital and healthy, while the five south-facing transplants became partly dead or bleached. In the subsequent field experiment all thalli were transplanted to the northern side of the stems.

In this transplantation experiment acetone-rinsed thalli were able to resynthesize 30% of the original parietin level within 45 days in late autumn under natural conditions. The parietin content remained fairly constant during the winter, but increased to 50% of the control level in spring (Fig. 3). During the transplantation there were no significant changes in either gross photosynthetic capacity or quantum yield for photosynthetic O_2 evolution measured at room temperature (Fig. 3), even during winter at temperatures well below freezing.

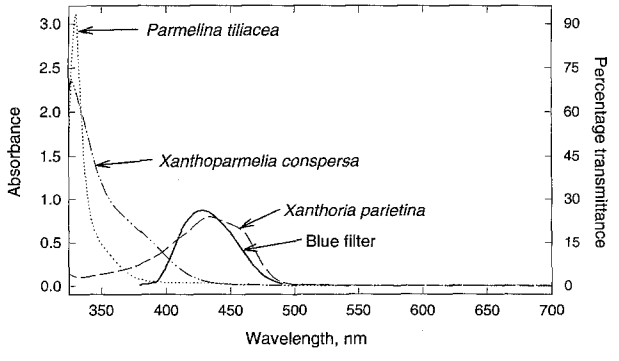

Fig. 1 Absorption spectra of acetone extracts from *Xanthoria parietina*, *Xanthoparmelia conspersa* and *Parmelina tiliacea*. Transmittance spectrum for the blue Kodak filter used for quantum yield determinations is also shown

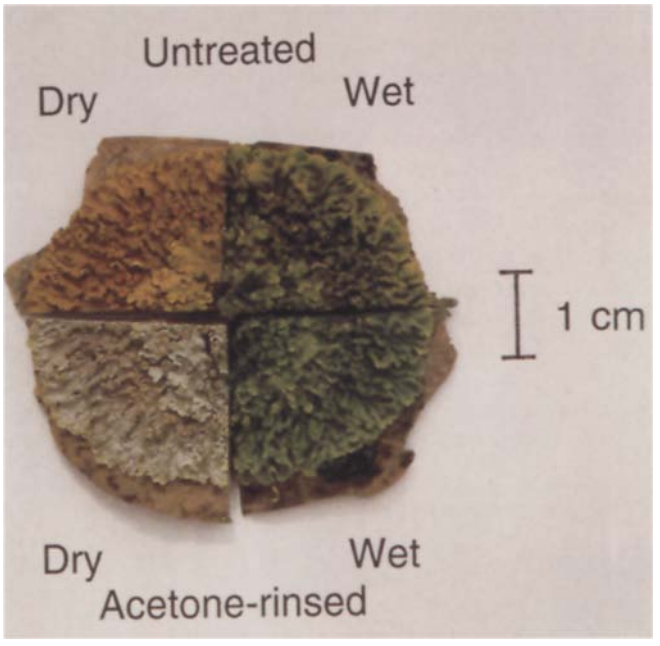

Fig. 2 One thallus of *Xanthoria parietina* divided into four parts to show the contrasting colours after acetone rinsing and/or wetting

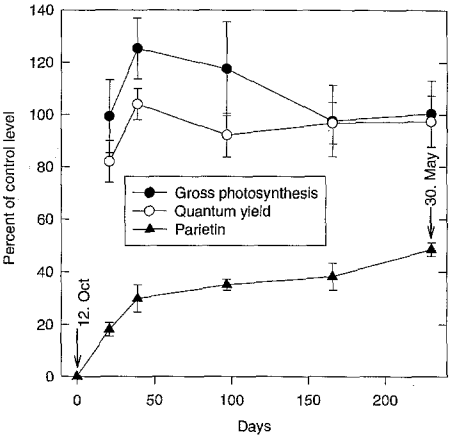

Fig. 3 Time-course of gross photosynthesis, quantum yield of photosynthetic O_2 evolution and parietin content for *Xanthoria parietina* thalli that had been transplanted to *Populus tremula* stems. The values are expressed as percent of control levels before acetone rinsing and are means of 5 measurements±1 SE

Effects of thallus water content

No harmful effects of acetone-rinsing on F_v/F_m were observed at a thallus water content below 25%. There was then a gradual decrease in F_v/F_m to zero as thallus water content immediately before acetone-rinsing increased from 25 to 100% of oven-dry weight (Fig. 4). During this transitional stage, a gradient in damage developed across the thallus. The thin lobe tips dried first and showed normal F_v/F_m upon rewetting, while central and thicker parts of the same thallus were wet and were damaged or killed during acetone-rinsing. This transitional

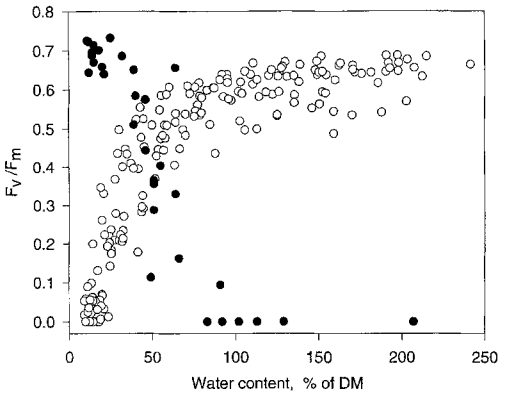

Fig. 4 Maximum quantum yields of photosystem II (F_v/F_m) as a function of water content for 20 *Xanthoria parietina* thalli measured repeatedly during a 2-h drying cycle (*open symbols*), and F_v/F_m after acetone-rinsing of thalli with different water contents (*closed symbols*)

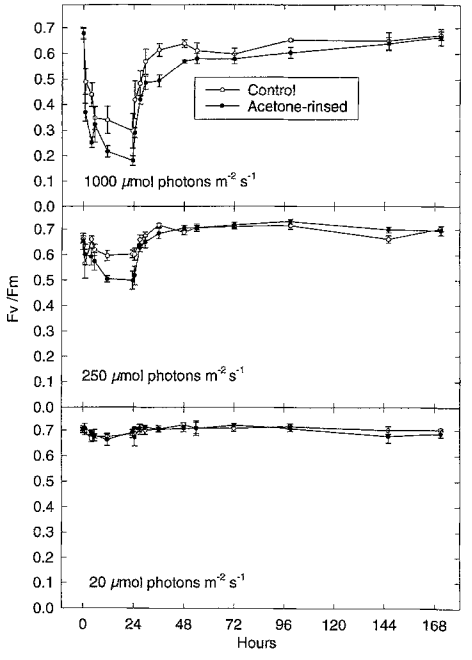

Fig. 5 F_v/F_m in hydrated *X. parietina* thalli exposed to 1000, 250 or 20 µmol photons m^{-2} s^{-1} for 24 h. After 24 h all thalli were kept at 20 µmol photons m^{-2} s^{-1}. Each point represents the mean of measurements on 5 thalli±1 SE

water content range also represented the onset and following steep rise in F_v/F_m (Fig. 4). F_v/F_m increased with thallus water content from 25 to 100%.

Effects of acetone-rinsing on bryophytes

F_v/F_m in *Polytrichum commune* rinsed in acetone was 0.812±0.005 (n=5) compared with 0.813±0.007 for the untreated samples, and in acetone-rinsed *Hypnum cupressiforme* F_v/F_m was 0.727±0.022 (n=5) compared with 0.722±0.030 in untreated samples.

Table 1 Apparent quantum yields (Φ) for acetone-rinsed and control thalli of *Xanthoria parietina* in red light from light-emitting diodes and in blue light (see Fig. 5). The percentage reduction in quantum yields in blue light compared with red light is shown. Each value is the mean of 8 measurements ±1 SE. The significance levels (t-test) for comparison with the controls are shown

	Control	Acetone-rinsed
Φ in red light	0.0557±0.0048	0.0580±0.0048 n.s.
Φ in blue light	0.0282±0.0024	0.0428±0.0027 **
% Reduction in Φ	50.4±2.3	25.1±2.6 ***

** P<0.01, *** P<0.001, n.s. not significant

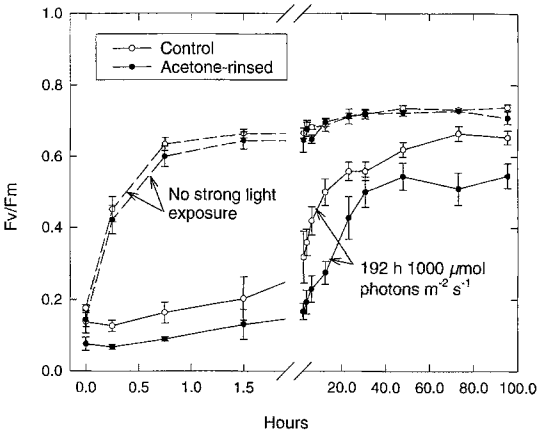

Fig. 6 Recovery of F_v/F_m at 20 µmol photons m^{-2} s^{-1} for hydrated thalli of *Xanthoria parietina* after a 192 h exposure in dry condition to 1000 or 20 µmol photons m^{-2} s^{-1}, subsequently sprayed with water at time 0. Each point is the mean of measurements on 5 thalli±1 SE

Light exposure of hydrated thalli

A 24-h exposure of hydrated thalli of *X. parietina* to 250 or 1000 µmol photons m^{-2} s^{-1} for 24 h caused a rapid and deep decrease in F_v/F_m (Fig. 5). The depression in F_v/F_m was greatest in parietin-free thalli at both high-light levels. Ultimately there was an almost complete recovery from photoinhibition at low light levels (20 µmol photons m^{-2} s^{-1}) for both acetone-rinsed and control thalli, although recovery from exposure to 1000 µmol photons m^{-2} s^{-1} was slower for acetone-rinsed thalli (Fig. 5).

Apparent quantum yield for *X. parietina* was 50% lower in blue light than in red light for parietin-containing thalli, while the reduction in quantum yield in blue light compared with red light was only 25% in parietin-free thalli (Table 1).

Light exposure of dry thalli

Acetone-rinsed *X. parietina* thalli, stored air-dry at 20 µmol photons m^{-2} s^{-1} for 8 days, reached normal F_v/F_m values within 1 h after wetting like control thalli (Fig. 6). However, thalli exposed to 1000 µmol pho-

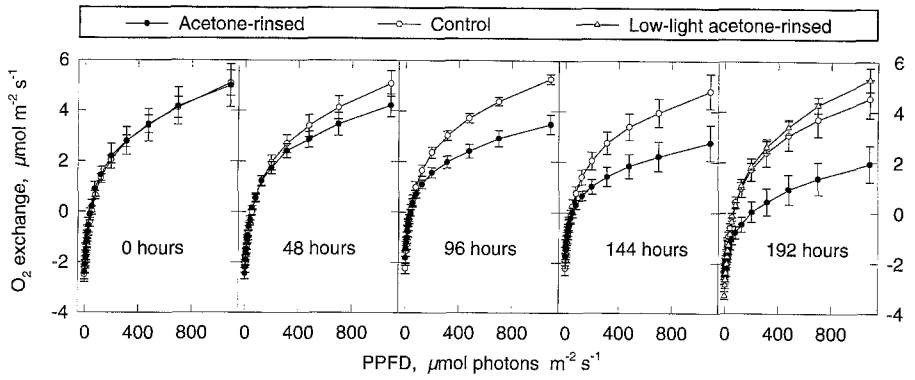

Fig. 7 Light response curves for photosynthetic O_2 evolution for acetone-rinsed and control thalli of *Xanthoria parietina* which had been exposed to various periods of 1000 μmol photons m^{-2} s^{-1} in the air-dry state. Low-light acetone-rinsed thalli were kept at 20 μmol photons m^{-2} s^{-1} for 192 h. Before mesurement all thalli were allowed to recover in hydrated condition for 2 days at 20 μmol photons m^{-2} s^{-1}. Each curve is the mean of light response curves for 5 thalli. Error bars show ±1 SE

Table 2 Apparent quantum yields of photosynthetic O_2 evolution with corresponding maximum quantum yields of photosystem II (F_v/F_m) values after recovery for *Xanthoria parietina* thalli exposed to increasing periods of 1000 μmol photons m^{-2} s^{-1}. The values are means of 5 measurements ±1 SE. The data are for the same thalli as in Fig. 7. Both F_v/F_m and Φ declined significantly ($P<0.001$) with duration of light exposure in acetone-rinsed thalli, while they did not change in control thalli (linear regression analysis). See the legend of Fig. 7 for details

Light exposure, hours	F_v/F_m		Quantum yield (Φ)	
	Control	Acetone	Control	Acetone
0	0.68±0.03	0.69±0.01	0.056±0.004	0.057±0.003
48	0.69±0.02	0.69±0.02	0.049±0.003	0.058±0.005
96	0.67±0.03	0.62±0.02	0.058±0.005	0.046±0.006
144	0.67±0.02	0.49±0.04	0.052±0.002	0.039±0.006
192	0.61±0.03	0.44±0.04	0.053±0.007	0.031±0.007

tons m^{-2} s^{-1} for 8 days showed substantially slower recovery of F_v/F_m. Recovery mainly took place within the first 48 h (Fig. 6). Therefore, in experiments with different duration of light exposure, 2 days exposure to 20 μmol photons m^{-2} s^{-1} was the standard recovery treatment before measurement of fluorescence and photosynthetic O_2 evolution. The recovery of F_v/F_m following a high-light treatment was slower for acetone-rinsed than for normal parietin-containing thalli of *X. parietina*. A 4-day recovery period under low light caused a 90% recovery of normal F_v/F_m values in control thalli compared with only a 70% recovery in parietin-free thalli (Fig. 6). F_v/F_m, quantum yield for O_2 production and light response curves for photosynthetic O_2 production were not permanently affected in normal parietin-containing thalli subjected even to 8 days of exposure to high light levels (Fig. 7, Table 2). However, for thalli without parietin there was a gradual decrease in all these parameters with increasing duration of exposure to high light levels (Fig. 7, Table 2).

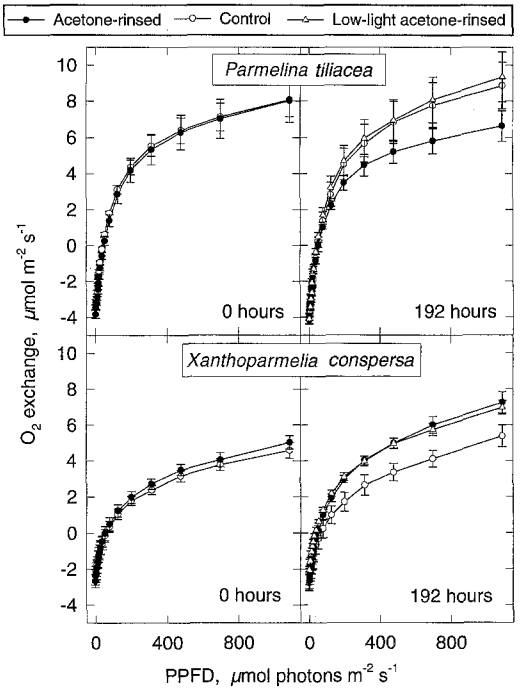

Fig. 8 Effects of exposure to 1000 μmol photons m^{-2} s^{-1} for 192 h in dry condition on photosynthetic O_2 evolution light response curves for acetone-rinsed and control thalli of *Parmelina tiliacea* and *Xanthoparmelia conspersa*. Low light acetone-rinsed thalli were kept at 20 μmol photons m^{-2} s^{-1} for 192 h. Before mesurement all thalli were allowed to recover in hydrated condition for 2 days at 20 μmol photons m^{-2} s^{-1}. Each curve is the mean of light response curves for 5 thalli. Error bars show ±1 SE

Exposure to 1000 μmol photons m^{-2} s^{-1} for 8 days did not significantly affect photosynthetic O_2 production in either *Parmelina tiliacea* or *Xanthoparmelia conspersa* (Fig. 8), while F_v/F_m was slightly lower after exposure to high light levels in acetone-rinsed thalli (Table 3). Acetone rinsing had no effects on O_2 production or F_v/F_m in control thalli of these two lichens kept at low light (Fig. 8, Table 3). The acetone extract from *Parmelina tiliacea* and *Xanthoparmelia conspersa* had low absorbance at wavelengths that are important for photosynthesis (Fig. 1).

Table 3 F_v/F_m±1 SE for *Parmelina tiliacea* and *Xanthoparmelia conspersa* measured just before measurements of light response curves for photosynthetic O_2 evolution. The data are for the same thalli as in Fig. 8. Values with different letters are significantly different at 5% level (Student-Newman-Keuls multiple comparison test)

Treatment	Light	*Parmelina tiliacea*	*Xanthoparmelia conspersa*
Control	0	0.769±0.007 a	0.732±0.010 ab
Acetone-rinsed	0	0.785±0.004 a	0.745±0.007 a
Control	192 h 1000 µmol	0.719±0.019 b	0.717±0.008 ab
Acetone-rinsed	192 h 1000 µmol	0.722±0.016 b	0.709±0.008 b
Acetone-rinsed	192 h 20 µmol	0.772±0.007 a	0.748±0.010 a

Discussion

This study failed to find any adverse effects of acetone rinsing on air-dry lichen thalli. Since acetone damage started to occur as water content reached the critical lower level for photosystem II (PSII) activity, the photosynthetic apparatus must be inactive for the lichen to tolerate acetone rinsing (Fig. 4). Experiments with the old forest lichen *Usnea longissima* (data not shown) and with air-dry bryophytes and the poikilohydric fern *Polypodium vulgare* (K. Solhaug and Y. Gauslaa, unpublished work) also failed to find any adverse effects of rinsing with 100% acetone. Dry seeds can be soaked in acetone without damage (Milborrow 1963; Tao and Khan 1974). In general, desiccation-resistant plant tissues are apparently not adversely affected by 100% acetone in an air-dry state. Acetone-rinsing of air-dry lichens is a standard method for extracting secondary lichen products in chemotaxonomic studies (Culberson and Kristinsson 1970), but has also a highly interesting but unused potential for testing the various hypotheses reviewed in Fahselt (1994) on the potential ecological significance and physiological implications of the numerous secondary products that have been reported in lichens (Culberson 1969; Culberson et al. 1977). The photobiont of air-dry *X. parietina* shows a remarkable resistance in surviving annual fumigations with methyl bromide and naphthalene for several years (Keller et al. 1995), and cryofixed and sputtercoated lobes of *X. parietina* survived examination in a low-temperature scanning electrone microscope (Honegger 1995).

Parietin-free lichen thalli did not fully resynthesize the quantity of parietin removed within the transplantation period (Fig. 3). However, the period with active metabolism might have been too short, since the temperature during most of the winter was below freezing. The final increase in parietin content in late spring (Fig. 3) indicates that the production of parietin occurs during periods of active growth. The similar levels of parietin in lichen thalli on the north- and south-facing sides of *Populus tremula* stems, unexpected in view of the reported correlation between parietin content and light intensity (Hill and Woolhouse 1966), could be due to the free-standing position of trees which might allow the northern and southern sides to receive similar amounts of radiation after flushing of leaves at a high latitude. However, on branches within the canopy, the parietin content seems to be much lower since specimens were considerably less pigmented, so only a narrow part of the light intensity gradient reported by Hill and Woolhouse (1966) was sampled around our stems.

Light transmission through the upper fragile cortex of *X. parietina* thalli could not be measured directly. The large reduction in apparent quantum yield of blue light compared with red light for normal thalli shows that parietin absorbs significant amounts of blue light in moistened thalli (Table 1). The distinct change in colour of air-dry thalli from orange to grey after acetone rinsing also indicates that parietin contributes significantly to light absorbance in air-dry thalli (Fig. 2). The upper surface reflectance of visible sunlight from parietin-containing *X. parietina* increases from 10 to 16% during a drying cycle (calculated from the original dataset of Gauslaa 1984), so the combined light screening of tiny reflecting air spaces (Stocker 1927) and absorbtion by parietin is considerably more efficient in an air-dry than a hydrated thallus.

Photoinhibited hydrated *X. parietina* thalli were able to recover (Fig. 5) in a similar way to higher plants (e.g. Öquist and Huner 1991). The parietin reduced photoinhibition of the moist photobiont (Fig. 5). Photoinhibition measured as a short-term reduction in F_v/F_m is considered to be a short-term down-regulation of PSII that protects the photosynthetic apparatus against strong light (e.g. Adams et al. 1995). Parietin-free thalli developed a greater down-regulation of PSII and recovered at a slower rate, but F_v/F_m returned to normal level after some days indicating no permanent damage. A recovery period of at least 48 h should therefore be used as a standard procedure to detect more permanent adverse effects of high-light. However, lichens are poikilohydric organisms that seem to require alternating drying and rehydration periods for optimal growth (see Kershaw 1985). Photosynthesis in lichens normally recovers rapidly upon resoaking (Ried 1960), and *X. parietina* thalli exposed to low light levels rapidly restored the normal level of F_v/F_m (Fig. 6). Lichens are usually exposed to the highest light intensities in dry conditions when repair mechanisms are probably inactive. Air-dry thalli may be protected against high light levels by a functional interruption of energy transfer between the light harvesting chlorophyll *a/b* pigment complex and PSII (Bilger et al. 1989). However, exposure to high light levels during dry periods causes severe damage in some species (Valladares et al. 1995; Gauslaa and Solhaug 1996). Prolonged and incomplete recovery after rehydration (Fig. 6) indi-

cates that air-dry *X. parietina* is susceptible to damage by high light levels. High light levels inevitably imply a high degree of desiccation in lichens. However, desiccation alone does not seem to result in reduced F_v/F_m values since even thalli of old forest lichens like *Lobaria pulmonaria*, *Usnea longissima* and *Evernia divaricata* stored in a desiccator for 3 weeks showed no reduction in F_v/F_m (K. Solhaug and Y. Gauslaa, unpublished work). The increased susceptibility to high light of air-dry thalli of parietin-free *X. parietina* (Figs. 4 and 5, Table 2) shows that parietin has a photoprotective role.

The sensitivity of the green-algal photobiont to high light levels may be a heritage from its evolutionary origin in water. The photoprotective function of parietin suggests that there really is a mutualistic relationship between the two symbionts in *X. parietina,* allowing the photobiont to survive prolonged periods of sunshine in the air-dry state despite the generally inherent parasitic tendency in the fungal partner stressed by Kappen (1994).

The acetone-soluble substances from *Parmelina tiliacea* and *Xanthoparmelia conspersa* did not protect these lichens against high-intensity visible light (Fig. 8, Table 3). However, these two lichens have cortical pigments or constituents that are not extracted by acetone and that hide the chlorophyll colour of their photobionts even in the moist condition. Therefore their cortex probably provides a fairly high degree of photoprotection. The acetone-soluble substances of these two species, which strongly absorb UV radiation, probably protect against UV damage.

References

Adams WW, Demmig-Adams B, Verhoeven AS, Barker DH (1995) 'Photoinhibition' during winter stress: involvement of sustained xanthophyll cycle-dependent energy dissipation. Aust J Plant Physiol 22:261–276

Ahmadjian V (1993) The lichen symbiosis. Wiley, New York

Bilger W, Rimke S, Schreiber U, Lange OL (1989) Inhibition of energy transfer to photosystem II in lichens by dehydration: different properties of reversibility with green and blue-green phycobionts. J Plant Physiol 134:261–268

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

Culberson CF (1969) Chemical and botanical guide to lichen products. University of North Carolina Press, Chapel Hill

Culberson CC, Kristinsson H (1970) A standard method for the identification of lichen products. J Chromatogr 46:85–93

Culberson CF, Culberson WL, Johnson A (1977) Second supplement to chemical and botanical guide to lichen products. American Bryological and Lichenological Society, Missouri Botanical Garden, St. Louis

Demmig-Adams B, Máguas C, Adams WW III, Meyer A, Kilian E, Lange OL (1990) Effect of high light on the efficiency of photochemical energy conversion in a variety of lichen species with green and blue-green phycobionts. Planta 180:400–409

Ertl L (1951) Über die Lichtverhältnisse in Laubflechten. Planta 39:245–270

Fahselt D (1994) Secondary biochemistry of lichens. Symbiosis 16:117–165

Gauslaa Y (1984) Heat resistance and energy budget in different Scandinavian plants. Holarct Ecol 7:1–78

Gauslaa Y, Solhaug KA (1996) Differences in the susceptibility to light stress between epiphytic lichens of ancient and young boreal forest stands. Funct Ecol 10:344–354

Hill DJ, Woolhouse HW (1966) Aspects of the autecology of *Xanthoria parietina* agg. Lichenologist 3:207–214

Honegger R (1995) Experimental studies with foliose macrolichens: fungal responses to spatial disturbance at the organismic level and to spatial problems at the cellular level during drought stress events. Can J Bot 73 suppl 1:S569–S578

Huneck S (1973) Nature of lichen substances. In: Ahmadjian V, Hale ME (eds) The lichens. Academic Press, London, pp 495–522

Kappen L (1994) The lichen, a mutualistic system – some mainly exophysiological aspects. Cryptogam Bot 4:193–202

Keller P, Mukhtar A, Galun M (1995) Resistance: are there limits to resistance in lichens? Symbiosis 18:87–88

Kershaw KA (1985) Physiological ecology of lichens. Cambridge University Press, Cambridge

Lawrey JD (1986) Biological role of lichen substances. Bryologist 89:111–122

Milborrow BV (1963) Penetration of seeds by acetone solutes. Nature 199:716–717

Öquist G, Huner NPA (1991) Effects of cold acclimation on the susceptibility of photosynthesis to photoinhibition in Scots pine and in winter and spring cereals, a fluorescence analysis. Funct Ecol 5:91–100

Richardson DHS (1967) The transplantation of lichen thalli to solve some taxonomic problems in *Xanthoria parietina* (L.) Th. Fr. Lichenologist 3:386–391

Ried A (1960) Stoffwechsel und Verbreitungsgrenzen von Flechten. II. Wasser- und Assimilationshaushalt, Entquellungs- und Submersionsresistenz von Krustenflechten benachbarter Standorte. Flora 149:345–385

Rundel PW (1969) Clinal variation in the production of usnic acid in *Cladonia subtenuis* along light gradients. Bryologist 72:40–44

Rundel PW (1978) The ecological role of secondary lichen substances. Biochem Syst Ecol 6:157–170

Steiner M, Hauschild G (1970) Die Anthrachinone von Caloplacaceae und Teloschistaceae (Lichenes). Dtsch Bot Ges Neue Folge 4:23–34

Stocker O (1927) Physiologische und ökologische Untersuchungen an Laub- und Strauchflechten. Flora 121:334–415

Tao KL, Khan AA (1974) Penetration of dry seeds with chemicals applied in acetone. Plant Physiol 54:956–958

Tobler F (1925) Zur Physiologie der Farbunterschiede bei *Xanthoria.* Ber Dtsch Bot Ges 43:301–305

Valladares F, Sanchez-Hoyos A, Manrique E (1995) Diurnal changes in photosynthetic efficiency and carotenoid composition of the lichen *Anaptychia ciliaris*: effects of hydration and light intensity. Bryologist 98:375–382