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APPLICATION OF FLUOROMETRIC METHODS TO MEASUREMENTS OF LICHEN PHOTOSYNTHETIC RESPONSES TO CHANGING TEMPERATURE AND THALLUS HYDRATION

MILOŠ BARTÁK, JOSEF HÁJEK, JAN GLOSER

Masaryk University, Department of Plant Physiology and Anatomy, Kotlářská 2, 61137 Brno, Czech Republic

ABSTRACT

The paper deals with estimation of photosynthesis in three low-temperature adapted lichen species: *Cladonia digitata, Cetraria islandica, Hypomnia physodes* using two methods of chlorophyll fluorescence: (1) Basic chlorophyll fluorescence parameters determined from Kautsky kinetics measured with a pulse-amplitude modulated fluorometer, (2) Rapid light response curves of photosynthesis determined from quantum yield of photochemical reactions of photosystem II (Φ_{II}). Both method were applied on lichens transferred to a laboratory and exposed to descending air temperature (22-2 °C) and thallus hydration (0-90 % of water saturation deficit - WSD). In *C. digitata* and *H. physodes*, decrease in temperature induced decrease in the following chlorophyll fluorescence parameters: F_V/F_M, Φ_{II}, qP. In all species studied, continuous thallus dehydration caused no change in F_V/F_M, Φ_{II} , qP within 50-100 % WSD but induced dramatic decrease in the above parameters within the range of 40-80 % WSD. Within the same range, an increase of qN was observed. Photosynthesis declined with decrease in temperature and thallus hydration. Significant differences found between the three species in their responses to temperature and moisture conditions are discussed. We can conclude, that the chlorophyll fluorescence methods proved as a valuable tool for studies of stress physiology of lichens, and that they may be especially advantageous for field measurements, even under harsh alpine or Antarctic environments.

KEY WORDS: low temperature photosynthesis - *Cladonia digitata* - *Cetraria islandica* - *Hypogymnia physodes*

INTRODUCTION

Over last two decades, measurements of induced chlorophyll fluorescence in intact plants has become a very useful method for the basic research of the photosynthetic mechanism, as well as for the ecophysiological study of plant responses to a variety of environmental factors. In contrast to traditional gasometric methods (measurement of photosynthetic activity from gas exchange rate connected with biochemical part of photosynthesis), fluorometry is based on estimation of changes in primary (biophysical and physico-chemical) part of photosynthesis. This may be an advantage for deeper analysis of photosynthetic processes, but, on the other hand, some limitations of its use for ecophysiological study of carbon metabolism and carbon balance are obvious. Nevertheless, some new experimental procedures developed recently has broadened the scope of fluorometry. This is particularly true in the case of derivation of so-called light response curves of photosynthesis (= dependences of net photosynthetic CO_2 uptake rate on incident

photosynthetically active radiation) from measurements of induced chlorophyll fluorescence, described by White *et* Critchley (1999). Fluorometric methods are non-invasive, very fast and more robust than vulnerable gasometric approaches, which make them particularly suitable for measurements in field conditions. They are now widely used in ecophysiological and stress studies in higher plants, but, unfortunately, they are much fewer applied in ecophysiological study of lichens. As a part of our methodical preparation of a proposed complex ecological research in coastal Antarctic oasis, we have assessed the validity of using fluorometry in stress physiological research of several species of cold-adapted lichens. In addition to the measurements of basic fluorescence parameters (connected with elaborating of an optimal protocol for their determination), newly modified method for determination of photosynthetic light curves was tested.

MATERIAL AND METHODS

Experimental plants

Samples of *Cladonia digitata* (L.) Hoffm. and *Hypogymnia physodes* (L.) Nyl. were collected in the Krkonoše Mountains (the Giant Mountains, N Bohemia, Czech Republic) in the location Růžová hora (1380-1420 m a.s.l.) and Bucharova cesta (1370-1410 m a.s.l.). Samples of *Cetraria islandica* (L.) Ach. were collected in the West Tatra Mts. (N Slovakia) in the location Bystrá dolina (2000 m a.s.l.). The samples were transferred to a laboratory where they were stored in dark at 5 °C.

Before measurements, thalli of the species studied were fully saturated with water for 2 h in dark at room temperature (22 °C). Then, a set of chlorophyll fluorescence measurements were done on the lichen species in response do changing light (0-600 μmol m^{-2} s^{-1}), stepwise lowering of air temperature (from 22 to 2 °C) or thallus dehydration. Status of dehydration was calculated as water saturation deficit (WSD).

$$WSD = 100 * (FMmax - DMt - DMs) / (FMa - DMt - DMs) \quad (1),$$

where FMmax is water-saturated fresh mass of thallus, FMa is actual fresh mass of thallus, DMt is dry mass of thallus, DMs is dry mass of substrate). Chlorophyll fluorescence data were used for the estimation of light-response curves of photosynthesis (see below) and the photosystem II functioning (see below).

Chlorophyll fluorescence parameters

A set of chlorophyll fluorescence parameters was determined from an analysis of slow Kautsky kinetics (recorded by PAM-2000 fluorometer) supplemented with saturation pulses. On dark-adapted (10 min) thalli of lichens, a weak light of 0.5 μmol m^{-2} s^{-1} was applied accompanied with a saturation pulse (5000 μmol m^{-2} s^{-1}). From this, basic chlorophyll fluorescence (F_0) and maximum capacity of PS II (F_V/F_M) were determined. Then, actinic light (10 μmol m^{-2} s^{-1}) was applied for 5 min until steady-state chlorophyll fluorescence (Fs) was reached, followed by saturation pulse. This enabled to determine quantum yield of photochemical reactions of PS II (Φ_{II}), photochemical quenching (qP), and non-photochemical quenching (qN).

$$\Phi_{II} = (F'_M - F_S) / F'_M \quad (2),$$
$$qP = (F'_M - F_S) / (F'_M - F_0) \quad (3),$$
$$qN = (F_M - F'_M) / (F_M - F_0) \quad (4),$$

where F_M is maximum chlorophyll fluorescence on dark-adapted thalli, and F'_M is maximum chlorophyll fluorescence on light-adapted thalli (under actinic light). Nomenclature of chlorophyll fluorescence parameters and their abbreviation was used according to van Kooten *et* Snell (1990).

Rapid light response curves of photosynthesis (RLCs)

RLCs were recorded using a PAM-2000 (Germany) fluorometer and a method originally developed by White *et* Critchley (1999) in modification of Barták (unpublished). On dark-adapted thalli, ascending light was applied (actinic light) in nine steps using halogen lamp as a source. Photosynthetic photon flux density (PPFD) of the actinic light was 37, 51, 66, 100, 135, 195, 270, 400, and 600 μmol m^{-2} s^{-1}. At each PPFD level, saturation pulse of strong white light (5000 μmol m^{-2} s^{-1}) was applied in order to determine quantum yield of photochemical reactions of PS II (Φ_{II} - see Chlorophyll fluorescence parameters) and, consequently electron transport rate (ETR).

$$ETR = \Phi_{II} * 0.5 * A * PPFD \quad (5),$$

where 0.5 is a numeric coefficient reflecting the fact that two quanta are required per one electron transported, A is an apsorptance of thalli estimated to 0.8, and PPFD is an amount of photosynthetically active radiation incident on the top of thalli. Apparent rate of gross CO_2 assimilation (GP) was calculated using the equation:

$$GP = ETR * \Phi_{CO2} \quad (6),$$

where Φ_{CO2} is quantum yield of CO_2 fixation. Numeric value of Φ_{CO2} was assumed 0.125 (Krall *et* Edwards, 1992) considering that minimum 8 quanta is required per one molecule of CO_2 fixed. Rapid light-response curves were constructed by plotting GP against PPFD used as an actinic light and maximum GP (GP_{max}) was calculated as an asymptotic value of exponential fit (Potvin *et* al., 1990).

RESULTS AND DISCUSSION

Response to low temperature

Lowering of air temperature induced decline in F_V/F_M, Φ_{II}, and qP (Fig. 1) that showed similar trend in *C. digitata* and *H. physodes*. Decline of F_V/F_M and Φ_{II} indicate decreased maximum capacity of photochemical reactions of PS II and decreased effectivity of energy transfer in PS II. Gradual decrease of qP with decresing temperature reflects increasing number of gradually "closed" reaction centres of PS II. This is a typical response of higher plants and lichens with green algae as primary photobionts (Sundberg *et* al., 1997a). Different response was observed in case of qN, which increased with temperature lowering in *C. digitata* while it decreased in *H. physodes*. In green algal lichens, obviously increase of qN is reported with temperature decrease (Sundberg *et* al., 1997a).

Analysis of rapid light-response curves of GP (Fig. 2) revealed that lowering of air temperature from 22 to 9 °C had no effect on GP and GP_{max} in *C. digitata* and *H. physodes*. Further lowering of air temperature, however, induced a decrease of about 25 %. The results indicate that temperature optimum of photosynthesis of the two species studied was rather low (9 °C or below). This was particulary true for *C. islandica*. In other words, long-term adapted lichens to low-temperature keep their photosynthetic activity high within a wide temperature interval, going down to at least 9 °C. GP_{max} reached the values of 2.5 and 2.0 μmol CO_2 m^{-2} s^{-1} in *C. digitata* and *H. physodes*, respectively. This is well comparable to the experimental evidence in many lichen species (*e.g., Lobaria pulmonaria, Platismatia glauca*, Sundberg *et* al., 1997b). GP_{max} in lichens rarely exceeds the value of 5 μmol CO_2 m^{-2} s^{-1} due to low concentration of algae cells and chlorophyll molecules, respectively in lichen thallus (0.5-1.5 μmol g^{-1} (DW), Palmqvist et Sundberg, 2000).

Response to water saturation deficit

During continuous dehydration of fully saturated thalli from 0 to 50 % of water saturation deficit (WSD), F_V/F_M, Φ_{II} , and qN remained constant (keeping their maximum values) - Fig. 3. Further dehydration led to the decrease in F_V/F_M, Φ_{II} , while qN increased indicating involvement of non-radiative dissipation of absorbed light energy. Enhanced non-radiative energy dissipation during severe desiccation of lichen thalli protects reaction centers of photosystem II of algal photobiont from negative effects such as degradation of protein components of photosystem II. Recently, two protective mechanisms in dehydrated lichen thalli are considered: disconnection of the photochemical apparatus (Bilger *et* al., 1989) and conversion of violaxanthin to zeaxanthin, *i.e.* xanthophyll cycle energy dissipation (Calatayud *et* al., 1997).

Minimum F_V/F_M, Φ_{II}, values were reached in 90 % of WSD in both species studied (*C. digitata, H. physodes*). Minimum values of F_V/F_M in 90 % of WSD were caused by close-to-zero values of variable fluorescence (Fv) which indicated loss of photosystem II ability to transfer electron from donor side to an acceptor quinone (Q_A). This may be caused either by the accumulation of radical P_{680+} or the inhibition of primary charge separation (Sass *et* al., 1995). Severe dehydration of thalli, however, did not cause any damage or destruction of pigment-protein complexes of PS II because after several tens of minutes of rehydration maximum values of F_V/F_M were reached. No damage of PS II with ongoing desiccation is generally attributed to the detachment of light harvesting chlorophyll a/b antenna from the PS

II complex or redistribution of excitation energy from PS II to PS I (for review *see* Palmqvist, 2000). In lichens that have a green alga *Trebouxia* sp. as photobiont, however, some additional mechanism protecting their photosynthetic apparatus from desiccation-induced damage is expected, *e.g.* presence of compounds that can substitute for water and stabilize proteins and membranes. As these substances, lichen polyols, ribitol, arbitol and mannitol may serve (Farrar, 1988). Another group of compouds serving in lichen desiccation tolerance are antioxidants, *e.g.* glutathione (Kranner *et* Grill, 1995).

Shape of rapid light response curves of photosynthesis in dehydrated thalli showed similar response in the two species (Fig. 4). Partial dehydration (35 % of WSD) had no effect on GP and GPmax (about 2.5 μmol CO_2 m^{-2} s^{-1}) while severe dehydration (65 % of WSD) led to dramatic decrease in GP and GPmax. *H. physodes* were foud more sensitive to dehydration than *C. digitata,* because it did not show positive GP at 65 % of WSD while the latter species still had positive GP values. In both species studied, similar value of light saturating photosynthesis was found (PPFD = 350 μmol m^{-2} s^{-1}) indicating that the thalli were well-acclimated to low light, typical for their natural habitats.

CONCLUSIONS

Both fluorometric method tested proved as easily aplicable to determination of photosynthetic parameters in lichens adapted to low temperature. They were sensitive enough to reflect changes in physiological status of lichen thalli when exposed to (1) descending air temperatures and (2) continuous dehydration. Rapid light curves of phytosynthesis based on Φ_{II} measurements under ascending PPFD distinguished species-specific differences in thalli photosynthesis. The use of Φ_{II} in the estimation of lichen photosynthes might be, however, accompanied with some minor inaccuracies. Generally, the relation between Φ_{II} and GP slightly differs from linearity in such a way that it slightly underestimates GP under low PPFDs and slightly overestimates GP in high PPFDs (Sundberg *et* al, 1997a). In the field, satisfactorial estimation of GP is obtained when linearity of the relationship is assumed. For more precise laboratory GP estimation, however, chlorophyll fluorescence measurements (Φ_{II} determination) might be supplemented with the gazometric method mesuring either CO_2 consumption or O_2 evolution of a lichen thallus.

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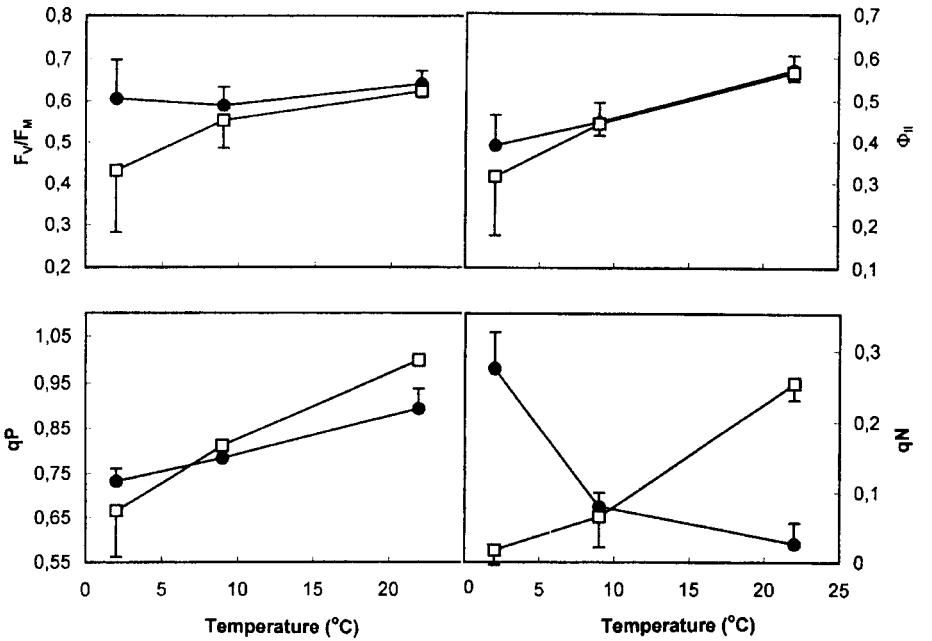

Fig. 1 Temperature dependence of basic chlorophyll fluorescence parameters recorded on fully hydrated lichen thalli: F_V/F_M (upper left panel), quantum yield of photochemical reactions of PS II - Φ_{II} (upper right panel), photochemical quenching - qP (lower left), nonphotochemical quenching - qN (lower right). *Cladonia digitata* (•), *Hypogymnia physodes* (□). Data points are means of at least three mesurements ± standard deviations.

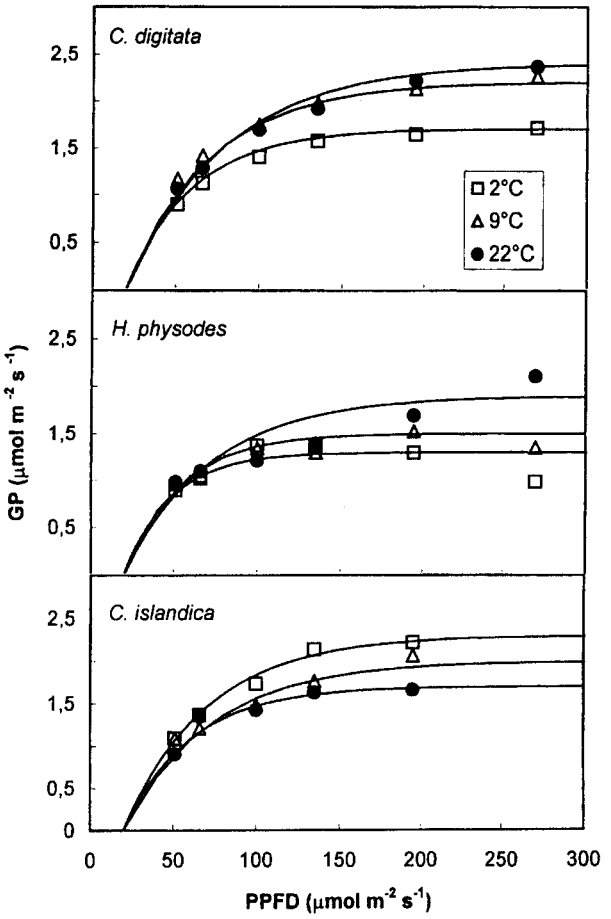

Fig. 2 Rapid light response curves of gross photosynthesis (GP) of fully hydrated lichen thalli (water saturation deficit WSD = 0 %) recorded at 22 (•), 9 (Δ), and 2 (□) °C. Measurements were made under constant CO_2 concentration (400±5 μmol CO_2 mol^{-1}). Data points are means of at least three measurements.

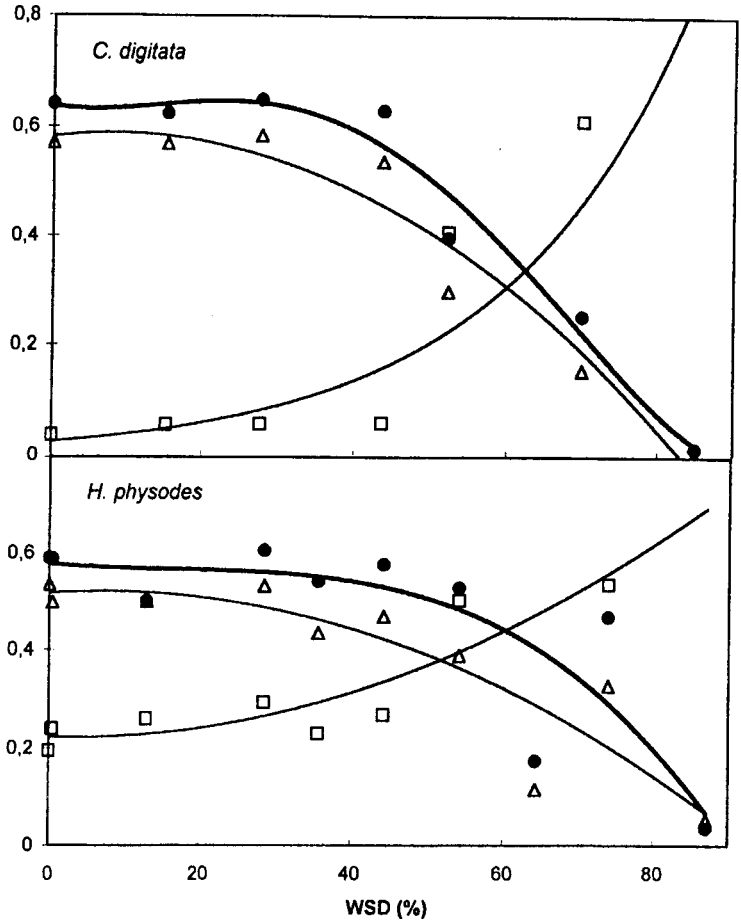

Fig. 3 Dependence of basic chlorophyll fluorescence parameters of lichen thalli on water saturation deficit (WSD): F_V/F_M (•), quantum yield of photochemical reactions of PS II - Φ_{II} (Δ), non-photochemical quenching - qN (□). Measurements were made at 22 °C and under constant CO_2 concentration (400±5 μmol CO_2 mol^{-1}).

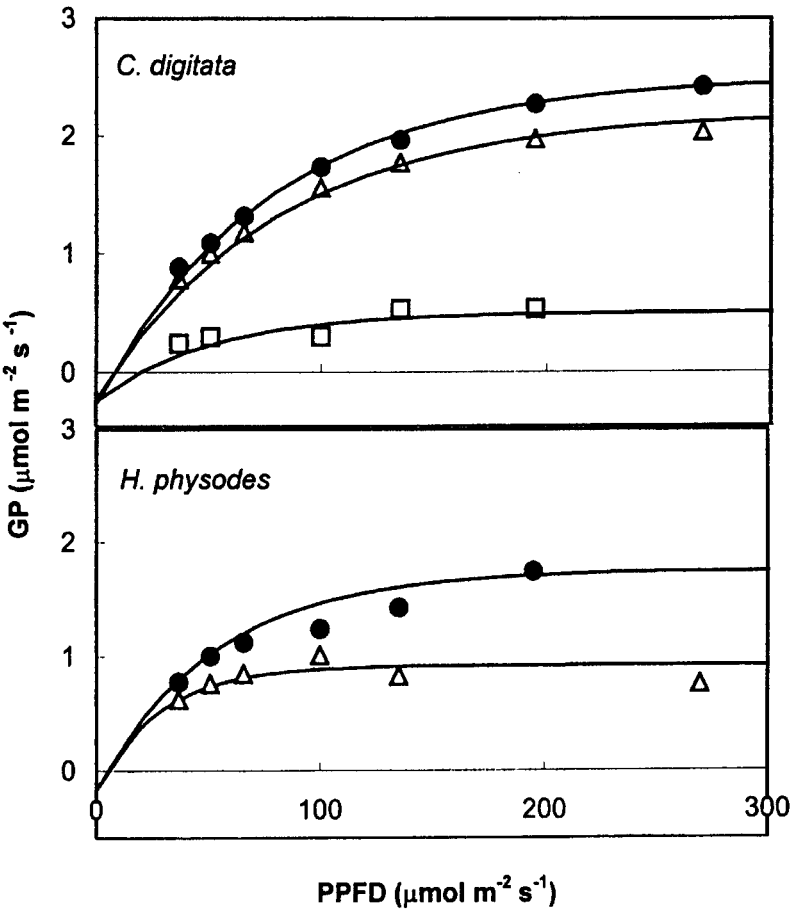

Fig. 4 Rapid light response curves of gross photosynthesis (GP) as dependent on dehydration of lichen thalli recorded at 22 °C. WSD of 0 % (•), WSD of 35 % (Δ), WSD of 65 % (□). Measurements were made under constant CO_2 concentration (400±5 μmol CO_2 mol^{-1}). Data points are means of at least three mesurements.