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Heterogeneity of chlorophyll fluorescence over thalli of several foliose macrolichens exposed to adverse environmental factors: Interspecific differences as related to thallus hydration and high irradiance

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Abstract

Spatial heterogeneity of chlorophyll (Chl) fluorescence over thalli of three foliose lichen species was studied using Chl fluorescence imaging (CFI) and slow Chl fluorescence kinetics supplemented with quenching analysis. CFI values indicated species-specific differences in location of the most physiologically active zones within fully hydrated thalli: marginal thallus parts (*Hypogymnia physodes*), central part and close-to-umbilicus spots (*Lasallia pustulata*), and irregulary-distributed zones within thallus (*Umbilicaria hirsuta*). During gradual desiccation of lichen thalli, decrease in Chl fluorescence parameters (F_0 - minimum Chl fluorescence at point O, F_P – maximum Chl fluorescence at P point, Φ_2 - effective quantum yield of photochemical energy conversion in photosystem 2) was observed. Under severe desiccation (>85 % of water saturation deficit), substantial thalli parts lost their apparent physiological activity and the resting parts exhibited only a small Chl fluorescence. Distribution of these active patches was identical with the most active areas found under full hydration. Thus spatial heterogeneity of Chl fluorescence in foliose lichens may reflect location of growth zones (pseudomeristems) within thalli and adjacent newly produced biomass. When exposed to high irradiance, fully-hydrated thalli of *L. pustulata* and *U. hirsuta* showed either an increase or no change in F_0, and a decrease in F_P. Distribution of Chl fluorescence after the high irradiance treatment, however, remained the same as before the treatment. After 60 min of recovery in the dark, F_0 and F_P did not recover to initial values, which may indicate that the lichen used underwent a photoinhibition. The CFI method is an effective tool in assessing spatial heterogeneity of physiological activity over lichen thalli exposed to a variety of environmental factors. It may be also used to select a representative area at a lichen thallus before application of single-spot fluorometric techniques in lichens.

Additional key words: chlorophyll fluorescence imaging; desiccation; high irradiance; high light; *Hypogymnia physodes*; *Lasallia pustulata*; lichen; photosynthetic parameters; *Umbilicaria hirsuta*.

Introduction

The method of chlorophyll (Chl) fluorescence imaging (Lang *et al.* 1994) has already met an increasing number of applications in the laboratory and in the field (*e.g.*, Lichtenthaler and Miehé 1997, Lichtenthaler *et al.* 1997). Using Chl fluorescence imaging (CFI), several problems have been studied in higher plants, *e.g.*, heterogeneity of intercellular CO_2 concentration (C_i) (Meyer and Genty 1998), patchiness of stomatal closure during ongoing water stress (Meyer and Genty 1999), early detection of water and temperature stress (Lang *et al.* 1996), photoinhibition of photosynthesis (Osmond *et al.* 1999), effects of herbicides (Lichtenthaler *et al.* 1997) and viral infection (Osmond *et al.* 1998) on decrease of leaf photosynthetic performance. While in higher plants heterogeneity of Chl fluorescence and net photosynthetic rate over a leaf blade has been documented many times (Šesták and Šiffel 1997), information on the heterogeneity in lower plants and lichens is almost lacking. Jensen and

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Symbols and abbreviations: CFI – chlorophyll fluorescence imaging; Chl – chlorophyll; F_P – chlorophyll fluorescence at P point of Chl fluorescence kinetics, F_V – variable chlorophyll fluorescence; P_G – gross photosynthetic rate, PPFD – photosynthetic photon flux density; PS2 – photosystem 2; WSD – water saturation deficit; Φ_2 – effective quantum yield of photochemical energy conversion in photosystem 2.

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Siebke (1997) used CFI for screening of Chl presence in lichen thalli and simple assessment of physiologically active thalli parts. Another attempt to estimate Chl fluorescence heterogeneity in the field was made by Schroeter *et al.* (1997) who reported significant differences in quantum yield of photochemical energy conversion of photosystem 2 (Φ_2) between central and marginal parts of *Buellia frigida* thallus. To our best knowledge, no attempt has yet been made to estimate changes in Chl fluorescence heterogeneity over a lichen thallus as induced by changes in physical factors of environment (*e.g.*, air temperature, water status of thallus). Since lichens are organisms without specialised water conducting tissue and efficient control mechanisms of water loss, one may expect higher heterogeneity in Chl fluorescence distribution over a lichen thallus than in a leaf blade of higher plants. Also the non-uniform distribution of photobiont cells (unicellular algae or cyanobacteria) within lichen thallus and the age-related differences in optical properties of upper cortex may contribute to the Chl fluorescence heterogeneity.

Considering the above facts, we hypothesised that heterogeneity of Chl fluorescence over lichen thalli should be high even when measured under standardised conditions (optimum hydration of thallus, constant air temperature). Another working hypothesis to prove was that there should be inter-specific differences in Chl fluorescence distribution over lichen thalli that reflect different growth patterns. In addition, the changes in Chl fluorescence distribution over thalli ongoing with desiccation and high irradiance stress were recorded and analysed. The aim of our paper was to test the above hypotheses and to enlarge the applicability of CFI to stress physiology in lichens.

Materials and methods

Lichens: Thalli of three foliose lichen species were collected from field sites in the naturally desiccated state in January 2000. *Hypogymnia physodes* was collected from bark of orchard trees in close vinicity to Moravany village, 5 km south of Brno, Czech Republic. Thalli of *Lasallia pustulata* and *Umbilicaria hirsuta* were collected from perpendicular granitic rock walls in the Oslava river valley near the village Ketkovice, 30 km west of Brno, Czech Republic. All collected thalli were transferred to a laboratory and stored at 5 °C. Prior to the measurements, the thalli were covered by wet paper and re-hydrated for 2 h at room temperature.

Chl fluorescence was measured on dark-adapted (10 min), fully hydrated lichen thalli, and after gradual desiccation on thalli showing a different water saturation deficit (WSD). The Chl fluorescence signal was measured using a kinetic fluorometric CCD camera *FluorCam* (*Photon Systems Instruments*, Brno, Czech Republic). Chl fluorescence was induced by imposing a 1 s pulse of weak excitation irradiance ($\lambda = 650$ nm, 120 μmol m^{-2} s^{-1}) on the lichen thallus. Fast changes in Chl fluorescence of the whole thallus during the irradiance-induced Chl fluorescence kinetics (Kautsky effect) were recorded with the time resolution of 0.04 s and stored as an image file using a *FluorCam* software. Further analysis of the image file consisted of re-colouring original monochrome image (grey scale) into false colours and identification of maximum and minimum Chl fluorescence signal within a single thallus. For this purpose, the peak Chl fluorescence F_P was chosen. The minimum-to-maximum span of F_P was divided into four classes each of which represented certain false colour (from high to low F_P: red, yellow, green, and blue) found within the image of lichen thallus. Location and area of each of the four Chl fluorescence classes was determined using image analysis system *LUCIA*, as well as the pertinent F_P values from induction curves of Chl fluorescence (*FluorCam* software).

Using the above technique, sub-areas of high to low Chl fluorescence signal were distinguished on the thalli. In the zones exhibiting extremely high and low F_P, slow Chl fluorescence induction kinetics were measured, supplemented with a saturation pulse (0.8 s, 5000 μmol m^{-2} s^{-1}) at steady-state Chl fluorescence reached after 5 min of irradiance (*PAM-2000*, *H. Walz*, Effeltrich, Germany). The effective quantum yield of photochemical energy conversion in PS2 (Φ_2, Φ_{II} – for nomenclature see Roháček and Barták 1999) was calculated as $\Delta F/F'_M$ (Genty *et al.* 1989), where ΔF is the difference between maximum Chl fluorescence after saturation pulse applied on light-adapted lichen sample and steady-state Chl fluorescence. The rate of gross photosynthesis (P_G) was then estimated using the formula (Sundberg *et al.* 1997):

$$P_G = \mathrm{ETR} \times \Phi_{CO2} \quad (1),$$

where ETR is electron transport rate and Φ_{CO2} is quantum yield of CO_2 fixation. Numeric value of Φ_{CO2} was assumed as 0.125 (Krall and Edwards 1992) considering that minimum 8 quanta are required per one molecule of CO_2 fixed. ETR was calculated as:

$$\mathrm{ETR} = \Phi_2 \times 0.5 \times a \times \mathrm{PPFD} \quad (2),$$

where 0.5 is a numeric coefficient reflecting the fact that two quanta are required per one electron transported, a is an absorptance of thalli estimated to 0.8, and PPFD is amount of photosynthetically active radiation (actinic irradiance from fluorometer) incident on the top of the thalli.

High irradiance treatment: Fully hydrated thalli of *L. pustulata* and *U. hirsuta* were exposed for 40 min to a PPFD of 2000 μmol m^{-2} s^{-1} in order to induce photoinhibition. Before and after the high irradiance treatment, Chl fluorescence distribution over thalli was measured on dark-adapted (10 min) samples using the method described above. The differences in Chl fluorescence distribution were evaluated using a *LUCIA* image analysis software. At the thalli area exhibiting maximum F_P, Chl fluorescence kinetics were recorded before, immediately after, and in 30 min intervals after high irradiance treatment. The kinetics was analysed in order to determine F_V changes during high irradiance treatment and subsequent recovery.

Results and discussion

Heterogeneity in Chl fluorescence under full hydration: There were substantial differences in F_P over the area of fully hydrated lichen thalli with striking species-specific pattern. In *H. physodes*, the highest F_P was found in marginal thalli parts and to a lower extent in few minor parts near the thalli centre (Fig. 1). The finding well fits to the anatomy of *H. physodes*. It is a typical laciniate foliose lichen forming lobes, at the end of which youngest and newly grown thalli parts are located. They can be found either at peripheral thalli parts or within the thalli where they form overlapping structures (Büdel and Scheidegger 1996). In *L. pustulata*, F_P reached maximum near the central point (umbilicus) and in well-separated spots arranged in irregular circles around it. The distribution of maximum F_P corresponded well with the anatomic features of the species because the location of the spots showing F_P maximum was identical with verrucoide outgrowths formed at the upper side of the thalli by intercalary pseudomeristems (Honegger 1996). Higher physiological activity at these spots is very probable but some effect of thallus morphology on Chl fluorescence signal could interfere. The edges of verrucoide outgrowths were not perpendicularly oriented to the excitation radiance source that could diminish their absorption characteristics and, consequently, Chl fluorescence intensity. In contrast to the other two species, the flat *U. hirsuta* had a patchy distribution of F_P within the thalli without any regular pattern. Maximum F_P values were found at relatively large areas, the size and location of which was sample-specific. This may be attributed to a patchy distribution of intercalary pseudomeristems producing new thalli parts that result in irregular intrathalline gradients of physiological activity. This type of heterogeneity is typical for the majority of lichens having umbilicate thallus morphology (Honegger 1993).

The heterogeneity of Chl fluorescence over fully hydrated lichen thalli was reflected also in the shape of Chl fluorescence induction kinetics recorded by a modulated fluorometer either at central and marginal parts of thalli. Differences were found in F_V, F'_M, and consequently in Φ_2 values. Since Φ_2 is the main input parameter for P_G estimation, there were also differences in P_G between investigated parts of thalli (Fig. 3).

Heterogeneity of gross photosynthetic rate (P_G): Due to the small size of the thalli of the studied lichen species and the relatively large dimensions of the fluorometric probe, P_G was studied only at central and marginal parts (Fig. 3). The highest P_G was found in physiologically more active parts of thalli showing highest F_V, as indicated previously by an analysis of CFI. In *U. hirsuta*, a difference in P_G of about 13.3 % was found between physiologically more and less active parts. The difference in P_G between central and marginal thalli parts of *H. physodes* was less pronounced than in the two other species. This might be attributed to the young age and small size of the thalli that still exhibited a high physiological activity in central parts (values not shown). With pronounced age and size of the *H. physodes* thallus, differences in P_G between central and marginal thalli parts might be expected. In *L. pustulata*, high F_V did not necessarily correspond to a high Φ_2 and a high P_G.

Heterogeneity of Chl fluorescence during desiccation: In the course of desiccation of thalli from 0 to 90 % WSD all species studied exhibited gradual decrease in F_P (Fig. 1) throughout whole thalli. This was reflected by a decrease of the thalli area showing the highest Chl fluorescence and by an increase in area of the lowest fluorescence (Fig. 2). At WSD of 90 %, some parts of thalli showed no Chl fluorescence at all and the remaining thalli parts had very low Chl fluorescence. The effect of desiccation was more expressed in a decrease of F_0 value and F_V at any time of the Chl fluorescence induction, and in a decrease of maximum Chl fluorescence after a saturation pulse (F'_M derived from Chl fluorescence induction kinetics – data not shown here). These changes are documented for lichens (*e.g.*, Sass *et al.* 1995, Jensen *et al.* 1999, Barták *et al.* 2000a) and explained as consequences of a functional disconnection of PS2 antennae from the PS2 core. F_0 decreased with desiccation which could be caused partially by the changes of optical properties of the upper cortex due to the higher presence of reflective air bubbles (Scheidegger and Schroeter 1995). Other mechanisms contributing to the F_0 decrease with thalli desiccation may be connected with redistribu-tion of excitation radiation to PS1 (Chakir and Jensen 1999)

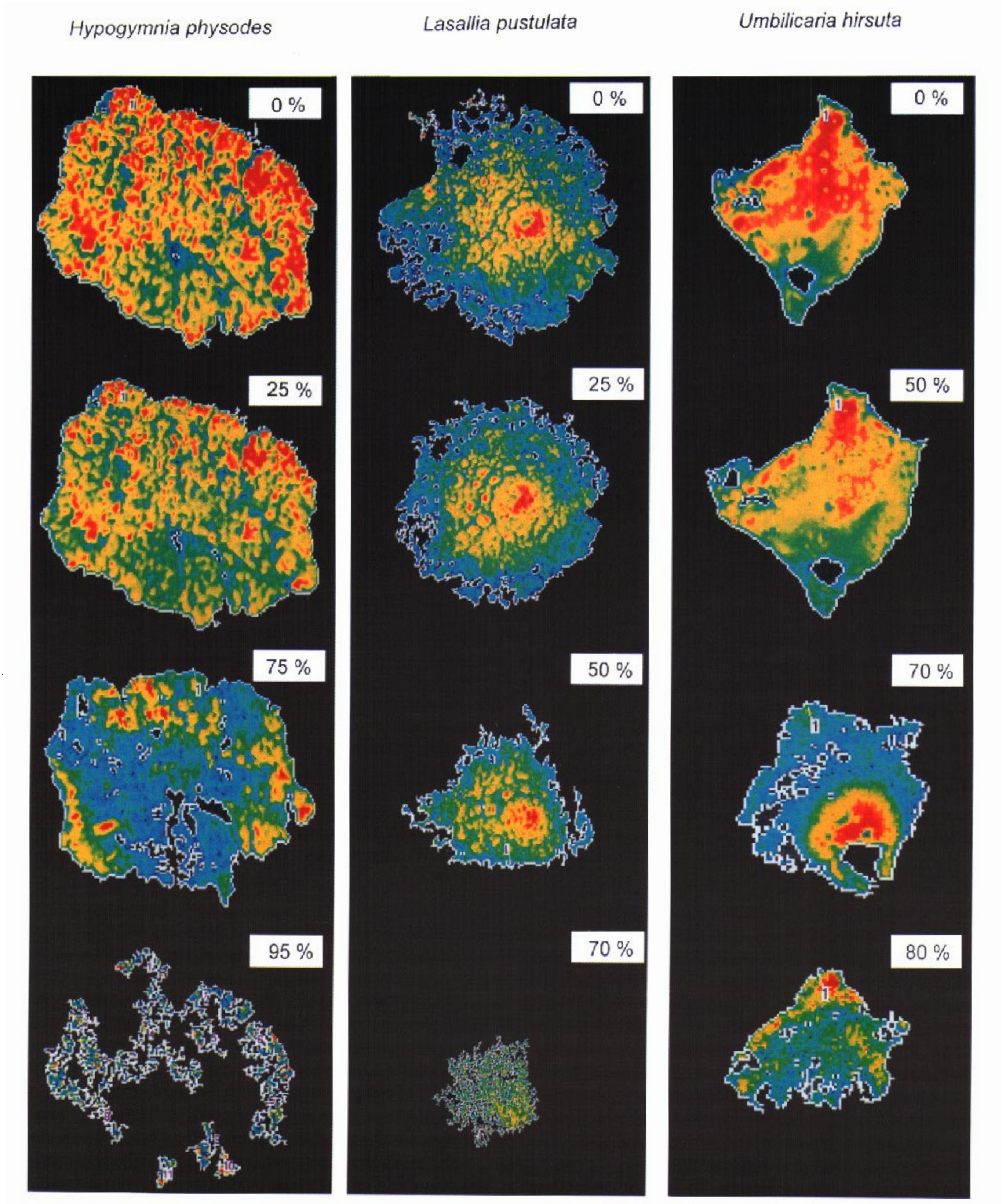

Fig. 1. Heterogeneity of chlorophyll (Chl) fluorescence over thalli of three lichen species under increasing water saturation deficit [%]. Chl fluorescence is given in false colour in the following order (from maximum to minimum): red, yellow, green, blue.

and with Chl fluorescence re-absorption caused by antennae complex aggregation (Horton *et al.* 1996) which was documented for various poikilohydric mosses and lichens (*e.g.*, Bartošková *et al.* 1999a,b, Takács *et al.* 2000).

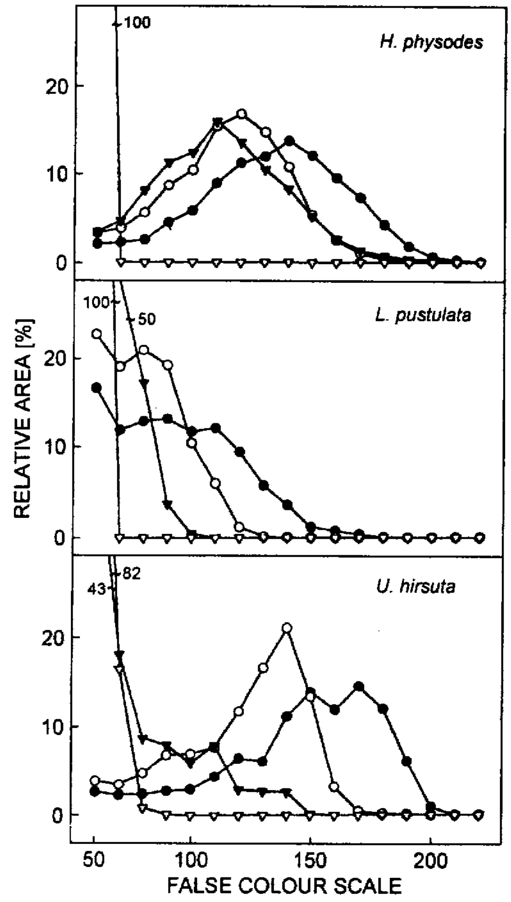

Fig. 2. Relative areas taken by false colours (256 colour scale) within the whole chlorophyll (Chl) fluorescence image (for source *see* Fig. 1) of lichen thalli under increasing water saturation deficit (WSD) during desiccation from a fully hydrated state. The higher the number of false colours, the higher the Chl fluorescence signal. WSD for *Hypogymnia physodes*: ● 0 %, ○ 70 %, ▼ 76 %, ▽ 95 %; for *Lasallia pustulata*: ● 0 %, ○ 71 %, ▼ 85 %, ▽ 95 %; for *Umbilicaria hirsuta*: ● 0 %, ○ 63 %, ▼ 72 %, ▽ 96 %.

During desiccation, the species studied showed different pattern of the loss of Chl fluorescence signal. *H. physodes* had more or less uniform loss of F_P at all thallus parts but under high dehydration (WSD 95 %) the Chl fluorescence signal was measurable only in marginal thallus parts. This pattern probably reflects the distribution of the youngest thallus parts that are in *H. physodes* located at the end of lobes. In *L. pustulata*, the fastest decrease of F_P during desiccation was observed at the thallus margin. Under severe desiccation, only the central part of thallus around the umbilicus remained active. In *U. hirsuta*, an irregular pattern of the Chl fluo-rescence signal with ongoing desiccation was found. The most sensitive were margins of the thallus followed by the very central part while the rest of thalli exhibited some activity even at 80 % of WSD. This behaviour can be explained by thallus morphology. Marginal thallus parts are thinner than central parts and thus more susceptible to dehydration. The central thallus parts were likely capable to retain water for a longer time and lost their activity later than margins. Thallus parts showing some physio-logical activity even at a WSD of 80 % were located in the same zones where the highest Chl fluorescence signal was detected at full hydration (*cf.* Fig. 1).

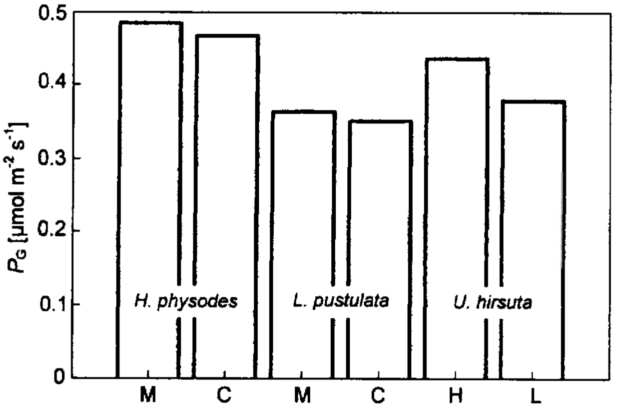

Fig. 3. Gross photosynthetic rate (P_G) at full hydration, CO_2 concentration of 400±5 μmol(CO_2) mol^{-1}, PPFD of 15 μmol m^{-2} s^{-1}, and air temperature of 22 °C for central (*C*) and marginal (*M*) parts in thalli of *Hypogymnia physodes* and *Lasallia pustulata*, and the parts showing high (*H*) and low (*L*) physiological activity in *Umbilicaria hirsuta*.

Responses to high irradiance: High irradiance treatment of fully hydrated thalli of *L. pustulata* and *U. hirsuta* led to a decrease of F_P in all thalli parts (*see* Fig. 4). The extent of the decrease was, however, species-specific: when considering the whole thallus, it reached about 75 % of the initial F_P value in *L. pustulata* and 97 % in *U. hirsuta*. F_0 showed a different response to high irradiance treatment: it increased in *U. hirsuta* but did not increase in *L. pustulata*. During recovery from high irradiance treatment in the dark, F_P as well as F_V did not reach the initial values even after 60 min (Fig. 4). High irradiance treatment of *L. pustulata* and *U. hirsuta* led to an apparent decrease of F_V which together with the irradiance-induced decrease in F_V/F_M and Φ_2 (see Barták *et al.* 2000b) could be caused by photoinhibitory changes in photosynthetic apparatus. In *L. pustulata* and *U. hirsuta*, the F_V decrease was induced under laboratory conditions closely simulating outdoor environment at the beginning of spring (full hydration of thallus, high irradiance). However, two contradictory opinions exist in the literature whether the photoinhibitory changes appear in lichens under field conditions. Kappen *et al.* (1998) reported that photoinhibition may not occur under field conditions, especially in high irradiance-adapted lichens. Valladares *et al.* (1997), on the contrary, reported that photoinhibition was probable in lichen species having a limited pool of xanthophyll cycle pigments. In shade-adapted lichens, undoubtedly, photoinhibition was documented (Gauslaa and Solhaug 1996, 1999). In our opinion, photoinhibition probably occurs in the field at least under specific combination of some environmental factors (*e.g.*, full thalli hydration, extreme temperature, high irradiance).

In conclusion, we proved the potential of the CFI method as a non-intrusive tool for the analysis of photosynthesis in poikilohydric organisms. In foliose and crustose lichens, the assessment of spatial heterogeneity of Chl fluorescence may help to find a mean thallus activity and location of the area within a lichen thallus that is representative for the whole thallus. When using fluorometry to estimate the apparent electron transport through PS2, effective quantum yield (Φ_2), or maximum rate of P_G, selection of the most suitable and representative area for the placement of the fluorometric probe is crucial. To avoid errors in estimation of physiological status of lichen thallus caused by a non-representative probe placement, CFI should be used prior to spot measurements.

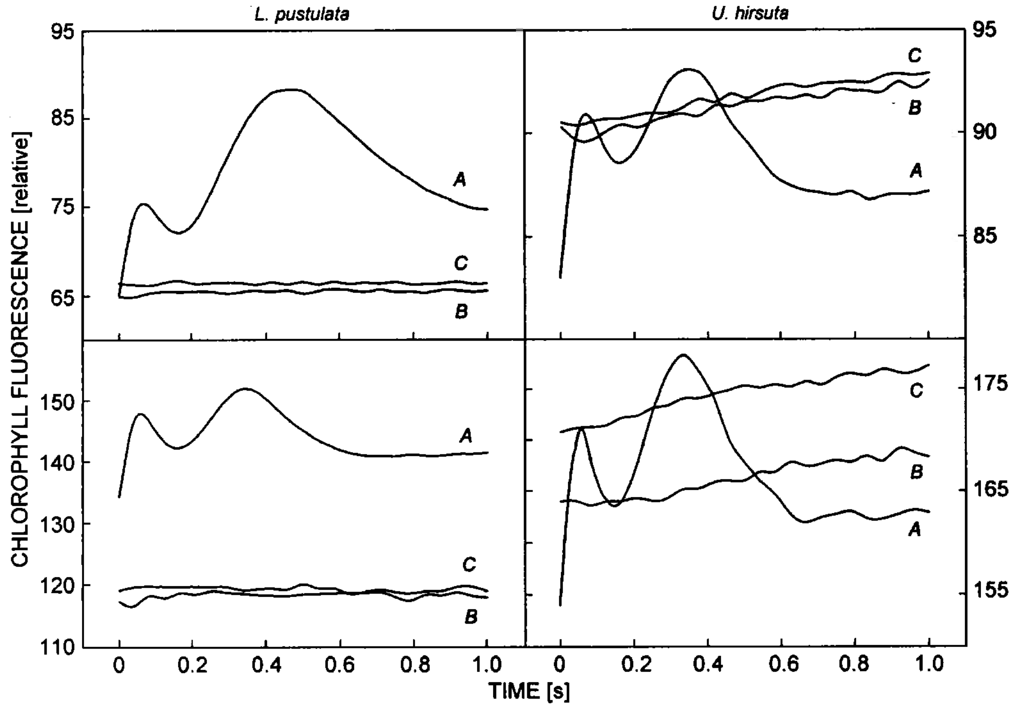

Fig. 4. Fast induction kinetics of chlorophyll fluorescence in *Lasallia pustulata* (*left*) and *Umbilicaria hirsuta* (*right*) before (*A*) a high irradiance treatment and 10 (*B*) or 60 min (*C*) after it. Kinetics either for the most physiologically active zones (spots) within the thalli (*lower panel*) and for whole thalli (*upper panel*).

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