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Cell compartmentation of UV-absorbing compounds in two aquatic mosses under enhanced UV-B

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Abstract – The effects of enhanced UV-B radiation on UV-absorbing compounds (UVAC), the maximum quantum yield of photosystem II (F_v/F_m), DNA integrity, and the sclerophylly index, were analyzed in the mosses *Bryum pseudotriquetrum* and *Fontinalis antipyretica*. The study was performed for 31 days under laboratory conditions. Enhanced UV-B increased the bulk level of the vacuolar soluble UVAC (SUVAC) in both mosses and the concentration of two different soluble kaempferols in *B. pseudotriquetrum*. However, enhanced UV-B had no effect on the bulk level of cell wall-bound insoluble UVAC (WUVAC) in both mosses and the concentration of insoluble *p*-coumaric acid in *F. antipyretica*. Thus, the insoluble fraction would be less UV-B-responsive than the soluble one. This probably happened because (1) the constitutively high bulk level of WUVAC (and noticeably higher than that of SUVAC) would already provide a sufficiently effective protection; and (2) WUVAC would be relatively immobilized in the cell wall, which would limit the reaction capacity of these compounds to UV-B. The protective mechanisms developed by both mosses could not totally prevent UV-B damage, which was indicated by the modest decrease of F_v/F_m and the increase in DNA damage. We discuss the ecological and phylogenetic implications of the differences in UVAC compartmentation between liverworts and mosses.

UV-B radiation / UV-absorbing compounds / Ozone depletion / Mosses / Compartmentation / Vacuoles / Cell walls

INTRODUCTION

Ultraviolet (UV) radiation is a minority part of solar radiation, but it has important biological effects on the morphology and physiology of photosynthetic organisms, including bryophytes (Boelen *et al.*, 2006; Newsham & Robinson, 2009; Martínez-Abaigar & Núñez-Olivera, 2011). The amount of solar UV-B (280-315 nm) radiation reaching the ground has increased due to the anthropogenic ozone reduction. At northern mid-latitudes, ozone loss has been estimated around 6% in the last 30 years, which might have resulted in a UV-B increase of up to 12% (McKenzie *et al.*, 2003). The future trend for UV-B radiation is notably uncertain, in particular due to the influence of climate change factors (McKenzie *et al.*, 2007;

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Hegglin & Shepherd, 2009). In addition, ozone mini-holes may cause up to 33% decreases in ozone and consequent transitory UV-B increases between 43 and 75% (Antón *et al.*, 2007). Thus, the study of UV-B effects on organisms will be an important issue in the coming several decades.

In photosynthetic organisms, increased UV-B may trigger diverse damage, repair and acclimation processes, so that the plant can cope with the new situation (Jansen *et al.*, 1998). Thus, damage to DNA and the photosynthetic apparatus (pigment degradation, photoinhibition, and decreases in photosynthetic rates and enzyme activities) may be counteracted by protective mechanisms, such as DNA repariring, antioxidant systems and the accumulation of UV-absorbing compounds (UVAC), mainly phenolics.

The bulk UV absorbance of methanolic plant extracts has been the most used variable to globally measure UVAC and the associated UV protection capacity. This variable has been notably valuable in the context of UV research, being the one that most consistently responded to enhanced UV-B in tracheophytes and bryophytes (Searles *et al.*, 2001; Newsham & Robinson, 2009). Nevertheless, this variable can be usefully complemented by the measurement of the specific individual UVAC contributing to that bulk UV absorbance, since each compound may respond to UV-B differently (Kotilainen *et al.*, 2008). Furthermore, Schnitzler *et al.* (1996) measured both the bulk UV absorbance and specific UVAC in two different cell compartments of pine needles: the soluble fraction (mainly located in the vacuole), and the insoluble cell wall-bound fraction. Diacylated flavonol glycosides, such as 3'',6''-di-*p*-coumaroyl-isoquercitrin and 3'',6''-di-*p*-coumaroyl-astragalin, were present in the soluble fraction, whereas 4-coumaric acid and ferulic acid were found in the insoluble fraction. The differentiation between soluble and insoluble fractions is relevant, especially in the monostratified leaves of bryophytes, because cell wall-bound insoluble UVAC (WUVAC) would provide a more efficient UV screen than intracellular soluble UVAC (SUVAC), and thus could enhance UV-B tolerance (Clarke & Robinson, 2008).

The effects of UV-B on bryophytes are not properly understood yet, in spite of the important work carried out mainly in the last decade (see recent reviews in Boelen *et al.*, 2006; Newsham & Robinson, 2009; Martínez-Abaigar & Núñez-Olivera, 2011). In particular, UVAC compartmentation between vacuoles and cell walls has been little studied (Taipale & Huttunen, 2002; Clarke & Robinson, 2008; Snell *et al.*, 2009; Fabón *et al.*, 2010; Lappalainen, 2010). This issue may be important in such structurally simple plants as bryophytes, since their UVAC compartmentation is much simpler than that of tracheophytes and thus easier to model. In addition, it has been hypothesized that liverworts and mosses may show different compartmentations: liverworts would have a higher bulk level of SUVAC than of WUVAC (Fabón *et al.*, 2010), whereas the inverse pattern would be found in mosses (Clarke & Robinson, 2008; Lappalainen, 2010). More studies on the compartmentation of UVAC in bryophytes and its responses to enhanced UV-B are needed to test this hypothesis.

The aim of the present study was to examine, under laboratory conditions, the response of the UVAC compartmentation of two mosses (*Bryum pseudotriquetrum* and *Fontinalis antipyretica*) to enhanced UV-B radiation, given that previous studies with these mosses (Martínez-Abaigar *et al.*, 2003; Núñez-Olivera *et al.*, 2004; Martínez-Abaigar *et al.*, 2009) did not take into account this important issue. We measured the bulk levels of SUVAC and WUVAC, together with specific UVAC belonging to the two main types of UV-protective phenolics, hydroxycinnamic acids and flavonoids, that can be located in both the soluble and

cell wall fractions (Dixon & Paiva, 1995; Schmitz-Hoerner & Weissenbock, 2003). We also measured the maximum quantum yield of photosystem II (F_v/F_m), DNA integrity, and the sclerophylly index, which could indicate the damage effects caused by enhanced UV-B on the photosynthetic apparatus, DNA, and growth. The simultaneous evaluation of damage and protection mechanisms would serve to a better understanding of the tolerance and acclimation to enhance UV-B in the mosses used, and also to accumulate information on the different strategies developed by bryophytes regarding UVAC compartmentation.

MATERIALS AND METHODS

Experimental design

Unshaded and submerged samples of two aquatic mosses, *Bryum pseudotriquetrum* (Hedw.) P.Gaertn. *et al.* and *Fontinalis antipyretica* Hedw., were collected on 15 November 2007 at the first-order stream Lumbreras (1903 m altitude, 42º00'30'' N, 02º38'40'' W), in La Rioja (northern Spain). The material was rinsed with stream water and transported to the laboratory in a portable icebox. Species were identified using a standard key (Casas *et al.*, 2006). *Bryum pseudotriquetrum* is an acrocarpous moss which forms green, reddish or brown tufts composed by several cm tall shoots covered in its basal part with a brown mat of rhizoids. Leaves are lanceolate and decurrent, and have a reddish base, a border of narrow cells and a thick and shortly excurrent nerve. *Fontinalis antipyretica* is a large pleurocarpous moss that forms dangling submerged masses ranging in colour from bright green to brownish. Its ovate and strongly keeled leaves, which lack a nerve, give the shoots a characteristic three-sided appearance.

For each species, green healthy apices were selected and distributed into 15 plastic tubes provided with a basal net which prevented material losses. The 30 tubes were placed in a circulating bath system within a growth chamber. The bath was filled with air-bubbled stream water (pH 6.8, conductivity 21 μS cm^{-1}) which was maintained at a constant temperature of 10ºC using an immersion chiller. The radiation was provided by a Hönle SOL 1200RF2 lamp (Dr. Hönle AG UV-Technologie, Gräfelfing/Munich, Germany) and Sylvania Coolwhite (Osram-Sylvania, Madrid, Spain) and True-lite full spectrum (True Sun, Steubenville, OH, USA) fluorescent tubes. Spectral characteristics have been published elsewhere (Gröniger *et al.*, 1999). By covering the tubes with specific UV cut-off foils, three radiation regimes were randomly assigned to the tubes (in five replicates for each regime):

– P (photosynthetically active radiation, PAR, alone), using Ultraphan 395 (Digefra GmbH, Munich, Germany), which cut off all UV radiation.

– PA (PAR + UV-A), using Folex 320 (Folex GmbH, Dreieich, Germany), which cut off UV-B and UV-C radiation.

– PAB (PAR + UV-A + UV-B), using Ultraphan 295 (Digefra GmbH, Munich, Germany), which cut off UV-C radiation.

The filters were pre-irradiated for one hour and replaced every week. Samples were cultivated with a 12:12 photoperiod for 31 days, replacing weekly the water in the culture. The UV source (Hönle lamp) was switched on around noon for 7 h per day (square-wave). Table 1 shows the radiation conditions in the

Table 1. Radiation conditions in the three radiation regimes (P, PA, and PAB) under which the samples were cultivated. UV_{BE} (biologically effective UV radiation) were calculated on the basis of the action spectra specified.

	P	*PA*	*PAB*
PAR (μmol m^{-2} s^{-1})	522	512	543
PAR (W m^{-2})	97	98	100
UV-A (W m^{-2})	2.61	32.6	35.2
UV-B (W m^{-2})	0.006	0.010	1.21
UV_{BE} (W m^{-2}) (Caldwell, 1971)	0.00	0.00	0.36
UV_{BE} (W m^{-2}) (Setlow, 1974)	0.00	0.00	0.34
UV_{BE} (W m^{-2}) (Flint & Caldwell, 2003)	0.01	0.46	0.83

three different regimes. The plants under the PAB regime received a daily UV-B dose of 28.2 kJ m^{-2}, which was required to mimic a 20% ozone depletion as calculated with a computer model (Björn & Teramura, 1993). The biologically effective UV irradiance (UV_{BE}) was estimated using classic and modern action spectra: the generalized plant damage action spectrum normalized at 300 nm (Caldwell, 1971), the DNA damage spectrum (Setlow, 1974), and that of Flint & Caldwell (2003). Plants were submerged at 1-2 cm depth, which attenuated less than 0.01% the photosynthetic and UV wavelengths. The spectral irradiances were measured using a spectroradiometer (Macam SR9910, Macam Photometrics Ltd, Livingstone, Scotland), and PAR was measured with a quantum sensor (LI-190SA, LI-COR, Lincoln, NE, USA).

Physiological measurements

The sclerophylly index (SI) was calculated at the end of the experiment, as the quotient between the dry mass (DM: 60ºC for 24 h) and the surface area of the prostrate apex (LI-COR LI-3000 area meter, Lincoln, NE, USA). Previously, fresh mass was measured. The maximum quantum yield of PSII (F_v/F_m) was periodically measured at predawn with a portable pulse amplitude modulation fluorometer (MINI-PAM, Walz, Effeltrich, Germany) following Núñez-Olivera *et al.* (2004). DNA damage was evaluated by detection of thymine dimers, using UV-C irradiated DNA of the plasmid pBSK for calibration (for details, see Otero *et al.*, 2006). Samples for the analysis of DNA damage were taken at the end of the light period on the final day of the experiment.

In both species, the bulk level of UV-absorbing compounds (UVAC) was analyzed by spectrophotometry (Perkin-Elmer λ35 spectrophotometer, Perkin-Elmer, Wilton, CT, USA) in the soluble fraction (SUVAC, presumably mainly located in the vacuoles) and the insoluble cell wall-bound fraction (WUVAC). For these analyses (Fabón *et al.*, 2010), fresh shoot apices were frozen in liquid N_2 and ground in a TissueLyser (Qiagen, Hilden, Germany). Then, 5 ml of methanol: water: 7M HCl (70:29:1 v/v/v) was added for extraction (24 h at 4ºC in the dark). The extract was centrifuged and the supernatant and pellet were considered the source of SUVAC and WUVAC, respectively (Clarke & Robinson, 2008). In

the supernatant, the bulk level of SUVAC was measured, in arbitrary units, as the area under the absorbance curve in the interval 280-400 nm ($AUC_{280-400}$) per unit of DM. The pellet was hydrolysed with NaOH, acidified with HCl and extracted three times with ethyl acetate. After evaporation, the material was dissolved in methanol and the bulk level of WUVAC was spectrophotometrically measured as previously described for the bulk level of SUVAC.

Individual UVAC were measured in both species by high-performance liquid chromatography (Agilent HP1100 HPLC system, Agilent Technologies, Palo Alto, CA, USA), following Arróniz-Crespo *et al.* (2006). In the soluble fraction of *B. pseudotriquetrum*, the flavonols kaempferol-3-*O*-glucoside and kaempferol-3,7-*O*-diglucoside were found and measured. These are two of the major flavonoids of this species (Webby *et al.*, 1996). Their quantification was made by calibration curves with the commercial external standard kaempferol (Sigma-Aldrich, St. Louis, MO, USA). In the insoluble fraction of *F. antipyretica*, *p*-coumaric acid was detected and quantified. The individual UVAC found in *B. pseudotriquetrum* were not detected in *F. antipyretica*, and viceversa.

Statistical analysis

In each moss species, the effects of the radiation regime and culture period on the responses of F_v/F_m and UVAC-related variables were tested using a two-way analysis of variance (ANOVA), with repeated measures for the culture period, once proved that the data met the assumptions of normality and homoscedasticity. In the case of significant differences, means were then compared by Tukey's test. The effect of the radiation regime on SI and the amount of thymine dimers of each species at the end of the experiment was tested using a one-way ANOVA. All the statistical procedures were performed with SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Representative absorption spectra of the insoluble and soluble fractions of the extracts of *B. pseudotriquetrum* and *F. antipyretica* are shown in Fig. 1. Insoluble fractions of both species showed several absorption peaks in the 268-316 nm region (mainly in the UV-B band), whereas the soluble fractions showed well-defined unique peaks in the UV-C band (at 267 nm in *B. pseudotriquetrum* and 269 nm in *F. antipyretica*). In the soluble fraction of *B. pseudotriquetrum*, a shoulder around 330 nm could also be noted.

At the beginning of the experiment, the bulk level of WUVAC was higher than that of SUVAC in both species, in terms of the area under the absorbance curve in the interval 280-400 nm ($AUC_{280-400}$) per mg DM: 28.5 ± 2.4 *vs.* 14.9 ± 0.5 in *B. pseudotriquetrum*, and 41.1 ± 3.0 *vs.* 4.5 ± 0.1 in *F. antipyretica*. Something similar occurred along the course of the experiment, where the ranges of the bulk level of WUVAC and SUVAC in *B. pseudotriquetrum* were, respectively, 27.5-45.3 and 9.9-19.2, while in *F. antipyretica* they were 29.3-42.1 and 2.9-5.9 (Figs 2-3).

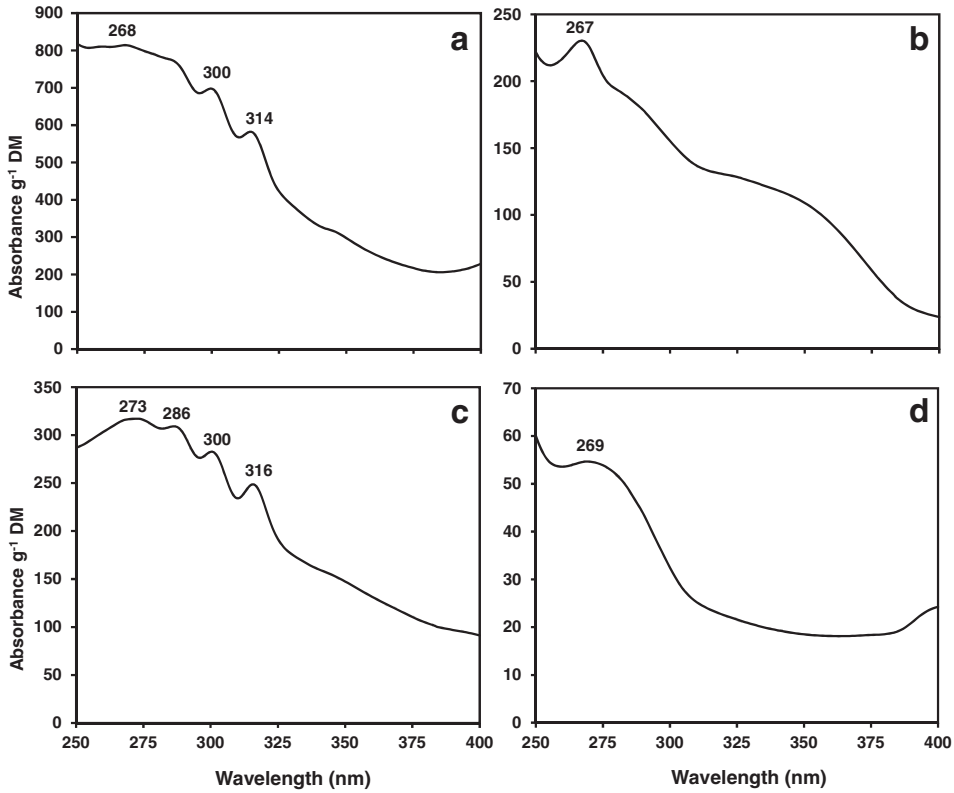

Fig. 1. Representative absorption spectra of the insoluble (a, c) and soluble (b, d) fractions of the extracts of *Bryum pseudotriquetrum* (a, b) and *Fontinalis antipyretica* (c, d).

All the variables measured several times along the experiment were significantly affected by the culture period, except the concentration of *p*-coumaric acid in *F. antipyretica*, and most of these variables (except the bulk level of WUVAC in both species and *p*-coumaric acid in *F. antipyretica*) were also affected by the radiation regime (Table 2).

In *B. pseudotriquetrum* (Fig. 2), SUVAC increased around 25% in PAB samples and then maintained relatively stable levels, showing significantly higher values in PAB than in P samples. Kaempferol-3-*O*-glucoside slightly increased in P samples in the first days of the experiment and then progressively decreased until the final days, whereas in PAB samples it increased in the first days and then remained stable. Kaempferol-3,7-*O*-diglucoside progressively decreased in P samples, whereas in PAB samples it slightly decreased in the first days and then remained fairly stable. Both flavonols showed significantly higher values in PAB than in P samples. F_v/F_m increased in all the radiation regimes in the first days of culture (more steeply in P and PA than in PAB samples), and then decreased until the end of the experiment (more smoothly in P than in PAB samples). F_v/F_m values were significantly higher in P than in PAB samples.

Table 2. Overall effects of the culture time and radiation regime on the variables measured in *Bryum pseudotriquetrum* and *Fontinalis antipyretica* along the course of the experiment, tested by a 2-way ANOVA (with repeated measures for the culture time), and interactions between both main factors. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, NS non-significant. SUVAC and WUVAC: respectively, soluble and cell wall-bound UV-absorbing compounds.

	Culture time	*Radiation regime*	*Interaction*
Bryum pseudotriquetrum			
Bulk level of SUVAC	***	*	**
Bulk level of WUVAC	***	NS	NS
Kaempferol-3-*O*-glucoside	*	**	NS
Kaempferol-3,7-*O*-diglucoside	**	***	*
F_v/F_m	*	*	NS
Fontinalis antipyretica			
Bulk level of SUVAC	***	***	**
Bulk level of WUVAC	***	NS	NS
p-Coumaric acid	NS	NS	NS
F_v/F_m	**	**	NS

In *F. antipyretica* (Fig. 3), the bulk level of SUVAC showed unclear temporal trends in the three radiation regimes, but its values were significantly higher in PA and PAB than in P samples. Temporal trends were also ill-defined in the bulk level of WUVAC and *p*-coumaric acid, whose values were not significantly different between the three radiation regimes. With respect to F_v/F_m, it remained fairly stable in P and PA samples along the culture period (except for a final decrease in PA samples), whereas it progressively decreased in PAB samples. PAB samples showed significantly lower F_v/F_m values than P and PA samples.

In both species, PA samples frequently showed temporal trends and values of the variables that were intermediate between P and PAB samples (Figs 2-3).

Significant interactions between culture time and radiation regime were found only in a few variables: the bulk level of SUVAC in both species and the concentration of kaempferol-3,7-*O*-diglucoside in *B. pseudotriquetrum* (Table 2).

At the end of the culture period, SI was affected ($P < 0.05$) by the radiation regime in *B. pseudotriquetrum* (Fig. 4), showing significantly higher values in P than in PA samples (respectively, 3.44 ± 0.18 and 2.88 ± 0.11 mg cm^{-2}), while PAB samples showed intermediate values (3.11 ± 0.13 mg cm^{-2}). In *F. antipyretica*, SI was not significantly affected by the radiation regime (Fig. 4). In both species, the amount of thymine dimers at the end of the experiment was significantly affected by the radiation regime ($P < 0.001$), showing higher values in PAB than in P and PA samples (Fig. 4).

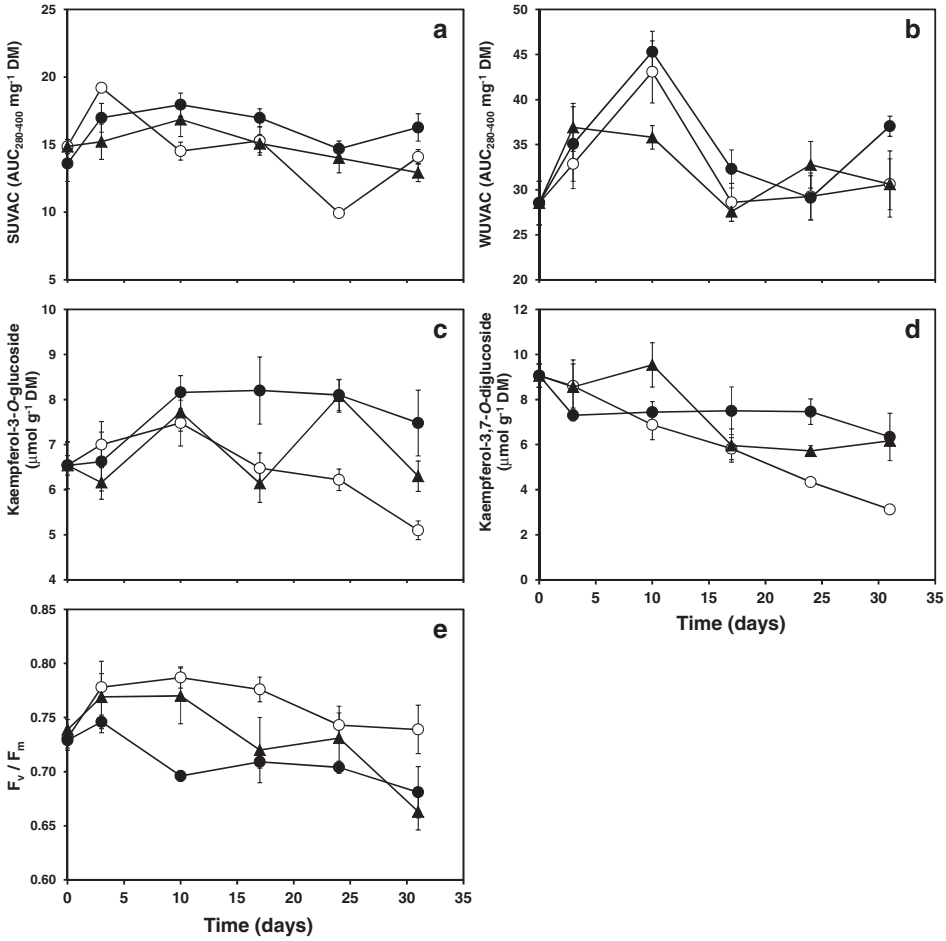

Fig. 2. Temporal changes, over the culture period, of the following variables of *Bryum pseudotriquetrum*: a) the bulk levels of soluble UV-absorbing compounds (SUVAC), as the area under the absorbance curve in the interval 280-400 nm ($AUC_{280\text{-}400}$) per unit of DM; b) the bulk levels of cell wall-bound UV-absorbing compounds (WUVAC), in $AUC_{280\text{-}400}$ per unit of DM (note the different scale); c) the concentration of kaempferol-3-*O*-glucoside present in the soluble fraction; d) the concentration of kaempferol-3,7-*O*-diglucoside present in the soluble fraction; and e) the maximum quantum yield of PSII (F_v/F_m). Values (mean ± SE) measured in the different radiation regimes (P, white circles; PA, black triangles; PAB, black circles) are shown.

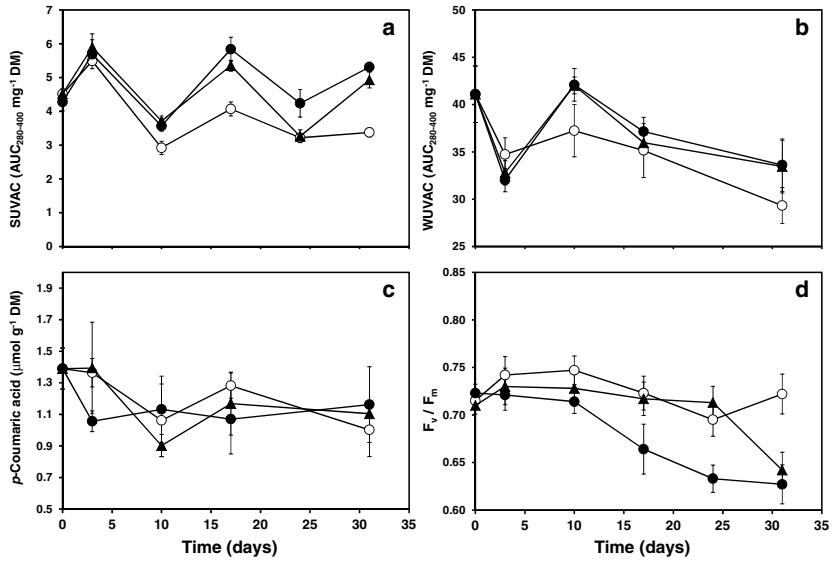

Fig. 3. Temporal changes, over the culture period, of the following variables of *Fontinalis antipyretica*: a) the bulk levels of soluble UV-absorbing compounds (SUVAC), as the area under the absorbance curve in the interval 280-400 nm ($AUC_{280-400}$) per unit of DM; b) the bulk levels of cell wall-bound UV-absorbing compounds (WUVAC), in $AUC_{280-400}$ per unit of DM (note the different scale); c) the concentration of *p*-coumaric acid present in the insoluble fraction; d) the maximum quantum yield of PSII (F_v/F_m). Values (mean ± SE) measured in the different radiation regimes (P, white circles; PA, black triangles; PAB, black circles) are shown.

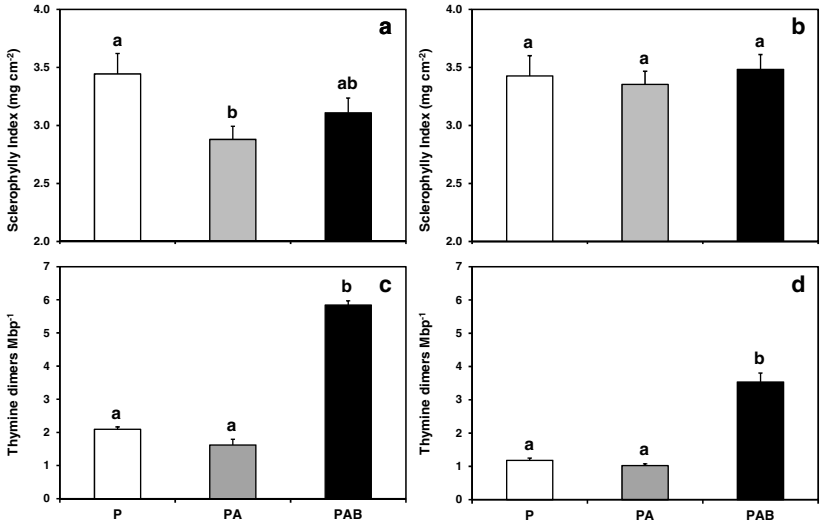

Fig. 4. Sclerophylly Index (SI) and number of thymine dimers (means ± SE) in *Bryum pseudotriquetrum* (a, c) and *Fontinalis antipyretica* (b, d) under the three radiation regimes (P, PA and PAB) at the end of the culture period. For each species and variable, different letters mean significant differences (at least $P<0.05$) between the radiation regimes (one-way ANOVA).

DISCUSSION

On the basis of the known absorption maxima of common types of phenolic compounds (Waterman & Mole, 1994), the absorption spectra of the insoluble fraction of *B. pseudotriquetrum* and *F. antipyretica* (Fig. 1) might mainly be due to the presence of hydroxycinnamic acids, one of which has already been identified in the latter species (*p*-coumaric acid). In the soluble fraction of *B. pseudotriquetrum*, the shoulder around 330 nm suggested the presence of flavonoids, among which two flavonols (kaempferol-3-*O*-glucoside and kaempferol-3,7-*O*-diglucoside) were identified. The absorbance in the soluble fraction of *F. antipyretica* was very low and no compound could be identified.

In the two species studied, the bulk level of SUVAC was comparable to the values found in these species in a 3-year study under field conditions (8.9-21.1 $AUC_{280\text{-}400}$ mg^{-1} DM in *B. pseudotriquetrum* and 2.1-5.7 in *F. antipyretica*: Núñez-Olivera *et al.*, 2010). In comparison with other mosses, the bulk level of SUVAC was relatively high in *B. pseudotriquetrum* and average in *F. antipyretica*, but in both cases lower than that found in most of the liverworts studied (Arróniz-Crespo *et al.*, 2004; Otero *et al.*, 2008). This suggests that the bulk level of SUVAC strongly depends on the species and the type of bryophyte considered. In this last sense, mosses and liverworts seem to show different protection mechanisms against UV radiation, given that liverworts accumulate a higher amount of SUVAC than mosses. Comparative data regarding the bulk level of WUVAC (as $AUC_{280\text{-}400}$ mg^{-1} DM) in bryophytes are much more scarce, but it is interesting to note that the liverwort *Jungermannia exsertifolia* subsp. *cordifolia* showed lower values (9-16 $AUC_{280\text{-}400}$ mg^{-1} DM: Fabón *et al.*, 2010) than the two mosses studied here (27-45). In addition, the bulk level of WUVAC was 2 to 3-fold higher than that of SUVAC in *B. pseudotriquetrum* along the experiment, while in *F. antipyretica* it was 6 to 13-fold higher. In Antarctica, the bulk levels of WUVAC and SUVAC showed rather similar values in *B. pseudotriquetrum*, whereas in *Ceratodon purpureus* and *Schistidium antarctici* the bulk level of WUVAC was up to 9-fold higher than that of SUVAC (Clarke & Robinson, 2008). In line with this, in sub-Arctic *Pleurozium schreberi* and *Polytrichum juniperinum*, the bulk level of WUVAC was 10-12 times higher than that of SUVAC (Lappalainen, 2010). These findings in mosses contrast with the fact that, in the only liverwort analyzed to date in this regard (*Jungermannia exsertifolia* subsp. *cordifolia*), the bulk level of SUVAC was about 2.5-fold higher than that of WUVAC (Fabón *et al.*, 2010). This would support the hypothesis that liverworts and mosses show different variants of UV protection, with higher bulk SUVAC in liverworts and higher bulk WUVAC in mosses. Given that WUVAC may provide a more spatially uniform and effective UV screen than SUVAC (Clarke & Robinson, 2008), it could be speculated that mosses as a group would be better adapted to colonize UV-rich sun-exposed environments than liverworts. Further investigation in more species is needed to confirm these hypotheses and their ecological relevance.

In our study, the response of the bulk level of both SUVAC and WUVAC to enhanced UV-B was evaluated in mosses for the first time, because previous studies (1) only took into account the bulk level of SUVAC (Martínez-Abaigar & Núñez-Olivera, 2011), (2) analyzed the responses to ambient (not enhanced) UV-B (Clarke & Robinson, 2008), or (3) were dedicated to liverworts (Fabón *et al.*, 2010).

The bulk level of WUVAC in both species, together with the concentration of the cell wall-located compound *p*-coumaric acid in *F. antipyretica*, showed

unclear temporal variations and did not respond to enhanced UV-B. This could be due to the fact that levels of WUVAC were constitutively high in these mosses, as occurred in three Antarctic mosses including *B. pseudotriquetrum* (Clarke & Robinson, 2008). These constitutive levels of WUVAC would already provide a notably effective protection against UV-B, and there would be no need to increase them when the mosses were exposed to artificially enhanced (this study) or naturally increasing (Clarke & Robinson, 2008) UV-B levels. However, in the liverwort *Jungermannia exsertifolia* subsp. *cordifolia*, both the bulk level of WUVAC and *p*-coumaric acid from the cell wall were induced by enhanced UV-B (Fabón *et al.*, 2010). This would additionally support the different behaviour of mosses and liverworts regarding their protective mechanisms against UV-B.

The bulk level of SUVAC in both species, together with the concentrations of the two flavonols measured in the soluble fraction of *B. pseudotriquetrum*, were higher in PAB than in P samples. Thus, the compounds in the soluble fraction may be more UV-B-responsive than those in the insoluble one, probably because the former would be more directly connected with the cell metabolism than the latter, that would be more or less immobilized in the cell wall. Nevertheless, only the bulk level of SUVAC and the concentration of kaempferol-3-*O*-glucoside in *B. pseudotriquetrum* increased along the experiment with respect to the initial values, and thus only these variables seemed to be clearly inducible by enhanced UV-B. The bulk level of SUVAC had not responded to enhanced UV-B in previous laboratory studies using both *B. pseudotriquetrum* (Martínez-Abaigar *et al.*, 2009) and *F. antipyretica* (Martínez-Abaigar *et al.*, 2003; Núñez-Olivera *et al.*, 2004). These negative results could have been caused, at least in *B. pseudotriquetrum*, because the samples had been collected near the summer solstice, when their natural protection against UV-B would be sufficient and would not have been increased in the laboratory culture. However, in the present study, the samples were collected in November, a period of the year in which the natural protection of the samples would be lower and could have been increased when exposed to the UV-B levels applied in the experiment. The collection date near the summer solstice could also have been the reason underlying the negative results obtained in one of the previous laboratory studies with *F. antipyretica* (Núñez-Olivera *et al.*, 2004), but not in the other one (Martínez-Abaigar *et al.*, 2003), in which samples were collected in December. The bulk level of SUVAC in *F. antipyretica* did not either respond to naturally fluctuating UV-B under field conditions (Núñez-Olivera *et al.*, 2010), whereas it responded in *B. pseudotriquetrum* (Dunn & Robinson, 2006; Núñez-Olivera *et al.*, 2010), although not always (Clarke & Robinson, 2008). Thus, the bulk level of SUVAC is more UV-B responsive in *B. pseudotriquetrum* than in *F. antipyretica*, both in the laboratory and the field. The lack of response reported in some studies using these species might be due to an inadequate collection date or other unkown factors whose elucidation needs further investigation. In other mosses tested, the bulk level of SUVAC was UV-B-responsive in *Ceratodon purpureus* (Clarke & Robinson, 2008) but not in *Schistidium antarctici*, *Brachythecium rivulare* or *Racomitrium aciculare* (Clarke & Robinson, 2008; Martínez-Abaigar *et al.*, 2009), while the same variable in liverworts responded to UV-B in most cases (Newsham *et al.*, 2005; Núñez-Olivera *et al.*, 2009; Fabón *et al.*, 2010; Martínez-Abaigar & Núñez-Olivera, 2011). Therefore, species-specific characteristics and the fact of being a moss or a liverwort notably influence the responsiveness of the bulk level of SUVAC to UV-B in bryophytes.

The two flavonols measured in the soluble fraction of *B. pseudotriquetrum* decreased in P samples from the beginning to the end of the experiment, whereas

in PAB samples increased (kaempferol-3-*O*-glucoside) or remained stable (kaempferol-3,7-*O*-diglucoside). The decrease in P samples could be due to the experimental elimination of any UV radiation incident on those plants, which resulted in a relaxation of the UV-induced stress they would have been suffering in the field and a consequent diminution of the concentration of UV-protective compounds. In contrast, the intensification of UV-stress in PAB samples led to the maintenance or increase of the levels of those compounds. It should be noted that kaempferols may act not only as UV absorbers (Dixon & Paiva, 1995), but also as potent antioxidants (Sroka, 2005).

In both species, F_v/F_m was significantly lower in PAB than in P samples. In *F. antipyretica*, this was expected in the light of previous results (Martínez-Abaigar *et al.*, 2003; Núñez-Olivera *et al.*, 2004). However, in a prior study with *B. pseudotriquetrum*, F_v/F_m did not change when the moss was exposed to enhanced UV-B (Martínez-Abaigar *et al.*, 2009). As discussed above, this could happen because the samples for this previous study had been collected near the summer solstice and would be better protected than the samples used in the present study, that were collected in November. Under a general perspective, it is not surprising that F_v/F_m decreases under enhanced UV-B, because this decrease indicates a stress situation and, in particular, the phenomenon of photoinhibition (Maxwell & Johnson, 2000). Photoinhibition is caused through damage of a well-known target of UV-B radiation: the protein D1 in photosystem II (Jansen *et al.*, 1998). In our study, the decrease in F_v/F_m in PAB samples was modest (7% in *B. pseudotriquetrum* and 13% in *F. antipyretica*). This suggests a dynamic and reversible photoinhibition, and consequently a moderate photosynthetic damage.

DNA damage was much stronger in PAB than in P and PA samples. This is congruent with previous results obtained after exposing *B. pseudotriquetrum* and other mosses and liverworts to enhanced UV-B in both the laboratory (Lud *et al.*, 2002; Otero *et al.*, 2006; Turnbull *et al.*, 2009; Fabón *et al.*, 2010) and the field (Lud *et al.*, 2002, 2003). All these results are consistent with the fact that DNA is a specific target of UV-B (Jansen *et al.*, 1998). However, no DNA damage was found when *B. pseudotriquetrum*, *F. antipyretica* or the liverwort *Jungermannia exsertifolia* subsp. *cordifolia* were exposed to ambient UV-B levels under field conditions (Núñez-Olivera *et al.*, 2009, 2010). This suggests that efficient DNA repairing mechanisms occur in nature, and that DNA damage takes place only when bryophytes are exposed to higher than ambient UV-B. To date, only Turnbull & Robinson (2009) have demonstrated DNA damage in bryophytes (the mosses *B. pseudotriquetrum*, *Ceratodon purpureus* and *Schistidium antarctici*) exposed to naturally increased ambient UV-B levels in the field, but it should be taken into account that this study was conducted in the Antarctic, where the extremely harsh environmental conditions might prevent DNA repair.

SI was not affected by enhanced UV-B in any of the species studied. Given that SI may be considered as an indirect indicator of growth, because newly grown shoots are softer (less sclerophyllous) than old shoots (Martínez-Abaigar *et al.*, 2003), no difference in growth between UV-B exposed and non-exposed samples could be established at the end of the culture period. This contrasts with the increase in SI found in *B. pseudotriquetrum* and *F. antipyretica* exposed to enhanced UV-B in previous laboratory studies, which would mean a lower growth in UV-B exposed samples (Martínez-Abaigar *et al.*, 2003, 2009). No convincing explanation can be offered for this discrepancy, apart from the fact that the specific samples used in this study were tolerant, in terms of growth, to the experimental radiation conditions (UV-B dose, proportions of PAR, UV-A and UV-B, etc.) that were applied.

UV-A radiation did not show specially defined effects in any of the species studied, and the values of the variables (and their temporal trends) in PA samples were frequently intermediate between P and PAB samples (Figs 2-3). This is in line with the diversity of results previously obtained in bryophytes, since UV-A radiation may have effects similar to PAR (Niemi *et al.*, 2002; Martínez-Abaigar *et al.*, 2003) or to UV-B (Fabón *et al.*, 2010). The ill-defined effects of UV-A in our study may be logical taking into account the relatively moderate UV-A doses used, in comparison with the annual range measured in the collection site (Núñez-Olivera *et al.*, 2010). In tracheophytes and algae, UV-A may have significant effects on the induction of phenolics and photoinhibition (Kotilainen *et al.*, 2008; Figueroa *et al.*, 2009; Hakala-Yatkin *et al.*, 2010), and it has been included in the modern action spectra in the context of UV research (Flint & Caldwell, 2003). In bryophytes, the precise effects of UV-A are still poorly known and require further study.

In conclusion, the two mosses studied had noticeably higher bulk levels of WUVAC than SUVAC, which confirms previous results in other mosses (Clarke & Robinson, 2008; Lappalainen, 2010). In addition, enhanced UV-B increased the bulk level of SUVAC in the two species and the concentration of two kaempferols in the soluble fraction of *B. pseudotriquetrum*, but had no effect on the bulk level of WUVAC in the two species and the concentration of *p*-coumaric acid in the insoluble fraction of *F. antipyretica*. Thus, the insoluble fraction would be less UV-B-responsive than the soluble one, probably because the constitutively high bulk level of WUVAC would already provide a notably effective protection against UV-B. Also, WUVAC would be less responsive due to their relative immobilization in the cell wall with respect to SUVAC. Taking into account that soluble and insoluble fractions may provide different UV screening mechanisms (Clarke & Robinson, 2008), it is convenient to analyze separately both the bulk levels and the specific compounds of the two fractions to better evaluate the protection of bryophytes against UV-B radiation. In summary, the two mosses studied were constitutively protected against enhanced UV-B by a high bulk level of WUVAC, and *B. pseudotriquetrum* was additionally protected by a UV-B-inducible mechanism, the increase of both the bulk level of SUVAC and the concentration of soluble kaempferol-3-*O*-glucoside. Nevertheless, these protection mechanisms could not totally prevent UV-B damage in the mosses, that was evident in a modest decrease of F_v/F_m and an increase in the amount of thymine dimers.

Our data suggest that the protection strategies of mosses and liverworts may be different, since Fabón *et al.* (2010) reported that liverworts show a higher accumulation of SUVAC than WUVAC, and equally UV-B-responsive SUVAC and WUVAC. It may be speculated that these differerences have ecological, evolutionary and phylogenetic implications: (1) if WUVAC were more efficient UV screens than SUVAC (Clarke & Robinson, 2008), mosses as a group would be more competitive than liverworts in UV-rich environments; (2) these two different plant evolutionary lineages would have acquired different strategies to cope with higher UV-B levels since the water-to-land transition; and (3) this difference could be an additional evidence of the phylogenetic distance between mosses and liverworts, that nowadays is considered to be deeper than previously thought (Qiu *et al.*, 2007).

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REFERENCES

ANTÓN M., CANCILLO M.L., SERRANO A., VAQUERO J.M. & GARCÍA J.A., 2007 — Ozone mini-hole over southwestern Spain during January 2004: Influence over ultraviolet radiation. *Geophysical research letters* 34: 1-5.

ARRÓNIZ-CRESPO M., NÚÑEZ-OLIVERA E., MARTÍNEZ-ABAIGAR J. & TOMÁS R., 2004 — A survey of the distribution of UV-absorbing compounds in aquatic bryophytes from a mountain stream. *The bryologist* 107: 202-208.

ARRÓNIZ-CRESPO M., NÚÑEZ-OLIVERA E., MARTÍNEZ-ABAIGAR J., BECKER H., SCHER J., ZAPP J., TOMÁS R. & BEAUCOURT N., 2006 — Physiological changes and UV protection in the aquatic liverwort *Jungermannia exsertifolia* subsp. *cordifolia* along an altitudinal gradient of UV-B radiation. *Functional plant biology* 33: 1025-1036.

BJÖRN L.O. & TERAMURA A.H., 1993 — Simulation of daylight ultraviolet radiation and effects of ozone depletion. *In:* Young A.R. (ed.), *Environmental UV Photobiology*. New York, Plenum Press, pp. 41-71.

BOELEN P., DE BOER M.K., DE BAKKER N.V.J. & ROZEMA J., 2006 — Outdoor studies on the effects of solar UV-B on bryophytes: overview and methodology. *Plant ecology* 182: 137-152.

CALDWELL M.M., 1971 — Solar UV irradiation and the growth and development of higher plants. *In:* Giese A.C. (ed.), *Photophysiology: current topics in photobiology and photochemistry, Vol. 6*. New York, Academic Press, pp. 131-177.

CASAS C., BRUGUÉS M., CROS R.M. & SÉRGIO C., 2006 — *Handbook of mosses of the Iberian Peninsula and Balearic Islands*. Barcelona, Institut d'Estudis Catalans.

CLARKE L.J. & ROBINSON S.A., 2008 — Cell wall-bound ultraviolet-screening compounds explain the high ultraviolet tolerance of the Antarctic moss, *Ceratodon purpureus*. *New phytologist* 179: 776-783.

DIXON R.A. & PAIVA N.L., 1995 — Stress-induced phenylpropanoid metabolism. *Plant cell* 7: 1085-1097.

DUNN J.L. & ROBINSON S.A., 2006 — Ultraviolet B screening potential is higher in two cosmopolitan moss species than in a co-occurring Antarctic endemic moss: implications of continuing ozone depletion. *Global change biology* 12: 2282-2296.

FABÓN G., MARTÍNEZ-ABAIGAR J., TOMÁS R. & NÚÑEZ-OLIVERA E., 2010 — Effects of enhanced UV-B radiation on hydroxycinnamic acid derivatives extracted from different cell compartments in the aquatic liverwort *Jungermannia exsertifolia* subsp. *cordifolia*. *Physiologia plantarum* 140: 269-279.

FIGUEROA F.L., KORBEE N., CARRILLO P., MEDINA-SANCHEZ J.M., MATA M., BONOMI J. & SANCHEZ-CASTILLO P.M., 2009 — The effects of UV radiation on photosynthesis estimated as chlorophyll fluorescence in *Zygnemopsis decussata* (Chlorophyta) growing in a high mountain lake (Sierra Nevada, Southern Spain). *Journal of limnology* 68: 206-216.

FLINT S.D. & CALDWELL M.M., 2003 — A biological spectral weighting function for ozone depletion research with higher plants. *Physiologia plantarum* 117: 137-144.

GRÖNIGER A., HALLIER C. & HÄDER D.P., 1999 — Influence of UV radiation and visible light on *Porphyra umbilicalis*: Photoinhibition and MAA concentration. *Journal of applied phycology* 11: 437-445.

HAKALA-YATKIN M., MANTYSAARI M., MATTILA H. & TYYSTJARVI E., 2010 — Contributions of Visible and Ultraviolet Parts of Sunlight to Photoinhibition. *Plant and cell physiology* 51: 1745-1753.

HEGGLIN M.I. & SHEPHERD T.G., 2009 — Large climate-induced changes in ultraviolet index and stratosphere-to-troposphere ozone flux. *Nature geoscience* 2: 687-691.

JANSEN M.A.K., GABA V. & GREENBERG B.M., 1998 — Higher plants and UV-B radiation: balancing damage, repair and acclimation. *Trends in plant science* 3: 131-135.

KOTILAINEN T., TEGELBERG R., JULKUNEN-TIITTO R., LINDFORS A. & APHALO P.J., 2008 — Metabolite specific effects of solar UV-A and UV-B on alder and birch leaf phenolics. *Global change biology* 14: 1294-1304.

LAPPALAINEN N., 2010 — *The response of ectohydric and endohydric mosses under ambient and enhanced ultraviolet radiation*. Doctoral Thesis. Oulu, University of Oulu.

LUD D., MOERDIJK T.C.W., VAN DE POLL W.H., BUMA A.G.J. & HUISKES A.H.L., 2002 — DNA damage and photosynthesis in Antarctic and Arctic *Sanionia uncinata* (Hedw.) Loeske under ambient and enhanced levels of UV-B radiation. *Plant, cell and environment* 25: 1579-1589.

LUD D., SCHLENSOG M., SCHROETER B. & HUISKES A.H.L., 2003 — The influence of UV-B radiation on light-dependent photosynthetic performance in *Sanionia uncinata* (Hedw.) Loeske in Antarctica. *Polar biology* 26: 225-232.

MARTÍNEZ-ABAIGAR J., NÚÑEZ-OLIVERA E., BEAUCOURT N., GARCÍA-ÁLVARO M.A., TOMÁS R. & ARRÓNIZ M., 2003 — Different physiological responses of two aquatic bryophytes to enhanced ultraviolet-B radiation. *Journal of bryology* 25: 17-30.

MARTÍNEZ-ABAIGAR J., OTERO S., TOMÁS R. & NÚÑEZ-OLIVERA E., 2009 — Effects of enhanced ultraviolet radiation on six aquatic bryophytes. *Cryptogamie, Bryologie* 30: 157-175.

MARTÍNEZ-ABAIGAR J. & NÚÑEZ-OLIVERA E., 2011 — Aquatic bryophytes under ultraviolet radiation. *In:* Tuba Z., Slack N. & Stark L. (eds), *Bryophyte Ecology under Changing Climate*. Cambridge, Cambridge University Press, pp. 17-30.

MAXWELL K. & JOHNSON G.N., 2000 — Chlorophyll fluorescence - a practical guide. *Journal of experimental botany* 51: 659-668.

MCKENZIE R.L., BJÖRN L.O., BAIS A. & ILYASD M., 2003 — Changes in biologically active ultraviolet radiation reaching the Earth's surface. *Photochemical and photobiological sciences* 2: 5-15.

MCKENZIE R.L., AUCAMP P.J., BAIS A.F., BJÖRN L.O. & HYAS M., 2007 — Changes in biologically-active ultraviolet radiation reaching the Earth's surface. *Photochemical and photobiological sciences* 6: 218-231.

NEWSHAM K.K., GEISSLER P., NICOLSON M., PEAT H.J. & LEWIS-SMITH R.I., 2005 — Sequential reduction of UV-B radiation in the field alters the pigmentation of an Antarctic leafy liverwort. *Environmental and experimental botany* 54: 22-32.

NEWSHAM K.K. & ROBINSON S.A., 2009 — Responses of plants in polar regions to UVB exposure: a meta-analysis. *Global change biology* 15: 2574-2589.

NIEMI R., MARTIKAINEN P.J., SILVOLA J., SONNINEN E., WULFF A. & HOLOPAINEN T., 2002 — Responses of two *Sphagnum* moss species and *Eriophorum vaginatum* to enhanced UV-B in a summer of low UV intensity. *New phytologist* 156: 509-515.

NÚÑEZ-OLIVERA E., MARTÍNEZ-ABAIGAR J., TOMÁS R., BEAUCOURT N. & ARRÓNIZ-CRESPO M., 2004 — Influence of temperature on the effects of artificially enhanced UV-B radiation on aquatic bryophytes under laboratory conditions. *Photosynthetica* 42: 201-212.

NÚÑEZ-OLIVERA E., OTERO S., TOMÁS R. & MARTÍNEZ-ABAIGAR J., 2009 — Seasonal variations in UV-absorbing compounds and physiological characteristics in the aquatic liverwort *Jungermannia exsertifolia* subsp. *cordifolia* over a three-year period. *Physiologia plantarum* 136: 73-85.

NÚÑEZ-OLIVERA E., OTERO S., TOMÁS R., FABÓN G. & MARTÍNEZ-ABAIGAR J., 2010 — Cyclic environmental factors only partially explain the seasonal variability of photoprotection and physiology in two mosses from an unforested headwater stream. *The bryologist* 113: 277-291.

OTERO S., NÚÑEZ-OLIVERA E., MARTÍNEZ-ABAIGAR J., TOMÁS R., ARRÓNIZ-CRESPO M. & BEAUCOURT N., 2006 — Effects of cadmium and enhanced UV radiation on the physiology and the concentration of UV-absorbing compounds of the aquatic liverwort *Jungermannia exsertifolia* subsp. *cordifolia*. *Photochemical and photobiological sciences* 5: 760-769.

OTERO S., CEZÓN K., MARTÍNEZ-ABAIGAR J. & NÚÑEZ-OLIVERA E., 2008 — Ultraviolet-absorbing capacity of aquatic bryophytes from Tierra del Fuego (Argentina). *Journal of bryology* 30: 290-296.

QIU Y.L., LI L.B., WANG B., CHEN Z.D., DOMBROVSKA O., LEE J., KENT L., LI R.Q., JOBSON R.W., HENDRY T.A., TAYLOR D.W., TESTA C.M. & AMBROS M., 2007 — A nonflowering land plant phylogeny inferred from nucleotide sequences of seven chloroplast, mitochondrial, and nuclear genes. *International journal of plant sciences* 168: 691-708.

SCHMITZ-HOERNER R. & WEISSENBÖCK G., 2003 — Contribution of phenolic compounds to the UV-B screening capacity of developing barley primary leaves in relation to DNA damage and repair under elevated UV-B levels. *Phytochemistry* 64: 243-255.

SCHNITZLER J.P., JUNGBLUT T.P., HELLER W., KÖFFERLEIN M., HUTZLER P., HEINZMANN U., SCHMELZER E., ERNST D., LANGEBARTELS C. & SANDERMANN H.J., 1996 — Tissue localization of u.v.-B-screening pigments and of chalcone synthase mRNA in needles of Scots pine seedlings. *New phytologist* 132: 247-258.

SEARLES P.S., FLINT S.D. & CALDWELL M.M., 2001 — A meta-analysis of plant field studies simulating stratospheric ozone depletion. *Oecologia* 127: 1-10.

SETLOW R.B., 1974 — The wavelengths in sunlight effective in producing skin cancer: a theoretical analysis. *Proceedings of the national academy of sciences of the United States of America* 71: 3363-3366.

SNELL K.R.S., KOKUBUN T., GRIFFITHS H., CONVEY P., HODGSON D.A. & NEWSHAM K.K., 2009 — Quantifying the metabolic cost to an Antarctic liverwort of responding to an abrupt increase in UVB radiation exposure. *Global change biology* 15: 2563-2573.

SROKA Z., 2005 — Antioxidative and antiradical properties of plant phenolics. *Zeitschrift für Naturforschung Section C – Journal of biosciences* 60: 833-843.

TAIPALE T. & HUTTUNEN S., 2002 — Moss flavonoids and their ultrastructural localization under enhanced UV-B radiation. *Polar record* 38: 211-218.

TURNBULL J.D. & ROBINSON S.A., 2009 — Accumulation of DNA damage in Antarctic mosses: correlations with ultraviolet-B radiation, temperature and turf water content vary among species. *Global change biology* 15: 319-329.

TURNBULL J.D., LESLIE S.J. & ROBINSON S.A., 2009 — Desiccation protects two Antarctic mosses from ultraviolet-B induced DNA damage. *Functional plant biology* 36: 214-221.

WATERMAN P.G. & MOLE S., 1994 — *Analysis of phenolic plant metabolites*. Oxford, Blackwell Scientific Publications.

WEBBY R.F., MARKHAM K.R. & SMITH R.I.S., 1996 — Chemotypes of the Antarctic moss *Bryum algens* delineated by their flavonoid constituents. *Biochemical systematics and ecology* 24: 469-475.