plant biology

Plant Biology ISSN 1435-8603

RESEARCH PAPER

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

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**Keywords**
Absorbance; chlorophyll fluorescence parameters; lichens; UV spectra; UV-B resistance.

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**Editor**
R. Leegood

Received: 22 January 2018; Accepted: 6 June 2018

doi:10.1111/plb.12855

## 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 content 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. Lichens from two different locations in Cordoba, Argentina, were selected: (i) high altitude and dry plots at (Los Gigantes) and (ii) lowland high salinity plots (Salinas Grandes).
- 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 screen content was observed. Chlorophyll fluorescence parameters showed a UV-B-induced decline in $F_V/F_M$, $\Phi_{PSII}$ and qP indicating limitation of primary photosynthetic processes in photosystem II (PSII) of symbiotic alga, more pronounced in Salinas Grandes samples. Protective mechanism of PSII were activated by the UV-B treatment to a higher extent in samples from Salinas Grandes (NPQ 0.48) than in Los Gigantes samples (NPQ 0.26).
- We concluded that site-related characteristics, and in particular different UV-B radiation regimen, had a strong effect on resistance of the photosynthetic apparatus of *P. austrosinense* to UV-B radiation.

## INTRODUCTION

In lichens growing in open and unshaded habitats, several photoprotective mechanisms exist. These minimise negative effects of photosynthetically active radiation (PAR) and UV radiation on the photosynthetic apparatus of lichen photobionts. Several compounds are involved into photoprotection, *e.g.* UV-B screens, pigments and enzymes with antioxidative activity. In lichens, more than 100 UV absorbing compounds have been described (for review see *e.g.* Nguyen *et al.* 2013). These are mainly secondary metabolites produced by the fungal partner. In addition to 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. The majority of UV-B absorbing compounds, *e.g.* phenolics, depsides, depsidones, depsones, anthraquinones, dibenzofurans, usnic acid and its derivatives and xanthones, belong to the acetyl polymalonyl pathway (see Elix & Stocker-Wörgötter 2008). The mevalonic pathway is used in the synthesis of terpenes and steroids. The shikimic acid pathway produces terphenylquinones and pulvinic acids derivatives.

In a single lichen species, the 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 and among the thalli of a lichen populations growing under contrasting light conditions (sun *versus* shade habitats). Different doses of sunlight (Bjerke & Dahl 2002) and different water supply that modify intrathalline reflection of radiation (Solhaug *et al.* 2009) may affect UV-B screen production and allocation in lichens. Lichens from high altitudes cope better with higher UV-B doses than those from lowlands. Thus, they contain 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 (*e.g.* Jayalal *et al.* 2013; Perez & Guzmán 2015). In Argentina, lichens of the genus *Parmotrema* are quite frequent also in mountainous (Estrabou 1999) and central parts of Argentina (Rodriguez *et al.* 2016). In Cordoba province, they grow in several habitats, ranging from lowland to mountain areas. Within the last decades, research has focused on various ecological and biological aspects of the *Parmotrema* genus, addressing species tolerance to air pollution (Estrabou *et al.* 2011), oxidative stress in the 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) activity. 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) and biological characteristics of the *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 microclimate factors related to altitude. Similarly, Armaleo *et al.* (2008) focused on light intensity effects on depsides and depsidones in *P. hypotropum*. Natural content of depsidones was investigated by Duong *et al.* (2015) in *P. tsavoense.* Sensitivity of *Parmotrema* species to UV-B radiation and changes in secondary metabolites in response to altered UV-B is still unknown. Similarly, only limited knowledge exists on the 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 curve parameters. We hypothesised that *P. austrosinense* from mountainous locations, exposed to a higher amount of incident UV-B radiation, would have more resistance to UV-B than samples from lowland locations.

## MATERIAL AND METHODS

### Species description

*Parmotrema austrosinense* has a foliose thallus, 4 to 12 cm in diameter. The thallus is lobate, individual lobes are narrow to broad and flat with sub-rotund apices. Thallus colour differs 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 in woodlands. The species is reported (Jayalal *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 *P. austrosinense* (Zahlbr.) Hale were collected from two different habitats in Cordoba province. The first sampling site is located in the foothills of Los Gigantes, Sierras Grandes, 70 km west of Cordoba city. This is a pristine area, around 100–1200 m a.s.l., with xerophilic vegetation, mostly perennial shrubs. The other collection site was Salinas Grandes region, 200 km north of Cordoba city, in the semi-desert climate domain, with <400 mm rainfall per year, where the vegetation, including lichens, is adapted to dry conditions and typically halophilic. Altitude varies between 150 m a.s.l. on the salt beaches to 300 m a.s.l. on the margins. Lichen thalli were collected from tree trunks or rocks; they were cleaned from adhering pieces of rock or substrate and stored in the laboratory at room temperature.

### Morphological characteristics

To characterise *P. austrosinense* morphology and anatomy, cross-sections were analysed 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 h while illuminated by a light source (LED panel, 400–750 nm, 15 μmol $m^{-2}$ $s^{-1}$ PAR; Tron, Brno, CZ) at 10 °C. Then, thin slices of lichen samples were taken and photographed in 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 in Petri dishes and covered with a UV-B transparent plastic foil to prevent changes in thallus hydration status. Optimally hydrated lichen thalli (tested before and selected on the basis of maximum constant $F_V/F_M$ reached after 48 h of hydration) were exposed to PAR (10 μmol $m^{-2}$ $s^{-1}$; Li-1400 radiometer; Li-Cor, Lincoln, NB, USA) supplemented with 3.0 W·$m^{-2}$ UV-B radiation for 10 days. During exposure, temperature was kept at 20.0 ± 0.4 °C (measured with a HOBO thermosensor and datalogger; OnSet Computers, USA). The thalli were exposed to PAR + UV-B continuously, *i.e.* there was no dark period). 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 (290–315 nm). In preexperiments, we alos tested 0.6 and 1.4 W·$m^{-2}$ (measured with a SpectroSense unit equipped with a UV-B sensor; Skye Instruments, UK), however, since these two UV-B doses induced a very limited response of chlorophyll fluorescence parameters, they were excluded and only a dose of 3.0 W·$m^{-2}$ was used for the experiment. During exposure, 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 days of supplemental UV-B) were dried in a lyophilizator for 24 h. then homogenized with a ball mill (Retsch MM 2000, Germany). 100 mg of the homogenized material were extracted with ethanol and absorbances within 190–700 nm were measured with a UV-VIS spectrophotometer (Specord 205; Analytik, Jena, Germany). Absorbance at 280 and 310 nm was measured for UV-B screens

according to Buffoni-Hall *et al.* (2002); in addition, absorbance at 470, 649 and 664 nm was measured to analyse 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 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 $F_V/F_M$, $\Phi_{PSII}$, qP and NPQ were measured on day 0, control). Then, the same fluorescence parameters were measured after 24, 48 and 72 h of exposure 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 chlorophyll fluorescence signals recorded (Fig. 1). First, background chlorophyll fluorescence ($F_0$) was measured on the sample exposed to low (measuring) light. Then thalli were exposed to a saturating pulse of light to induce maximum chlorophyll fluorescence ($F_M$). After which thalli were light-adapted for 5 min until 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 ($\varphi_{PSII}$) were calculated. Equations 1 and 2 in the FluorCam7 software (Photon Systems Instruments, Czech Republic) were used for this purpose.

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

$$F_V/F_M = (F_M - F_0)/F_M \quad (1)$$

$$\Phi_{PSII} = (F'_M - F_S)/F'_M \quad (2)$$

$$qP = (F'_M - F_S)/(F'_M - F_0) \quad (3)$$

$$NPQ = (F_M - F'_M)/F'_M \quad (4)$$

Time courses of the chlorophyll fluorescence parameters were constructed for the lichens from Los Gigantes and Salinas Grandes and the differences are 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 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 Table 1 legend), relative fluorescence decrease (Rfd; Lichtenthaler *et al.* 2005) and the time at which the M1 peak was reached ($t_{M1}$, for M1 details see Fig. 1 legend).

### Statistical analysis

Differences in chlorophyll fluorescence parameters recorded after 24, 48 and 72 h of UV-B exposure, and differences related to locality (Salinas Grandes, Los Gigantes) were evaluated by factorial ANOVA (STATISTICA, StatSoft version 13) with the Fisher LSD test. Statistically significant differences were considered at $P = 0.05$.

## RESULTS

### Thallus anatomy

*Parmotrema austrosinense* showed large variability in morphometric parameters of thalli (Table 2). However some parameters, *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 (214.3 μm) thallus parts. Medulla, as well as lower cortex thickness were 32.4% and 30.2%, respectively, larger in central than marginal thallus parts.

### Photosynthetic pigments

The carotenoid content decreased after 24 h of UV-B treatment, followed by an increase after 10 days of treatment (Table 3). These changes were, however, not statistically significant. In thalli from both sampling sites (Salinas Grandes and Los Gigantes), carotenoid levels were the same or higher after UV-B treatment (10 days) compared with initial values. Chl *a* content in thalli followed a similar trend over the experimental time course, *i.e.* a decrease after 24 h of treatment followed by an increase. Due to high variability in raw data for 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 observations for carotenoids. In thalli from Los Gigantes, Chl *a* content was significantly higher after 10 days of UV-B exposure than after 24 h. Chl *b* content exhibited different patterns in thalli from contrasting localities. There was a decrease followed by an increase in samples from Salinas Grandes, while increased Chl *b* values was found in thalli from Los Gigantes. These UV-B exposure- and locality-related differences were, however, not statistically significant.

### 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 changes within the first 24 h of UV-B exposure (*e.g.* $F_{M1}$, $F_o$, $F_p$, $F_s$ in Los Gigantes sample) or gradual accumulated stress with duration of UV-B treatment (*e.g.* $F_p/F_{pp}$ and $F_{M1}/F_s$ for both localities). The main difference inshape of the slow chlorophyll fluorescence kinetics was in relation to steady state ($F_S$) to background chlorophyll fluorescence ($F_0$ at O point). Typically $F_s$ was lower than $F_0$ in control thalli but 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 $F_P/F_{PP}$ all decreased with duration of UV-B treatment. Most of these

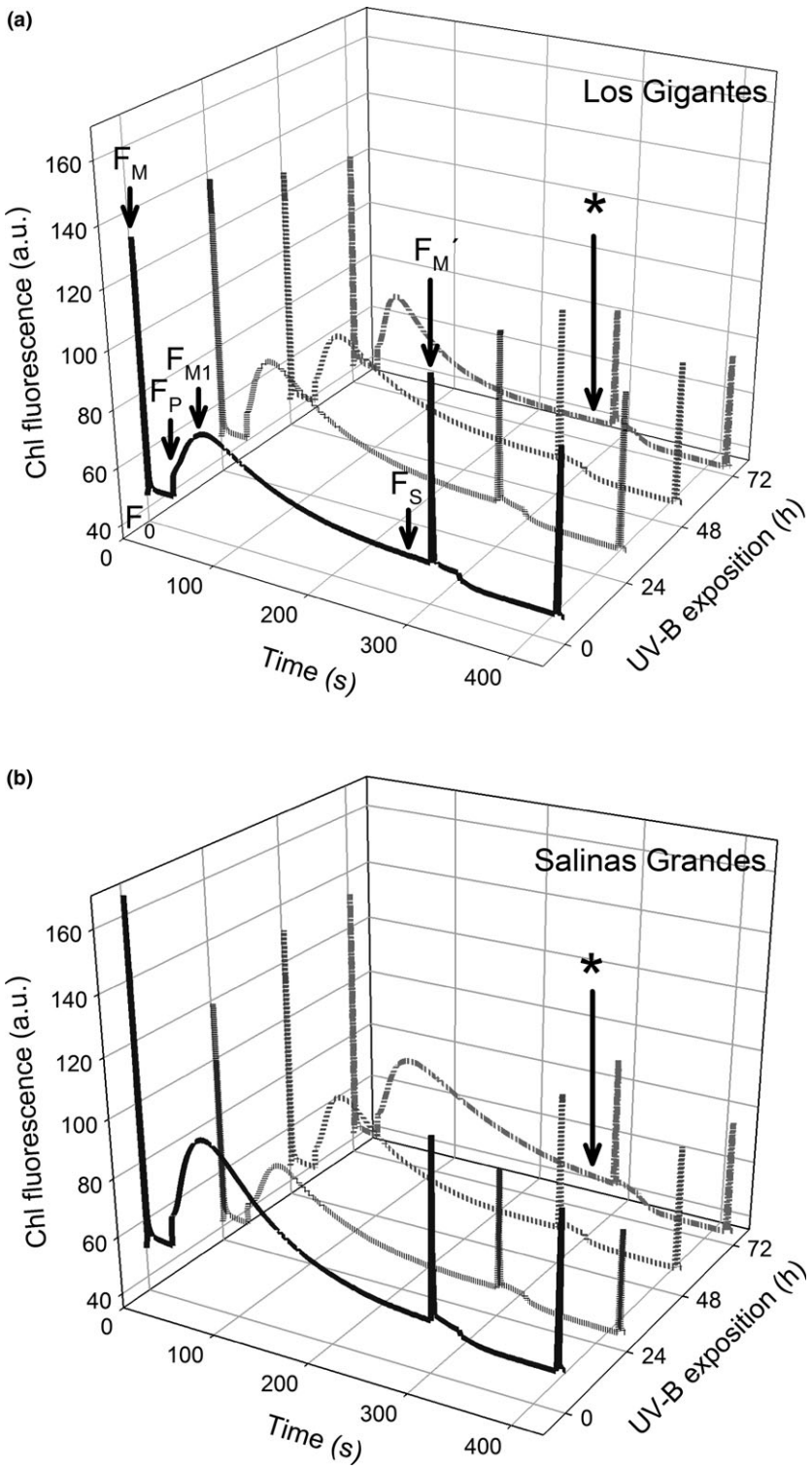


**Fig. 1.** Slow Kautsky kinetics of chlorophyll fluorescence supplemented with the saturation pulse method (quenching analysis) recorded in *P. austrosinense* before (control) and after 24, 48 and 72 h of UV-B treatment (3.0 $W \cdot m^{-2}$): (a) thalli collected at Los Gigantes; (b) thalli collected 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 curves are means of three replicates. An asterisk indicates steady-state chlorophyll fluorescence reached in the Los Gigantes samples ($F_s$ = constant) but not in Salinas Grandes (chlorophyll fluorescence signal still shows a decline).

**Table 1.** 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 days, respectively) in *P. austrosinense* thalli collected at two different sampling sites (Salinas Grandes and Los Gigantes. $F_O$, $F_P$, $F_{PP}$, $F_{M1}$, $F_S$: chlorophyll fluorescence signals (for definition, see Fig. 1) – $F_{PP}$ is $F_P$ from 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.

| | $F_{M1}$ | $t_{M1}$ | $F_P$ | $F_S$ | $F_P/F_S$ | $F_P/F_{PP}$ | $F_{M1}/F_S$ | Rdf ($Fd/F_S$) | $F_O$ |
|---|---|---|---|---|---|---|---|---|---|
| Salinas Grandes | | | | | | | | | |
| 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 days | 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 | | | | | | | | | |
| 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 days | 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 |

changes were, however, not statistically significant. In contrast, the thalli from Los Gigantes showed either an 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 a decrease ($t_{M1}$, $F_P/F_{PP}$) with UV-B treatment (Table 1) but 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 ($\varphi_{PSII}$) quantum yield of photosynthetic processes in PSII (Fig. 2). During the 0–48 h exposure interval, $F_V/F_M$ decrease was faster and more pronounced in thalli from Salinas Grandes than Los Gigantes. With UV-B exposure, $\Phi_{PSII}$ decreased in both Los Gigantes and Salinas Grandes samples. For both localities, $\Phi_{PSII}$ values recorded after 72 h exposure decreased significantly compared to initial values. After 72 h, however, $\Phi_{PSII}$ values were not significantly different (Salinas Grandes and Los Gigantes samples). The photochemical quenching coefficient (qP), which reflects the proportion of open PSII reaction centres used in energy conversion of absorbed light, declined with duration of UV-B exposure in lichens from both sampling sites. The decline, however, was not statistically significant. Similarly, differences in qP between Los Gigantes and Salinas Grandes thalli were no significant. Non-photochemical quenching (NPQ), which is attributed to activation of protective mechanisms in chloroplasts, increased from 0.2 to 0.26 in Los Gigantes thalli within the 24 h of UV-B exposure and then remained more or less constant. Thalli from Salinas Grandes, however, besides an increase within the 24 h exposition, showed a slight increase in NPQ between 24 and 72 h of exposure. The NPQ differences between Salinas Grandes and Los Gigantes, as well as between NPQ in control and after 72 h of UV-B treatment (for a locality) were statistically significant.

### UV-B absorbing compounds

Long-term (10 days) exposure to a 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 in compounds absorbing within the range 220–370 nm (Fig. 3, left). 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 220–540 nm. This increase in UV-B absorbing compounds was even higher after 24 h of UV-B treatment (see Table 4). The absorbance peaks were not well distinguished due to a noisy signal in thalli from Los Gigantes, however, they were evident in the ranges 220–230 and 270–280 nm.

## DISCUSSION

### Thallus anatomy

The results on thallus anatomy are comparable with data reported by Bissacot Barbosa & Marcelli (2010). The algal layer thickness in *P. austrosinense* was similar to values found for *P. perlatum* (22 μm; Carniel *et al.* 2015). Also, values were similar to data reported for other *Parmeliaceae* species, *e.g. Parmelia sulcata* (45–85 μm; Bennett 2002). Our results on algal layer

**Table 2.** Anatomical parameters in *P. austrosinense* thalli. Values are means of 520 measurements in 30 cross-sections.

| Mean ± SD | 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 |

Means of three replicates replicates ± SD are presented. Letters indicate statistically significant differences (ANOVA, LSD Fisher test, $P = 0.05$).
UC = upper cortex thickness; PL = photobiont layer thickness; M = thickness of medula; LC = lower cortex thickness; TT = thallus thickness.

**Table 3.** Content ($mg \cdot g^{-1}$ DW) of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoids (Car) in *P. austrosinense* after UV-B treatment ($3.0\ W \cdot m^{-2}$ for 24 h and 10 days, respectively).

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

Mean of three replicates replicates ± SD are presented. Letters indicate statistically significant differences (ANOVA, LSD Fisher test, $P = 0.05$).

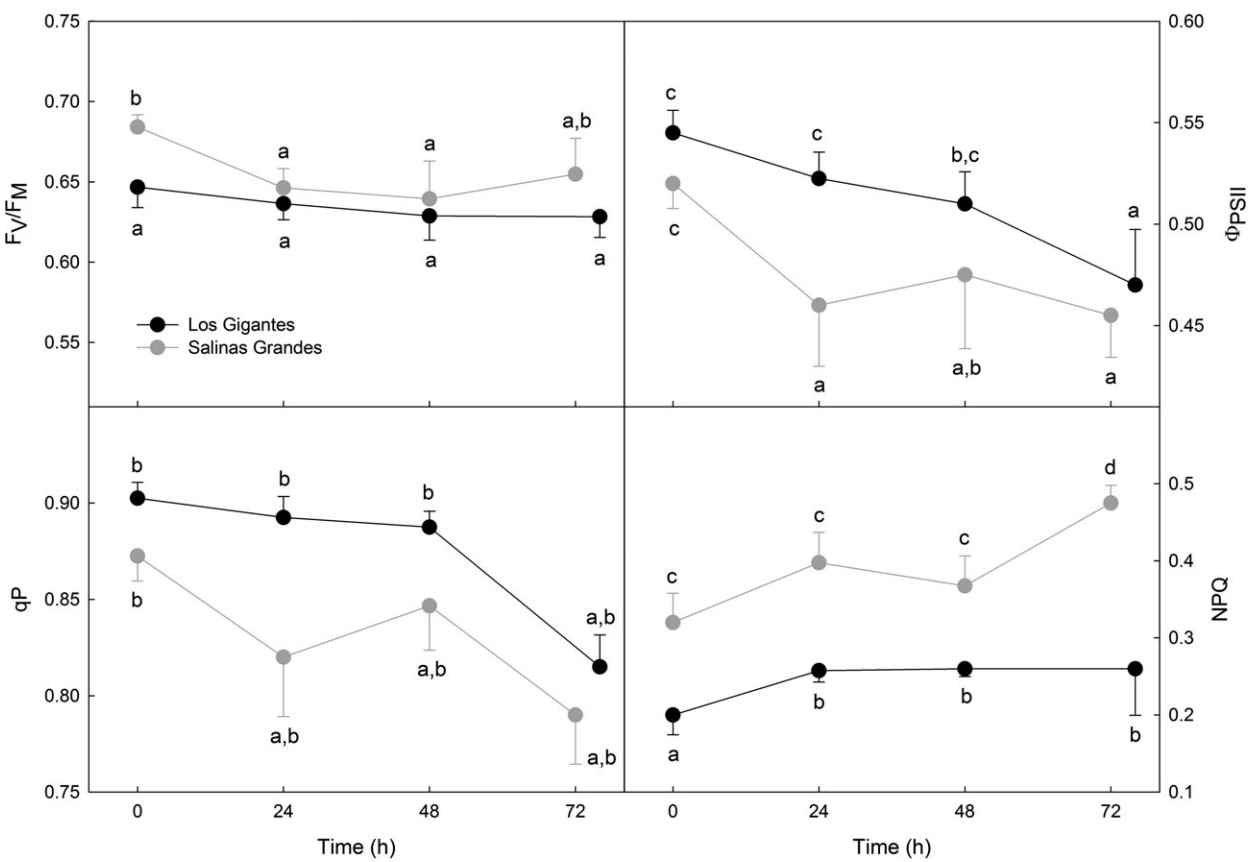


**Fig. 2.** Time courses of chlorophyll fluorescence parameters ($F_V/F_M$ – potential quantum yield of photochemical processes in PSII; $\Phi_{PSII}$ – effective quantum yield of photochemical processes in PSII; qP – photochemical quenching; NPQ – non-photochemical quenching) in *P. austrosinense* thalli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

thickness in *P. austrosinense* are comparable to data obtained for foliose lichens. Similar photobiont layer thickness is reported for *Dermatocarpon polyphilizum* (25–160 μm; M. Marečková, M. Barták, J. Hájek, unpublished results) and *Umbilicaria antarctica* (44.2 μm; M. Marečková, unpublished data). In a cyanolichen (*Peltigera* sp.), however, the photobiont

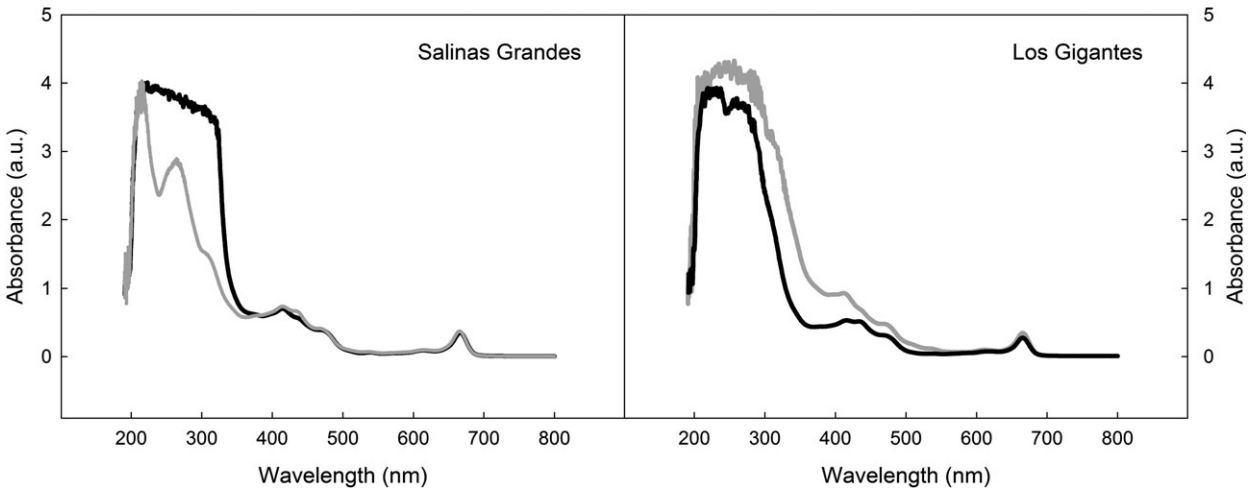


**Fig. 3.** Spectral absorbance curves (mean of three replicates) of ethanol extracts from *P. austrosinense* after 10 days of treatment with 3.0 W·m$^{-2}$ UV-B radiation in samples collected from Salinas Grandes (left) and Los Gigantes (right). Black line represents untreated control. Grey line represents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

**Table 4.** Content 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 days, respectively).

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

Means of three replicates ± SD are presented. The letters indicate statistically significant differences (ANOVA, LSD Fisher test, $P = 0.05$).

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 (19–88 μm) thickness. Overall, these results suggest that multiple independent environmental variables, *e.g.* light regimen of the habitat, may be responsible for differences in intrathalline morphological characteristics of *P. austrosinense,* such as algal layer thickness.

### Chlorophyll fluorescence kinetics

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

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

### UV-B absorbing compounds

The UV-B-induced damage to UV-B screens was demonstrated in *P. austrosinense* from Salinas Grandes after 10 days of UV-B treatment. The decrease in absorbance values within the range 180–350 nm, as well as 280 and 300 nm is comparable to data of Estêvão (2015) who reported an initial increase of UV-B

screens followed by a decrease after 6 days of treatment; more pronounced at 3.0 than at 1.5 $W \cdot m^{-2}$. This effect indicates destruction of the screens and a decrease of absorbance, 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 (peak absorption at 230, 280 and 320 nm; Plsíková *et al.* 2014), and/or lecanoric acid with similar peak absorption (213, 270 and 304 nm; BeGora & Fahselt 2001; Luo *et al.* 2009). An increase in absorbance in *P. austrosinense* from Los Gigantes found after 10 days of UV-B treatment, indicating a high capacity of the thalli grown at high altitude to synthesise UV-B absorbing compounds and, therefore, higher constitutive resistance to negative effects of UV-B. Higher values of $\Phi_{PSII}$ 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 *versus* low altitude). Indeed, climatological measurements and data reported by Luccini *et al.* (2006) indicates a 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 $F_V/F_M$, $\Phi_{PSII}$, 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, which protects lichen photobionts against excess PAR (*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 destruction of pigment–protein complexes forming the photosynthetic apparatus of the symbiotic alga and, consequently, to a decrease in $\Phi_{PSII}$ (Fig. 2). In our study, however, the UV-B-induced decline in $\Phi_{PSII}$ did not lead to full inhibition, indicating that photosynthetic processes in *P. austrosinense* are quite resistant to high UV-B doses. Such resistance has been documented for 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 the photobiont do not seem to be critical for growth and survival as 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).

## 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.

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