Chlorophyll fluorescence characteristics of the cyanobacterial lichen *Peltigera rufescens* under field conditions

I. Seasonal patterns of photochemical activity and the occurrence of photosystem II inhibition

JOHANNA M. R. LEISNER, WOLFGANG BILGER and OTTO L. LANGE

Julius-von-Sachs-Institut für Biowissenschaften, Universität Würzburg, Mittlerer Dallenbergweg 64, D-97082 Würzburg, Germany

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Summary

Photosystem (PS) II fluorescence of the cyanobacterial lichen *Peltigera rufescens*, together with microclimate parameters (light, temperature, relative humidity, and rainfall), was recorded over a complete year (September 1992 – August 1993). Measurements were made on thalli at two quasi-natural growing sites in a xerothermic steppe formation in the Botanical Garden, Würzburg. The sites spanned the natural habitat range for the species, one being partly shaded whilst we increased exposure at the other by removing the steppe canopy. Chlorophyll fluorescence parameters were automatically determined at 20 minute intervals using a PAM-2000 fluorometer. From the fluorescence data, metabolically active phases of the poikilohydrous lichen could clearly be distinguished from dormancy. In the majority of cases, dormancy could be attributed to desiccation. During winter, frost inhibited activity completely at temperatures below -5°C. Metabolic activity of the lichen occurred over a wide range of temperatures and light conditions including periods of very high light when photoinhibitory damage might have been expected. However, values of the optimal quantum efficiency of PS II (determined under low light at dawn) showed no depression (photoinhibition) except when metabolic activity of the lichen had been severely curtailed by frost and/or extended drought. The inhibition was reversed after even brief periods of normal metabolic activity. *Peltigera rufescens*, therefore, seemed to be well adapted to its natural environment and showed little photoinhibition as long as it frequently hydrated and became metabolically active.

Key words: *Peltigera rufescens*, cyanobacteria, lichen, chlorophyll fluorescence, metabolic activity, light, photoinhibition, stress, quantum yield

1. Introduction

Lichens are poikilohydric and their thallus hydration is, therefore, strongly dependent on the water status of their environment. In most habitats lichens dry out frequently, and their metabolic activity is confined to time periods of favourable thallus water content which, consequently, effectively determines primary productivity. Many investigations have been undertaken to study the hydration-dependent photosynthetic performance of lichens in the field under different climate conditions (KAPPEN 1988). In the temperate region of Europe, CO₂ exchange and water relations were measured by SCHULZE & LANGE (1968) of *Hypogymnia physodes* in its natural habitat in northern Germany, BRUNS-STRENGE & LANGE (1991) and LANGE & BRUNS-STRENGE (1991) studied the performance of *Cladonia portentosa* on its sand dune site on a North Sea island, and HAHN et al. (1989) investigated several lichen species in a local xerothermic steppe formation near Würzburg. However, for technical reasons, CO₂ exchange measurements in the field are only possible for limited periods of time (days or weeks). As a consequence, no long-term studies of lichen metabolic performance obtained with this method exist in the literature.

A recently developed alternative method, modulated fluorometry, has made it possible to monitor photosystem (PS) II fluorescence of plants automatically and reliably for long periods. The PAM-2000 fluorometer has been especially designed for measurements under field conditions (BILGER et al. 1995) and is excellently suited for work with lichens (SCHROETER et al. 1992). As
a result, continuous observation of lichen activity in the field has become possible. Data acquired with a PAM-2000 fluorometer allow not only detection of the activity state of a lichen but also access to information about light use efficiency and possible inhibition of the photochemical apparatus (for reviews see Krause & Weis 1991; Schreiber & Bilger 1993). Schoeter & Schulz (1995), in the maritime Antarctic, were the first to use fluorescence measurements to estimate annual production of lichens.

The terricolous cyanobacterial lichen Peltigera rufescens (Weis) Humb. is a widespread foliose species. Its habitat range stretches from the arctic through the boreal, temperate, and mediterranean zones, and from sea level to the alpine region (Vitikainen 1994). According to Klement (1955), P. rufescens characterises the phytosociological order of the European soil lichen communities (Epigaeetalia Klem.), and is frequently encountered as a member of the lichen communities Fulgensietum fulgantis Gams and Cladonietum convolueae Müller within the locally occurring Franconian xerothermic steppe formations around Würzburg.

The sparse canopy of higher vegetation in this type of habitat allows considerable light penetration to plants growing on the ground. Accordingly, any terricolous lichen must possess mechanisms by which high light stress can either be avoided or tolerated. Lichens often become desiccated during high irradiation, and this could be considered a simple way to avoid photoinhibition. Short-term laboratory studies have shown that lichens are much more sensitive to high light in the hydrated state (Demming-Adams et al. 1990). Consequently, the occurrence of photoinhibition in a lichen under natural conditions might not solely depend on light exposure but rather on a complex interplay between several microclimatic factors which determine thallus hydration.

The long-term study reported here was carried out to clarify both lichen performance and the possible occurrence of photoinhibition under natural conditions. PS II fluorescence of P. rufescens, together with microclimate parameters, was recorded over a complete year at a quasi-natural growing site in a xerothermic steppe formation in the Botanical Garden, Würzburg. In this paper, we describe characteristic seasonal patterns of lichen activity with respect to microclimate, and focus special attention on conditions expected to lead to inhibition of PS II quantum yield efficiency. In a following publication, different diel courses will be examined, and their distribution during the course of the year discussed; in parallel to these measurements, also the seasonal changes in carotenoid content of the lichen were studied (see also Leisner et al. 1994; for further details see Leisner 1995).

2. Materials and methods

2.1. Site description and time period of measurements

Peltigera rufescens was studied in the “xerothermic steppe formations of Central Europe” section of the Botanical Garden, Würzburg. Natural populations can be found which grow over moss within the open phanerogamic vegetation. Studies were made of P. rufescens at two sites that spanned a large part of the species natural habitat range (see Wirth 1995). One site was left undisturbed (i.e. it remained “partially shaded”), while an adjacent site had the higher vegetation canopy opened in order to provide an unshaded, “open” habitat. Microclimate data were recorded simultaneously at both sites. PS II fluorescence measurements were taken alternately at the two sites. Over the period Sep., Oct., and Nov. 92, the measuring site was changed once a month; then, from Dec. 92 through Aug. 93, twice a month. In December 92 and January 93 several days of fluorescence measurements (9 and 6, respectively) were lost because of equipment (laptop computer) failure due to strong frost. Otherwise, recording of microclimate and fluorescence performance of the lichen took place continuously from September 2nd 92 through August 27th 93.

2.2. Microclimate measurements

At each of the two measuring sites, incident photon flux density (PFD) was measured using one horizontal quantum sensor (LI-190 SB, Li-Cor, Lincoln, Nebraska, USA) placed about 5 cm from the lichen thallus on which fluorescence was monitored. Thallus temperature was measured by a copper-constantane thermo-couple (0.3 mm wire diameter) pushed into the lichen from below. About 50 cm from the two sites, a Thermoelement-Pychrometer (System Černuska, Innsbruck, Austria) was installed 5 cm above ground for measurement of ambient air temperature and relative humidity (rh). At a distance of about 5 m from the station, a rain gauge (Tipping Bucket Raingauge DRG 3, 0.2 mm/tp, Campbell Scientific Inc., Logan, Utah, USA) measured rainfall. Microclimate data were recorded with a micrologger (21X-Micrologger, Campbell Scientific Inc.). Temperature, PFD and rh were sampled every 30 s and stored as 10 min means. Rainfall was recorded as 10 min sums. The data were transferred at weekly intervals into a storage module (Solid-State Storage Module SM 192, Campbell Scientific Inc.) and subsequently into a computer for further processing.

2.3. PS II fluorescence under field conditions

A portable, modulated fluorometer (PAM-2000, Walz, Effeltrich, FRG) was installed at the measuring station. The PAM-2000 was operated by a laptop computer which also calculated and stored the data. Both of these instruments were supplied with line power. The fibreoptic of the PAM-2000 was firmly fixed in a perspex holder (Fig. 1) so that a constant
angle (60°) and distance (1 to 2 cm), with respect to the lichen thallus, was maintained during the measurements. Always a thallus lobe that was predominantly horizontal and flat was used for measurement, and the equipment was placed so that it did not shade the lichen at any time of the day.

A weak, modulated measuring beam of less than 1 μmol m⁻² s⁻¹ PFD was operated continuously so that steady state fluorescence yield could be determined (F₀ at night and F during the day; nomenclature of the fluorescence parameters follows van Kooten & Snel 1990). Every 20 min, a saturation pulse (PFD of 3000 μmol m⁻² s⁻¹ and 1 s duration) was applied to measure maximal fluorescence yield (Fₘ at night and Fₘ' in the daytime). The time interval of 20 min was chosen as a reasonable compromise between a need for continuous monitoring of PS II activity and the minimizing of any alteration to the natural environmental conditions. From these data, quantum yield of linear electron transport through PS II (Φₑ) was calculated as Fₚ/Fₘ = (Fₘ - F₀)/Fₘ, nighttime; and Φₑ = Fₘ' - F)/Fₘ', daytime (Genty et al. 1989). During the field measurements, when the lichen was dry, fluorescence signals became so low that the absolute value was of the same order of magnitude as the normal internal variability of the instrument. On those occasions Φₑ values became unreliable, and such data points were omitted from the plots of diel courses. The sensitivity of the PAM-2000 is affected by ambient temperature (WALZ NEWS 2, Jan. 92, Walz). This only concerns measurements of absolute fluorescence yields (e.g. F₀) but does not alter the relative parameters calculated here.

2.4. PS II fluorescence in the laboratory

In addition to the field studies, the dependence of the fluorescence parameters of P. rufescens on PFD and thallus hydration were measured under controlled conditions. A PAM 101 fluorometer (including a FL 103 unit, Walz) was used with saturation light pulses provided by a halogen light source (KL 1500, Schott, Mainz, FRG). Light response curves of Φₑ were produced by adjusting actinic light intensities (to 6, 12, 24, 55, 115, 150, 350, 800 μmol m⁻² s⁻¹ PFD) with neutral density filters (NG series, Schott) in a LS 2 light source (Hansatech, Bachofen, Reutlingen, FRG; 6 and 12 μmol m⁻² s⁻¹) or in a second halogen light source (KL 1500, Schott; all other intensities).

A dry thallus of P. rufescens collected on April 20th 93 was moistened, cleaned, and reactivated at 20°C and 10 μmol m⁻² s⁻¹ PFD for 1 hour. A well hydrated lobe was then placed on a moist sponge inside a light-tight brass cuvette which had an apertue sized to take the fluorometer fibreoptic. This cuvette remained at a constant (room-) temperature. After darkening the sample for 5 min in the cuvette, the pulsed measuring beam was switched on and a saturation pulse was applied (measuring F₀ and Fₘ, respectively). The actinic light was then turned off for 2 min and, after this recovery period, a saturation pulse was applied (to determine FₘD = the maximal fluorescence yield after brief darkening following a light period). The minimal fluorescence yield during this period was taken as F₀'.'

The dependence of the fluorescence parameters on thallus hydration was investigated by placing a cleaned and well hydrated (sprayed but not supersaturated) lobe of P. rufescens thallus on a computer operated, automatic balance (PM 480 Delta Range, Mettler-Toledo AG, Greifensee, Switzerland). The fluorometer fibreoptic was fixed above the thallus and, with a constant actinic PFD of 150 μmol m⁻² s⁻¹, saturation pulses were applied at 100 s intervals. The weight of the sample was recorded as it slowly dried out and, subsequently, the dry weight (DW) of the thallus was determined (3 days at 70°C) to calculate the weight loss of the thallus hydration.

3. Results

3.1. PS II fluorescence of Peltigera rufescens: dependence on hydration and incident PFD

The PS II fluorescence of the lichen determined under controlled laboratory conditions is portrayed in Fig. 2 for thallus hydration, and Fig. 3 for PFD dependence. In thallus hydration studies (Fig. 2), maximal Φₑ was typically reached at water contents (WC) of around 150–170%. Φₑ started to decline when WC decreased below 150%, and, together with the absolute fluorescence yields (F and Fₘ) declined rapidly at WC below 100%. At WC lower than 60%, while the absolute fluorescence signals were measurable and still declining, Fₘ had decreased to the level of F, causing Φₑ to become 0. At WC of 20 to 30% the absolute fluorescence signals had reached a constant low level about one tenth of that of a well hydrated lichen. These results for
optimal and suboptimal WC are in agreement with the findings of Lange et al. (1995), who measured CO₂ exchange in two species of the genus Peltigera. Suprasaturating WC was not tested in the present study, however, Φₑ was not found to be altered at suprasaturating WC by Lange et al. (1996).

The light dependence of the fluorescence parameters in a well hydrated lichen (Fig. 3) show, in general and as found for leaves of higher plants (Genty et al. 1989; Schreiber & Bilger 1993), that Φₑ decreased with increasing PFD. However, in contrast to higher plants, maximum quantum yield of PS II was not reached in the dark pre-adapted state, but at low PFD (Fig. 3a, see Rouag & Dominy 1994; Schreiber et al. 1995). The exact PFD producing such a maximal Φₑ changed slightly with the overall light adaptation of the lichen, i.e. it became higher for lichens adapted to higher growth PFD (data not shown). The rise of Φₑ at low PFD was partially due to F₉ being higher than F₉', and also to a lowering of steady state fluorescence in the light (F) relative to F₀ (Figs. 3b and c). This suggests a reduction of Qₐ in darkness. After exposure to higher PFDs, F₉ and F₉' remained more or less similar but, after low actinic light, F₉ was considerably lower than F₉' and also slightly lower than F₉ (Fig. 3b). Minimal fluorescence in darkness subsequent to illumination (F₀') was also substantially lowered following the measurements at low light but remained at a relatively constant level of about 70% of F₀ after periods of higher PFD (Fig. 3c).

![Fig. 3. Dependence of fluorescence parameters of a well hydrated thallus of P. rufescens on incident PFD. During measurements the lichen was kept at room temperature and ambient air. PS II quantum yield was calculated as ΔF/F₉'. F₉ was determined after a dark period of 2 min subsequent to the illumination at the indicated PFD.](image)

3.2. Metabolic and photochemical activity of Peltigera rufescens, and microclimate under field conditions over a full year

Microclimate data from the two study sites, one shaded and one open, showed that PFD incident on the lichen could differ between them by a factor of up to 3 on clear days, but that air temperature differences were only slight. The activity patterns of P. rufescens were found to depend much more strongly on the general weather situation than on the differences in microclimate between these sites. The data sets from both sites were, therefore, suitable to be combined to provide a continuous record over the entire year on the lichens metabolic performance under field conditions. For simplicity,
one or two series of five consecutive days for each of the four seasons will be presented. These series are examples of typical microclimatic conditions for the respective season, showing representative patterns of activity and photochemical efficiency for the lichen.

The first series, autumn 1992, was selected from the second half of September (22nd through 26th), when relatively warm and dry weather still prevailed (Fig. 4). During this period, the lichen showed diel cycles of hydration and desiccation similar to those found throughout most of the summer (see also Figs. 8 and 9). Day one (Sept. 22nd) was bright and sunny, PFD reached 1200 μmol m⁻² s⁻¹ around noon, and thallus temperature rose to 45°C, 17 K above air temperature. The lichen was dried out and, as a result, no variable chlorophyll fluorescence was measurable, and quantum yield was zero. It should be noted that high thallus temperatures well above air temperature were typical for dry thalli at high PFD. In contrast, under the same conditions, moist thalli were only slightly above air temperature or, under low PFD, even slightly below it. At night, thallus temperature tracked air temperature much more closely. On day two (Sept. 23rd), dew condensation probably occurred around sunrise and hydrated the thallus. Dewfall was not measurable with the automatic rain gauge but its occurrence became obvious by a slow and steady rise in quantum yield at that time. The day was cloudy, humid, and not too warm. Φₑ sharply decreased shortly before noon, indicating that the lichen had dried out at this point. Light rainfall in the afternoon resulted in a second rise in Φₑ, after which the lichen became dehydrated again and showed no more diurnal photochemical activity. The response pattern was repeated on the later days of this series, and only differed on Sept. 25th when another rainfall event occurred leading to a rapid rise in Φₑ, in contrast to the slow rises during dew hydration.

The second series, also in autumn, was selected from the second half of November (19th through 23rd). This was a period of cool and rainy weather, and on these overcast days PFD did not exceed 80 μmol m⁻² s⁻¹ (Fig. 5, note the PFD scale being 10% of Fig. 4). Rainfall was reported on every day, and the lichen did not dry out at all. Under these conditions of continuous hydration, P. rufescens maintained a high quantum yield which was only influenced by the PFD through most of the meas-

Fig. 4. Time course of quantum yield of PS II (bottom panel), and of the microclimate at the growing site of P. rufescens in the Botanical Garden of Würzburg from Sept. 22nd through Sept. 26th 1992 at the open growing site. Depicted are relative humidity (rh, dashed line), and temperatures of air (thick line) and lichen thallus (thin line, top panel), and PFD (thin line, center panel), as 10 min means, and rainfall (black columns, center panel) as 10 min sums (scaled from top to bottom). Quantum yield of PS II fluorescence at night (filled diamonds) and during the light phase (open diamonds) were measured once every 20 min. The lichen showed frequent hydration/desiccation during the depicted time period.
uring period. In this series, the enhanced $\Phi_e$ at low light (see also Fig.3) could be observed very clearly at dawn as well as at dusk. During the remainder of the light phases, $\Phi_e$ showed the normal variation associated with changes in incident PFD. In the night from Nov. 20th to Nov. 21st, quantum yield declined for the hydrated thallus when temperatures fell below 0°C. After this event, quantum yield during the following dawn/dusk and night remained slightly lower than before. This suggests a cold-induced inhibition of the photochemical apparatus which was not immediately reversible.

One series from Dec. 29th 1992 through Jan. 2nd 1993 was chosen to characterize frosty winter conditions (Fig. 6). Every night, air temperature fell below −10°C, and it rose to about 0°C only for a few hours per day around noon. The thalli of *P. rufescens* were obviously not desiccated, but contained frozen water. This can be concluded from the onset of photochemical activity in the absence of precipitation, as soon as thallus temperature rose above −2°C. Depressed $\Phi_e$ at temperatures below 0°C had already been observed in the November series (Fig. 5). When thallus temperature fell below −5 to −6°C, quantum yield became zero (Fig. 6). This presumably indicates dehydration of the cyanobiont by extracellular ice formation (SCHROETER et al. 1994). The short phases of photochemical activity around noon suggest that at least some of the ice melted and (partially) rehydrated the cyanobionts. As temperatures decreased during this series of days, a progressive shortening of the active phases was observed together with lower values of $\Phi_e$ at almost identical PFD. This could have resulted either from an accumulating inhibition of PS II or from lower cyanobiont hydration due to lower temperatures. Over the whole period, the lichen showed hardly any activity in the dark. This could have limited repair and adaptational processes. On Jan. 2nd, temperatures remained below freezing for the entire day, and $\Phi_e$ remained zero. In contrast to the autumn series, thallus temperature of the lichen was approximately 5 K above air temperature during the nights, but stayed slightly below air temperature around noon. The close contact of the lichen to the ground possibly resulted in some conductive heat transfer which led to the change in temperature pattern.

Early spring of 1993 was very dry, and is well represented by a series from the second half of March (20th through 24th; Fig. 7). Prior to this period there had been 11 days of dry and sunny weather during which the lichen had only once been slightly moistened by dewfall. In the evening of March 21st some rainfall was reported at the weather station of the Deutscher Wetterdienst Würzburg 2.5 km away from the Botanical Garden (the tipping bucket rain gauge being out of order at the time). This should have been sufficient to thoroughly hydrate
Fig. 6. As Fig. 4, but from Dec. 29th 1992 through Jan. 2nd 1993 at the partially shaded growing site. At subzero temperatures rh was not determined. Metabolic activity of the lichen was severely limited by frost.

Fig. 7. As Fig. 4, but from March 20th through March 24th 1993 at the open growing site. During this period the automatic rain gauge was out of order. The indicated rainfall was reported at the weather station of the Deutscher Wetterdienst Würzburg, which is 2.5 km away from the Botanical Garden. The lichen had been dried out for several days, and a strong inhibition of PS II was evident on the occasions when the thallus was hydrated.
Fig. 8. As Fig. 4, but from July 16th through July 20th 1993 at the open growing site. The lichen showed frequent hydration/desiccation cycles due to repeated precipitation. Rainfall provided thorough thallus hydration on most days, and the lichen remained photochemically active far into the days, receiving rather high PFDs prior to and during desiccation.

Fig. 9. As Fig. 4, but from Aug. 21st through Aug. 25th 1993 at the open growing site. The lichen’s metabolic activity was severely limited by drought during this period. The sparse hydration by one slight rainfall and one occasion of dewfall did not lead to optimal photochemical activity.
the lichen since, at the same time, quantum yield increased suddenly in a manner typical for rehydration after rain (see Fig. 4). \( \Phi_e \) initially remained at a very low level until dawn, after which it rose slightly until the lichen dried out. The occurrence of such a low quantum yield in a well hydrated lichen indicates a severe inhibition of PS II efficiency. This was possibly caused by the preceding periods of extended and severe desiccation and frosts (see Fig. 10). On the following day a very similar course of events occurred. On March 23rd, quantum yield both in the dark and the light was somewhat higher than on March 22nd. It appears that a certain amount of recovery had occurred when the lichen had had one period of substantial hydration allowing activity for several hours in the dark and in moderate light.

A series from mid-July (16th through 20th) was selected to illustrate one particular aspect of lichen performance during summer (Fig. 8). The weather was warm and sunny but unstable with frequent rainfall. Except for July 18th, \( P. \) rufescens had always been well hydrated from rainfall during the night, and the typical enhanced \( \Phi_e \) in low light was seen at sunrise. The lichen then dried out every day during the light phase but usually not before PFD had substantially exceeded 1000 \( \mu \)mol m\(^{-2}\) s\(^{-1}\). Nevertheless, during this period when the lichen became hydrated regularly, desiccation under high PFD did not lead to an inhibition of the photochemical apparatus. Instead, quantum yield of the well hydrated lichen reached very high values every day at dawn (and also at dusk on July 19th).

A further summer series was selected from the second half of August (21st through 25th), when sunny, hot, and dry weather prevailed (Fig. 9). The thalli remained desiccated during most of this period except for Aug. 23rd when a slight rainfall occurred. The photochemistry of the lichen was reactivated for a few hours by this rain, and also during the following night when slight hydration by dew took place. The suppressed quantum yields on these two occasions may indicate either suboptimal hydration or long lasting inhibition of PS II quantum yield.

3.3. Quantum yield efficiency of \( Peltigera \) rufescens and the occurrence of inhibition of PS II

We carefully examined the entire data set to find any indications of lasting inhibition of PS II photochemical efficiency. For this investigation, all those days were selected on which \( P. \) rufescens was apparently well hydrated around sunrise but not frozen. On such days, the typical increase in \( \Phi_e \) in low light over dark quantum

![Fig. 10. Annual course of dawn PS II quantum yield at low light, and microclimate data, monitored under field conditions on \( P. \) rufescens. Circles: elevated dawn \( \Phi_e \) of well hydrated but not frozen lichen thalli growing in the open (open symbols) or in partial shade (filled symbols). Narrow columns: integrated PFD during lichen metabolic activity per day. Marked below are the days on which the thalli were desiccated during the entire light phase (Thallus desicc.), and those on which temperatures below 0°C occurred (Frost).](image-url)
yield (see e.g. Fig. 3) could be observed. When $\Phi_e$ is elevated in this manner during low light, quantum yield cannot be reduced by incomplete thallus or cyanobiont hydration, and respiratory and/or light induced reduction of the electron transport chain are minimal. Therefore, these values give the best nondestructive indication of the functional state of PS II for a cyanobacterial lichen in the field. All selected values of such dawn $\Phi_e$ are depicted in Fig. 10 (open circles, open site values; solid circles, shaded site values).

We further examined the possible influence of light on inhibition of PS II by calculating the integrated daily totals for light received during periods when the lichen was photochemically active (Fig. 10, narrow bars). This set of data contains more values than the quantum yield data measured on the well hydrated lichen because days with subsaturating thallus hydration, or when activation of the lichen occurred after sunrise, are included. In addition to excessive PFD, we also considered other possible stress factors by indicating days when subzero temperatures occurred, and days without photochemical activity because of thallus desiccation.

Peltigera rufescens, whether growing at the open or the partially shaded site, had dawn $\Phi_e$ values between 0.48 and 0.68 during most of the annual cycle. There was a small tendency for the exposed samples to show a lower $\Phi_e$ than partially shaded thalli, possibly indicating slight photoinhibition. However, in July (see Fig. 8), despite receiving much higher PFD when hydrated, the lichen at the exposed site had $\Phi_e$ that were as high as those from thalli at the partially shaded site.

Severely lowered dawn $\Phi_e$, indicating PS II inhibition, occurred only during February and March 93 for thalli growing at the open site. Previous to and during those times, lichen metabolic activity had been strongly inhibited by frost (e.g. see Fig. 6). In addition, March 93 was very dry, and active phases were rare for the lichen (Fig. 7). After similar periods of prolonged thallus desiccation at other times, dawn $\Phi_e$ of P. rufescens also showed distinct depressions (e.g. mid July, Fig. 10). It is uncertain if these were caused by incident light or were a consequence of extended dehydration.

4. Discussion

This investigation had two main objectives: first, to attempt to monitor the photosynthetic activity of a lichen over a calendar year using a non-contact measuring system, the PAM-2000 modulated fluorometer; second, to discover how often, and when, the cyanobacterial lichen P. rufescens showed evidence of PS II inhibition. Both objectives were successfully attained. The fluorometer measured the PS II state of the lichen every 20 minutes for twelve months, the only interruptions being when the machine was moved between sites, and a brief period in which the laptop computer was affected by severe frost. Because the fluorometer fibreoptic was several mm from the lichen, the thallus could fully equilibrate with the environment, an important requirement when dealing with a poikilohydric plant. One possible problem might arise from the small scale variation in irradiation that could have occurred due to the inhomogeneous surface of the lichen. The PAM-2000 measures average $F_M$ and $F$ over an area of about 0.5 cm$^2$, and thallus movement during drying and hydration might have caused changing areas to be measured at different times. This uncertainty will always persist. The consistency of the results, however, suggests that it caused probably only minor deviations. Certainly this study agrees with the results of SCHROETER et al. (1992) who used a similar system to monitor lichen activity in the Antarctic. Because the obtained data allow the active phases of the lichen to be quantified they offer the opportunity to estimate productivity over chosen periods. This aspect will be presented in a forthcoming paper with a detailed analysis of the daily response patterns of P. rufescens under field conditions.

The fluorometer measured the quantum efficiency of PS II so that any inhibition could be detected. Interpretation of inhibition requires some understanding of factors likely to affect it. Preliminary laboratory studies confirmed that, under temperatures around the optimum for P. rufescens, $\Phi_e$ was affected by both thallus water content and PFD level. When temperatures were above zero, a low or zero $\Phi_e$ and strongly decreased absolute fluorescence yields indicated that the lichen had become desiccated and was effectively dormant. A high $\Phi_e$ in darkness indicated that the lichen was potentially photosynthetically active, i.e. was hydrated. These results are in agreement with LANGE et al. (1996) who did a comparison of gas exchange and fluorescence parameters for the closely related cyanobacterial lichen Peltigera neckeri. Also, by taking the $\Phi_e$ values at times of low PFD, dawn or sunset, it was possible to judge the intrinsic efficiency of PS II. A maximal quantum yield of around 0.6 is considered normal for non-inhibited cyanobacterial lichens (DEMMIG-ADAMS et al. 1990).

Utilising these guidelines it is possible to analyze the data presented here, especially taking into account the time sequence of events, in order to evaluate the influence of important environmental factors on the quantum yield of the lichen in the field. First we consider those factors that have a direct effect on quantum yield but an effect that is reversible.

Water content: The WC of the lichens was the factor which caused the largest changes in $\Phi_e$ and which limited photochemical activity the most. There is little doubt that the major proportion of observed reductions of $\Phi_e$ to zero can be attributed to drying since such
symptoms, with one exception documented below, were always correlated with a lack of water availability. Where the drought had not lasted too long, rainfall restored \( \Phi_e \) with kinetics in the order of time resolution of our measurements (e.g. Fig. 8).

**Temperature effects, especially frost:** High temperatures were recorded for the thalli but only during high PFD and when dry. Temperatures of moist lichens were close to ambient and never approached levels likely to produce heat damage. However, subzero temperatures always resulted in depressed \( \Phi_e \). At temperatures of \(-5^\circ C\) and below, activity had completely stopped. As the temperature fell from zero to \(-5^\circ C\) there was a sharp decline in \( \Phi_e \). It is presumed that, below \(-5^\circ C\), the lichen has frozen, and transfer of water to extracellular ice has dehydrated the cyanobacteria. At subzero temperatures above \(-5^\circ C\) the decline in \( \Phi_e \) probably indicates either a direct effect of low temperature or a progressive dehydration due to transfer of water to external ice.

**Light intensity:** When the lichen was thoroughly hydrated after rain or during humid weather conditions (e.g. in November) the WC was not limiting \( \Phi_e \), and the influence of incident PFD was obvious. Under natural conditions it was similar to what had been observed in the laboratory (see above). Under high PFD \( \Phi_e \) was reduced due to limitation of activity of PS II by energy consuming processes. However, \( \Phi_e \) never fell below 0.3 as long as the lichen remained well hydrated and no substantial inhibition of PS II had already occurred (see Figs. 8 and 5). At low PFD at dawn and dusk, \( \Phi_e \) was actually enhanced in comparison to the values observed at night; a contrast to what has been found for higher plants. This phenomenon was clear in both field and laboratory measurements (Fig. 3), and is already known from laboratory studies with non-lichenized cyanobacteria (e.g. Rouag & Dominy 1994). Cyanobacteria, and obviously also cyanobacterial lichens, are in a low fluorescing state (state 2; Williams & Allen 1987) in the dark because, in contrast to higher plants, part of their electron transport chain not only serves light driven but also respiratory electron transport (for a review see Scherer 1990). Therefore, in the dark, their plastoquinone pool is reduced to an extent which is sufficient for the induction of state 2 in which excitation energy is preferentially distributed to PS I, and state 1 is only induced by low PFD (Dominy & Williams 1987; Mullineaux & Allen 1990; Mi et al. 1992). This leads, in an experimentally produced light response curve, to the occurrence of a maximal quantum yield at low PFD (Fig. 3), and, in field measurements, to an elevated low light \( \Phi_e \) at dawn and dusk while the lichen thallus was well hydrated (e.g. Fig. 5). In this study, the regular 20 min determinations were frequent enough to track the maximal values of \( \Phi_e \) just after dawn which could then be routinely used as an indicator of intrinsic PS II efficiency.

**Sustained inhibition of PS II:** Changes in intrinsic PS II efficiency that could indicate irreversible effects on PS II including photoinhibition were sought in the data. Of particular interest was the effect of high PFD which, under short term laboratory conditions, has been shown to cause strong photoinhibition in cyanobacterial lichens (Demmg-Adams et al. 1990). Throughout the annual course, *P. rufescens* showed dawn values of \( \Phi_e \) varying between 0.48 and 0.68 both at the open and the partially shaded site (Fig. 10). The exposed samples revealed a tendency to lower values in comparison to the thallus in partial shade, possibly indicating slight photo-inhibition. Nevertheless, even exposed lichens appeared to be largely resistant to high PFD during the warmer season. At that time, dry lichens typically became hydrated at night and dried out during the light phase of the next day (see Figs. 4 and 7 through 9). Depending on the extent of thallus hydration and the weather conditions, *P. rufescens* could be subject to quite high PFD during photochemical activity, at least shortly before desiccation. However, this was apparently not sufficient to lead to a persistent depression in PS II quantum yield. This is in accordance with results of Hahn et al. (1993) who found no impairment of photosynthetic gas exchange in rehydrated *P. malacea* which had dried out in PFDs of up to 1500 \( \mu \)mol m\(^{-2}\) s\(^{-1}\) in the field. In fact, it appeared that all occurring depressions of PS II quantum yield became reversed as soon as *P. rufescens* was intermittently hydrated for a few days in a row without temperatures below 0°C, no matter under what incident light intensities.

Desiccation of the lichen during strong irradiation could have conferred photoprotection. Demmg-Adams et al. (1990) had shown that dry lichens were insensitive to photodamage, at least in the short term. In experiments where we kept *P. rufescens* thalli well hydrated over several days in full sunshine, no unusually strong reduction of \( \Phi_e \) became apparent (Leisner et al. 1995). This indicates that in summer the lichens were inherently tolerant to high PFD.

We detected severe PS II inhibition only during February and March 93 in thalli growing at the open site (e.g. see Fig. 7). Before and during that time, extended periods of (deep) frost had greatly inhibited lichen metabolic activity (e.g. see Fig. 6). In early spring, the frozen thallus often thawed in the late morning in a PFD of up to 500 \( \mu \)mol m\(^{-2}\) s\(^{-1}\) (data not shown). Shortly after dusk they froze again. Reactivation of photochemical electron transport during the light phase could well have contributed to the risk of PS II overexcitation and subsequent damage even under comparatively low PFDs. Similar conditions are known to induce photoinhibition in higher plants (Farage & Long 1991; Krause 1994).
However, even when frost-induced limitation of $\Phi_e$ was alleviated in the dark, a slight depression in quantum yield was evident during the following 24 hrs (see Fig. 5). Hence, the strong and persistent inhibition of PS II in February possibly was a cumulative effect of repeated freezing/thawing cycles. This effect was especially severe in the open habitat where incident PFD was much higher than at the partially shaded site. In addition, the frost-caused limitation of metabolically active phases in the dark may have restricted recovery processes. March 93 was very dry so that active phases of the lichen were rare all together (see Figs. 7 and 10). Therefore, $P. rufescens$ may have had little opportunity to recover from the previous frost-promoted inhibition.

Apparently, the occurrence of sustained inhibition following frost or frost combined with desiccation has at lease three possible explanations which, at the moment, cannot be distinguished. Extended desiccation alone, either from normal drying or through freezing, might be sufficient to produce the effect. Alternatively, extended desiccation with associated high PFD might be needed, and finally, inhibition through desiccation of the ability to recover from previous stress might be the determinant. In the case of the latter suggestion it is worth noting that during summer the influence of high PFD was very small when regular recovery activity was possible.

Our measurements documented the large variation of the quantum efficiency of PS II in a cyanobacterial lichen during the different seasons of a year. The diel changes of dawn $\Phi_e$ reflected the impact of constantly changing environmental conditions on $P. rufescens$. They clearly revealed the activity pattern of the lichen and could provide a method by which the overall productivity of the system might be estimated. Based on the available data on maximal PS II efficiency, photoinhibition did not play a role as long as no other environmental stress factors (like frost and/or extended times of thallus desiccation) severely limited metabolic activity of the lichen. High incident PFD during photochemical activity did not, on its own, cause inhibition of PS II in $P. rufescens$. We conclude from these year-long measurements that, under natural conditions, persistent photoinhibition was rare. When PS II inhibition did occur, it could not be entirely explained by excess light.

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References


