DR. JOSEF HÁJEK (Orcid ID : 0000-0002-2679-1707)

DR. ANA CAROLINA MATEOS (Orcid ID : 0000-0002-6787-0153)

Article type : Research Paper

Editor : R Leegood

Corresponding author mail id: jhajek@sci.muni.cz

Effect of UV-B radiation on the contents of UV-B absorbing compounds and photosynthetic parameters in *Parmotrema austrosinense* from two contrasting habitats

M. Barták[1], E. Pláteníková[1,▪], H. Carreras[2], J. Hájek[1,*], J. Morkusová[1], A. C. Mateos[2] & M. Marečková[1]

[1]*Department of Experimental Biology, Laboratory of Photosynthetic Processes, Faculty of Science, Masaryk University, University Campus – Bohunice, Kamenice 5, 625 00 Brno, Czech Republic*

[2] *Instituto Multidisciplinario de Biología Vegetal, CONICET and Chemistry Department, FCEFyN, Universidad Nacional de Cordoba, Av. Velez Sarsfield, 1611, X5016 GCA, Cordoba, Argentina*

▪ *recent address: Slovanské náměstí 7, 612 00 Brno, Czech Republic*

* Corresponding author

Abstract

- We studied the resistance of *Parmotrema austrosinense* to UV-B stress. We focused on the effects of a high dose UV-B radiation on the contents of (chlorophylls, carotenoids) and UV-B screening compounds.

- Photosynthetic parameters were measured by chlorophyll fluorescence (potential and effective quantum yields, photochemical and non-photochemical quenching) and evaluated in control and UV-B treated lichens as well. Lichens from two different locations in Cordoba, Argentina were selected: (1) high altitude and dry plots at (Los Gigantes) and (2) lowland high salinity plots (Salinas Grandes).

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/plb.12855

- UV-B treatment led to a decrease in the content of photosynthetic pigments and UV-B screens (absorbance decrease in 220-350 nm), in the samples from Salinas Grandes, while in Los Gigantes samples, an increase in UV-B screens contents was observed. Chlorophyll fluorescence parameters showed UV-B induced decline in F_V/F_M, Φ_{PSII} and qP indicating limitation of primary photosynthetic processes in photosystem II of symbiotic alga, more pronounced in Salinas Grandes samples. Protective mechanism of PS II were activated by the UV-B treatment to a higher extent in the samples from Salinas Grandes (NPQ of 0.48) than in Los Gigantes samples (NPQ of 0.26).

- We concluded that site-related characteristics and in particular different UV-B radiation regimen, had a strong effect on resistance of photosynthetic apparatus of *P. austrosinense* to UV-B radiation.

Key words: lichens; UV-B resistance; chlorophyll fluorescence parameters; UV spectra; absorbance.

Acknowledgements:

The authors thank the MOBILITY project No. 7AMB15AR021 for support. The Argentinian-Czech project allowed joint work of team members from the University of Cordoba (Argentina) and the Masaryk University, Brno (Czech Republic). The work reported in this study, digital microscopy in particular, was supported also by the ECOPOLARIS project (CZ.02.1.01/0.0/0.0/16_013/0001708) provided by the Czech Ministry of Education, Health and Youth.

Running Title: Resistance of *Parmotrema* to UV-B

Introduction

In lichens growing in open and unshaded habitats, several photoprotective mechanisms exist. They minimize negative effects of photosynthetically active radiation and UV radiation to photosynthetic apparatus of lichen photobionts. Several compounds are involved into photoprotection, e.g. UV-B screens, pigments and enzymes with antioxidative activities. In lichens, more than 100 UV absorbing compounds described (for review, see *e.g.*, Nguyen *et al.* 2013). They are mainly secondary metabolites produced by the fungal partner. In addition to a strong absorption of radiation in the UV-B region, these compounds also have the ability to scavenge free radicals and reactive oxygen species formed during photoinhibiton (PAR/UV-B).

Lichen UV-B absorbing compounds are products of three principal synthetic pathways: acetyl polymalonyl pathway, shikimic acid pathway and mevalonic acid pathway. Majority of UV-B absorbing compounds, such as *e.g.*, phenolics, depsids, depsidones, depsons, antraquinones,

dibenzophurans, usnic acid and its derivates, antraquinones, xanthons, belong to the acetyl polymalonyl pathway (see Elix & Stocker-Wörgötter 2008). Mevalonic pathway is used in the synthesis of terpens and steroids. Shikimic acid pathway produces terphenylchinones and pulvic acids derivates.

In a single lichen species, amount of UV-B absorbing compounds may vary within a single thallus and between thalli of a lichen population. These differences in the amount of UV-B compounds are age- and morphology-related. In the complex thallus arrangement, different thallus parts may experience different light quantities which may result in intrathalline variability in UV-B absorbing compounds (Bjerke *et al.* 2002; 2004). Such individual and intra-population variability in the amount of UV-B absorbing compounds is smaller than differences between among the thalli of a lichen populations growing under contrasting light conditions (sun vs. shade habitats). Different doses of sunlight (Bjerke & Dahl 2002) and different water supply that modifies intrathalline reflection of radiation (Solhaug *et al.* 2009) may affect UV-B screens production and allocation in lichens. Lichens from high altitudes cope with higher UV-B doses than those from lowlands. Thus, they exhibit a higher amount of UV-B screening compounds (Rancan *et al*. 2002). Together with other climatological factors this leads to seasonality in the amount of UV-B absorbing compounds in lichen thalli.

Parmotrema is one of the largest genera in the Parmeliaceae family, with more than 220 out of 350 known species distributed mainly in tropical regions (see *e.g*. Jayalal *et al*. 2013; Perez & Guzmán 2015). In Argentina, the lichens of genus *Parmotrema* are quite abundant also in the mountainous (Estrabou 1999) and central parts of Argentina (Rodriguez *et al.* 2016). In Cordoba province, it grows in several habitats ranging from lowland to mountain areas. Within the last decades, research focused on various ecological and biological aspects of the *Parmotrema genus* addressing species tolerance to air pollution (Estrabou *et al*. 2011), oxidative stress in lichen photobiont (Caviglia & Modenesi 1999; Sharma & Kalikotay 2012) and intrathalline content of secondary metabolites with antioxidative (Ghate *et al*. 2013) and antibacterial (Jain & Jain 2016) activities. In other studies, spectral properties of the lichen thallus in response to its hydration status (Barták *et al*. 2016), herbicide effects on anatomical characteristics (Modenesi 1993), biological characteristics of *in-vitro* cultivated mycobiont (Fazio *et al.* 2009) have been assessed. Sensitivity to other ecological factors, however, has been scarcely studied. Shukla *et al*. (2016) investigated protective secondary compounds in *P. reticulatum* in relation with microclimatic factors related to altitude. Similarly, Armaleo *et al*. (2008) focused on light intensity effects on depsides and depsidones in *P. hypotropum*. Natural content of depsidons was investigated by Duong *et al*. (2015) in *P. tsavoense.* Sensitivity of *Parmotrema* species to UV-B radiation and changes in secondary metabolites to altered UV-B is still unknown. Similarly, only limited knowledge exists on UV-B effect on photosynthetic parameters. To fill this gap, we exposed *P. austrosinense* to UV-B radiation under laboratory conditions and evaluated changes in photosynthetic pigments, UV-B absorbing compounds, chlorophyll fluorescence parameters as well as spectral absorbance curves parameters. We hypothesized that *P. austrosinense* from mountainous locations, exposed to a higher amount of incident UV-B radiation, would exhibit more resistance to UV-B treatment than samples from lowland locations.

Material and Methods

Species description

Parmotrema austrosinense has a foliose thallus, 4 to 12 cm diameter. The thallus is lobate, individual lobes are narrow to broad, and plane with subrotund apices. Thallus color differ between the upper and lower thallus surface, ranging from pale green to yellowish grey on the upper surface while the lower surface is pale brown to black. The lower surface is also rich in black rhizines. The species grows on bark of trees or shrubs, rock surfaces, in open habitats and woodlands. The species is reported (Jaylal *et al*. 2013) to have several secondary metabolites: upper cortex with atranorin and chloroatranorin; medulla with lecanoric acid (http://lichenportal.org/).

Site description and lichen collection

Thalli of *Parmotrema austrosinense (Zahlbr.)* Hale were collected from two different habitats in Cordoba Province. The first sampling site is located at the foothills of Los Gigantes, Sierras Grandes, 70 km west from Cordoba city. This is a pristine area, around 100-1200 m a.s.l., with xerophile vegetation, mostly perennial shrubs.

The other collection site was Salinas Grandes region, 200 km north from Cordoba city. Altitude varies between 150 m a.s.l. on the salt beaches and 300 m a.s.l. on the margins. It belongs to the Semi-desert climatic domain, with less than 400 mm rainfall per year, being the driest region of Córdoba province. The vegetation, including lichens, is adapted to dry conditions and typically halophilic.

Lichen thalli were collected from tree trunks or rocks; they were clean from adhering pieces of rock or substrate and were stored in the laboratory at room temperature.

Morphological characteristics

To characterize *P. austrosinense* morphology and anatomy, cross-sections were analyzed using the digital microscope WHX-900F linked to a full HD LCD monitor (Keyence, Japan). Before analysis, the lichen samples were allowed to rehydrate for at least 24 hours while illuminated by a light source (LED panel produced by Tron, Brno, CZ, spectrum of 400-750 nm, 15 μmol m^{-2} s^{-1} of PAR) at 10 °C. Then, thin slices of lichen samples were taken and photographed with a small amount of demineralised water to prevent drying. Thickness of lichen thalli, upper cortex, photobiont layer and lower cortex were measured and analysed using the Keyence WHX software.

UV-B Treatment

Hydrated thalli of *P. austrosinense* were placed into Petri dishes and covered by a UV-B transparent plastic foil to prevent changes in thallus hydration status. Optimally-hydrated lichen thalli (tested before and selected on basis of maximum constant F_V/F_M value reached after 48 h hydration) were exposed to photosynthetically active radiation (10 μmol m^{-2} s^{-1} PAR, measured by a Li-1400 radiometer, Li-Cor, USA) supplemented by 3.0 W m^{-2} of UV-B radiation for 10 d. During the exposition, temperature was kept at 20.0±0.4 ^{o}C (measured by a HOBO thermosensor and datalogger, OnSet Computers, USA). Within the period, the thalli were exposed to PAR + UV-B continuously, i.e. there was no dark subperiod). As UV-B source, a broadband TL lamp (Phillips, the Netherlands, type: TL 20W/12 RS SLV) was used which emitted radiation in the 'B' bandwidth of the UV spectrum (290 to 315 nm). In pre-experiments, we tested also 0.6 and 1.4 W m^{-2}(measured by a SpectroSense unit equipped with a UV-B sensor, Skye Instruments, Wales, UK), however, since these two UV-B doses induced only very limited response of chlorophyll fluorescence parameters, they were excluded and only the dose of 3.0 W m^{-2} was used for the experiment. During exposition, chlorophyll fluorescence parameters were measured after 24, 48, and 72 h to evaluate the effect of supplemental UV-B on photosynthetic processes.

UV-B screens and pigment content

Control and UV-B treated thalli (10 d of supplemental UV-B) were dried out in a lyophilizator for 24 h. and then homogenized with a ball mill (Retsch MM 2000, Germany). 100 mg of the homogenized vegetal material were extracted with ethanol and the absorbances within 190-700 nm wavelength range were measured with a UV-VIS spectrophotometer (Specord 205, Analytik Jena, Germany). The absorbances at 280 and 310 nm were measured for UV-B screens, according to Buffoni-Hall *et al*. (2002); in addition, the absorbances at 470, 649, and 664nm were measured to analyze the content of for carotenoids (Car), chlorophyll a (Chl a), and b (Chl b) according to Lichtenthaler & Wellburn (1983).

Chlorophyll fluorescence

Prior to chlorophyll fluorescence measurements, dry thalli of *P. austrosinense* were rehydrated for 48 h over wet filter paper placed in Petri dishes under a dim light at 5°C. After rehydration and full activation of primary photochemical processes (maximum F_V/F_M – data not shown), chlorophyll fluorescence parameters F_V/F_M, Φ_{PSII}, qP, and NPQ were measured day 0, control). Then, the same chlorophyll fluorescence parameters were measured after 24, 48, and 72 h of exposition to supplemental UV-B radiation. The measurements consisted of the following steps: thalli were pre-darkened for 10 min in the measuring compartment of a FluorCam HFC-010 fluorimeter (Photon Systems Instruments, Czech Republic). Then slow Kautsky kinetics supplemented with quenching analysis were measured and particular chlorophyll fluorescence signals recorded (Fig. 1). First, background chlorophyll fluorescence F_0 was measured on the sample exposed to low (measuring) light. Then, the thalli were exposed to a saturating pulse of light to induce maximum chlorophyll fluorescence (F_M). Afterwards, thalli were light-adapted for 5 min until the steady state chlorophyll fluorescence (F_S) was reached. At this moment, thalli were exposed to another saturating pulse of

light and the value for maximum chlorophyll fluorescence on light-adapted sample (F_M') was obtained. To evaluate UV-B effects on photochemical processes of photosynthesis in *P.* austrosinense, potential (F_V/F_M) and effective quantum yield (Φ_{PSII}) were calculated. The eqns. 1, 2 in the FluorCam7 software (Photon Systems Instruments, Czech Republic) were used for this purpose.

Similarly, coefficient of photochemical (qP), and non-photochemical quenching (NPQ) related to the proportion of absorbed light energy utilized in photosynthetic and non-photosynthetic protective processes, respectively, were calculated using the eqn. 3, and 4 preprogrammed in the FluorCam7 software.

$$F_V/F_M = (F_M - F_0)/F_M \quad \text{Eqn.1,}$$

$$\Phi_{PSII} = (F_M' - F_S)/F_M' \quad \text{Eqn.2,}$$

$$qP = (F_M' - F_S)/ (F_M' - F_0) \quad \text{Eqn.3,}$$

$$NPQ = (F_M - F_M')/ F_M' \quad \text{Eqn.4.}$$

Time courses of the chlorophyll fluorescence parameters were constructed for the lichens from Los Gigantes and Salinas Grandes and the differences discussed. Additional chlorophyll fluorescence parameters were calculated from chlorophyll fluorescence values (F_0, F_{M1}, F_P, and F_S signals) of the slow Kautsky kinetics (for the signals specification, see Fig. 1). The parameters were specific ratios F_P/F_S, F_P/F_{PP}, F_{M1}/F_S (for explanation, see the Table 4 legend), relative fluorescence decrease Rfd (Lichtenthaler *et al*. 2005), and the time at which the M1 peak was reached (t_{M1} - for M1 details, Fig. 1 legend).

Statistical analysis

Differences in chlorophyll fluorescence parameters recorded after 24, 48, and 72 h of UV-B exposure, and the differences related to locality (Salinas Garndes, Los Gigantes) were evaluated by factorial ANOVA (STATISTICA, StatSoft v. 13) by LSD Fisher Test. The statistically significant differences were considered at P 0.05.

Results

Thallus anatomy

P. austrosinense showed a large variability in morphometrical parameters of thalli (Table 1). However some parameters, such as *e.g*., upper cortex thickness did not differ between central and marginal parts of thalli. Photobiont layer thickness was much larger in central (48.3 μm) than

marginal (38.2 μm) thallus parts. Similarly, overall thallus thickness was larger in central (271.4 μm) than marginal thallus parts (214.3 μm). Medulla, as well as lower cortex thickness were 32.4%, and 30.2%, respectively larger in central than marginal thallus parts.

Photosynthetic pigments

The levels of carotenoids showed a decrease after 24 h of UV-B treatment followed by an increase after 10 d of treatment (Table 3). These changes were, however, statistically insignificant. In thalli from both sampling sites (Salinas Grandes and Los Gigantes, respectively), carotenoids levels were the same or higher after UV-B treatment (10 d) compared with the initial values. Chl a contents in the thalli followed a similar trend over the experimental time course, *i.e*. a decrease after 24 h treatment followed by an increase. Thanks to a high variability in raw data on Chl a, the trend was not statistically significant. The final Chl a content was more or less comparable to initial values or higher, similarly to what was observed with carotenoids. In thalli from Los Gigantes, the Chl a contents was significantly higher after 10 d exposition than that recorded after 24 h exposition. Chl b content exhibited different pattern in the thalli from contrasting localities. It showed a decrease followed by an increase samples from Salinas Grandes, while an increase in Chl b values was found in thalli from Los Gigantes. These UV-B exposition- and locality-related differences were, however, statistically insignificant.

Chlorophyll fluorescence kinetics

The shape of slow chlorophyll fluorescence kinetics and the parameters derived from the curves were affected by UV-B. The effects, were time-dependent reflecting either the changes happening within the first 24 h of UV-B exposition (e.g. chlorophyll fluorescence signal FM1, Fo, Fp, Fs in Los Gigantes sample) or gradual accumulated stress with the time of the UV-B treatment (e.g. parameters Fp/Fpp and FM1/Fs for both localities). The main difference of a shape of the slow chlorophyll fluorescence kinetics was in the relation of steady state (F_S) to backround chlorophyll fluorescence (F_0 at O point). Typically Fs which was lower than F_0 in control thalli but was found higher than F_0 in UV-B exposed thalli. In *P. austrosinense* collected in Salinas Grandes, F_{M1}, F_P, F_S, F_0, F_P/F_S, F_{M1}/F_S, Rdf, t_{M1}, and F_P/F_{PP} showed a decrease with the time of UV-B treatment. Majority of these changes were, however, statistically insignificant. Contrastingly, thalli from Los Gigantes showed either increase (F_P/F_S, F_{M1}/F_S, Rdf) or an increase followed by a decrease (F_{M1}, F_P, F_S, F_0) or decrease (t_{M1}, F_P/F_{PP}) with UV-B treatment (Table 4). The changes were not statistically significant.

Effects on photosynthetic parameters

The exposure of *P. austrosinense* thalli to UV-B (3 W m^{-2}) led to a gradual decrease in potential (F_V/F_M) and effective (Φ_{PSII}) quantum yields of photosynthetic processes in photosystems II (see Fig. 2). Within 0 - 48 h exposure interval, the F_V/F_M decrease was faster and more pronounced in the thalli from Salinas Granded than Los Gigantes. With UV-B exposition, Φ_{PSII} decreased in both Los Gigantes and Salinas Grandes samples. For both localities, the Φ_{PSII} values recorded after 72 h

exposition decreased significantly compared to control (initial values). After 72h of exposition, however, Φ_{PSII} values were not significantly different (Salinas Grandes compared to Los Gigantes samples). Photochemical quenching coefficient (qP) which reflects the proportion of open PSII reaction centres used in energy conversion of absorbed light, declined with the time of UV-B exposition in lichens retrieved from both sampling sites. The decline, however, was not statistically significant. Similarly, the differences in qP between Los Gigantes and Salinas Grandes thalli were found insignificant. Non-photochemical quenching (NPQ) which is generally attributed to an activation of protective mechanism in chloroplastic apparatus, increased from 0.2 to 0.26 in the Los Gigantes thalli within the 24 h UV-B exposition and then remained more or less constant with the time of exposition. The thalli from Salinas Grandes, however, showed, besides the increase within the 24 h exposition, a slightly increasing trend in NPQ within the period from 24 to 72 h exposition. The NPQ differences between Salinas Grandes and Los Gigantes samples, as well as between NPQ in control and after 72 h of UV-B treatment (for a locality) were found statistically significant.

UV-B absorbing compounds

Long-term (10 d) exposition to high dose of UV-B (3.0 W m^{-2}) led to a decrease in UV-B absorbing compounds in *P. austrosinense* from Salinas Grandes (Fig. 3). The decrease was found in the compounds absorbing within the range of 220-370 nm (Fig. 3, left). The absorbance peaks were evident at 220, 272, and 320 nm in the spectral curve of UV-B. In control thalli, no such peaks were distinguishable. In thalli from Los Gigantes, an increase in UV-B absorbing compounds was evident within the range of 220-540 nm. The increase in UV-B absorbing compounds was even higher after 24 h of UV-B treatment (see Table 2). The absorbance peaks were not well distinguished due to noisy signal in thalli from Los Gigantes, however, they were fairly evident at the ranges 220-230, and 270-280nm.

Discussion

Thallus anatomy

The results on thallus anatomy are well comparable with data reported by Bissacot Barbosa and Marcelli (2010). The algal layer thickness in *P. austrosinense* was similar to the values found for *P. perlatum* (22 μm, Carniel *et al*. 2015). Also, the values were similar to data reported for other Parmeliaceae species, such as *e.g*., *Parmelia sulcata* (45-85 μm, Benett, 2002). Our results on algal layer thickness in *P. austrosinense* are well comparable the data obtained for foliose lichens. Similar photobiont layer thickness is reported for *Dermatocarpon polyphilizum* (25-160 μm, Marečková *et al*. 2017) and *Umbilicaria antarctica* 44.2 μm (Marečková, unpublished data). In cyanolichen (*Peltigera* sp.), however, photobiont layer was thicker (70.8 μm, unpublished data). Other thallus measurements were similar to previous data from *P. perforatum* regarding thallus (156-362 μm) and upper cortex thickness (19-88 μm), as well. *Overall, these* results suggest that multiple independent environmental variables such as *e.g*., light regimen of the habitat, may be responsible for differences in intrathalline morphology characteristics of *P. austrosinense,* such as algal layer thickness.

Chlorophyll fluorescence kinetics

For slow Kautsky kinetics of chlorophyll fluorescence, F_{M1} were found higher than F_P in control and UV-B treated *P. austrosinense.* The apparent secondary peak of chlorophyll fluorescence (M1) recorded during actinic light period is typical for some lichens species (Conti *et al*. 2014) and attributed either to less effective reoxidation of plastochinone pool or to contribution of F_0 (during actinic light) to the overal chlorophyll fluorescence signal (Roháček and Barták, 1999). The time at which M1 peak was reached decreased with UV-B treatment similarly to the evidence reported by Estêvão (2015) for *Xanthoria elegans.*

Chlorophyll fluorescence signal changes during slow Kautsky kinetics record comprise combined effect of photochemical and non-photochemical processes in photosynthetic apparatus. Since both photochemical and non-photochemical processes are simultaneous, the slow Kautsky kinetics curves show a polyphasic character, in which the presence and relative height of M1 peak might be attributed to negative effects of stress action in photosynthetic apparatus and activation of protective mechanisms (non-photochemical quenching). In our data, non-photochemical quenching was induced by supplemental UV-B, since an increase in NPQ with UV-B exposition time was apparent for both localities (see Fig. 2). Similarly, the rate of chlorophyll fluorescence signal decline from M1 to a steady state (F_S – see Fig.1) may support the conclusion of UV-B –induced stress to photosynthetic apparatus because M1 to Fs decline is indicative of initial phase of inhibition of primary photosynthetic processes and an increase in non-photochemical processes (see NPQ in Fig. 2). The fast decline from M1 and early reach of the steady state chlorophyll fluorescence indicates non-stressed plant (Fs constant after 72 h of UV-B treatment, Los Gigantes samples) while slow decline is indicative of stress. In the latter case, steady state is not reached after 72 h of UV-B exposition (cf Fig. 1, Fs is not constant at 300 s of actinic light) - samples from Salinas Grandes).

UV-B absorbing compounds

The UV-B induced damage to the UV-B screens was demonstrated in *P. austrosinense* from Salinas Grandes after 10 d of UV-B treatment. The decrease in absorbance values within the range of 180-350 nm, as well as 280 and 300 nm wavelengths is well comparable to data of Estêvão (2015) who reports initial increase of UV-B screens followed by a decrease after 6 d treatment, more pronounced at 3.0 than 1.5 W m^{-2}. This effect indicates destruction of the screens and the decrease of absorbance is similar to the situation when UV-B screens are removed from the thallus by acetone rinsing (Martic 2016). The decrease in absorbance might be attributed mainly to usnic acid destruction, since the spectrum remaining after UV-B treatment might be atranorin (having the peak absorption in 230, 280, and 320 nm, Plsíková *et al*. 2014), and/or lecanoric acid with similar peak absorption (213, 270 and 304 nm, Luo *et al*. 2009; BeGora & Fahselt 2001). Increase in absorbance in *P. austrosinense* from Los Gigantes found after 10 d of UV-B treatment indicates a high capacity of the thalli grown at high altitude to synthetize UV-B absorbing compounds and, therefore, a higher constitutive resistance to UV-B negative effects. Higher values of Φ_{PSII}, and qP (Fig. 2), together with lower NPQ values, found in thalli from Los Gigantes (control, i.e initial values before UV-B treatment) compared to those from Salinas Grandes support the conclusion that primary photosynthetic

processes in thalli collected from high altitudes are less affected by high doses of UV-B than low-altitude thalli. The differences in UV-B absorbing compounds and chlorophyll fluorescence parameters found between *P. austrosinense* from the two collection sites might be attributed to different UV-B to which the lichens are exposed in their contrasting habitats (high vs. low altitude) . Indeed, measurements reported by Luccini *et al*. (2006) indicates UV Index of 12-14 and 8-10 for January in Los Gigantes and Salinas Grandes, respectively.

Chlorophyll fluorescence parameters

The effects of UV-B on F_V/F_M, Φ_{PSII}, qP, and NPQ presumably result from the UV-B dependent synthesis and destruction of sun-screening compounds. Protective effects of these compounds in lichens are well documented for *e.g*., UV-B induced synthesis of parietin that protects lichen photobionts against excessive photosynthetically active radiation (*e.g*., Gauslaa & Solhaug, 2004). Therefore, increased UV-B causes increased PAR-screening, and thus leads to a pigment-dependent reduction in light amount available at the photobiont level beneath the increasingly coloured upper cortex. Such responses are, however, caused by natural physiological amounts of UV-B. The effects of higher than physiological UV-B doses, such as those used in our study, lead to dectruction of pigment-protein complexes forming the photosynthetic apparatus of symbiotic alga and, consequently to a decrease in Φ_{PSII} (Fig. 2). In our study, however, the UV-B induced decline in Φ_{PSII} did not lead to full inhibition indicating that photosynthetic processes in *P. austrosinense* are quite resistant to high UV-B doses. Such resistance was already documented for the lichen species growing in open habitats such as *Xanthoria elegans* (Nybakken *et al*. 2004). In lichens treated with high UV-B doses, photosynthetic processes in a photobiont do not seem to be a critical point for growth and survival since the mycobiont is reported to be more susceptible to UV-B and thus responsible for the reduction in biomass production and growth limitation in lichens (Chowdhury *et al*. 2017).

References

Armaleo D., Zhang Y., Cheung S. (2008) Light might regulate divergently depside and depsidone accumulation in the lichen *Parmotrema hypotropum* by affecting thallus temperature and water potential. *Mycologia,* **100**, 565-576.

Barták M., Hájek J., Amarillo A. C., Hazdrová J., Carreras H. (2016) Changes in spectral reflectance of selected Antarctic and South American lichens caused by dehydration and artificially-induced absence of secondary compounds. *Czech Polar Reports,* **6**, 221-230.

BeGora M. D., Fahselt D. (2001) Usnic acid and atranorin concentrations in lichens in relation to bands of UV irradiance. *The Bryologist,* **104**, 134-140.

Bennett, J. P. (2002) Algal Layer Ratios as Indicators of Air Pollutant Effects in *Parmelia sulcata*. *The Bryologist,* **105**, 104-110.

Bissacot Barbosa S., Marcelli M. (2010) Comparative thallus anatomy of two *Parmotrema* (Parmeliaceae, lichenized Ascomycota) with reticulate maculae. *Acta Bot. Brasilica,* **24**, 803-811.

Bjerke J. W., Dahl T. (2002) Distribution patterns of usnic acid-producing lichens along local radiation gradients in West Greenland. *Nova Hedwigia,* **75**, 487-506.

Bjerke J. W., Joly D., Nilsen L., Brossard T. (2004) Spatial trends in usnic acid concentrations of the lichen *Flavocetraria nivalis* along local climatic gradients in the Arctic (Kongsfjorden, Svalbard). *Polar Biology,* **27**, 409-417.

Bjerke J. W., Lerfall K., Elvebakk A. (2002) Effects of ultraviolet radiation and PAR on the content of usnic and divaricatic acids in two arctic-alpine lichens. *Photochemical & Photobiological Sciences,* **1**, 678-685.

Buffoni-Hall R. S., Bornman J. F., Bjorn L. O. (2002) UV-induced changes in pigment content and light penetration in the fruticose lichen *Cladonia arbuscula* ssp. Mitis. *Journal of Photochemistry and Photobiology B: Biology*, **66**, 13-20.

Carniel F.C., Zanelli D., Bertuzzi S., Tretiach M. (2015) Desiccation tolerance and lichenization: a case study with the aeroterrestrial microalga *Trebouxia* sp. (Chlorophyta). *Planta,* **242**, 493-505.

Caviglia A. M., Modenesi P. (1999) Oxidative stress and ascorbic acid contents in *Parmotrema reticulatum* and *Parmelia sulcata* thalli. *Lichenologist,* **31**, 105-110.

Conti S., Hazdrová J., Hájek J., Očenášová P., Barták M., Skácelová K., Adamo P. (2014) Comparative analysis of heterogeneity of primary photosynthetic processes within fruticose lichen thalli: Preliminary study of interspecific differences. *Czech Polar Reports,* **4**, 149-157.

Chowdhury D. P, Solhaug K.-A., Gauslaa Y. (2017) Ultraviolet radiation reduces lichen growth rates. *Symbiosis,* **73**, 27-34.

Duong T.-H., Chavasiri W., Boustie J., Nguen K.-P.-P. (2015) New *meta*-depsidones and diphenyl ethers from the lichen *Parmotrema tsavoense* (Krog & Swinscow) Krog & Swinscow, Parmeliaceae. *Tetrahedron,* **71**, 9684-9691.

Elix J. A., Stocker-Wörgötter E. (2008) Biochemistry and secondary metabolites. In *Lichen Biology* (ed. T. H. Nash III.), pp. 1–8. Cambridge University Press, Cambridge, Cambridge, UK.

Estrabou C. (1999) La familia Parmeliaceae (s. str.) en la provincia de Córdoba. Análisis sistemático y biogeográfico. Tesis Doctorado en Ciencias Biológicas, Universidad Nacional de Córdoba, inédita.

Estrabou C., Filippini E., Soria J. P., Schelotto G., Rodriguez J. M. (2011) Air quality monitoring system using lichens as bioindicators in Central Argentina. *Environmental Monitoring and Assessment,* **182**, 375-383.

Estêvão D. M. M. (2015) Production of UV-B screens and changes in photosynthetic efficiency in Antarctic *Nostoc commune* colonies and a lichen *Xanthoria elegans* depend on a dose and duration of UV-B stress. *Czech Polar Reports,* **5**, 55-68.

Fazio A. T., Bertoni M. D., Adler M. T., Ruiz L. B., Rosso M. L., Muggia L., Hager A., Stocker-Wörgötter E., Maier M. S. (2009) Culture studies on the mycobiont isolated from *Parmotrema reticulatum* (Taylor) Choisy: metabolite production under different conditions. *Mycological Progress*, **8**, 359-365.

Gauslaa Y., Solhaug K.-A. (2004) Photoinhibition in lichens depends on cortical characteristics and hydration. *The Lichenologist,* **36**, 133-143.

Ghate N. B., Chaudhuri D., Sarkar R., Sajem A. L., Panja S., Rout J., Mandal N. (2013) An antioxidant extract of tropical Lichen, *Parmotrema reticulatum*, induces cell cycle arrest and apoptosis in breast carcinoma Cell Line MCF-7. *PlosOne,* **8**, e82293.

Jain P. K., Jain A. P. (2016) Effect of lichens on dermal wound healing with possible *Parmotrema reticulatum* antioxidant and antibacterial mechanism. *Asian Journal of Pharmacy and Pharmacology,* **2**, 10-14.

Jayalal U., Divakar P. K., Joshi S., Oh S.-O., Koh Y. J., Hur J.-S. (2013) The Lichen genus *Parmotrema* in South Korea. *Mycobiology,* **41**, 25-36.

Lichtenthaler H. K., Wellburn A. R. (1983) Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions,* **11**, 591-592.

Lichtenthaler H. K., Buschmann C., Knapp M. (2005) How to correctly determine the different chlorophyll fluorescence parameters and the chlorophyll fluorescence decrease ratio R-Fd of leaves with the PAM fluorometer. *Photosynthetica,* **43**, 379-393.

Luccini E., Cede A., Piacentini R., Villanueva C., Canziani P. (2006) Ultraviolet climatology over Argentina. *Journal of Geophysical Research*, **111**: 1-15.

Luo H., Yamamoto Y., Kim J. A., Jung J. S., Koh Y. J., Hur J.-S. (2009) Lecanoric acid, a secondary lichen substance with antioxidant properties from *Umbilicaria antarctica* in maritime Antarctica (King George Island). *Polar Biology,* **32**, 1033-1040.

Marečková M., Barták M., Hájek J. (2017 – subm.) Temperature effects on photosynthetic performance of Antarctic lichen *Dermatocarpon polyphyllizum*. Chlorophyll fluorescence study. *Polar Biology*, submitted.

Martic L. (2016) Lichen secondary metabolites in *Umbilicaria antarctica* evaluated by acetone rinsing. *Czech Polar Reports,* **6**, 186-190.

Modenesi P. (1993) An SEM study of injury symptoms in *Parmotrema reticulatum* treated with paraquat or growing in sulphur dioxide-polluted air. *The Lichenologist,* **25**, 423-433.

Nguyen K.-H., Chollet-Krugler M., Gouault N., Tomasi S. (2013) UV-protectant metabolites from lichens and their symbiotic partners. *Natural Product Reports*, **30**, 1490.

Nybakken L., Solhaug K.-A., Bilger W., Gauslaa Y. (2004) The lichens *Xanthoria elegans* and *Cetraria islandica* maintain a high protection against UV-B radiation in Arctic habitats. *Oecologia,* **140**, 211-216.

Perez R. E., Guzmán G. (2015) *Parmotrema* species in a cloud forest region turned into an urban zone in Xalapa, Veracruz, Mexico. *Bosque*, **36**, 357-362.

Plsíková J., Štěpánková J., Kašpárková J., Brabec V., Bačkor M., Kozurková M. (2014) Lichen secondary metabolites as DNA-interacting agents. *Toxicology in vitro,* **28**, 182-186.

Rancan F., Rosan S., Boehm K., Fernández E., Hidalgo M. E., Quihot W., Rubio C., Boehm F., Piazena H., Oltmanns U. (2002) Protection against UVB irradiation by natural filters extracted from lichens. *Journal of Photochemistry and Photobiology B: Biology,* **68**, 133-139.

Rodriguez J. M., Hernandez J. M., Fillipini E., Canas M., Estrabou C. (2016) Nuevas citas de macrolíquenes para Argentina y ampliaciones de distribución en el centro del país. *Boletín de la Sociedad Argentina de Botánica*, **51**, 405-417.

Roháček K., Barták M. (1999) Technique of the modulated chlorophyll fluorescence: basic concepts, useful parameters, and some applications. *Photosynthetica,* **37**, 339-363.

Sharma B. Ch., Kalikotay S. (2012) Screening of antioxidant activity of lichens *Parmotrema reticulatum* and *Usnea* sp. from Darjeeling hills, India. *IOSR Journal of Pharmacy,* **2**, 54-60.

Shukla V., Patel D. K., Bajpai R., Semwal M., Upreti D. K. (2016) Ecological implication of variation in the secondary metabolites in Parmelioid lichens with respect to altitude. *Environmental Science and Pollution Research,* **23**, 1391-1397.

Solhaug K.-A., Lind M., Nybakken L., Gauslaa Y. (2009) Possible functional roles of cortical depsides and medullary depsidones in the foliose lichen *Hypogymnia physodes*. *Flora,* **204**, 40-48.

Legends to Figs. and Tables

Table 1. Anatomical parameters in *P. austrosinense* thalli. The values are means of 520 measurements done in 30 cross sections. Means of three replicates replicates ± standard deviations are presented. The letters indicate statistically-significant differences (ANOVA, LSD Fisher test, P = 0.05). Key to the abbreviations: UC- upper cortex thickness, PL – photobiont layer thickness, M – thickness of medula, LC – lower cortex thickness, TT – thallus thickness, Std – standard deviation. Central part of thallus is reported for the TT range of 251 - 338 μm. Marginal part of thallus exhibited TT between 139 and 250 μm.

Table 2. Contents of UV-B screening compounds in thalli of *P. austrosinense* collected at two different sampling sites, after UV-B treatment (3.0 W m^{-2} for 24 h, and 10 d, respectively). Means of three replicates ± standard deviations are presented. The letters indicate statistically-significant differences (ANOVA, LSD Fisher test, P = 0.05).

Table 3. Contents (mg. g^{-1} DW) of chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoids (Car) in *P. austrosinense* after UV-B treatment (3.0 W m^{-2} for 24 h, and 10 d, respectively).). Means of three replicates replicates ± standard deviations are presented. The letters indicate statistically-significant differences (ANOVA, LSD Fisher test, P = 0.05).

Table 4. Chlorophyll fluorescence parameters calculated from slow Kautsky kinetics of chlorophyll fluorescence after UV-B treatment (3.0 W m^{-2} for 24 h and 10 d, respectively) in *P. austrosinense* thalli collected at two different sampling sites (Salinas Grandes and Los Gigantes).) F_0, F_P, F_{PP}, F_{M1}, F_S: chlorophyll fluorescence signals (for definition, see Fig.1) – F_{PP} means F_P from the kinetics recorded before UV-B treatment (time = 0); F_P/F_S, F_P/F_{PP}, F_{M1}/F_S: chlorophyll fluorescence ratios; Rdf: relative decline of chlorophyll fluorescence; t_{M1}: time at which the M1 peak was reached).

Fig. 1. Slow Kautsky kinetics of chlorophyll fluorescence supplemented with saturation pulse method (quenching analysis) recorded in *P. austrosinense* before (control), and after 24, 48, and 72 h of UV-B treatment (3.0 W m^{-2}): a) thalli collected at Los Gigantes; b) thalli collected at Salinas Grandes F_0: background chlorophyll fluorescence signal; F_P: chlorophyll fluorescence signal at point P; F_{M1}: chlorophyll fluorescence signal at point M1; F_S: steady state chlorophyll fluorescence; F_M and $F_{M'}$: maximum chlorophyll fluorescence signals reached after the application of saturation pulse in dark- and light-adapted state, respectively. The particular curves are means of three replicates. An asterisk indicates steady-state chlorophyll fluorescence reached in the Los Gigantes samples (Fs = constant) but not in Salinas Grandes (chlorophyll fluorescence signal still shows decline).

Fig. 2. Time courses of chlorophyll fluorescence parameters (F_V/F_M – potential quantum yield of photochemical processes in PSII, Φ_{PSII} – effective quantum yield of photochemical processes in PSII, qP – photochemical quenching, NPQ – non-photochemical quenching) in *P. austrosinense* thalli collected at two different sampling sites (Salinas Grandes – grey symbols, Los Gigantes – black symbols). Data points represent means of 3 replicates +/- standard deviations (error bars). The letters indicate statistically-significant differences (ANOVA, LSD Fisher test, P = 0.05).

Fig. 3. Spectral absorbance curves (means of three replicates) of ethanol extracts from *P. austrosinense* after 10 days of treatment with 3.0 W m^{-2} UV-B radiationin samples collected from Salinas Grandes (left) and Los Gigantes (right). Black line represents untreated control. Grey line represents UV-B treated lichen thalli.

Mean±Std	**UC**(µm)	**PL**(µm)	**M**(µm)	**LC**(µm)	**TT**(µm)
All thalli	**25.9**±7.2	**40.7**±14.2	**128.5**±30.4	**33.3**±14.0	**228.3**±34.1
Thallus thickness (central part)	**25.2**±6.8 *a*	**48.3**±14.6 *b*	**157.5**±26.8 *b*	**40.4**±16.9 *b*	**271.4**±18.9 *b*
Thallus thickness (marginal part)	**26.1**±7.3 *a*	**38.2**±13.2 *a*	**119.0**±25.0 *a*	**31.0**±12.0 *a*	**214.3**±25.0 *a*

Table 1

	Salinas Grandes			**Los Gigantes**	
	A_{280}	A_{310}		A_{280}	A_{310}
Control	**3.683**±0.179 *a,b*	**3.689**±0.348 *b,c*		**3.350**±0.567 *a,b*	**1.927**±0.203 *a,b*
24 h	**3.848**±0.447 *a,b*	**3.576**±0.059 *b,c*		**4.290**±1.117 *b*	**4.081**±2.563 *c*
10 d	**2.326**±0.177 *a*	**1.464**±0.135 *a*		**4.695**±1.996 *b*	**2.517**±0.558 *a,b,c*

Table 2

	Salinas Grandes			Los Gigantes		
	Chl a ($mg*g^{-1}$)	Chl b ($mg*g^{-1}$)	Car ($mg*g^{-1}$)	Chl a ($mg*g^{-1}$)	Chl b ($mg*g^{-1}$)	Car ($mg*g^{-1}$)
Control	**0.961**±0.218 *b,c*	**0.309**±0.046 *a,b*	**0.313**±0.061 *b*	**0.784**±0.022 *a,b*	**0.237**±0.023 *a*	**0.288**±0.036 *a,b*
24 h	**1.018**±0.191 *b,c*	**0.298**±0.045 *a,b*	**0.277**±0.049 *a,b*	**0.598**±0.057 *a*	**0.261**±0.096 *a,b*	**0.190**±0.051 *a*
10 d	**1.024**±0.112 *c*	**0.326**±0.044 *a,b*	**0.324**±0.040 *b*	**0.905**±0.077 *b,c*	**0.357**±0.071 *b*	**0.298**±0.096 *b*

Table 3

Salinas Grandes									
	F_{M1}	t_{M1}	F_P	F_S	F_P/F_S	F_P/F_{PP}	F_{M1}/F_S	Rdf (Fd/F_S)	F_0
Control	**93.22**±12.11 *b*	**70.02**±6.63 *b*	**65.05**±6.31 *b*	**53.11**±4.63 *b*	**1.22**±0.11 *ab*	**1.0**±0.10 *a*	**1.76**±0.03 *e*	**0.23**±0.02 *d*	**57.36**±5.97 *b*
24 h	**67.84**±7.18 *a*	**68.02**±6.71 *ab*	**51.25**±5.19 *a*	**45.03**±20.60 *a*	**1.14**±0.02 *ab*	**1.08**±0.01 *b*	**1.51**±0.03 *ab*	**0.14**±0.02 *ab*	**46.04**±4.89 *a*
48 h	**76.15**±5.75 *a*	**62.02**±5.66 *ab*	**56.37**±6.07 *ab*	**48.33**±4.20 *ab*	**1.17**±0.01 *ab*	**1.05**±0.02 *ab*	**1.58**±0.03 *c*	**0.17**±0.02 *bc*	**49.97**±4.28 *ab*
72 h	**75.34**±7.85 *a*	**70.02**±6.83 *b*	**53.07**±5.67 *a*	**47.14**±3.80 *ab*	**1.13**±0.01 *ab*	**1.09**±0.02 *b*	**1.60**±0.03 *c*	**0.13**±0.02 *ab*	**46.17**±4.90 *a*
10 d	**67.84**±0.01 *a*	**68.02**±6.70 *ab*	**51.25**±4.55 *a*	**45.03**±4.03 *ab*	**1.14**±0.02 *b*	**1.08**±0.02 *b*	**1.51**±0.03 *ab*	**0.14**±0.02 *ab*	**46.04**±4.89 *a*

Los Gigantes									
	F_{M1}	t_{M1}	F_P	F_S	F_P/F_S	F_P/F_{PP}	F_{M1}/F_S	Rdf (Fd/F_S)	F_0
Control	**71.81**±7.80 *a*	**72.02**±7.29 *b*	**53.91**±6.04 *a*	**49.22**±4.97 *b*	**1.10**±0.05 *a*	**1.0**±0.10 *a*	**1.46**±0.03 *a*	**0.10**±0.02 *a*	**46.62**±5.16 *a*
24 h	**79.73**±8.80 *ab*	**60.02**±6.15 *ab*	**58.86**±5.67 *ab*	**51.23**±5.14 *b*	**1.15**±0.01 *ab*	**0.92**±0.08 *ab*	**1.56**±0.03 *bc*	**0.15**±0.02 *b*	**49.81**±5.14 *ab*
48 h	**73.20**±7.51 *a*	**60.02**±5.99 *ab*	**54.61**±5.10 *ab*	**48.48**±4.95 *ab*	**1.13**±0.08 *ab*	**0.99**±0.06 *ab*	**1.51**±0.02 *ab*	**0.13**±0.01 *ab*	**46.85**±5.26 *a*
72 h	**72.90**±7.89 *a*	**56.02**±5.63 *a*	**52.18**±4.57 *a*	**43.08**±4.26 *ab*	**1.21**±0.05 *ab*	**0.98**±0.09 *ab*	**1.69**±0.03 *d*	**0.21**±0.02 *d*	**41.01**±3.87 *a*
10 d	**72.59**±8.12 *a*	**56.02**±5.56 *a*	**51.92**±4.63 *a*	**43.10**±4.29 *ab*	**1.20**±0.05 *ab*	**1.04**±0.04 *ab*	**1.68**±0.03 *d*	**0.20**±0.02 *cd*	**42.78**±4.99 *a*

Table4