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Leaf anatomy and photosynthetic efficiency of *Acrostichum danaeifolium* after UV radiation

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Abstract

This paper reports effects of ultraviolet B (UVB) radiation on leaf anatomy and contents of chlorophyll and carotenoids, as well as photosynthetic parameters, in young sporophytes of *Acrostichum danaeifolium* Langsd. & Fisch. (Polypodiopsida, Pteridaceae) exposed to UV radiation treatments for 1 h daily for six weeks. The leaves showed large aerenchyma and present chloroplasts in both epidermises. After cultivation under PAR + UVA + UVB, leaves showed curling and malformed stomata on the abaxial face. After the UV treatment, chloroplasts in leaves were arranged against the inner wall of the epidermal cells. Transmission electron microscopy analysis showed some dilated thylakoids and plastoglobuli in chloroplasts and vesicles containing phenolic compounds in the cytoplasm. Differences were not observed between control and UV-treated plants in their contents of chlorophylls, carotenoids, and photosynthetic parameters. *A. danaeifolium* grown in sunny mangrove environment seems to have mechanisms preventing photosystem damage.

Additional keywords: giant leather fern; mangrove; photoresponse; young sporophyte.

Introduction

Five mangrove ecosystems are found on the island of Santa Catarina (Brazil). This marks their southernmost distribution along the southwestern Atlantic coast. Therefore, the structural characterization of this vegetation is a valuable tool in identifying the responses of the ecosystem to environmental stresses for conservation strategies.

Acrostichum aureum L. and *Acrostichum danaeifolium* Langsd. & Fisch. (Polypodiopsida, Pteridaceae) (Smith *et al*. 2006) are mangrove ferns, both cited for Santa Catarina (Reitz 1961). *Acrostichum* L. is recognized for its pinnate leaves 2–4 m tall, with reticulated and sporangial ribs that cover the entire leaf surface of the pinnae (Moran 1995). According to Mehltreter and Palacios-Rios (2003), the phenology of *A. danaeifolium* is subject to climatic and environmental fluctuations. *A. danaeifolium* ("giant leather fern") is close to herbaceous species, but even as a halophyte plant, it appears to be dependent on direct sunlight for reproduction in saline substrates and generally grows when exposed to full sunlight (Gomez 1983, Janzen 1985). It is endemic to mangrove vegetation (Hietz 2010).

The quantity and quality of exposure to UV radiation depends on the time of year, latitude, altitude, clouds, and aerosols (McKenzie *et al*. 2007). Ultraviolet radiation, consequently, provides information about plant environment. However, when exposed to higher doses of UV radiation or UV radiation of shorter wavelengths, plants can be damaged (Aphalo *et al.* 2012). Researchers are primarily interested in the effects of UVB radiation on plants and other organisms exposed to UV radition as a result of ozone depletion in the stratosphere, mainly from the release of chlorofluorocarbons (CFCs) (Aphalo *et al*. 2012).

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Abbreviations: A – absorbance; AL – actinic light; AT-O – toluidine blue; Chl – chlorophyll; DAC – days of cultivation; E_k – saturating irradiance; ETR – electron transport rate; ETR_{max} – maximum electron transport rate; F_m' – maximum fluorescence of a light-adapted leaf; F_s – steady state fluorescence obtained at light-acclimated samples; F_{II} – ratio of chlorophyll *a* associated with PSII; FM – fresh mass; LM – light microscopy; PAM – pulse-amplitude modulation; RLC – rapid light curves; SEM – scanning electron microscopy; SL – saturating light pulses; SP – single saturating pulse; Φ_{PSII} – actual photochemical efficiency of PSII; TEM – transmission electron microscopy; UV – ultraviolet; UVA – ultraviolet A radiation; UVB – ultraviolet B radiation; $Y_{(II)}$ – effective quantum yield; α_{ETR} – photosynthetic efficiency.
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UVB radiation induces negative physiological effects, causing changes in plant transpiration, pigments related to photosynthesis, as well as morphology and anatomy. It decreases photosynthetic capacity and causes DNA damage, while, at the same time, eliciting plant protective mechanisms (Greenberg *et al*. 1989, Tevini and Teramura 1989, Hilal *et al*. 2004, Schulze *et al*. 2005, Ranjbarfordoei *et al*. 2009).

Williamson *et al*. (2014) predicted that erythemal UV exposure would increase by 2050 in the tropics by 3 and 8% as a result of decreased cloud cover and ozone depletion. They further commented that the effects of ozone depletion on climate are becoming increasingly apparent, highlighting the complexity of the Earth's climate system. Ozone depletion over Antarctica has caused the poleward shift in the Southern hemisphere circulation, resulting in increased precipitation in the subtropics (15–35°S). However, as the stratospheric ozone recovers, an opposing effect is expected and subtropical regions are likely to become drier.

Since *A. danaeifolium* plants are dependent on full sunlight for their development, they need to adapt to additional stresses that result from climate change. Therefore, this study aimed to analyze the effects of chronic exposure to high levels of UVB radiation in young sporophytes of *A. danaeifolium* in order to understand the effects of radiation stress and the possible adaptive strategies of this plant.

Material and methods

Plant collection: In America, *Acrostichum* is common in open salt marshes, on alluvial banks of estuaries and along ditches. Occasionally, it grows in slightly shaded places such as thickets and has been reported in fresh water somewhat above the sea (Tryon and Tryon 1982). Fertile leaves of *Acrostichum daneifolium* were collected on 21 August 2012 (winter) in the Permanent Preservation Area (PPA) of Itacorubi Mangrove (150 hectares) situated on the Santa Catarina Island at 27°34'13"–27º35'31" south and 48º30'07"–48º31'33" west. According to the Köppen classification system, the climate is Cfa. It is a humid subtropical climate characterized by hot summers with an annual mean temperature of 21°C. The relative humidity is high, around 80%, with annual rainfall of 1,527 mm, evenly distributed throughout the year (Alvares *et al.* 2013). Sporophylls were dried at room temperature on filter paper in order to induce dehiscence. The spores were removed and separated from sporangia by filtering through lens paper and later stored in glass jars under refrigeration at 7 ± 1ºC.

PAR + UVA and PAR + UVA + UVB treatments: The nominal values of PAR in the germination chamber were obtained with a *LI-COR LI-250A* light meter (*LI-COR,* Lincoln, NB). UBV radiation in the field and in the growth chamber were also obtained with a *LI-COR LI-250A* light meter (PAR) and radiometer model *IL 1400A* (*International Light*, Newburyport, MA, USA) (UV). Spore germination and sporophyte cultivation were carried out in a germination chamber provided with PAR + UVA and PAR + UVA + UVB throughout the experimental period of 10 months. PAR + UVA was obtained using four 20 W white fluorescent lamps with emission of 13 µmol(photon) m^{-2} s^{-1}. The temperature was adjusted to 25°C and 12-h photoperiod. Daily treatments of PAR + UVA + UVB were performed using two UVB emission lamps (*UVB Broadband TL20W/12RS SLV 25, Philips*, Holland). The average emission of UVB on the growth room shelf was 0.73 W m^{-2}. Trays were numbered, and their positions were changed weekly. UVB radiation was provided for 1 h daily during six weeks between 12:00 and 13:00 h. The integral UVB radiation for six weeks was 110.88 kJ m^{-2} and the integral UVA radiation for six weeks was 131.46 kJ m^{-2}. On 17 November 2012, UVB radiation was measured in the field between 10:00 to 14:00 (*see* text table). Data obtained in the field was used to calculate the UVB radiation employed in this work. Biologically effective radiation doses were calculated for chloroplast and DNA damage according to Jones and Kok (1966) and Setlow (1974) (*see* text table).

Values of UVB radiation measured in the field on a sunny day in November 2012 in Florianópolis, SC, Brazil.

Time [h]	UVB [W m^{-2}]	[KJ m^{-2} h^{-1}]
10:00	0.77	2.77
11:00	1.38	4.96
12:00	1.69	6.08
13:00	1.85	6.66
14:00	1.74	6.26

Spore germination and sporophyte cultivation: For the germination assay (PAR + UVA and PAR + UVA + UVB), spores of *A. danaeifolium* were inoculated in four transparent polyethylene boxes filled with 180 g of autoclaved mangrove soil and covered with a thin layer (60 ml) of mineral solution (Dyer 1979) with addition of 0.8% agar. After solidification of the medium, 20 mg of spores were inoculated on the substrate surface.

Most gametophytes germinated under UVB radiation died after 30 days of cultivation (DAC). The gametophyte development in PAR + UVA was monitored weekly by optical microscopy until the onset of sporophytes. The first sporophytes were observed after 85 DAC. After nine months of cultivation in the growth chamber, 36 sporophytes were transplanted to vases containing autoclaved mangrove soil.

Values of absolute irradiance, effective irradiance, absolute dose, and effective dose of PAR, UVA, and UVB for treatments. UVA absolute dose values correspond to the effective dose of 12 h of total exposure to light. Ai – absolute irradiance, Ei – effective irradiance, Ad – absolute dose, Ed – effective dose.

Spectrum	Ai [W m^{-2}]	Ei [W m^{-2}]		Ad [kJ m^{-2}]	Ed [kJ m^{-2}]	
		DNA	Chl		DNA	Chl
PAR (12 h)	13.26	-	-	572.69	-	-
PAR (12 h) for 42 days	556.925			22,792. 98		
UVB (1 h)	0.73	0.04	0.36	2.64	0.16	1.28
UVB (1 h)- 42 days	30.66			110.88		
UVA (1 h) under UVB lamps	0.62	-	0.11	2.23	-	0.82
UVA (1 h) under UVB lamps - 42 days	26.04			93.66		
UVA (11 h) under PAR lamps	0.25	-	0.01	0.9		
UVA (11 h) under PAR lamps - 42 days	10.5			37.8		
Total UVA - 42 days	11.12			131.46		

The height of the plants ranged from 1 to 1.5 cm, and they presented three to four small leaves (0.5 cm in length). The vases were placed inside boxes covered with transparent PVC plastic film that allows the permeation of most UVB radiation.

Light microscopy (LM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM): Samples of the middle region of young leaves of *A. danaeifolium* grown under PAR + UVA or PAR + UVA + UVB for six weeks were collected for microscopic analyses.

For LM, samples were fixed in 2.5% glutaraldehyde in sodium phosphate buffer (0.1 M, pH 7.2), overnight (Feder and O'Brien 1968), washed in the same buffer, dehydrated in ethanolic series, and preserved in ethanol 70%. After that, they were dehydrated in ethanolic series up to 96% and infiltrated in methacrylate (historesin) (*Leica Historesin*, Heidelberg, Germany) according to the manufacturer's instructions. The blocks were then sectioned into sections of 5 μm in thickness by rotary microtome (*Leica RM 2125 RT*, *Leica*, Germany), and the sections were stained with different histochemical techniques. Toluidine blue (TB-O) (O'Brien *et al*. 1965) was used to identify acidic polysaccharides by metachromatic reaction. The slides were mounted in transparent glass varnish (Paiva *et al*. 2006).

For SEM analyses, samples previously fixed and preserved in ethanol 70% were dehydrated in ethanolic series up to 100% and subjected to critical point of CO_2 in a *Leica EM CDP 030* critical point drier (*Leica,* Heidelberg, Germany). The samples were then adhered to an aluminum support with double-sided carbon tape and metalized with 20-nm gold particles in a *Bal-Tec CED 030* metalizer (*Bal-Tec AG*, Balzers, Liechtenstein). A scanning electron microscope (SEM) (*JEOL JSM–6390 LV*, *JEOL Ltd.,* Tokyo, Japan) at 10 kV was used to analyze and document the samples.

For TEM analyses, samples were fixed in glutaraldehyde, 2% sucrose, and 0.1 M cacodylate (pH 7.2) overnight at 4°C (Schmidt *et al.* 2009). Afterwards, they were washed three times in the same buffer, post-fixed in 0.1 M cacodylate buffer containing 1% OsO_4 for 4 h, rinsed in 0.1 M cacodylate buffer, dehydrated in acetone series, and infiltrated with Spurr's resin (Spurr 1969). Semithin sections (700 nm) were stained with toluidine blue (TB-O) and studied under light microscopy. The ultrathin sections (70 nm) were contrasted with uranyl acetate and lead citrate according to Reynolds (1963). Samples were analyzed under TEM (*JEOL Model 1011*, *JEOL Ltd*., Tokyo, Japan) at 80 kv.

Quantification of photosynthetic pigments and photosynthetic efficiency: Chlorophylls (Chl) were analyzed in sporophytes of both treatments according to Hiscox and Israelstam (1979). Four leaf samples of 500 mg each were dried in liquid nitrogen and extracted in 5 ml of dimethylsulfoxide (DMSO) at 45°C for 45 min. The absorbance was analyzed using a *Hitachi 100-20* spectrophotometer (*Hitachi Co*., Japan). Contents of Chl and carotenoids (Car) were analyzed according to Wellburn (1994). The results were expressed in mg g^{-1}(dry mass, DM). Car were extracted following the protocol described by Kuhnen *et al*. (2009) with modifications. Three samples of 100 mg of leaves were immersed in liquid nitrogen, soaked in 1 mL of methanol and incubated for 1 h in the dark. Contents of Car were analyzed according to Wellburn (1994). The results were expressed in mg g^{-1}(DM).

The emission of Chl *a* fluorescence was obtained with a *Diving-PAM*, underwater fluorometer (*Walz*, Effeltrich, Germany) equipped with an optical fiber 5.5 mm in diameter and a blue diode (470 nm) as the light source. Rapid light curves (RLC) (Ralph and Gademann 2005) of *A. daneifolium* sporophytes were registered after one, two, five, and six weeks of treatment ($n = 36$ plants) by applying a series of eight pulses of saturating light (SL) with a duration of 0.8 s each, followed by 0.8 s of exposure to increasing actinic light (AL) [2–2,250 μmol(photon) m^{-2} s^{-1} PAR] (Genty *et al*. 1989, White and Critchley 1999, Ritchie 2008). Prior to each SL, a value for steady-state fluorescence obtained at light-acclimated samples (F_s) was recorded. Afterwards, a value for maximal fluorescence of light-acclimated samples (F_m') was obtained (White and

Critchley 1999, Maxwell and Johnson 2000). These data were subsequently used to calculate the photochemical parameters. Effective quantum yield $Y_{(II)}$ was calculated as $Y_{(II)} = (F_m' - F_s)/F_m'$. With $Y_{(II)}$ values after each SP, it was possible to obtain ETR values, such as ETR = $Y_{(II)} \times$ PAR × A × FII, where PAR is the incident AL utilized sequentially at RLCs, and A is the absorbance, which was estimated as 0.89 for PAR + UVA and 0.92 for PAR + UVA + UVB. These values were obtained following Figueroa *et al*. (2003). Finally, F_{II} corresponds to the ratio of Chl *a* associated to PSII, *i.e*., 0.5 for green algae and land plants. Different parameters were estimated in RLCs with the fitting model of Platt *et al*. (1980): ETR_{max} and α_{ETR}, which, respectively, are maximum electron transport rate and photosynthetic efficiency. Saturating irradiance (E_k) was calculated as $E_k = ETR_{max}/\alpha_{ETR}$. PAM parameters were calculated with the *WinControl* software (*V.3.18*, *Walz*, Germany), using the default settings for fast cornering lights (RLC) (Genty *et al*. 1989).

Statistical data analysis: Mean and standard deviation were calculated by *Excel for Windows* (*Microsoft*). Data were compared by analysis of variance (*ANOVA*), followed by the *Tukey*'s test (5%) (Zar 1996). Results were analyzed by *Excel ASSISTAT 7.0* and *BioEstat 5.0* (5% probability, $p<0.05$). To compare RLCs quantitatively, using parametric statistics, the parameters were calculated by the *Platt*'s equation (Platt *et al*. 1980).

Results

Leaf anatomy: The sporophytes of *A. daneifolium* kept under PAR were well hydrated, showed expanded leaves, with homogeneous coloration (Fig. 1*A*), while plants exposed for six weeks to PAR + UVA + UVB were wrinkled and necrotic in the areas most affected by UVB (Fig. 1*B*).

SEM images of epidermal cells of the abaxial surface of *A. daneifolium* leaves cultivated under PAR showed very sinuous anticlinal walls and convex periclinal walls, the stomata were the anomocytic type, measuring about 48 μm ($n = 20$) with homogeneous guard cells. Pores were quite evident in the images (Fig. 2*A*), while the epidermal cells of the abaxial face of *A. daneifolium* leaves exposed to PAR + UVA + UVB for six weeks showed several abnormal stomata, sometimes without ostiole, or very small in size, measuring around 20 μm ($n = 20$) (Fig. 2*B*). SEM images of epidermal cells of the adaxial face of *A. danaeifolium* leaves cultivated under PAR showed sinuous anticlinal walls and convex periclinal walls (Fig. 2*C*), while the epidermal cells of the abaxial face of *A. daneifolium* leaves exposed to PAR + UVA + UVB showed less sinuous anticlinal walls, along with some damaged cells and crystals, probably waxes (Fig. 2*D*).

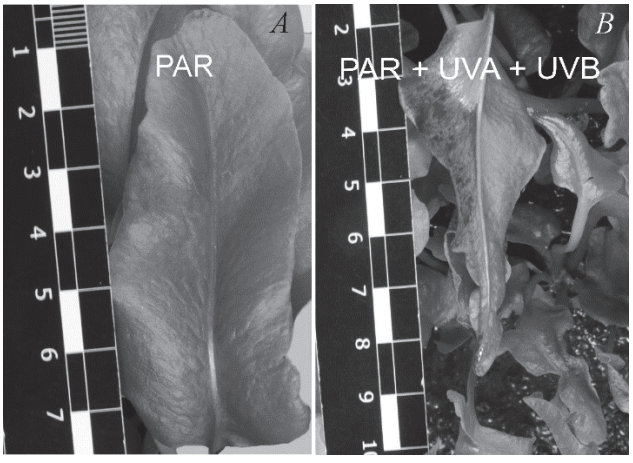

Fig. 1. Sporophytes of *Acrostichum danaeifolium* under PAR were well hydrated, showed expanded leaves, with homogeneous coloration (*A*). Sporophytes exposed for six weeks to PAR + were wrinkled and necrotic in areas most affected by UVB (*B*).

Cross sections of the rachis of *A. danaeifolium* leaves stained with TB-O (Fig. 3*A–F*) revealed three vascular bundles with unicellular epidermises, both adaxial and abaxial, presenting rounded and isodiametric cells under both PAR + UVA and PAR + UVA + UVB (Fig. 3*A–D*). The parenchyma cells were large and thin-walled, with large vacuoles, and large intercellular spaces (Fig. 3*C*,*D*). Next to the adaxial face, cells tended to be homogeneous and smaller than the cells near the abaxial face. These cells were variable in size and seemed to be larger in the plants cultivated under PAR (Fig. 3*C*) compared to the plants exposed for six weeks to PAR + UVA + UVB (Fig. 3*D*). For both treatments, vascular bundles were collateral and enclosed by the endoderm with very homogeneous cells, followed by a layer of pericycle cells. The vascular bundle showed a strong reaction to TB-O (Fig. 3 *D–F*), indicating a strong metachromatic reaction of the acidic polysaccharides in cell walls.

Cross sections of *A. danaeifolium* leaf blade showed unicellular epidermises, both adaxial and abaxial. Epidermis was composed of cells with different sizes and shapes, mainly on the adaxial surface of the plants exposed to PAR + UVA + UVB (Fig. 4*A*,*B*). The mesophyll cells, which also showed different sizes and shapes, formed large aerenchyma that might occupy the entire leaf thickness in both treatments (Fig. 4*A–D*). Chloroplasts were observed in the epidermal cells of both faces (Fig. 4*C–F*). The epidermal cells of the abaxial face of the plants cultivated under PAR + UVA (Fig. 4*E*) showed nucleus and chloroplast homogeneously distributed around the vacuole.

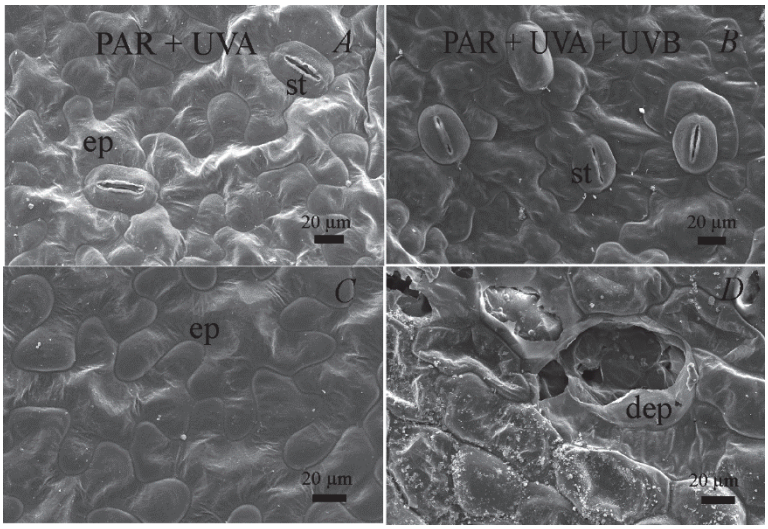

Fig. 2. Scanning electronic microscopy of epidermis of *Acrostichum danaeifolium* leaves cultivated under PAR and PAR + UVB for six weeks. Abaxial epidermis under PAR (*A*); abaxial epidermis under PAR + UVB showed abnormal stomata (st), (*B*); adaxial epidermis under PAR (*C*); adaxial epidermis under PAR showed damaged cells (dep) (*D*).

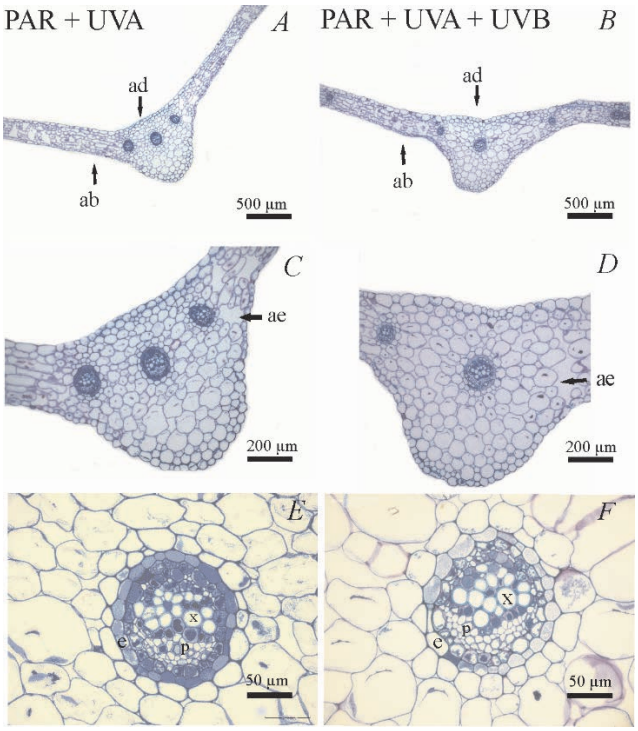

Fig. 3. Light microscopy (LM) of cross sections of the rachis of *Acrostichum danaeifolium* leaves cultivated under PAR and PAR + UVB for six weeks and stained with toluidine blue. Leaves cultivated under PAR (*A,B,C*). Leaves exposed to UVB for six weeks (*D,E,F*). ad – adaxial surface, ab – abaxial surface, ae – aerenchyma, e – endodermis,p – phloem, x – xylem.

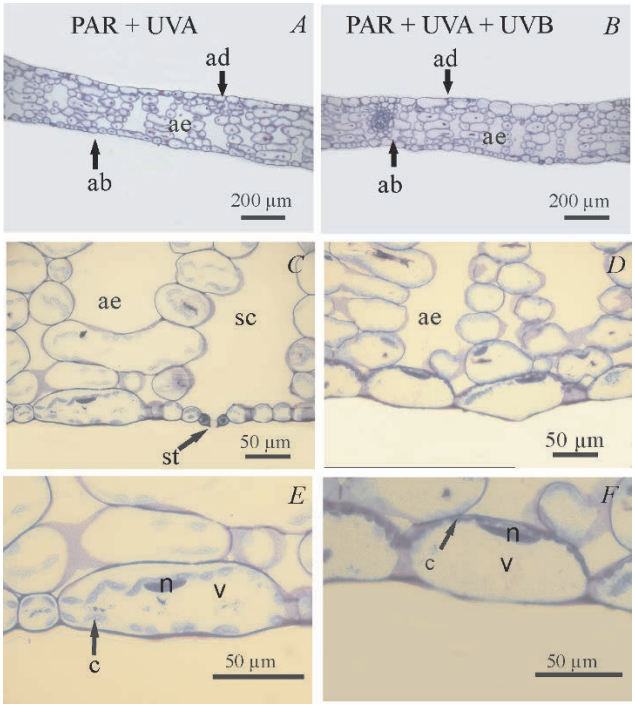

Fig. 4. Light microscopy (LM) of cross sections of *Acrostichum danaeifolium* leaf blades. Adaxial epidermis of leaves under PAR (*A*); under PAR + UVB, the epidermal cells on the adaxial surface (*B*); aerenchyma observed in both treatments (*A,B*). Details of aerenchyma in both treatments (*C,D*). Abaxial epidermis under PAR (*E*). Cells exposed to PAR + UVB (*F*). ad – adaxial surface, ab – abaxial surface, ae – aerenchyma, c – chloroplast, st – stomata, sc – substomatic chamber, n – nucleus, v – vacuole.

On the other hand, in the cells on the abaxial epidermis of plants exposed to PAR + UVA + UVB, the chloroplasts and nuclei were more densely stained and seemed to be distributed against the inner cell wall (Fig. 4*F*).

Cell analyses of *A. danaeifolium* by TEM showed that the control group exhibited an organization similar to that observed by light microscopy (Fig. 5*A–D*). Cells were surrounded by thin cell wall (Fig. 5*A,B*). The chloroplast were large and showed a typical well-developed grana and stromal thylakoids (Fig. 5*B*). The compact stroma contained a few starch grains (Fig. 5*A,B*). In the chloroplasts, small plastoglobuli were observed between the thylakoids (Fig. 5*C*). In the cytoplasm, mitochondria with

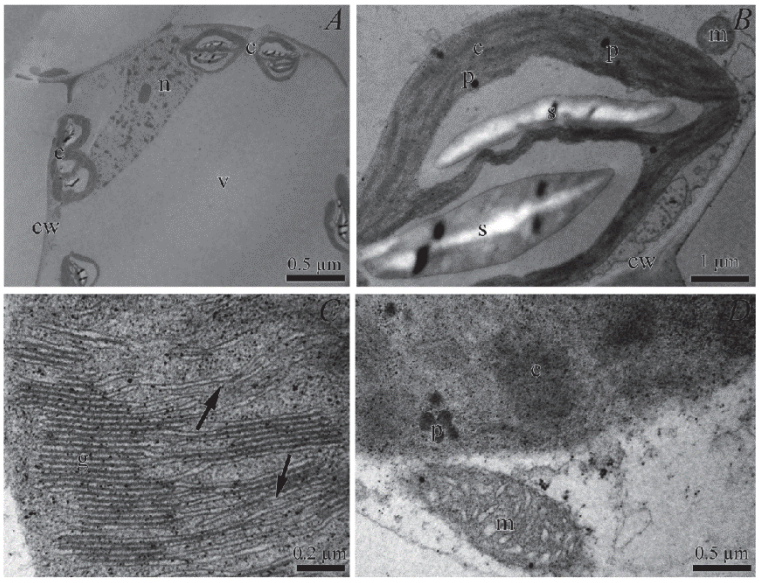

Fig. 5. Transmission electron microscopy (TEM) of *Acrostichum danaeifolium* leaf blade cultivated under PAR. Detail of cell filled with chloroplast, nucleus, large vacuole and thin cell wall (*A*). Chloroplast with plastoglobuli and the presence of starch grains (*B*). Mitochondria with associated chloroplast. Magnification of previous figure showing detail of grana and stromal thylakoids (*arrows*) (*C*). Mitochondria with well-developed cristae membranes (*D*). c – chloroplast, cw – cell wall, g – grana, m – mitochondria , n – nucleus, p – plastoglobule, s – starch grains, v – vacuole.

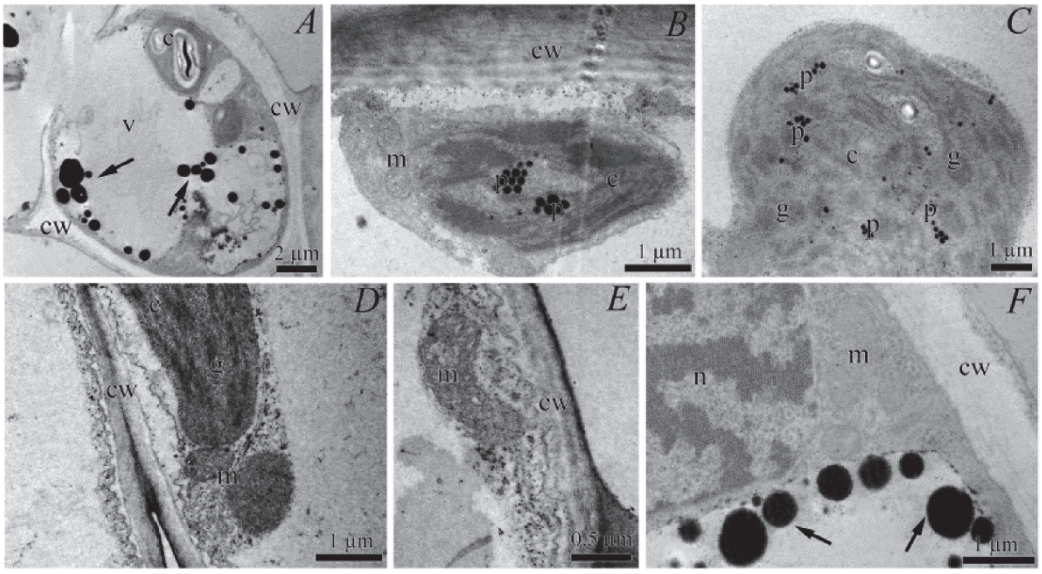

Fig. 6. Transmission electron microscopy (TEM) of *Acrostichum danaeifolium* leaf blade cultivated with PAR + UVB for six weeks. Detail of cell filled with chloroplast, large vacuole and thin cell wall (*A*). Vesicles (arrows) containing phenolic compounds. Plastoglobuli in chloroplast and associated mitochondria (*B*). Thylakoid membranes with irregular morphology and grana in the chloroplast (*C, D*). Detail of disrupted mitochondria (*E*). Vesicles (arrows) with phenolic compounds (*F*). c – chloroplast, cw – cell wall, g – grana, m – mitochondria , n – nucleus, p – plastoglobule, s – starch grains, v – vacuole.

well-developed cristae membranes were observed (Fig. 5*D*). However, after exposure to PAR + UVA + UVB for six weeks, the cells of *A. danaeifolium* showed a few ultrastructural changes (Fig. 6*A–F*). The thylakoid membranes did not show dramatic ultrastructural damage (Fig. 6*B–D*). Some thylakoids appeared dilated and with irregular morphology. However, plastoglobuli seemed to increase in the chloroplasts (Fig. 6*B*,*C*). Some mitochondria appeared to be disrupted and irregular in shape (Fig. 6*E*). A large quantity of vesicles containing phenolic compounds was observed in the cytoplasm (Fig. 6*A*,*F*).

Photosynthetic parameters by fluorescence of Chl *a*: Similar values of ETR_{max}, α_{ETR}, and α_{ETR} were observed for sporophytes of *A. danaeifolium* cultivated under PAR + UVA and those cultivated under PAR + UVA + UVB, respectively, which did not differ from each other after one, two, five, and six weeks of the treatments (Table 1). No differences were observed in the contents of Chl *a*, *b*, and Car between sporophytes cultivated under PAR + UVA and those cultivated under PAR + UVA + UVB after six weeks (Table 2).

Discussion

The leaves of young sporophytes of *A. daneifolium* showed a peculiar anatomy that has not yet been described for this species. Specifically, the leaves showed large aerenchyma and chloroplasts in the epidermal cells of both faces. *Sensu lato,* aerenchyma is simply parenchymatous tissue with a large volume of intercellular space (Drew *et al*. 2000). These small plants are continually subjected to changes in tides, flooding, and deprivation of CO_2, which can explain the existence of aerenchyma in the leaves as a mechanism that could store CO_2 at night. In the aquatic plant *Lobelia dortmanna* L. (Campanulaceae), Pedersen and Sand-Jensen (1992) showed that the concentration of CO_2 in its lacunae increases 23-fold over that of ambient atmospheric concentration, ranging from about 0.3% during the day to about 0.7% at night. Similar results were obtained for *Typha latifolia* L. (Typhaceae) (Constable *et al.* 1992), where gas in the leaf aerenchyma ranged from ambient CO_2 contents around noon to about 0.6% of CO_2 (18 times higher than the ambient content) in the early morning. Stomata of the anomocytic type were observed only in abaxial epidermis. Leaves did not exhibit palisade parenchyma.

When sporophytes of *A. danaeifolium* were exposed for six weeks to PAR + UVA + UVB treatment, they showed curled leaves with some necrotic areas. Leaf winding of plants exposed to UVB radiation may indicate a strategy to protect the abaxial face against UV radiation. According to Boeger and Poulson (2006) and de Almeida *et al.* (2013), these changes in leaf morphology due to UV are responses to stress caused in the process of development and cellular metabolism. Several studies have reported winding of leaves when plants are exposed to UVB, including, for example, *Arabidopsis thaliana* (L.) Heynh (Brassicaceae) (Boeger and Poulson 2006), *Larix occidentalis* Nutt. (Pinaceae), *Picea glauca* var. *engelmannii* (Parry ex Engelm.) B. Boivin (Pinaceae) (Krol *et al*. 2000), and *Brassica napus* L. (Brassicacea) (Wilson and Greenberg 1993). The latter authors cite three possible causes for such a winding. The first reason involves triggering a UVB receptor, while the second one involves auxin degradation, and the third cause is connected to degradation of proteins, DNA or photosystems (Wilson and Greenberg 1993; Saibo et al 2003).

The leaves of *A. danaeifolium* exposed for six weeks to PAR + UVA + UVB radiation showed abnormal stomata, often small in size and sometimes without ostiole. For light microscopy, chloroplasts and nuclei on the abaxial epidermis were densely stained by TB-O and were arranged parallel to the light source. Orientation and movement of chloroplasts are well known to occur in many groups: algae, mosses, ferns, and higher plants (Haupt and Scheuerlein 1990). The orientation responses and stationary arrangements of chloroplasts depend on the incident light. In weak light, they move toward the face arrangement, occupying the cell walls that are perpendicular to the light direction. In strong light, chloroplasts assume a profile arrangement such that they gather at the cell walls parallel to the direction of light (Augustynowicz and Gabrys 1999).

Our results showed that young sporophytes of *A. danaeifolium* adapted to high levels of UV radiation by curving leaves in a manner that reduced leaf area to light absorption, thus protecting adaxial epidermis cells against damage. In addition, the migration of chloroplasts to the opposite direction of the light source is a common strategy against high irradiation, as previously mentioned for several species.

After cultivation under PAR + UVA + UVB for six weeks, the leaves of *A. danaeifolium* showed some changes in ultrastructure, such as dilation of thylakoid

Table 1. Photosynthetic parameters of *Acrostichum daneifolium* after one, two, five, and six weeks of exposure to PAR+UVA and PAR + UVA + UVB for one hour a day. Data did not differ from each other ($n = 36$).

Week	Treatment PAR + UVA	PAR + UVA + UVB
	α_{ETR} [µmol(electron) m^{-2} s^{-1}]	
1	0.22 ± 0.02	0.22 ± 0.03
2	0.25 ± 0.03	0.25 ± 0.02
5	0.23 ± 0.04	0.28 ± 0.02
6	0.24 ± 0.04	0.28 ±0.02
F	0.81	
p	0.50	
	E_k [µmol(photon) m^{-2} s^{-1}]	
1	86.79 ± 3.22	100.97 ±13.97
2	101.60 ± 6.03	92.45 ±11.52
5	109.79 ± 11.91	97.52 ± 2.17
6	102.24 ± 12.35	102.44 ± 4.54
F	3.24	
p	0.03	
	ETR_{max} [µmol(electron) m^{-2} s^{-1}]	
1	18.95 ± 1.81	22.78 ± 4.81
2	25.74 ± 4.47	22.80 ± 4.22
5	25.90 ± 4.65	24.66 ± 3.84
6	25.90 ± 6.21	29.01 ± 3.23
F	1.6	
p	0.21	

Table 2. Contents of chlorophyll *a*, chlorophyll *b*, and carotenoids of *Acrostichum daneifolium* after 45 days in PAR and chronic exposure to PAR+UVB for one hour a day. Data did not differ from each other (*Student*'s *t*-test) ($n = 4$).

Treatment	Photosynthetic pigments [mg g^{-1}(DM)] Chl *a*	Chl *b*	Carotenoids
PAR	4.75 ± 0.11	4.93 ± 1.44	138.07 ± 21.20
PAR + UVB	4.75 ± 0.10	3.89 ± 0.37	134.55 ± 8.80
'*t*'	–0.0239	1.2115	0.2493

membranes and increase of plastoglobuli volume. Meanwhile, photosynthetic parameters after UVB exposure were preserved. ETR_{max} and α_{ETR} were not different between PAR + UVA and PAR + UVA + UVB treatments in leaves of *A. danaeifolium.* Similarly, ETR was not affected in three subarctic species of bryophytes after exposure to UVB (Arróniz-Crespo *et al*. 2011). Under TEM, images of chloroplasts did not reveal their location on the leaf blade. In these sections, we could see chloroplasts of both epidermises, as well as mesophyll chloroplasts. It is expected that the most affected chloroplasts are those exposed to higher irradiance. Our results suggest that the photosynthetic parameters were not affected because the chloroplasts of adaxial epidermis did not suffer damage and were able to compensate for photosynthesis damage in the chloroplasts, which were exposed to higher irradiation. On the other hand, *Azzola microphylla* Kaulf. (Salviniaceae), an aquatic fern, was exposed to UVB for 12 days and showed inhibition in PSII activity (Jayakumar *et al*. 2002). No differences were observed in the contents of Chl *a*, *b*, and Car in leaves of *A. danaeifolium* cultivated under PAR and PAR + UVA + UVB for six weeks. Randi *et al*. (2014) observed that Chl *a* decreased, while Chl *b* and trans-β-carotene increased in the gametophytes of *A. danaeifolium* cultivated for 30 d under PAR + UVA + UVB radiation, while the content of total phenolic compounds showed a two-fold increase. A large quantity of vesicles containing phenolic compounds in the cytoplasm was also observed in the present work in sporophytes of *A. daneifolium* after exposure to UVB radiation. This response to stress could also be a strategy to protect photosystems. Alterations in the contents of photosynthetic pigments and phenolic compounds after exposure to UVB radiation are quite variable among plants. *Crotalaria juncea* L. (Fabaceae) exposed to UVB radiation showed a decrease in photosynthetic pigments and an increase in anthocyanins and flavonoids (Balakrishnan *et al*. 2005). Ranjbarfordoei *et al*. (2009), working with *Prunus dulcis* (Miller) D. Webb (Rosaceae), observed a decrease of Car in plants exposed to UVB. Boeger and Poulson (2006) found higher concentrations of total Chl and phenolic compounds in *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae). Cuadra *et al*. (2004) observed a decrease in Chl and an increase in phenylpropanoids, compounds which are capable of absorbing UV radiation, in plants of *Jaborosa magellanica* Brisben (Solanaceae). Singh *et al*. (2011) observed a decrease in total Chl and the Chl *a*/*b* ratio in plants of *Dolichos lablab* L. (Fabaceae) exposed to UVB radiation. Although UVB radiation may have mild inhibitory effects on the biomass accumulation, Reboredo and Lidon (2012) concluded that photosynthesis is not significantly affected by changes in UVB radiation, when terrestrial plants grow under a protective environment, such as canopy. In a meta-analysis of polar plants, Newsham and Robinson (2009) concluded that photosynthetic parameters (F_v/F_m and Φ_{PSII}), Car and Chls were unaffected by exposure to UVB.

In the present study, sporophytes of *A. danaeifolium* exposed to PAR + UVA + UVB radiation showed curled leaves with some necrotic areas. The young leaves showed epidermal damage, as well as disturbance of the development and size of stomata. Chloroplasts showed some damage and assumed a profile arrangement after exposure to UVB radiation. However, photosynthetic pigments and photosynthetic parameters were preserved. Considering that *A. danaeifolium* is an endemic species of mangroves typically subjected to high levels of radiation during the summer, young sporophytes showed photoprotection mechanisms for preservation of photosynthetic activity, while damage occurred mainly in epidermal cells.

References

Almeida S.L., Schmidt É.C., Pereira D.T. *et al*.: Effect of ultraviolet-B radiation in laboratory on morphological and ultrastructural characteristics and physiological parameters of selected cultivar of *Oryza sativa* L. – Protoplasma **250**: 1303-1313, 2013.

Alvares C.A., Stape J.L., Sentelhas P.C. *et al*.: Köppen's climate classification map for Brazil. – Meteorol. Z. **22**: 711-728, 2013.

Aphalo P.J., Albert A., Björn L.O. *et al*.: Introduction. – In: Aphalo P.J, Albert A, Björn L O *et al.* (ed.): Beyond the Visible: A Handbook of Best Practice in Plant UV Photobiology. Pp. 1-34. University of Helsinki, Helsinki 2012.

Arróniz-Crespo M., Gwynn-Jones D., Callaghan T.V. *et al*.: Impacts of long-term enhanced UV-B radiation on bryophytes in two sub-Arctic heathland sites of contrasting water availability. – Ann. Bot.-London **108**: 557-565, 2011.

Augustynowicz J., Gabrys H.: Chloroplast movements in fern leaves: correlation of movement dynamics and environmental flexibility of the species. – Plant Cell Environ. **22**: 1239-1248, 1999.

Balakrishnan V., Venkatesan K., Ravindran K.C., Kulandaivelu G.: Protective mechanism in UV-B treated *Crotalaria juncea* L. seedlings. – Plant Protect. Sci. **41**: 115-120, 2005.

Boeger M.R.T., Poulson M.E.: [Effects of ultraviolet B radiaton on the foliar morphology of *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae).] – Acta Bot. Bras. **20**: 329-338, 2006. [In Portuguese]

Bouzon Z.L.: [Histochemistry and ultrastructure of the ontogenesis of tetrasporangia of *Hypnea musciformis* (Wulfen) J. V. Lamour. (Rhodophyta, Gigartinales)]. – Rev. Bras. Bot. **29**: 229-238, 2006. [In Portuguese]

Constable J.V.H., Grace J.B., Longstreth D.J.: High carbon dioxide concentrations in aerenchyma of *Typha latifolia.* – Am. J. Bot. **79**: 415-418, 1992.

Cuadra P., Herrera R., Fajardo V.: Effects of UV-B radiation on the Patagonian *Jaborosa magellanica* Brisben. – J. Photoch. Photobio. B. **76**: 61-68, 2004.

de Almeida S.L., Schmidt É.C., Pereira D.T. *et al*.: Effect of ultraviolet-B radiation in laboratory on morphological and ultrastructural characteristics and physiological parameters of selected cultivar of *Oryza sativa* L. – Protoplasma **250**: 1303-1313, 2013.

Drew M.C., He C.J., Morgan P.W.: Programmed cell death and

aerenchyma formation in roots. – Trends Plant Sci. **5**: 123-127, 2000.

Dyer A.F.: The Experimental Biology of Ferns. Pp. 657. Academic, London, 1979.

Feder N., O'Brien T.P.: Plant microtechnique: some principles and new methods. – Am. J. Bot. **55**: 123-142, 1968.

Figueroa F.L., Conde-Álvarez R., Gómez I.: Relations between electron transport rates determined by pulse amplitude modulated chlorophyll fluorescenceand oxygen evolution in macroalgae under different light conditions. – Photosynth. Res. **75**: 259-275, 2003.

Genty B., Briantais J.M., Baker N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. – Biochim. Biophys. Acta **990**: 87-92, 1989.

Gomez L.D.: *Acrostichum aureum.* – In: Janzen D.H. (ed.): Costa Rican Natural History. Pp. 185-187. Univ. Chicago Press, Chicago 1983.

Greenberg B.M., Gaba V., Canaani O. *et al.*: Separate photosensitizers mediate degradation of the 32-kDa photosystem II reaction center protein in the visible and UV spectral regions. – P. Natl. Acad. Sci. USA **86**: 6617-6620, 1989.

Haupt W., Scheuerlein R.: Chloroplast movement. – Plant Cell Environ. **13**: 595-614, 1990.

Hilal M., Parrado M.F., Rosa M. *et al.*: Epidermal lignin deposition in quinoa cotyledons in response to UV-B radiation. – Photochem. Photobiol. **79**: 205-210, 2004.

Hietz P.: Fern adaptations to xeric environments. – In: Mehltreter K., Walker L.R., Sharpe J.M. (ed.): Fern Ecology. Pp.140-176. Cambridge Univ. Press, Cambridge 2010.

Hiscox J.D., Israelstam G.F.: A method for the extraction of chlorophyll from leaf tissue without maceration. – Can. J. Bot. **57**: 1332-1334, 1979.

Jayakumar M., Eyini M., Lingakumar K., Kulandaivelu G.: Effects of enhanced ultraviolet-B (280-320 nm) radiation on growth and photosynthetic activities in aquatic fern *Azolla microphylla* Kaulf. – Photosynthetica **40**: 85-89, 2002.

Janzen D.H.: Mangroves: where's the understory? – J. Trop. Ecol. **1:** 89-92, 1985.

Jones L.W., Kok B.: Photoinhibition of chloroplast reactions. I. Kinetics and action spectra. – Plant Physiol. **41:** 1037-1043, 1966.

Johansen D.A.: Plant Microtechnique. Pp. 523. McGraw Hill, New York 1940.

Krol P.M., Ormrod D.P., Binder W.D., L'Hirondelle S.J.: Effects of ultraviolet-B radiation on needle anatomy and morphology of Western Larch, Interior Spruce and Lodgepole Pine. – J. Sustain. Forest. **10**: 141-148, 2000.

Kuhnen S., Lemos P.M.M., Campestrini L.H. *et al.*: Antiangiogenic properties of carotenoids: a potential role of maize as functional food. – J. Funct. Food. **1**: 284-290, 2009.

Maxwell K., Johnson G.N.: Chlorophyll fluorescence – a practical guide. – J. Exp. Bot. **51**: 659-668, 2000.

McKenzie R.L., Aucamp P.J., Bais A.F. *et al.*: Changes in biologically active ultraviolet radiation reaching the Earth's surface. – Photoch. Photobio. Sci. **6**: 218-231, 2007.

Mehltreter K., Palacios-Rios M.: Phenological studies of *Acrostichum danaeifolium* (Pteridaceae, Pteridophyta) at a mangrove site on the Gulf of Mexico. – J. Trop. Ecol. **19**: 155-162, 2003.

Moran R.C.: [Key to the families of pteridophytes.] – Flora Mesoamericana **1**: 1-2,1995. [In Portuguese]

Newsham K.K., Robinson S.A.: Responses of plants in polar regions to UVB exposure: a meta-analysis. – Glob. Change Biol. **15**: 2574-2589, 2009.

O'Brien T.P., Feder N., McCully M.E.: Polychromatic staining of plant cell walls by toluidine blue O. – Protoplasma **59**: 368-373, 1965.

Paiva J.G.A., Fank-de-Carvalho S.M., Magalhães M.P., Graciano-Ribeiro D.: [Lacquer stained glass 500®: an economically viable alternative for microscopic slides.] – Acta Bot. Bras. **20:** 257-264, 2006. [In Portuguese]

Pedersen O., Sand-Jensen K.: Adaptations of submerged *Lobelia dortmanna* to aerial life form: morphology, carbon sources, and oxygen dynamics. – Oikos **65**: 89-96, 1992.

Platt T., Gallegos C.L., Harrison W.G.: Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. – J. Mar. Res. **38**: 687-701, 1980.

Ralph P.J., Gademann R.: Rapid light curves: a powerful tool to assess photosynthetic activity. – Aquat. Bot. **82**: 222-237, 2005.

Randi A.M., Freitas M.C.A., Rodrigues A.C. *et al.*: Acclimation and photoprotection of young gametophytes of *Acrostichum danaeifolium* to UV-B stress. – Photosynthetica **52**: 50-56, 2014.

Ranjbarfordoei A., van Damme P., Samson R.: Elevated ultraviolet-B radiation influences photosynthetic pigments and soluble carbohydrates of sweet almond *Prunus dulcis* (Miller) D. Webb. – Electron. J. Envi. Agri. Food Chem. **8**: 1077-1084, 2009.

Reboredo F., Lidon F.J.C.: UV-B radiation effects on terrestrial plants – A perspective. – Emirates J. Food Agri. **24**: 502-509, 2012.

Reitz R.: [Vegetation of the maritime zone of Santa Catarina.] – Sellowia **13**: 17-115, 1961. [In Portuguese]

Reynolds E.S.: The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. – J. Cell Biol. **17:** 208-212, 1963.

Ritchie R.J.: Fitting light saturation curves measured using modulated fluorometry. – Photosynth. Res. **96**: 201-215, 2008.

Saibo N.J, Vriezen W.H, Beemster G.T, Van Der Straeten D.: Growth and stomata development of *Arabidopsis* hypocotyls are controlled by gibberellins and modulated by ethylene and auxins. – Plant J. **33**: 989-1000, 2003.

Schulze É.D., Beck E., Müller-Hohenstein K.: Plant Ecology. Pp. 702. Springer-Verlag, Berlin 2005.

Setlow R.B.: The wavelengths in sunlight effective in producing skin cancer: a theoretical analysis. – P. Natl. Acad. Sci. USA **71**: 3363-3366, 1974.

Schmidt É.C., Scariot L.A., Rover T., Bouzon Z.L.: Changes in ultrastructure and histochemistry of two red macroalgae strains of *Kappaphycus alvarezii* (Rhodophyta, Gigartinales), as a consequence of ultraviolet B radiation exposure. – Micron **40**: 860-869, 2009.

Singh R., Singh S., Tripathi R., Agrawal S.B.: Supplemental UV-B radiation induced changes in growth, pigments and antioxidant pool of bean (*Dolichos lablab*) under field conditions. – J. Environ. Biol. **32**: 139-145, 2011.

Smith A.R., Pryer K.M., Schuettpelz E. et al.: Classification for extant ferns. – Taxon **55**: 705-731, 2006.

Spurr A.R.: A low-viscosity epoxy resin embedding medium for electron microscopy. – J. Ultra. R. **26**: 31-43, 1969.

Tevini M., Teramura A.H.: UV-B effects on terrestrial plants. – Photochem. Photobiol. **50**: 479-487, 1989.

Tryon R. M., Tryon A. F.: Ferns and Allied Plants with Special Reference to Tropical America. Pp. 857. Springer, New York 1982.

Wellburn A.R.: The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. – J. Plant Physiol. **144**: 307-313, 1994.

Williamson C.E., Zepp R.G., Lucas R.M. *et al*.: Solar ultraviolet radiation in a changing climate. – Nat. Clim. Change **4**: 434-441, 2014.

Wilson M.I., Greenberg B.M.: Specificity and photomorphogenic nature of ultraviolet-B-induced cotyledon curling in *Brassica napus* L. – Plant Physiol. **102**: 671-677, 1993.

White A.J., Critchley C.: Rapid light curves: a new fluorescence method to assess the state of the photosynthetic apparatus. – Photosynth. Res. **59:** 63-72,1999.

Zar J.H.: Biostatistical Analysis, 3rd ed. Pp. 662. Prentice Hall, New Jersey 1996.