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HYGROPHILOUS OLD-GROWTH FOREST LICHENS ARE HIGHLY CAPABLE OF INSTANTANEOUS PHOTOSYNTHESIS ACTIVATION AFTER SHORT-TERM DESICCATION STRESS

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The vitality of lichens and their growth depend on the physiological status of both the fungal and algal partner. Many epiphytic lichens demonstrate high specificity to a habitat type and hygrophilous species are, as a rule, confined to close-to-natural forest complexes. Tolerance to desiccation stress and the rate of photosynthesis activation upon thallus hydration vary between species. Analyzes of chlorophyll fluorescence and photosynthesis efficiency have been widely applied to determine the viability of lichens. The aim of this study was to determine the activation photosynthesis rate upon hydration in epiphytic lichens exposed to short-term desiccation stress and to find potential links between their activation pattern and ecological properties. The results proved that even highly sensitive hygrophilous lichens, i.e., *Cetrelia cetrarioides*, *Lobaria pulmonaria*, *Menegazzia terebrata*, do not exhibit any delay in the restart of the photosynthesis process, compared to mesophytic or xerophytic ones. All examined lichens achieved nearly 100% of their maximum photosynthetic efficiency just one hour after they had been supplied with a relatively small quantity of water. Moreover, the increase in photosynthesis efficiency, measured at 20-minute intervals upon hydration, started from a relatively high level. In addition, the differences in the content of photosynthetic pigments and water holding capacity between species did not affect the general pattern of activation, which is comparable across various lichens. It can be concluded that healthy hygrophilous lichens do not require long hydration time to regain a high level of photosynthesis efficiency after a short rainless period. This fact supports the idea of applying chlorophyll fluorescence analysis in the field to assess vitality of lichens and the condition of their natural habitat.

Keywords: bioindicators, chlorophyll fluorescence, lichen ecophysiology, lichenized fungi, lichen photobiont properties, old-growth forest lichens, photosynthesis activation rate

INTRODUCTION

Lichens constitute symbiotic associations resulting from interactions among fungi (mycobionts), algae and/or cyanobacteria (photobionts), and specific elements of the bacterial microbiome combined within the lichen thallus (see Lücking et al., 2021). They have no special mechanism for active regulation of water content inside the thallus, therefore the processes responsible for the hydration status are almost entirely passive. In other words, the intra-body water content is closely related to the current humidity conditions of the local environment in which a lichen exists (Green at al., 2011). This feature causes lichens to be regularly exposed to desiccation and hydration cycles, while the regularity of these cycles largely depends on weather conditions and the specificity of the habitat (Jonsson Čabrajić et al., 2010). Generally, lichens have been classified as stress tolerators (Grime, 1979) and they are usually well adapted to climatic fluctuations within their natural habitat.

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They inactivate metabolic processes during unfavorable dry conditions and resume them when a hydration event occurs. Physiological flexibility in this regard depends on the species and the length of the drought period. There are both desiccation-sensitive and desiccation-tolerant lichens (Kranner et al., 2008). Similarly, species with radically different requirements for humidity and water supply conditions can be found among lichens (Munzi et al., 2019; Krzewicka et al., 2020).

Due to the close symbiotic relation, both algal and fungal components are involved in the growth of lichen thallus and the vitality of one of the partners depends on the physiological condition of the other. Ribitol produced as a result of the photosynthesis process becomes a precursor for-other sugar alcohols and fungal metabolites (Eisenreich et al., 2011). Activation of the process by the algal component strictly depends on the hydration state of the lichen thallus (Hovind et al., 2020). Water availability in the environment, the ability to use a variety of water sources (e.g., liquid form and water vapor), water holding capacity, and finally, capacity to rehydrate are crucial for lichen survival in a given habitat. Since the natural events leading to full hydration may be strongly intermittent and passive evaporation progresses rapidly, photosynthetic activation rate seems to be crucial to lichen growth performance and vitality. Photosystem activation kinetics upon hydration in humid air varies between species considerably and several hours may be required to regain efficient photosynthesis (Phinney et al., 2019; Hovind et al., 2020). Therefore, hygrophilous species are particularly sensitive to disruption of water conditions in the habitat (e.g., Bianchi et al., 2020).

Most epiphytic lichens demonstrate high specificity to non-forested or forest habitat (Kubiak and Osyczka, 2020; Osyczka and Kubiak, 2020). Many rare hygrophilous species, including the so-called relicts of ancient forests (Cieśliński et al., 1996), are strictly confined to the interior of close-to-natural forest complexes (Coppins and Coppins, 2002; Cieśliński, 2003; Motiejūnaitė et al., 2004; Nordén et al., 2007). Specific microclimatic conditions perceptible on a small landscape scale may decide on the presence or absence of a given lichen species (Kubiak and Osyczka, 2017). Therefore, all external factors as well as those internal attributes of the species that determine its physiological functioning are crucial for the persistence of sensitive hygrophilous lichens (Gauslaa et al., 2012; Gauslaa et al., 2019; Hovind et al., 2020; Jonsson Čabrajić et al., 2010).

Because of their poikilohydric nature, desiccation tolerance and the ability of lichens to revive from the dried state are currently arousing wide research interest in all aspects of their biological functioning. The stimulus for this study was to answer the basic question of how rapidly photobionts of healthy lichens exposed to short-term desiccation stress achieve high photosynthesis efficiency upon direct hydration and whether possible differences in the physiological response rate between various species relate to their ecological properties. The aim of the research was to evaluate potential links between the rate of photosynthesis activation in lichens and their ecological tolerance and habitat requirements. Moreover, the study was aimed at determining whether and to what extent the status of physiological parameters related to photosynthesis as well as water holding properties of thalli influences the activation rate in healthy chloro-lichens. The general hypothesis was set: temporarily dried hygrophilous lichens tend to be delayed in photosynthetic activation upon hydration compared to mesophytic or xerophytic ones. A secondary aim of the study was to evaluate the potential and possible limitations of chlorophyll fluorescence analysis performed on lichen samples directly in the field for research related to environmental assessment.

MATERIAL AND METHODS

SELECTED LICHEN SPECIES AND SAMPLING SITE

Nine epiphytic lichen species were selected for examination (abbreviations used later in the text are given in parentheses): *Cetrelia cetrarioides* (Duby) W.L. Culb. & C.F. Culb. (*Cet*), *Evernia prunastri* (L.) Ach. (*Eve*), *Flavoparmelia caperata* (L.) Hale (*Fla*), *Hypogymnia physodes* (L.) Nyl. (*Hyp*), *Lobaria pulmonaria* (L.) Hoffm. (*Lob*), *Menegazzia terebrata* (Hoffm.) A. Massal. (*Men*), *Parmelia sulcata* Taylor (*Par*), *Pseudevernia furfuracea* (L.) Zopf (*Pse*), *Tuckermanopsis chlorophylla* (Willd.) Hale (*Tuc*). They represent a wide spectrum of ecological tolerance in relation to moisture conditions of the habitat and they are confined to the interior of old forest complexes to varying degrees (Cieśliński, 2003; Wirth, 2010). These macro-lichens form relatively large-sized foliose to sub-fruticose thalli which are heteromerous on the

cross-section and contain trebouxioid green algae as the main photobiont (Smith et al., 2009). Brief characteristics of the species are provided in Supplementary data S1. Despite the differences in general distribution and specific ecological properties, all these lichens can be found in one geographic region or even within the same forest division (e.g., Kościelniak, 2013).

The lichen specimens intended for sample preparation and analyses were collected in the Bieszczady Mts. (Eastern Carpathians, south-east of Poland). The climate of this area has been assigned to Dfb type in accordance with the updated Köppen-Geiger classification (Kottek et al., 2006). The collection sites included the valley of the upper course of the San river and the Rzeczyca stream valley. All specimens were collected within one day (July 24, 2021); a five-day rainless period preceded the collection date. The weather conditions related to this period are presented in Supplementary data S2 (data obtained from the nearest local meteorological station; IMGW code 249220180). The samples were transported to the laboratory under dry and cool conditions the day after they had been collected in the field.

CHLOROPHYLL FLUORESCENCE ANALYSIS

The samples were cleaned from macroscopic foreign materials (remains of mosses, humus and tree bark) adhering to the thalli surfaces. Well-developed, relatively flat and regular-shaped parts of the thalli were assigned for measurements. The photosynthetic activity of lichens was measured in lab conditions based on chlorophyll *a* (Chl *a*) fluorescence. The analysis was performed using an advanced continuous excitation chlorophyll fluorimeter Handy PEA+ (Hansatech Instruments Ltd, Norfolk, England). The initial moisture content of the thalli before hydration did not exceed 10% (assessed using the impedance technique) and they were not photosynthetically active. Ten relatively large, equal sized and similar growth-stage patches/lobes of thalli of each species were sprayed with 1 ml of water under semi-dark conditions. The samples were inserted into leafclips with 4 mm diameter measuring aperture right after they had absorbed the water. The samples were measured at three 20-minute intervals after the water had been supplied, i.e., 20-min, 40-min and 60-min hydration time span. Between successive series of measurements, the open leafclips were transferred to a chamber providing high relative humidity of >95%. The Chl *a* fluorescence transients were induced by ultra-bright red-light (650 nm) provided by an array of three high-intensity LEDs. The light pulse intensity was 2400/mmol/m^2/s for 1 s, the gain of the PEA was 1.0. Prior to measurements, the samples were adapted to darkness for at least 15 min. The physiological indicator of photosynthetic efficiency F_V/F_M (see Maxwell and Johnson, 2000) was used to verify the activity of the lichen photobionts. The performance index PI_{ABS}, a global indicator that resumes the contribution of all fluorescence emission parameters (see Paoli et al., 2010), was also considered. The maximum efficiency of photosynthesis for photobionts of particular lichen species was determined based on measurements of samples that had been sprayed with water and stored in the humid chamber for 24 hours. Finally, the transient curves of fast fluorescence kinetic (sequence of steps called O-J-I-P; see Strasser et al., 2000) were generated for samples hydrated for 20, 40, 60 minutes and 24 hours.

PHOTOSYNTHETIC PIGMENTS ANALYSIS

Lyophilized lichen samples (ca 30 mg) were washed with $CaCO_3$-saturated 100% acetone; the washing procedure involved six 1-min rinses with 2 ml of bathing medium with gentle shaking. This was to remove substances capable of degrading chlorophyll to pheophytin during extraction and substances interfering with chlorophyll estimation (see Barnes et al., 1992). Subsequently, the pigments were extracted twice using 3 ml dimethyl sulfoxide (DMSO) with addition of 2.5 mg/ml polyvinylpolypyrrolidone (PVPP) for 45 min at 65°C in the dark with shaking at regular intervals at 70 rpm (New Brunswick, Innova 42, Eppendorf, Germany). After cooling to room temperature, final tubes containing 6 ml of extracts were centrifuged for 10 min at 3000 rpm (Centrifuge type MPW-340, Poland). The extracts were diluted 1 : 1 with fresh DMSO. The absorbance of the extracts was read at 665.1, 649.1, 480, 435, and 415 nm (DR 3800, Hach Lange, USA). Concentrations of Chl *a*, Chl *b* and total carotenoids were determined using Wellburn's equations (Wellburn, 1994). The pheophytinisation quotient (ratio between absorbance at 435 and at 415 nm, A_{435}/A_{415}) was also calculated (Garty, 2001). All steps were carried out in semi-dark conditions to avoid chlorophyll degradation. Four replicates for a single lichen specimen were measured and the mean value was considered to constitute one observation; sample size was n = 10 per each lichen species.

WATER HOLDING ESTIMATION

Ten thalli of each lichen species were air-dried for one week in a closed room providing stable conditions (temp.: ~18°C, RH: ~40%). Nearly equal weights (100 ± 4 mg) of whole fragments of dry thalli were sprayed with 1 ml of water and then transferred to a chamber with high relative humidity (>95%) to avoid water evaporation. After an hour, the samples were re-weighed. Water holding index (WH) was defined as follows: WH (g/g) = (wet weight – dry weight) / dry weight. Such treatment referred to the last series of chlorophyll fluorescence analysis, i.e., 60 min hydration time span.

DATA ANALYSIS

The normality of the distribution and the equality of variances were verified using the Kolmogorov-Smirnov test ($p > 0.05$) and Levene's test ($p > 0.05$), respectively. The data that did not meet the above assumptions were Box-Cox-transformed. A one-way analysis of variance (ANOVA) followed by Tukey's HSD test was used to test the differences in the parameters related to photosynthesis (contents of Chl *a*, Chl *b*, total carotenoids, Chl *a*+*b*, carotenoids/Chl ratio, A_{435}/A_{415} ratio, maximum values of F_V/F_M and PI_{ABS}) and WH across the examined lichens. A two-way analysis of variance (lichen species × hydration time span), followed by Tukey's (HSD) test, was performed to reveal significant differences in the F_V/F_M and PI_{ABS} values across particular lichen species and the period of time that had passed since thalli hydration. The ratios of the F_V/F_M or PI_{ABS} value obtained for an individual sample to the average maximum value of a given parameter specified for a particular species were included in the analysis (see Results section). The fluorescence transients (O-J-I-P curves) were plotted on a log-time axis based on the averaged data points ($n = 10$) double normalized to minimal (F_0) and maximal (F_M) levels for particular lichen species and examination series.

RESULTS

The physiological status of the photobionts regarding parameters related to the photosynthetic process as well as water holding properties of lichens is provided in Table 1. Since the presented values refer to specimens collected from their natural and undisturbed sites, the data reflect the normal physiological state of healthy individuals for particular species, which occurs in the middle of the growing season. The lichens differed significantly in terms of the content of photosynthetic pigments and the value of pheophytinisation quotient (Table 1). The species *Cet*, *Eve*, *Fla*, *Pse* and *Tuc* tended to have the lowest mean chlorophyll content, while the highest mean value was observed in *Men* and *Par*. Similarly, the lowest content of total carotenoids was recorded in *Cet*, *Eve*, *Fla*, *Pse* and *Tuc*. Apart from these species, *Lob* was included to the group with a relatively low content of carotenoids. The ratio A_{435}/A_{415} varied between species, the lowest mean value was noted in *Par* and *Tuc*, the highest in *Eve*. Despite the differences revealed, the content of pigments in all lichens was observed at a relatively balanced level; for example, the summed content of Chl *a*+*b*, as a rule, was in the range of 1.5 to 2.0 μg/mg of thallus dry matter. The lichens with a lower content of Chl *a* were usually characterized by correspondingly lower contents of Chl *b* and carotenoids. Additionally, increased values of the pheophytinisation quotient did not result in a considerable decrease in the content of Chl *a*, while the content of chlorophyll was always much higher than the content of carotenoids.

The maximum photosynthesis efficiency to be achieved by photobionts of particular lichen species was measured 24 hours after water had been supplied to their thalli (the assumption of achieving equilibrium state). Significant differences in the maximum F_V/F_M value between the species were revealed (Table 1). Exceptionally high values at the level of 0.8 in the case of *Hyp* and *Pse* were often recorded. Slightly lower maximum F_V/F_M ratios were noted in *Cet*, *Eve*, *Lob*, *Men* and *Tuc*. Nevertheless, very high values, always above the level of 0.7, were observed in all lichen samples regardless of species. This result proved that all collected lichen specimens were in a good physiological condition and were able to regain full photosynthetic efficiency after a hydration event. The PI_{ABS} parameter turned out to be highly species dependent and the value ranges were sometimes almost not overlapping between species (Table 1). The mean values determined in *Fla*, *Hyp*, *Lob*, *Par* and *Pse* were more than twice as high as those in the samples of *Eve* and *Tuc*.

The ability to passively absorb and store water in the thallus turned out to be very diverse among the tested lichens and a full range of propensity in

TABLE 1. Photobiont physiological parameters (mean ± SE, n = 10) related to photosynthesis and water holding index in examined lichens including the results of one-way ANOVA (F and p values are provided). Various letters indicate statistically significant differences ($p < 0.05$); for abbreviations of lichen species see Material and Methods.

Lichen	Parameters								
	Chl *a* (µg/mg DW)	Chl *b* (µg/mg DW)	Chl *a*+*b* (µg/mg DW)	Car (µg/mg DW)	Car/Chl	A_{435}/A_{415}	F_V/F_M (max)	PI_{ABS} (max)	Water holding index
Cet	1.14 ±0.04^{ab}	0.42 ±0.02^{ab}	1.57 ±0.04^{a}	0.34 ±0.02^{a}	0.22 ±0.01^{ab}	1.12 ±0.05^{ab}	0.74 ±0.01^{a}	0.42 ±0.04^{ab}	1.23 ±0.13^{abc}
Eve	1.10 ±0.05^{ab}	0.39 ±0.01^{a}	1.49 ±0.06^{a}	0.37 ±0.02^{a}	0.25 ±0.01^{b}	1.30 ±0.04^{b}	0.73 ±0.02^{a}	0.31 ±0.05^{a}	0.61 ±0.13^{a}
Fla	1.06 ±0.04^{a}	0.47 ±0.03^{ab}	1.53 ±0.05^{a}	0.34 ±0.02^{a}	0.22 ±0.01^{ab}	1.13 ±0.08^{ab}	0.77 ±0.01^{ab}	0.76 ±0.06^{b}	0.89 ±0.10^{ab}
Hyp	1.25 ±0.04^{abc}	0.49 ±0.03^{ab}	1.74 ±0.06^{ab}	0.41 ±0.04^{ab}	0.24 ±0.02^{ab}	1.23 ±0.10^{ab}	0.80 ±0.01^{b}	0.67 ±0.07^{b}	1.69 ±0.09^{c}
Lob	1.22 ±0.07^{abc}	0.49 ±0.02^{ab}	1.70 ±0.08^{ab}	0.32 ±0.03^{a}	0.19 ±0.02^{a}	1.26 ±0.04^{ab}	0.73 ±0.01^{a}	0.68 ±0.11^{b}	1.17 ±0.13^{abc}
Men	1.42 ±0.12^{c}	0.54 ±0.04^{b}	1.95 ±0.15^{b}	0.39 ±0.02^{ab}	0.20 ±0.01^{a}	1.14 ±0.04^{ab}	0.75 ±0.01^{a}	0.45 ±0.03^{ab}	1.31 ±0.13^{bc}
Par	1.35 ±0.08^{bc}	0.53 ±0.02^{b}	1.89 ±0.09^{b}	0.47 ±0.02^{b}	0.25 ±0.01^{b}	0.98 ±0.07^{a}	0.78 ±0.01^{ab}	0.76 ±0.09^{b}	1.57 ±0.17^{c}
Pse	1.09 ±0.06^{ab}	0.46 ±0.05^{ab}	1.56 ±0.09^{a}	0.35 ±0.01^{a}	0.23 ±0.01^{ab}	1.16 ±0.08^{ab}	0.80 ±0.01^{b}	0.69 ±0.07^{b}	0.86 ±0.20^{ab}
Tuc	1.05 ±0.04^{a}	0.42 ±0.03^{ab}	1.47 ±0.07^{a}	0.35 ±0.04^{a}	0.24 ±0.02^{ab}	1.04 ±0.04^{a}	0.74 ±0.02^{a}	0.32 ±0.06^{a}	1.00 ±0.11^{ab}
F value	**4.19**	**2.68**	**4.53**	**2.93**	**2.91**	**2.61**	**5.28**	**7.28**	**6.61**
p value	**< 0.001**	**< 0.05**	**< 0.001**	**< 0.02**	**< 0.02**	**< 0.02**	**< 0.001**	**< 0.001**	**< 0.001**

this respect was observed (Table 1). Water holding in *Eve*, for example, was about three times lower than in *Hyp*. Relatively low mean values of the water holding index, below or close to the level of 1.0, were calculated for *Fla*, *Pse* and *Tuc*. The capacities of thalli for water holding in the case of the remaining lichen species were higher and the indices calculated for individual samples usually exceeded the value of 1.0.

Since significant differences in the maximum F_V/F_M and PI_{ABS} values between species were indicated, value ratios (see Material and Methods section) were taken into account in determining the photosynthesis activation patterns upon thalli hydration across lichen species. This allowed for the comparison of lichens containing photobionts with different peculiar photosynthetic properties. The results of a two-way ANOVA proved that only 'hydration time span' had a significant effect ($p < 0.05$) on the photosystem II efficiency of photobionts. In contrast, factor 'lichen species' turned out not to be significant. Similarly, insignificant 'lichen species' × 'hydration time span' interaction resulted from the analysis (Table 2). Prompt activation of the photosynthesis process in all lichen species was observed; samples achieved over or almost 90% and over 98% of the maximum efficiency at 20 and 60 minutes after thalli hydration, respectively (Fig. 1a). Approaching max values of the PI_{ABS} parameter usually started from lower levels; however, after an hour, the level oscillating at 100% was reached in all examined lichens (Fig. 1b). Irrespective of lichen species and hydration time span, the fluorescence transient curves revealed the characteristic sequence of O-J-I-P steps with sigmoid character (Fig. 2). There were no strong distortions in the shape of the curves. Generally, the curves for 1 hour hydration almost coincided with the curves for 24 hours hydration in the case of each species.

TABLE 2. The results of two-way ANOVA for the effects of 'lichen species' (LS) and 'hydration time span' (HTS) on the photosystem II efficiency; value ratios (see Material and Methods section) were included in the analysis.

Parameter	Factors	SS (Sum of Squares)	MS (Mean Square)	DF (Degrees of Freedom)	η^2	F	p
F_V/F_M	LS	0.008	0.001	8	0.039	0.694	0.696
	HTS	0.072	0.036	2	0.269	**24.798**	**< 0.001**
	LS × HTS	0.011	0.001	16	0.054	0.483	0.952
	Error	0.196	0.001	135			
PI_{ABS}	LS	1.217	0.152	8	0.089	1.657	0.114
	HTS	2.369	1.184	2	0.160	**12.905**	**< 0.001**
	LS × HTS	0.908	0.057	16	0.068	0.618	0.864
	Error	12.392	0.092	135			

DISCUSSION

Lichens lack root systems, protective cuticles and have no special internal water regulation mechanisms. Therefore, they spontaneously absorb water from their surroundings in a passive way via the whole surface of the thalli. In an analogous way, water is lost through evaporation (Rundel, 1988). Therefore, the ability to perform photosynthesis closely depends on the hydration status of lichens, which in turn is determined by the availability of water and humidity of the habitat (Gasulla et al., 2021; Green et al., 2008; Kranner et al., 2003). Sufficiently frequent and efficient process of photosynthesis, as the first link in the lichen's metabolism, determines the performance and growth of lichens in the environment. Short-term fluctuations in water availability do not have serious harmful consequences, even in the case of hygrophilous lichens. Despite being deprived of water for several days, all old-growth forest indicators, such as *C. cetrarioides*, *L. pulmonaria* and *M. terebrata*, relatively quickly regained high photosynthetic efficiency, similarly to common mesophytic or xerophytic species (Fig. 1 and Fig. 2). There appears to be no relation between the ecological properties of lichens and the physiological response of their photobionts to hydration after a short rainless period, considering that the lichens developed in undisturbed conditions of a natural habitat. However, this does not contradict the fact that a small but notorious modification of the habitat parameters can be a noticeable limiting factor in the growth of sensitive lichens (Binachi et al., 2020). Furthermore, prolonged drought that causes permanent desiccation stress may prove lethal (Walters et al., 2005).

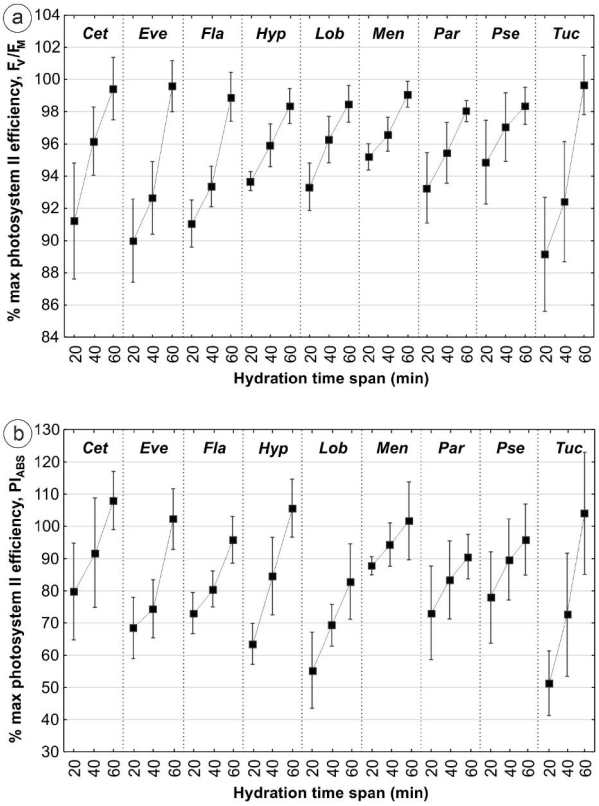

Fig. 1. Increase in photosynthesis efficiency (with regard to the F_V/F_M **(a)** and PI_{ABS} **(b)** parameters) determined in particular hydration time spans; for abbreviations of lichen species see Material and Methods.

All possible sources of water, such as rain, dew, surface flow, melting snow or water vapor, can lead to hydration of lichens. The advantages and effectiveness of these sources vary and different species are able to use them to different degrees

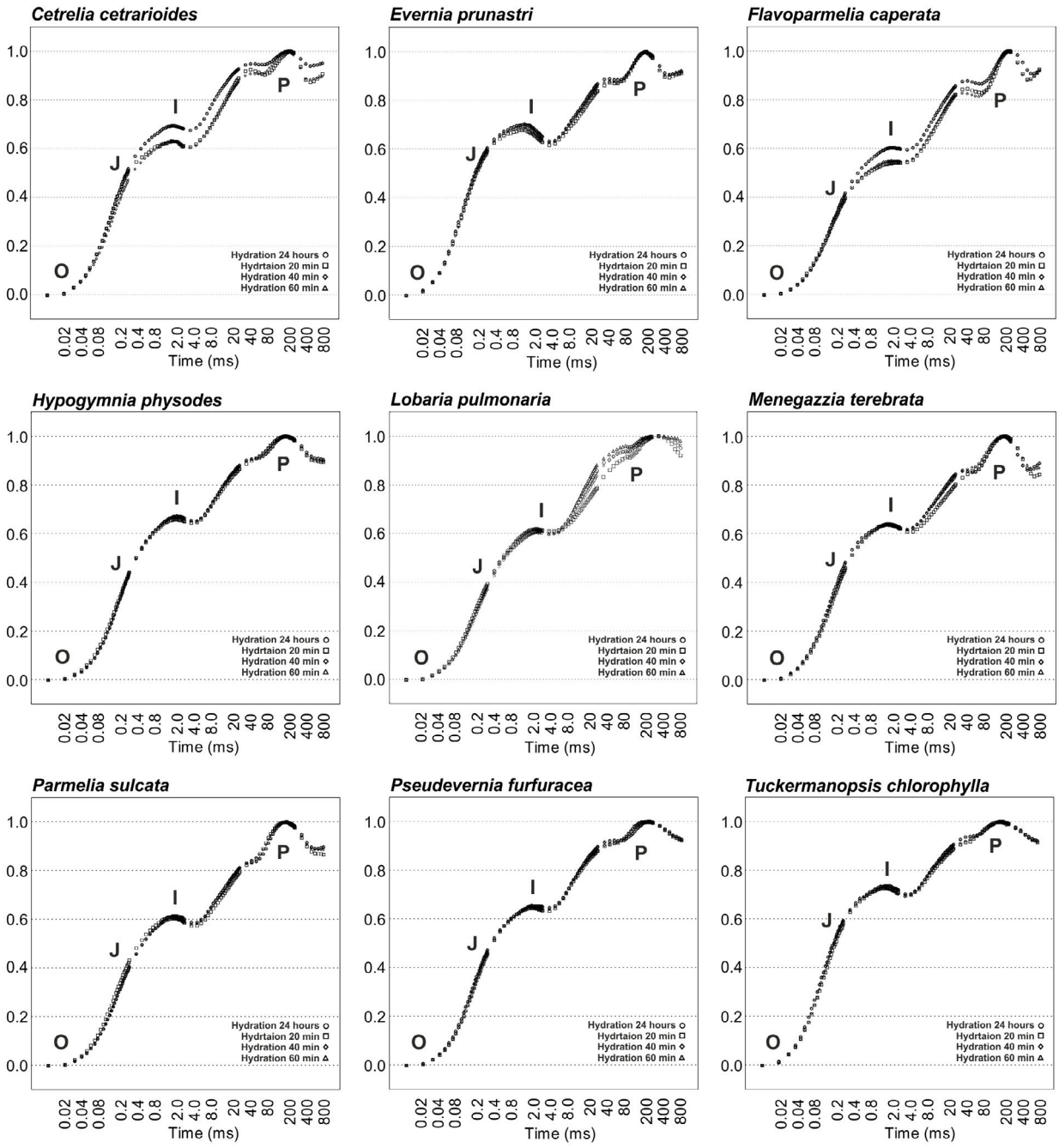

Fig. 2. Fluorescence O-J-I-P transients curves for particular lichens obtained from the thalli hydrated for 20, 40, 60 minutes and 24 hours. The curves are plotted based on the averaged data points (n = 10) double normalized to minimal (F_O) and maximal (F_M) levels.

(Rundel, 1988). Hydration by liquid water is almost immediate. Hydration with high air humidity is sluggish and less efficient and therefore generally induces a much slower photosynthesis activation, compared to direct exposure of thalli to liquid water (Jonsson et al., 2008; Lange et al., 1986). Effective hydration of the thallus and recovery of photosynthesis in highly humid air of RH close to

100% is possible, but it can take up to several hours (Hovind et al., 2020). In the temperate zone, such conditions rarely occur in natural sites, or are associated with nighttime or low-light periods of the day. Nevertheless, humid air is of great importance for lichens since it slows down evaporation and prolongs the hydration period induced by rain (Jonsson Čabrajić et al., 2010). Some of the examined epiphytes, for example *F. caperata*, *H. physodes*, *P. sulcata* and *P. furfuracea* achieved exceptionally high levels of photosynthesis efficiency after hydration with water and subsequent storage in high humidity for 24 hours (Table 1). Interestingly, very high values of the F_V/F_M and PI_{ABS} parameters concern mainly lichens with a wide ecological scale; although a high maximum of PI_{ABS} was also noted for *L. pulmonaria* (Table 1). High air humidity undoubtedly helps the lichens to obtain a state of full equilibrium.

Water holding capacity and the ability of the thalli to retain water are specific to species (Rundel, 1988). This feature influences lichen wet time and consequently the duration of photosynthesis. A fast hydration rate is frequently associated with a fast desiccation rate. The kinetics of these processes results primarily from morphological properties. The osmotic potential of the thallus, though to a lesser extent, may also be important (Hajek et al., 2006). The studied lichens clearly differ in terms of water absorbability (Table 1). The greatest weight gain upon one-hour of hydration in *H. physodes* and *P. sulcata* was observed, while the lowest in *E. prunastri*. Medulla in the first species is thick and loosened, the second species has a strongly developed lower surface of the thallus with a mass of rhizines, lobes of the last species are very thin. Nevertheless, regardless of the differences in the morphological structure and water holding, all examined lichens achieved nearly 100% of their maximum photosynthetic efficiency just one hour after they had been supplied with a relatively small quantity of water. Similarly, in all lichens, the increase in photosynthesis efficiency started from a relatively high level. Moreover, regardless of lichen species, the fluorescence transient curves typical of healthy or negligibly distorted lichens were obtained in the case of samples hydrated for only 20 minutes. Given this result, it can be assumed that at the algae cell level, the processes related to desiccation and hydration are similar in healthy lichens in the sense that the photobionts of hygrophilous lichens do not need either more water volume or longer hydration time to activate photosynthesis.

Because lichens do not change morphological form seasonally and are freezing tolerant (Solhaug et al., 2018), their metabolic activity is not necessarily limited to the vegetative season in a temperate climate. Nevertheless, lichens are exposed to the natural cycling in both chemical and climatic conditions. Therefore, their physiological parameters may vary across seasons (Malaspina et al., 2014). Fluctuations in parameters are not, in principle, regular and may concern both the fungal and algal partner. The contents of photosynthetic pigments in the examined lichen samples generally fall within the range given in analogous studies and can be considered as typical of healthy lichens. The basic chlorophyll content in terms of dry weight was different in the lichens at the time of the analysis. The same applied to the pheophytinisation quotient (Table 1). However, it cannot be inferred that differences at this level could have some effect on the rate of photosynthesis activation.

A lot of evidence has shown that some stenotopic lichens are particularly sensitive to any disturbance in their natural habitat (e.g., Nascimbene et al., 2016; Bianchi et al., 2020). The special attachment of hygrophilous epiphytes to the interior of old forests is reflected in their limited spatial distribution and sparse localities where they can be found. It seems that the short period of several days without hydration events is not a limiting factor for the physiological functioning of hygrophilous forest lichens. Such a natural situation generally does not lead to solid inhibition of the photosynthesis process in general and forest lichens do not require extra-long time (which could ensure moist and shady forest habitat) for reactivation, compared to mesophytic and xerophytic species (Fig. 1 and Fig. 2). Sensitive forest lichens, such as *C. cetrarioides*, *L. pulmonaria* and *M. terebrata* do not show any deceleration in this respect. Presumably, strong attachment to the forest habitat of many epiphytes in this functional context is associated with their low resistance to long-term desiccation stress. A forest habitat increases the chances of avoiding this stress or limiting its effects (e.g., Gauslaa et al., 2006; Renhorn et al., 1997).

CONCLUSIONS

As it turned out, healthy lichens growing under natural habitat conditions, regardless of their habitat requirements, ecological and water holding

properties, do not require long hydration time to regain a high level of photosynthetic efficiency. All examined lichen species being for a few days in dehydration state, achieved almost 100% of their maximum efficiency after an hour upon hydration (Fig. 1), starting at the level of about 90% with relatively well-shaped sigmoid fluorescence transient curves just after 20 minutes from supplying the thalli with water (Fig. 2). Analyzes of chlorophyll fluorescence have been widely used to assess the effect of potentially adverse factors on lichens vitality, also in the context of changes in the water balance and microclimatic conditions in a habitat (e.g., Gauslaa and Solhaug, 1996; Pirintsos et al., 2011; Jonsson Čabrajić et al., 2010; Atala et al., 2015). The protocol for monitoring with lichens (Nimis et al., 2002) recommends water spraying and rehydration of lichen thalli in the evening before the actual measurements (Jensen and Kricke, 2002). The results presented here support the idea of applying chlorophyll fluorescence analysis in the field to assess vitality of lichens, including highly sensitive species, and the condition of their natural habitat.

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