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Vitality of lichens under different light climates in an Araucaria forest (Pró-Mata RS, South Brazil) as determined by chlorophyll fluorescence

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ABSTRACT

The vitality of 64 lichen species (107 individual lichen thalli) growing under different light climates in an Araucaria forest in South Brazil was analyzed by chlorophyll fluorescence. Study sites were grouped according to their local light availability under full sunlight (about 2200 µmol m^{-2} s^{-1}): 1 = low light, up to 20 µmol m^{-2} s^{-1}; 2 = medium light, 20 to 100 µmol m^{-2} s^{-1}; and 3 = high light, more than 100 µmol m^{-2} s^{-1}. Maximum quantum yield of photosystem II, as shown by F_v/F_m of dark-adapted samples, was mainly between 0.3 and 0.7, with extremes of below 0.1 and up to 0.85. On average, yields were highest with low light availability (0.66). Groups 1 and 2 were not significantly different from each other, but groups 1 and 3, as well as groups 2 and 3 were. After dark adaptation, lichens were exposed to different light intensities by means of a chlorophyll fluorometer. The results show that low light-adapted lichens exhibit the highest sensitivity to excess light, as was also indicated by the data for non-photochemical quenching. Thus, shade-adapted lichens are obviously well protected from possible damage caused by excess light, which is important when exposed to sun flecks.

Keywords: Araucaria forest, chlorophyll fluorescence, heat dissipation, lichens, PAM fluorometer, photosynthetic yield

Introduction

Evaluation of photosynthetic performance is used as a vitality marker for photoautotrophic organisms. This is based on the functions of photosynthetic pigments, i.e. chlorophylls which, by photon-dependent electron transport, are involved in the formation of ATP and NADPH. If the electron transport system is somehow impaired, a surplus of light energy can be dissipated by fluorescence or heat production. The availability of portable fluorometers makes it possible to distinguish *in situ* between photosynthetic electron transport and alternative responses in a non-destructive way.

Most frequently the parameter F_v/F_m is used which is a measure of maximum photochemical quantum efficiency of photosystem II in dark-adapted plants (Bilger *et al*. 1995; Maxwell & Johnson 2000). F_v is calculated by subtracting F_o from F_m. Fo is the fluorescence when the reaction centers of photosystem II are fully open, Fm the maximum fluorescence which is obtained after applying a saturating light pulse which closes all reaction centers. In healthy higher plants maximum yield values are around 0.84. Another important parameter that can be calculated from the fluorescence data is nonphotochemical quenching (NPQ). NPQ indicates the proportion of absorbed (excess) light that is transformed into heat (Jensen & Kricke 2002). This can be taken as a measure for effective photoprotection.

Because of the ease of this technique it can be easily used for field studies and has also been applied for lichens (Gauslaa & Solhaug 1996; 2000; Jensen & Kricke 2002; Mayer &

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Hampp 2008). In contrast to plants, lichens are composite organisms made up by fungi (ascolichens; basidiolichens) and algae (chlorolichens) or/and cyanobacteria (cyanolichens). Here, the fungus delivers mainly water and nutrients while the photoautotrophic organism supplies carbohydrates. Fungal structures and compounds can also protect the algal partner from excess light and UV to varying degrees. Due to only little shadowing structures at rather dark sites (Ozenda & Clauzade 1970), the photobiont of lichens can make use of very low light intensities, but can also adapt to high ones when the fungal partner provides intense shade, especially when dry (e.g. *Dictyonema glabratum*; Mayer & Hampp 2008).

Lichens and mosses constitute a considerable floristic diversity and biomass among the epiphytes in the Araucaria forest which is still poorly investigated. Parts of the Planalto as well as the other highland of Rio Grande do Sul, the Serra do Sudeste (South Riograndensian Shield Plateau), were studied during the Regnellian Expedtion (Malme 1897), and the collected material, identified by recognized lichenologists, served as a basis for tropical lichenology (Lynge 1914; Marcelli 1998). Since 1974, Fleig and others have collected data about the lichen flora of the Planalto (Osorio & Fleig 1986; 1988; Fleig 1990; 1997; 1999; Eliasaro 1992; Fleig *et al*. 1995; Fleig & Grüninger 2000a; b; 2008; Käffer *et al*. 2009).

The climate at the site of investigation is characterized by humid air climbing from sea-level up to more than 900 m. There it cools off, causing frequent rainfall and fog. Drifting cyclones, a mixture of tropical and polar air masses, also cause rain and fog in autumn and winter. On longstanding average, there are 2,252 mm rain, and 92 foggy days per year (Bertoletti & Teixeira 1995). Occasionally, the influence of polar air is obvious in the cyclones. That is why temperatures in winter can very well dip below freezing (25 frosty days is the annual average). However, frost enters only faintly into the forest. Thus, numerous tropical lichens can be found.

Lichens of the Auracaria forest make use of a wide range of available light. Deep in the forest values down to 8 µmol photons m^{-2} s^{-1} contrast with up to 2,500 µmol photons m^{-2} s^{-1} in the open field. But due to light flecks also in dark places, high light intensities can occur for short times.

In order to investigate the adaptability of lichens to different light climates, we investigated the vitality of the photobiont at different locations in a residual Araucaria forest in Southern Brazil (Rio Grande do Sul, RS).

Materials and methods

Site of investigation

The Pró-Mata Nature Conservation and Research Center (CPCN) was created in 1991 by the PUCRS, with the support of Eberhard-Karls-University in Tübingen, Germany, and with the aim of encouraging research, environmental protection and sustainable regional development. With an area of 3.000 ha, it is located on the eastern border of the Araucarias Plateau, northeast of the state of Rio Grande do Sul (RS), between 29°27'-29°35'S 50°08'-50°15'W (Fig. 1), municipality of São Francisco de Paula in the State of RS (IMA/PUCRS & Bioma Consultoria Ambiental 2008).

The area climate is classified as super-humid to humid, conditioned by the temperature that characterizes the climate as subtropical with marine air masses that penetrate the continent. The vegetation of the area is classified as "Mixed Ombrophilous Forest", an exclusive vegetation of the Brazilian Southern Plateau, with disjunctions in elevated areas as Sea and Mantiqueira mountains. The tree formations of the Southern Plateau reflect specific situations of two floras found here: the Afro-Brazilian Tropical and the Austro-Brazilian Temperate flora, with *Araucaria angustifolia* as characterizing species.

CPCN Pró-Mata is located in the Paraná Province, in the Serra Geral Formation, which groups a thick sequence of vulcanites, mainly basaltic, containing interspersed acidic effusive rocks, which are more abundant at the top. This basalt results from the ascension of lavas from the Posadas-Torres tectonic line, the main emitter zone, around the end of the Jurassic and beginning of the Cretaceous or Neocretaceous, when the Gondwana continent broke south and formed the Atlantic Ocean. Thus, it constitutes the largest basaltic cover of the Earth, with 15 overlapping layers, reaching, in Três Forquilhas, the thickness of 1,025 m (Bertoletti & Teixeira 1995). The area is included in the eastern border of the geomorphological region "Planalto das Araucárias".

Sampling and experimental design

When a sampling site was selected, light intensity, position and orientation of the lichen on the trunk, and appearance of the thallus were recorded, and documented by photography. A specimen of the lichen was collected and protected in a paper bag for up to 3 hours at 20 to 25 °C before measurements started. For the determination of chlorophyll fluorescence, thalli were carefully sprayed with collected local rain water. Excess water was removed with paper towels. All steps of handling of the thalli were performed under low light (less than 80 µmol photons m^{-2} s^{-1}). Before starting a measurement, the thalli were kept under black velvet for at least 15 min. Sampling was always in the morning, fluorescence measurements were taken in the afternoon.

Determination of chlorophyll fluorescence parameters

Sites were chosen according to available light (photosynthetically active radiation, PAR; Licor, Lincoln, USA; Quantum Sensor, Li 190R) and the occurrence of lichens. At the period of sampling (March) maximum light intensities in the open field were about 2,200 to 2,500 µmol photons m^{-2} s^{-1}. PAR ranged from up to 20 to well above

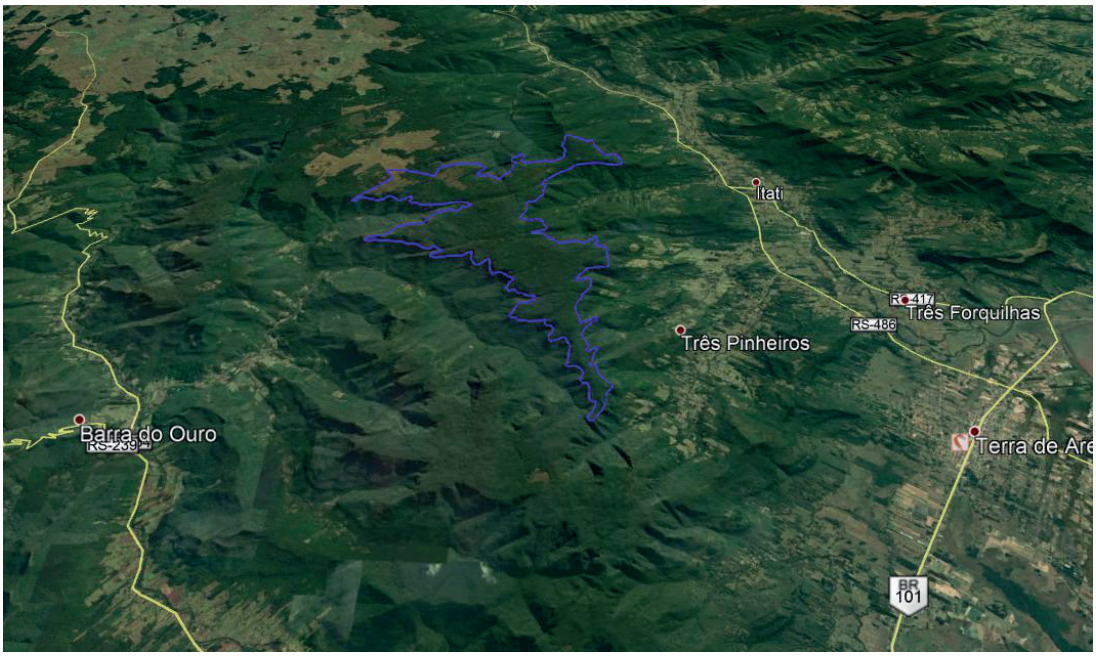

Figure 1. Localization map of the Pro-Mata CPCN area, in São Francisco de Paula, RS (IMA/PUCRS).

200 µmol photons m^{-2} s^{-1} inside the forest. Altogether, we investigated 107 lichen thalli (representing 64 different species) located on tree trunks at a hight of between 0.4 and 1.4 m (Tab. 1). They represented foliose and fructicose lichenized Ascomycota. Handling of the samples was essentially as described by Jensen & Kricke (2002). For standardized readings, we employed the Junior PAM (Walz, Effeltrich, Germany; www.walz.com) together with the "Rapid Light Curve function" program of WinControl-3 (Walz 2007) which employs eight increasing light levels with up to 840 µmol photons m^{-2} s^{-1}. All fluorescence measurements were carried out at least five times. For each successive reading, the glassfiber head was moved to a new position. For evaluation, we used the data given for maximum quantum yield of photosystem II (F_v/F_m) and "non-photochemical quenching", NPQ. F_v/F_m is generally used as a vitality marker and indicates the amount of photons used for driving photosynthetic electron transport. With higher plants, values go up to 0.84 (i.e. 84 % yield); lichens show lower yield values (Jensen & Kricke 2002; Nayaka *et al*. 2009). With dark-adapted lichens, we found values of up to 0.85. To obtain a comparable value for light sensitivity, we calculated the ratio of yields for illumination with 190 µmol photons m^{-2} s^{-1} (taken from the light curve) and dark-adapted samples.

NPQ (non-photochemical quenching) is calculated according to the equation $NPQ=(F_m-F_{m'})/F_{m'}$ ($F_{m'}$, maximal fluorescence yield of illuminated sample; WinControl-3; Walz 2007). NPQ shows the ability of the photobionts to handle excess light by heat dissipation. This means that the proportion of light which cannot by used for photosynthetic electron transport is transformed into heat. The higher the values are, the better is the detoxification of excess light. At light intensities below 100 µmol photons m^{-2} s^{-1}, values < 0.5 proof the absence of photoinhibition (Bilger *et al*. 1995; Jensen & Kricke 2002). We thus compared the ability for heat dissipation at two different light intensities, namely 190 and 66 µmol photons m^{-2} s^{-1} (also taken from the light curve), and present both the data itself and the respective ratio.

Statistics

Significance of difference between different sample values was verified with students t-test (Excel 2007).

Results and discussion

Lichens

Lichens were collected from trunks at a height of between 0.4 and 1.4 m. An overview of the lichens identified is given in Tab. 1, together with their photobionts, relative light incidence and maximum quantum yield of photosystem II.

Sites

Thirteen sites were selected according to available light at the interior or edge of the mixed Araucaria forest, ranging from deep shade (1) up to 20 µmol m^{-2} s^{-1}, through semi-shade (2) 20 to 100 µmol m^{-2} s^{-1}, to full sunlight (3) more than 100 µmol m^{-2} s^{-1} (Tabs. 2-4).

Table 1. Macrolichen specimens studied. No: number of collection; add "201603" in front of each four-digit number, ex. 1001->2016031001. Collections printed in bold are deposited in the herbarium of the PUCRS Porto Alegre/RS (MPUC). Gr/CY: photobiont cyanobacterium/green alga. RL: Relative Light intensity, given in % of PAR at full sunlight in the open field; 2,200 to 2,500 µmol photons m^{-2} s^{-1}). F_v/F_m: maximum quantum yield of photosystem II of dark-adapted samples (mean of five independent measurements).

	No.	Gr/Cy	% RL	F_v/F_m
Cladia aggregata (Swartz) Nylander	1001	Gr	98.5	-
Coccocarpia erythroxyli (Sprengel) Swinscow & Krog	0709	Cy	1.2	0.6
Coccocarpia palmicola (Sprengel) Arvidson & D.Galloway	1406	Cy	4.3	0.59
Coenogonium interplexum Nylander	0704/**1112**/1509	Gr	3.5/1.3/1.7	-/0.06/0.08
Coenogonium linkii Ehrenberger	1201/**1204**	Gr	2.7/1.1	0.06/0.04
Heterodermia casarettiana (Massalongo) Trevisan	**0801**/1104	Gr	3.7/1.1	0.31/0.20
Heterodermia galactophylla (Tuckerman) W.Culberson	0913	Gr	6.7	0.21
Heterodermia hypotraea (Vainio) Swinscow & Krog	**1303**A	Gr	47.5	0.52
Heterodermia japonica (Sato) Swinscow & Krog	1303B	Gr	47.5	0.44
Heterodermia leucomela (L.) Poelt ssp. *boryi* (Fée) Swinsc. & Krog	**0803**/1311	Gr	6.9/31.3	0.42
Heterodermia obscurata (Nylander) Trevisan	**0804**/0910/1101	Gr	4.5/10.7/10.0	0.37/0.32/0.33
Hypotrachyna consimilis (Vainio) Hale	1504	Gr	41.5	0.40
Hypotrachyna endochlora (Leigton) Hale	1506	Gr	17.7	0.41
Hypotrachyna laevigata (Smith) Hale	1306	Gr	46.9.6	0.54
Hypotrachyna livida (Taylor) Hale	1505	Gr	50.0	0.54
Leptogium austroamericanum (Malme) Dodge	1512	Cy	0.6	0.64
Leptogium azureum (Swartz) Montagne	**0609A**/1517	Cy	3.2/0.6	-/0.46
Leptogium cf.asperum Marcelli & Cunha	1113	Cy	2.7	0.45
Leptogium cyanescens (Rabenh.)Körb	1403	Cy	8.2	0.57
Leptogium cf.kalbii Marcelli & Cunha	0905	Cy	5.4	0.70
Leptogium cf.resupinans Nylander	0710	Cy	1.1	0.60
Leptogium sp.	1110	Cy	0.5	0.49
Leptogium isidiosellum (Riddle) Sierk	0701/0703/**0704**	Cy	3.7/4.5/4.5	0.32/0.32/0.27
Leptogium javanicum Montagne	0606/**1108**/	Cy	90.9/5.0	0.62/0.83
Leptogium moluccanum (Persoon) Vainio	0902	Cy	7.1	0.56
Lobaria cuprea (Müller Arg.) Zahlbr syn. *Ricasolia cuprea* M.Arg.	0908/1401/**1408**	Cy	1.0	0.30/0.69/0.66
Lobaria discolor (Bory) Hue syn. *Ricasolia discolor* (Bory) Nyl	0901/**0909**/1404	Gr	6.8	0.33/0.31/0.70
Lobaria erosa (Eschw.) Trevisan syn. *Ricasolia erosa* (Eschw.) Nyl.	0903/1107/ **1407**	Gr	5.4/3.1/2.1	0.36/0.19/0.69
Lobaria pseudolivacea Zahlb. syn. *Ricasolia olivacea* Sitzenb.	0707	Gr	3.6	0.24
Lobaria tenuis Vainio syn. *Ricasolia tenuis* (Vain.) Sitzenb.	0912	Gr	6.1	0.32
Pannaria rubiginosa (Acharius) Bory	1109	Cy	0.7	0.77
Parmotrema rampoddense (Nylander) Hale	1315B	Gr	25.0	-
Parmotrema bangii (Vainio) Hale	0610	Gr	3.2	0.45
Parmotrema cetratum (Ach) Hale	1315	GR	25.0	0.21
Parmotrema margaritatum (Hue) Hale	1102	Gr	2.3	0.26
Parmotrema melanothrix (Montagne) Hale	0911/1501/**1507**	Gr	14.3/27.7/23.1	0.33/0.33/0.33
Phyllopsora parvifolia (Persoon) Müller Arg. var. *parvifolia*	0906/**0907**	Gr	0.8/1.5	0.21/0.31
Pseudocyphellaria aurata (Acharius) Vainio	**1312**/1314	Gr	28.1	0.49/0.43
Pseudocyphellaria clathrata (De Notaris) Malme	0608/**0808**	Gr	3.2/2.4	0.49/0.49
Pseudocyphellaria kalbii Galloway	1103	Gr	1.1	0.34
Punctelia krogiae Marcelli & Canez	**0702**/0705	Gr	3.7/3.6	0.33/0.33
Punctelia microsticta (Müller Arg.) Krog	**0706**/0708	Gr	3.6/1.1	0.27/0.60
Punctelia subpraesignis (Nylander) Krog	0809	Gr	3.3	0.37
Rimelia cetrata (Acharius) Hale & Fletcher	0604/**0605/1315**	Gr	90.9/90.9/25.0	0.52/0.52/0.21
Rimelia homotoma (Nylander) Hale & Fletcher	0810	Gr	6.9	0.42
Rimelia reticulata (Taylor) Hale & Fletcher	1308/ **1315A**	Gr	71.9	0.44/
Rimelia simulans (Hale) Hale & Fletcher	1302/13**05**/1503	Gr	15.6/46.9/30.8	0.51/0.30/0.46
Rimeliella subsumpta (Nylander) Kurokawa	**1105** (*dieing back*)/1309	Gr	1.9/46.9	0.21/ 0.50
Sticta cf. *subcaperata* (Nylander) Nylander	0805	Cy	6.1	0.37
Sticta cf. *tomentosa* (Swartz) Acharius	0806	Cy	4.1	-

Table 1. Cont.

	No.	Gr/Cy	% RL	F_v/F_m
Sticta fuliginosa (Hoffman) Acharius	0607/1**402A**/1409	Cy	3.2/8.2/10.0	0.43/-/0.51
Sticta megapotamica Malme	**0904**/1206	Cy	5.4/1.3	-/0.70
Sticta sinuosa Persoon	1510/**1511**/1514	Cy	1.7/1.7/0.6	0.15/0.16/0.11
Sticta sp.	1402B	Cy	8.2 R	0.54
Sticta swartzii Galloway	**1205**/1313/1405	Cy	1.1/18.8/8.1	-/0.65/0.47
Sticta *tomentosa* (Swartz) Acharius	1111	Cy	1.3	0.63
Sticta *variabilis* Acharius	1202/**1203**/1513	Cy	0.7/1.3/0.6	0.08/0.11/ 0.18
Sticta *weigelii* (Acharius) Vainio	0807/1106/ 1316/1410	Cy	4.5/0.5/ 15.6/2.1	0.49/0.75/ 0.85/0.54
Teloschistes flavicans (Swartz) Norman	**0603**/1508	Gr	90.9/35.4	0.58/0.45
Usnea brasiliensis (Zahlbruckner) Motyka	1502	Gr	30.8	0.47
Usnea cf. *malmei* Motyka	1304	Gr	46.9	0.43
Usnea cf. *subscabrosa* Nylander ex Motyka	1307	Gr	71.9	0.43
Usnea poliotrix Krempelhuber	0601	Gr	90.9	0.43
Usnea steineri Krempelhuber	0602	Gr	90.9	0.40

Table 2. Fluorescence parameters of thalli exposed to weak light (up to 20 µmol photons m^{-2} s^{-1}). F_v/F_m, maximum quantum yield of photosystem II (dark-adapted), and ratio of quantum yield of photosystem II at 190 µmol photons m^{-2} s^{-1} versus dark adapted samples. NPQ, non-photosynthetic quenching of fluorescence (heat dissipation). Numbers in parantheses indicate different thalli of the same species.

tYield (mean)	Yield (ratio)	NPQ (ratio)	Species
(dark-adapted)	190 µ µmol m^{-2} s^{-1} / darkness	190/66 µmol m^{-2} s^{-1}	
0.64	0.35	3.8	*Punctelia microstictia* (1)
0.66	0.24	5.5	*Lobaria pseudolivacea*
0.54	0.6	3.9	*Punctelia microstictia* (2)
0.66	0.06	5.1	*Coenogonium interplexum* (1)
0.57	0.45	4.6	*Leptogium asperum*
0.69	0.39	3.6	*Pseudocyphellaria aurata*
0.67	0.06	4.4	*Coenogonium linkii*
0.70	0.08	14.9	*Sticta variabilis* (1)
0.72	0.11	9.0	*Sticta variabilis* (2)
0.73	0.04	5.5	*Coenogonium linkii*
0.56	-	8.5	*Sticta swartzii* (1)
0.55	0.70	3.8	*Hyotrachyna laeviagata*
0.64	0.08	3.7	*Coenogonium interplexum* (2)
0.68	0.15	6.3	*Sticta sinuosa* (1)
0.71	0.16	6.2	*Sticta sinuosa* (2)
0.61	0.64	3.8	*Leptogium austroamericanum*
0.63	0.18	2.0	*Sticta variabilis* (4)
0.71	0.11	5.8	*Sticta sinuosa* (3)
0.72	0.23	8.6	*Sticta variabilis* (3)
0.72	0.20	8.5	*Sticta sinuosa* (4)

Example for a full sunlight location

Figure 2 depicts an example for an isolated tree outside the forest in full sun light (2016-03-06; 13:50). PAR in the open field (reference light) was 2,200 µmol photons m^{-2} s^{-1}. According to the varying light intensity during day and under different weather conditions, light measurements are usually expressed as percentage of full sun light (100 % reference light, RL) in the open field, at the time. Lichens on the sunny side of the trunk, north exposition (southern hemisphere), received 2,000 µmol m^{-2} s^{-1}, i.e. about 90 percent of RL. In contrast, lichens in the shade (south exposition) obtained only 373 µmol m^{-2} s^{-1} (16 % RL). At the sun-exposed side, we collected three fruticose lichen species (*Usnea poliothrix*, *U. steineri*, *Teloschistes flavicans*) and two foliose ones (*Rimelia cetrata* 2x, *Leptogium javanicum*) (Tab. 1), some hepaticae and few mosses. The photobionts of all identified lichen species are green algae. When dry, the upper cortex of all species reflects light, this way probably minimizing light inhibition of photosynthesis. With their long hanging bushy thallus, the *Usnea* combs water out of the fog.

Table 3. Fluorescence parameters of thalli exposed to light intensities between 20 and 100 µmol photons m^{-2} s^{-1}. F_v/F_m, maximum quantum yield of photosystem II (dark-adapted), and ratio of quantum yield of photosystem II at 190 µmol photons m^{-2} s^{-1} versus dark adapted samples. NPQ, non-photosynthetic quenching of fluorescence (heat dissipation). Numbers in parantheses indicate different thalli of the same species.

Yield (mean)	Yield (ratio)	NPQ (ratio)	Species
dark-adapted	190 µmol m^{-2} s^{-1} /darkness	190/66 µmol m^{-2} s^{-1}	
0.62	0.49	-	*Pseudocyphelaria clathrata*
0.67	0.43	3.4	*Parmotrema bangii*
0.60	0.5	2.6	*Hypotrachyna protenta*
0.67	0.54	3.4	*Coccocarpia erythroxylii*
0.62	0.32	5.3	*Leptogium resupinans*
0.67	0.33	4.0	*Punctelia krogiae* (1)
0.49	0.32	3.3	*Leptogium isidiosellum* (1)
0.60	0.27	4.3	*Leptogium isidiosellum* (2)
0.65	0.46	3.5	*Punctelia krogiae* (2)
0.72	0.33	4.8	*Lobaria discolor* (1)
0.58	0.56	5.7	*Leptogium moluccanum*
0.71	0.36	5.0	*Lobaria erosa* (1)
0.69	0.21	3.1	*Phyllopsora parvifolia* (1)
0.71	0.31	3.0	*Phyllopsora parvifolia* (2)
0.71	0.30	3.9	*Lobaria cupea*
0.67	0.31	4.6	*Lobaria discolor* (2)
0.67	0.32	3.3	*Heterodermia obscurata*
0.67	0.33	4.0	*Parmotrema melanothrix* (2)
0.72	0.32	4.8	*Lobaria tenuis*
0.70	0.21	3.9	*Heterodermia galactophylla*
0.70	0.33	-	*Heterodermia obscurata*
0.68	0.26	3.4	*Parmotrema margaritatum*
0.72	0.34	5.1	*Pseudocyphellaria kalbii*
0.64	0.20	4.1	*Heterodermia casarettiana*
0.70	0.21	-	*Rimeliella subsumpta*
0.54	0.75	-	*Sticta weigelii*
0.73	0.19	5.5	*Lobaria erosa* (2)
0.58	0.83	4.5	*Leptogium javanicum*
0.58	0.77	-	*Pannaria rubiginosa*
0.61	0.49	-	*Leptogium* sp.
0.67	0.63	-	*Heterodermia leucomela*
0.68	0.28	4.9	*Pseudocyphellaria aurata* (1)
0.56	0.65	4.5	*Sticta swartzii*
0.69	0.43	5.3	*Pseudocyphellaria aurata* (2)
0.69	0.21	5.4	*Rimelia cetrata*
0.52	0.85	-	*Parmotrema rampoddense*

Example for a semi shade location

Figure 3 (2016-03-09. 08:45) gives an example for a dense secondary mixed forest with phorophytes of up to 10 m. The reference light varied from 280 (cloudy) to 2,200 µmol m^{-2} s^{-1}. Under sunny conditions, the diffuse light was about 58 µmol m^{-2} s^{-1} (2.6 % RL), while wandering sun Flecks provided 850 to 1,000 µmol m^{-2} s^{-1} (39 to 46 % RL). In reality, there is a variety of isolated spots, down to absolute shade which depends on branch density. This way true shade lichens and semi-shade ones may exist within a short distance. Here exclusively foliose lichens were found (Tab. 1): *Heterodermia galactophylla* (6.7 % RL), *H. obscurata* (4.5 % RL), *Leptogium moluccanum* (7.1 % RL), *L.* cf *kalbii* (5.4 % RL), *Lobaria cuprea* (1 % RL), *L. discolor* (6.8 % RL), *L. erosa* (2.1-5.4 % RL), *L. tenuis* (6.1 % RL), *Phyllospora parvifolia* var. *parvifolia* (0.8–1.5 % RL), *Sticta weigelii* (2.1-15.6 % RL), and *Rimelia subsumpta* (1.9 % RL). Of these, seven species are associated with green algae as photobionts, four with cyanobacteria. Only two species out of eleven are dark on their upper side, this way collecting sun radiation (*S. weigelii*, *L. moluccanum*).

Example for a deep shade location

This example shows a mixed forest with *Dicksonia sellowiana* of up to 15 m hight (Fig. 4; 2016-03-14; 10:15). A closed canopy of two stories strongly restricts penetration of light to the forest floor. This results in a dim moist environment around the lower tree trunks. In this case,

Table 4. Fluorescence parameters of thalli exposed to light intensities of more than 100 µmol photons m^{-2} s^{-1}. F_v/F_m, maximum quantum yield of photosystem II (dark-adapted), and ratio of quantum yield of photosystem II at 190 µmol photons m^{-2} s^{-1} versus dark adapted samples. NPQ, non-photosynthetic quenching of fluorescence (heat dissipation). Numbers in parantheses indicate different thalli of the same species.

Yield (mean)	Yield (ratio)	NPQ (ratio)	Species
dark-adapted	190 µmol m^{-2} s^{-1}/ darkness	190/66 µmol m^{-2} s^{-1}	
0.62	0.43	3.0	*Usnea poliothrix*
0.55	0.40	2.8	*Usnea steineri*
0.53	0.58	4.2	*Teloschistes flavicans* (1)
0.61	0.52	4.3	*Rimelia cetrata* (2)
0.61	0.62	4.5	*Leptogium javanicum* (1)
0.62	0.32	3.0	*Leptogium isidiosellum* (3)
0.69	-	3.8	*Heterodermia casarettiana* (1)
0.64	0.42	5.1	*Heterodermia leucomela* (1)
0.60	0.37	3.3	*Heterodermia obscurata* (2)
0.64	0.45	10.2	*Sticta subcaperata*
0.69	0.51	5.0	*Rimelia simulans*
0.70	0.52	5.3	*Heterodermia hypotrachea*
0.67	0.43	3.6	*Usnea malmei*
0.65	0.30	4.8	*Rimelia simulans* (1)
0.65	0.54	3.4	*Hypotrachyna laevigata*
0.66	0.43	2.8	*Usnea subscabrosa*
0.70	0.44	3.9	*Rimelia reticulata*
0.65	0.50	3.8	*Rimeliella subsumpta* (1)
0.47	0.83	-	*Sticta* sp.
0.54	0.49	-	*Sticta weigelii* (1)
0.69	0.24	5.1	*Pseudocyphellaria clathrata*
0.69	0.37	4.9	*Punctelia subpraesignis*
0.71	0.42	3.2	*Rimelia homotoma*
0.49	0.33	3.4	*Parmotrema melanothrix* (1)
0.62	0.47	3.6	*Usnea brasiliensis*
0.59	0.46	2.7	*Rimelia simulans* (2)
0.51	0.40	4.8	*Hypotrachyna consimilis*
0.48	0.40	4.6	*Hypotrachyna livida*
0.65	0.41	3.5	*Hypotrachyna endochlora*
0.67	0.33	3.5	*Parmotrema melanothrix* (2)
0.45	0.45	3.7	*Teloschistes flavicans* (2)

Figure 2. Sunny site. Isolated *Araucaria angustifolia* with *Usnea elongata*, *Usnea rubicunda*. Flowering *Tibouchina sellowiana* (Melastomataceae).

Figure 3. Dense secondary rainforest with medium incidence of light. On a trunk of *Brosimum gaudichii* an old thallus of *Lobaria discolor* is growing. Sun flecks with 38 to 6 % of relative light enter through gaps of the canopy (top left).

the reference light was 1,500 µmol m^{-2} s^{-1} (100 % RL) on a partly cloudy day, while the diffuse light was 58 µmol m^{-2} s^{-1} (3.9 % RL). The trees were predominantly covered by mosses. Lichens were found almost only on thin young trees. Three lichens of the foliose thallus type were selected, namely *Sticta megapotamica* (1.3-5.4 % RL), *Sticta swartzii* (1.1-18.8 % RL), *Sticta var*iabilis (0.6 to 1.3 % RL) (Tab. 1). All had a dark upper side in order to collect the sparse light. In all three *Sticta* species, the photobionts are cyanobacteria which have lower light saturation and light compensation points for photosynthesis compared to those of green algae in sun-adapted lichens (Green *et al*. 1993).

Leptogium (*L*. cf. *asperum* 2.7 % RL) and *Coenogonium* (*C. linkii* 1.1-2.7 % RL; *C. interplexum* 1.3-3.5 % RL) represent primitive types of thallus. They are highly shadow-adapted due to two peculiarities of their thallus: the biomass ratio of photobionts to mycobionts is markedly higher than in other macrolichens, and the light absorbing top coat of fungal hyphae above the photosynthesizing layer is extremely thin. In the thin black *Leptogium* species, the blue-green photobionts (*Nostoc*) are not confined to a distinct layer, but distributed in the whole transparent gelatinous thallus. The upper cortex forms a tiny single cellular layer. In the filamentous *Coenogonium* species, a relatively thick filament of the green alga *Trentepohlia* (10-24 µm in diameter) is enveloped by a tiny one-layer network of fungal hyphae.

Figure 4. Dim moist forest with 0.05 to 5 % of relative light. Trunks are covered with mosses. The photo shows a highly shade-adapted filamentous lichen, *Coenogonium* sp., with a diameter of about 10 mm.

Chlorophyll fluorescence data

Jensen & Kricke (2002) recommended a light intensity between 50 and 100 µmol photons m^{-2} s^{-1} of actinic light for NPQ studies with lichens. We have thus chosen 66 µmol photons m^{-2} s^{-1} (probably no photoinhibition) and 190 µmol photons m^{-2} s^{-1} (photoinhibition possible). Calculating a ratio of F_v/F_m and of NPQ induced by both light intensities, we aimed at getting a measure of light sensitivity of the different lichens. Tab. 2 to 4 arrange the lichens investigated according to the range of light intensities measured at their location. The values represent the local PAR during full sunlight in the open field (2,200 to 2,500 µmol photons m^{-2} s^{-1}). This is also given as relative light intensity in Tab. 1 (100 % RL represents full sunlight).

Values given for Yield and NPQ show the mean of five independent measurements with the same sample, but at changing thallus areas. In the dark-adapted state, all lichens present a rather high potential photosynthetic yield, with average values up to 0.66 (Tab. 5). This is in a range reported for healthy lichens (Jensen & Kricke 2002). In a similar study on Himalayan lichens, Nayaka *et al*. (2009) reported F_v/F_m values in a range from 0.023 to 0.655. Here, water availability and high light intensity were the major stressors. The lower values were mainly found with cyanolichens.

In the present study, lichens from low-light sites perform best (highest F_v/F_m – values on average, Tab. 5). The response to short-time illumination (20 s) with 190 µmol photons m^{-2} s^{-1} indicates the high light sensitivity of these lichens (largest decline in yield). This is shown by low 190 µmol photons m^{-2} s^{-1}/ darkness ratios (Tab. 2). Lichens from sites exposed to more than 20 µmol photons m^{-2} s^{-1} (medium and higher light intensities; Tabs. 3, 4) are more light-adapted. Here, the yield is less reduced under higher PAR. Table 5 summarizes the respective average values.

At the low-light sites (8 to 20 µmol photons m^{-2} s^{-1}), we could measure light flecks of more than 200 µmol photons m^{-2} s^{-1} which stayed in the minute-range. If this excess light causes some damage to the photosynthetic electron transport system, this is not persistent. Due to the very healthy dark values with yields up to 0.73, we assume efficient repair mechanisms (if photoinhibition occurred) which reconstitute the undamaged state. This is in accordance with observations reported by Barták *et al*. (2008). Comparing effects of duration and intensity of illumination showed that it is primarily the duration of light treatment rather than the intensity that causes damage. Thus, if light flecks occur at dark sites (and heat dissipation is not sufficient), their duration is obviously not long enough to cause longer lasting damage.

Table 6 summarizes the significance of the data. Accordingly, the maximum potential efficiency of photosystem II is highest in low light samples and significantly different from high light ones ($p = 0.03$). The same holds for medium light in comparison to high light samples.

After illumination with 190 µmol photons m^{-2} s^{-1}, the photosynthetic yield of low light samples is significantly lower than that of medium or high light lichens ($p = 0.005$ and 0.0006, respectively). There is no significant difference between medium and high light samples.

The values given for non-photochemical quenching (NPQ) give some idea about the handling of light which is in excess. These photons cannot be used for the already saturated electron transport and are thus dissipated as heat. Light-adapted organisms have generally a higher

Table 5. Fluorescence parameters of thalli exposed to different light intensities. Mean values and standard deviations of the data shown in Tables 2 to 4. F_v/F_m, maximum quantum yield of photosystem II (dark-adapted), and ratio of quantum yield of photosystem II at 190 µmol photons m^{-2} s^{-1} versus dark adapted samples. NPQ, non-photosynthetic quenching of fluorescence (heat dissipation) at 190 and 66 µmol photons m^{-2} s^{-1}, and ratio thereof.

Local light intensity	Yield (dark adapted)		Yield (ratio) 190 µmol m^{-2} s^{-1} / darkness		NPQ at 66 µmol m^{-2} s^{-1}		NPQ at 190 µmol m^{-2} s^{-1}		NPQ (ratio) 190/66 µmol m^{-2} s^{-1}	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD (n)
< 20 µmol m^{-2} s^{-1}	0.66	0.06	0.28	0.22	0.034	0.028	0.190	0.119	5.6	2.8 (22)
20 to 100 µmol m^{-2} s^{-1}	0.65	0.06	0.41	0.18	0.058	0.048	0.243	0.146	4.2	0.9 (36)
> 100 µmol m^{-2} s^{-1}	0.61	0.08	0.45	0.11	0.079	0.070	0.323	0.235	4.1	1.4 (32)

Table 6. Fluorescence parameters of thalli exposed to different light intensities. Significance of data (student´s t-test). Yield (mean) and yield (ratio) refer to the respective columns in Tables 2 to 4.

Yield data / origin of sample	P-value
Yield (mean), dark adapted. Low light versus medium light	0.7300
Yield (mean), dark adapted. Low light versus >100 µmol m^{-2} s^{-1}	0.0350
Yield (mean), dark adapted Medium light versus >100 µmol m^{-2} s^{-1}	0.0310
Yield (ratio), low light versus medium light	0.0050
Yield (ratio), low light versus >100 µmol m^{-2} s^{-1}	0.0006
Yield (ratio), medium light versus >100 µmol m^{-2} s^{-1}	0.3100

capacity for such photoprotection. In Tab. 2 to 4, we compare NPQ values at 66 and 190 µmol photons m^{-2} s^{-1} (see above, recommendations for NPQ studies with lichens). The rationale was: can lichens from illuminated sites cope better with excess light than those from rather dark sites? The data show a very high variation of the ratio of NPQ for both light intensities. Low-light adapted lichens respond with a rapid onset of protective heat dissipation. The ratio of NPQ at PAR 190 versus 66 mol m^{-2} s^{-1}) is 5.6 compared to 4.2 and 4.1 for more light-exposed lichens (Tab. 5). In addition, Tab. 5 gives the average values for NPQ at both light intensities. Even for 190 µmol photons m^{-2} s^{-1} they are well below 0.5. According to Jensen & Kricke (2002) such values are proof of absence for photoinhibition.

Pardow *et al*. (2010) compared the performance of lichens in the interior and at the edge of Atlantic rain forest fragments in Brazil. They distinguished cortical and non-cortical groups of lichens. Lichens in the interior (dominated by the cortical group) showed a higher proportion of PAR absorbed, called absorptivity, whereas the maximum quantum yield of photosystem II in the dark-adapted state was largely similar for both environments and groups (F_v/F_m = 0.58). Only non-cortical lichens (not considered in this study) in the forest interior had somewhat lower yields (0.53 on average). These values are considerably lower than those reported here (0.66) and by Jensen & Kricke (2002; 0.6 to 0.76).

Taken together, we found that lichens from dark as well as well illuminated sites exhibit similarly high values of potential photosynthetic yield, when determined in the dark-adapted state. However, when exposed to increasing photosynthetic active radiation, lichens from low light sites are highly sensitive and make full use of protective mechanisms (heat dissipation), Obviously heat dissipation was so effective, independent of light climate, that photoinhibition did not occur.

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