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Low-light recovery effects on assessment of photoinhibition with chlorophyll fluorescence in lichens

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Abstract: Chlorophyll *a* fluorescence is often used to estimate various types of damage in lichens. In order to optimize the output and improve interpretations of such measurements the protocol for pretreatment and measuring is important. To study the effects of measurement conditions, the lichens *Lobaria pulmonaria*, *L. scrobiculata*, *Xanthoria parietina* and *Parmelia sulcata* were first stressed by high light intensities at 600 or 1000 µmol photons m^{-2} s^{-1} for 4 h. Then various conditions during recovery or pretreatment were used to optimize the detection of more lasting damage. Recovery from photoinhibition was incomplete in darkness, whereas light as low as 0·2 or 1·0 µmol m^{-2} s^{-1} resulted in complete recovery if the recovery period was long enough. Additionally, low intensity light given for1·5 h after one day in darkness caused rapid and complete recovery. In conclusion, before measuring maximal PSII efficiency (F_v/F_m) with chlorophyll fluorescence, it is important to let lichens recover in low intensity light and not in darkness, to optimize recovery from photoinhibition; dark adaptation can only be recommended if the photoinhibition status of the lichens is of interest.

Key words: D1 protein, *Lobaria pulmonaria*, *Lobaria scrobiculata*, *Parmelia sulcata*, photosystem II, *Xanthoria parietina*

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Introduction

Excess excitation energy that cannot be used for photosynthesis may damage the photosynthetic apparatus and result in reduced efficiency of photosynthesis. Reduced efficiency of photosynthesis caused by high light intensities is called photoinhibition, and maximal photosystem II efficiency (F_v/F_m) has become the standard measure of photoinhibition in many studies (e.g. Adams *et al.* 2013). Photoinhibition can be divided into dynamic and chronic photoinhibition. Dynamic photoinhibition is related mainly to safe dissipation of excess excitation energy by the xanthophyll cycle whereas chronic photoinhibition is caused by damage, especially of the D1 protein in PSII (Osmond 1994). Photoinhibition in lichens also depends on their degree of hydration. Moist thalli can be more susceptible to photoinhibition because the cortex transmits more light when wet (Gauslaa & Solhaug 2001). However, moist thalli may recover by metabolic mechanisms during low light or dark periods (Gauslaa & Solhaug 1996). If thalli are exposed to intermediate light levels in the dry state for long periods they become photoinhibited (Gauslaa *et al.* 2012). Recovery by metabolic mechanisms is not possible in the dry state, and intermediate intensity light over long periods thus causes severe, chronic photoinhibition (Gauslaa *et al.* 2012). However, dry thalli have higher cortical screening than wet thalli and may also be protected by dissipation of energy in the photosystems, apparent as chlorophyll fluorescence quenching that does not seem to be associated with the xanthophyll cycle (Heber *et al.* 2010).

Chlorophyll *a* fluorescence is widely used for measuring photosystem II (PSII) efficiency, an indicator of stress caused by high light and other adverse environmental factors in photosynthetic organisms (e.g. Baker 2008). Maximal photosystem II efficiency has apparently become the most

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popular activity parameter in lichens. For the measurement of F_v/F_m, the sample needs to be dark-adapted before measurement to ensure that all reaction centres in PSII are fully oxidized. The recommended dark adaptation time in the fluorimeter manuals is often 15–30 minutes, and it is also stated that relaxation of F_m for accurate measurement of F_v/F_m is enhanced in moderate light intensities of 20–40 µmol m^{-2} s^{-1}. In the field, pre-dawn measurements of F_v/F_m might be the best way to achieve this (Maxwell & Johnson 2000; Baker 2008). However, if lichens are photoinhibited they might require a much longer time to recover from photoinhibition (e.g. Gauslaa & Solhaug 1996).

Photoinhibition measured as a decrease in F_v/F_m might have several causes. Conversion of violaxanthin to zeaxanthin in high light intensities will result in a decrease in F_v/F_m that would normally relax within seconds or minutes. However, irreversible damage to the D1 protein depends on D1 protein resynthesis in PSII which requires a longer time for relaxation (e.g. Maxwell & Johnson 2000; Nath *et al*. 2013). According to the model for D1 protein resynthesis, the damaged D1 protein must be degraded before the newly synthesized D1 protein is inserted into PSII (Nath *et al*. 2013). In the cyanobacterium *Cynechocystis*, recovery from photoinhibition is dependent on low light levels which seems to be important for a protease inhibitor which breaks down damaged protein before synthesis of new D1 protein (Singh *et al*. 2005). In pea plants, D1 protein degradation depends on low light levels. Therefore, photoinhibition after high light intensity treatment relaxes faster in low light levels than in darkness (Aro *et al*. 1994). Demmig-Adams *et al*. (1990*b*) showed that recovery from photoinhibition was faster in low light intensities (2–135 µmol photons m^{-2} s^{-1}) than in darkness. However, the recovery decreased with increasing light intensity from 2 up to 135 µmol photons m^{-2} s^{-1}. Low light levels are needed to recover F_v/F_m and oxygen production from photoinhibition in the red alga *Phyllophora truncata* (Hanelt *et al*. 1992). In the cyanobacterium *Spirulina platensis* recovery was much faster in low light and the recovery was inhibited by chloramphenicol, indicating that recovery depends on resynthesis of proteins (Vonshak *et al*. 1994). However, the light requirement necessary for optimal relaxation from photoinhibition is not known for lichens. Most lichen studies take lichens directly from the field or from specific stress exposures and place them in complete darkness before measuring F_v/F_m. If their D1 protein is damaged, relaxation of F_v/F_m can be highly inefficient in darkness.

The purpose of measuring F_v/F_m may vary. One aim might be to measure photoinhibition for lichens in the field or to measure the short-term effect of some photoinhibitory treatment under controlled conditions. In such cases long-term repair of, for example, damaged D1 protein in PSII reaction centres is undesirable. However, sometimes we aim to use uniform lichen material with maximal F_v/F_m before various stress experiments. Knowledge of treatment effects on recovery from natural stress or from stress under controlled conditions is therefore important.

This study aims to quantify the effect of various low light levels on the relaxation of F_v/F_m after photoinhibitory treatment in four lichen species in order to clarify the treatment of lichens before chlorophyll *a* fluorescence and adjust the measuring protocol accordingly.

Materials and Methods

Mature and healthy thalli of *Lobaria pulmonaria* were collected from twigs of *Picea abies* in Selnes, Namsos, Nord-Trøndelag, W Norway (64°25'N, 11°25'E) in March 2008 and thalli of *Lobaria scrobiculata* were sampled on trunks of *Salix caprea* in open *P. abies* forests in Horka, Overhalla, Nord-Trøndelag, W Norway (64°26'N, 11°47'E) in May 2008. The material was air-dried and stored at −18 °C until the experiment in September 2009. Well-developed thalli of *Parmelia sulcata* and *Xanthoria parietina* were collected in Ås, Norway (59°40'N, 10°45'E) in September 2009 from stems of *S. caprea* and *Populus tremula*, respectively. The material was air-dried and stored in the dark at room temperature until the start of the experiments a few days later.

All experiments were carried out at a constant temperature (18 °C). After 24 h acclimation at 5 µmol photosynthetically active photons m^{-2} s^{-1} from standard warm white fluorescent tubes, the thalli were

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photoinhibited for 4 h beneath a high intensity LED light panel (custom made SL3500, Photon Systems Instruments, Brno, Czech Republic). Thalli of *X. parietina* and *P. sulcata* were exposed to 1000 µmol photons m^{-2} s^{-1}, whereas *L. pulmonaria* and *L. scrobiculata* were exposed to 600 µmol photons m^{-2} s^{-1}. The red (λ_{max} = 637 nm), green (λ_{max} = 537 nm) and blue (λ_{max} = 463 nm) LEDs were set at equal irradiances during the photoinhibitory treatment.

Chlorophyll fluorescence

Maximal dark adapted PSII efficiency (F_v/F_m) was measured with a PAM 2000 fluorimeter (Heinz Walz, Effeltrich, Germany) after 15 min dark adaptation. F_v/F_m values were measured immediately before the photoinhibitory treatment and during recovery at 0, 1·5, 6, 18·5 and 20 h after the end of the photoinhibitory treatment. During recovery, half of the thalli were kept in total darkness and the remainder were kept under low light intensities of 0·2, 1, 5 or 30 µmol photons m^{-2} s^{-1} from standard warm white fluorescent tubes. The thalli maintained in total darkness during recovery were moved to 0·2, 1, 5 or 30 µmol photons m^{-2} s^{-1} for 1·25 h before a final measurement of F_v/F_m.

Statistical analysis

F_v/F_m data were analyzed with a one-way ANOVA followed by Tukey's test for pairwise comparisons using Minitab version 16.2.2.

Results

All lichens studied recovered completely from photoinhibition after 18·5 h recovery at 1, 5 or 30 µmol photosynthetically active photons m^{-2} s^{-1} (Fig. 1B, D, F & H). Even at 0·2 µmol photons m^{-2} s^{-1}, *Lobaria pulmonaria* and *Parmelia sulcata* recovered completely, whereas *L. scrobiculata* and *Xanthoria parietina* did not (Fig. 1B, D, F & H). After 1·5 h under low light levels, recovery was incomplete in all four species (Fig. 1A, C, E & G). However, recovery in *L. scrobiculata* gradually improved with increasing light intensity and returned after 1·5 h to the initial value at the highest light intensity during recovery (30 µmol photons m^{-2} s^{-1}; Fig. 1A). All light intensities between 0·2 and 30 µmol photons m^{-2} s^{-1} caused a similar level of recovery within both *L. pulmonaria* (Fig. 1E & F) and *P. sulcata* (Fig. 1C & D), whereas *X. parietina* required more than 0·2 µmol photons m^{-2} s^{-1} to recover (Fig. 1G & H). The cyanolichen *L. scrobiculata* did not recover in darkness while all green-algal lichens partially recovered in darkness and most of this recovery occurred within the first 1·5 h period (Figs 1 & 2). With respect to the kinetics in recovery after the high light exposure, the recovery was much weaker in all studied species kept in darkness (Fig. 2). However, at low light intensities (0·2–30 µmol photons m^{-2} s^{-1}) for just 1·25 h after the 18·75 h recovery in darkness, all species rapidly resumed normal F_v/F_m values, at least at the higher light levels (Fig. 2).

Discussion

Complete relaxation from photoinhibition in lichens clearly depends on low light, as shown previously for red algae (Hanelt *et al.* 1992), cyanobacteria (Vonshak *et al.* 1994; Singh *et al.* 2008) and higher plants (Greer *et al.* 1986; Aro *et al.* 1994). Efficient relaxation at as low a light level as just 0·2 µmol photons m^{-2} s^{-1} (Fig. 1) suggests that light during the recovery acts as a signal for relaxation and not as an energy source during the relaxation period. Singh *et al.* (2008) argue that recovery is linked to plastid gene regulation. The green-algal lichens recovered partially from photoinhibition in darkness whereas the cyanolichen *L. scrobiculata* did not recover at all. The fast partial relaxation of F_v/F_m in green-algal lichens probably depends on relaxation of dynamic photoinhibition by conversion of zeaxanthin to violaxanthin in darkness (e.g. Maxwell & Johnson 2000), whereas rapid relaxation does not occur in the cyanolichen *L. scrobiculata* because cyanobacteria lack the xanthophyll cycle (Demmig-Adams *et al.* 1990*a*).

In relaxed leaves of higher plants the F_v/F_m is remarkably stable at 0·80–0·83 (e.g. Baker 2008) but F_v/F_m values of $\geq$0·80 rarely occur in lichens sampled from their natural habitats. This suggests that slight photoinhibition is very common in lichens. However, severely photoinhibited *L. pulmonaria* thalli which were stored for 48 h at 5 µmol photons m^{-2} s^{-1} in a hydrated state did not fully recover from photoinhibition (Gauslaa & Solhaug 1996). It seems that variable levels of lasting photoinhibition are common for lichens under natural conditions (e.g. Gauslaa

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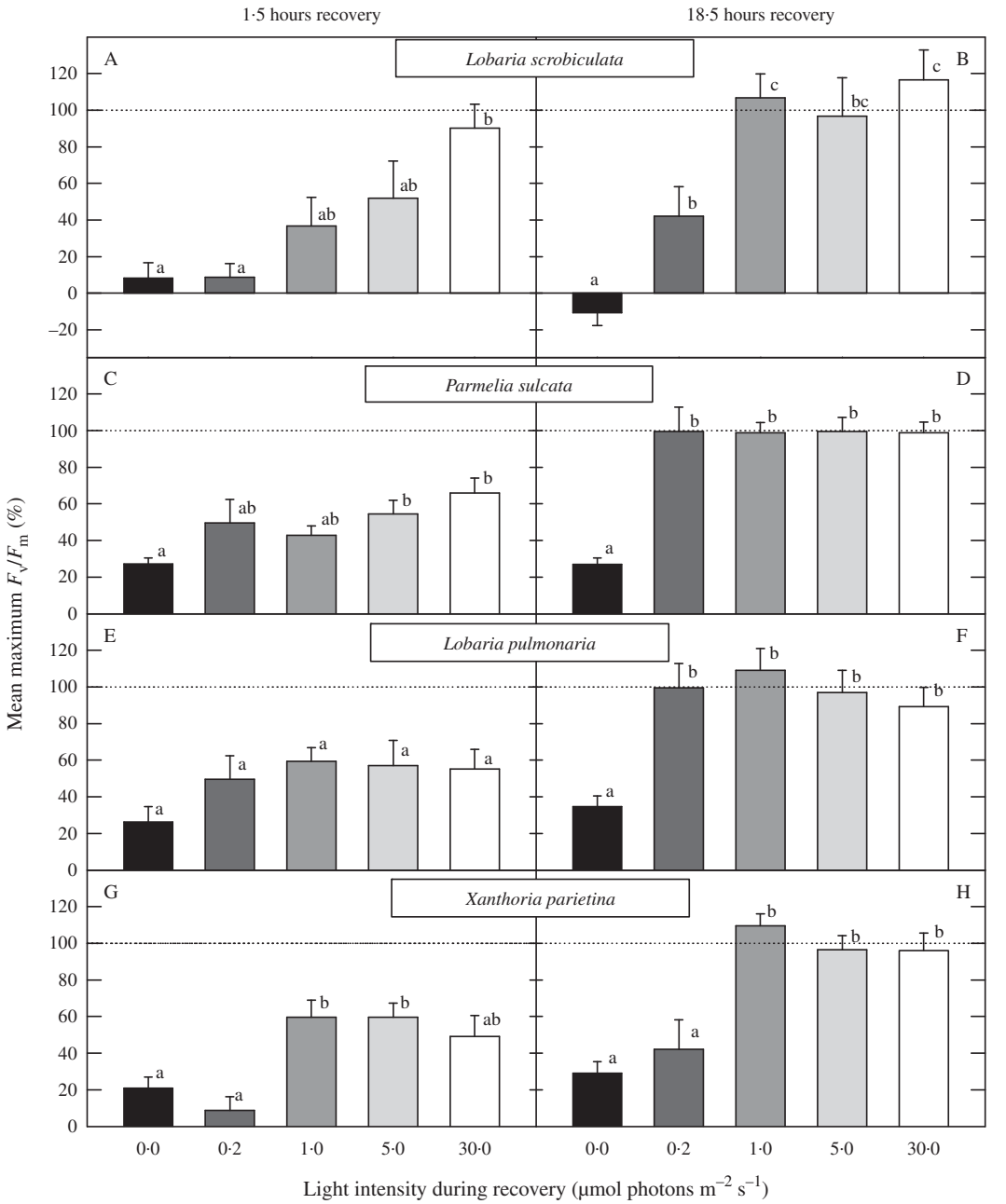

FIG. 1. Mean percentage recovery of F_v/F_m from photoinhibition after 1·5 h (A, C, E & G) and 18·5 h (B, D, F & H) in *Lobaria scrobiculata* (A & B), *Parmelia sulcata* (C & D), *L. pulmonaria* (E & F) and *Xanthoria parietina* (G & H) exposed to light intensities of 0, 0·2, 1·0, 5·0 and 30 μmol photons m^{-2} s^{-1}. Values with the same letter above the columns are not significantly different (Tukey's test for pairwise comparisons). Mean values ($n = 10$) are plotted +1SEM.

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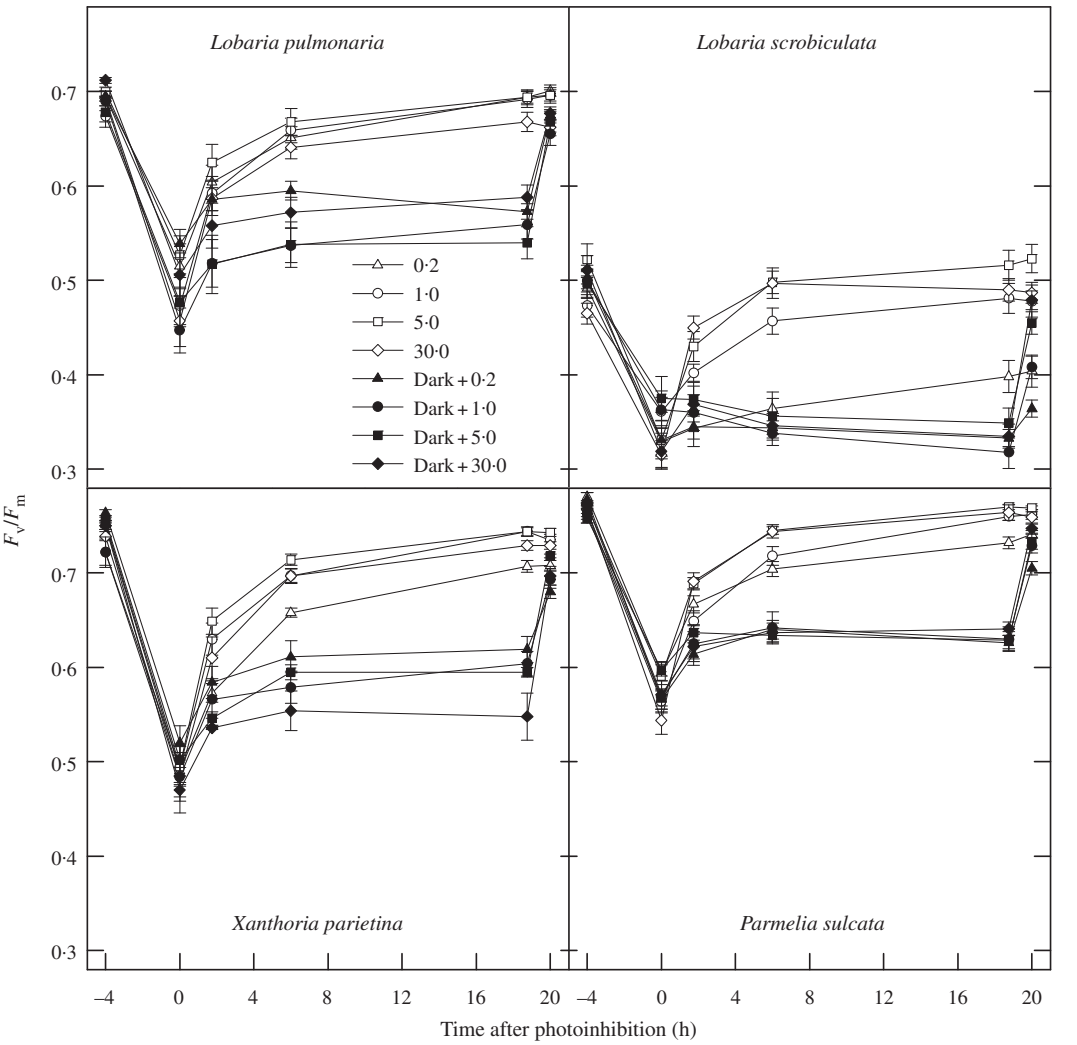

FIG. 2. Kinetics of maximal PSII efficiency (F_v/F_m) recovery after 4 h strong photoinhibitory light (600 µmol m^{-2} s^{-1} for *Lobaria scrobiculata* and *L. pulmonaria*, and 1000 µmol m^{-2} s^{-1} for *Parmelia sulcata* and *Xanthoria parietina*). F_v/F_m values were measured before high light treatment (−4 h), immediately after (0 h), and then after 1·5, 6 and 18·5 h during recovery in darkness (closed symbols) or under low light intensities of 0·2, 1·0, 5·0 and 30 µmol photons m^{-2} s^{-1} (open symbols). Before the last measurement (20 h) all thalli were kept under low light intensities for 1·25 h. Mean values ($n = 10$) are plotted ±1SEM.

et al. 2012; Färber *et al.* 2014) and that it varies with season (Vráblíková *et al.* 2006). In order to start experiments with more uniform and relaxed lichen material, it is important to acclimate lichens in low light, and not in darkness, for as long as 1–2 days to recover from natural photoinhibition before experiments. In cyanolichens, maximal F_v/F_m values are rarely higher than 0·6. These low values in cyanolichens may be caused by fluorescence from phycobilisomes contributing to increased F_o (Campbell *et al.* 1998). In addition, several compounds are shared between the photosynthetic and respiratory electron transport chains in cyanobacteria resulting in PSII not being fully oxidized in

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darkness (e.g. Binder 1982). Higher PSII yields than when dark-adapted can be achieved by exposure to low blue light that excites mainly PSI, resulting in more oxidized PSII (Solhaug *et al.* 2014).

Both the reduction in the effective quantum yield of PSII and F_v/F_m under photoinhibitory light and the recovery of these two parameters are similar in *L. pulmonaria* (Barták *et al.* 2006). Rapid recovery from photoinhibition under low light, as shown in Fig. 2, might be ecologically important. On a clear day, a lichen may become photoinhibited and it will desiccate during the day. Photoinhibition will stay constant or increase during the period in the dry state, depending on light exposure. The lichen thallus will regain moisture due to condensation during the night with a photosynthetic active period during the first part of the day (e.g. Green *et al.* 2008). The low light period at dawn might therefore be important for fast recovery of F_v/F_m and effective quantum yield of PSII before the period with photosynthesis in the early part of the day.

It seems that most green-algal lichens can gain F_v/F_m values close to 0·8 (Gauslaa & Solhaug 1996). However, F_v/F_m is often considerably lower. F_v/F_m values in the range of 0·45–0·65 have often been considered as normal under non-stressed conditions (Bačkor *et al.* 2006 and references therein). Such low F_v/F_m values are probably a result of long-term photoinhibition that slowly recovers. Lichens may have a more long-term downregulation of PSII efficiency similar to the sustained downregulation of PSII efficiency common in conifers during winter (Adams III *et al.* 2001). The ecological function of this sustained downregulation of PSII may be protection against high light during rapid changes in light levels.

In conclusion, this study shows that it is necessary to allow lichens to recover under low light intensities to optimize relaxation from photoinhibition. In darkness, relaxation is incomplete. If the purpose of an experiment is to measure natural photoinhibition, only dark adaptation should be used before F_v/F_m measurement, whereas if standardized lichen thalli without photoinhibition are needed, a long pretreatment under low light intensities is recommended.

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