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Desiccation protects Antarctic mosses from ultraviolet-B induced DNA damage

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Abstract

Antarctic mosses live in a frozen desert, and are characterised by the ability to survive desiccation. They can tolerate multiple desiccation-rehydration events over the summer growing season. As a result of recent ozone depletion, such mosses may also be exposed to ultraviolet-B radiation while desiccated. The ultraviolet-B susceptibility of Antarctic moss species was examined in a laboratory experiment that tested whether desiccated or hydrated mosses accumulated more DNA damage under enhanced ultraviolet-B radiation. Accumulation of cyclobutane pyrimidine dimers and pyrimidine (64) pyrimidone dimers was measured in moss samples collected from the field and then exposed to ultraviolet-B radiation in either a desiccated or hydrated state. Two cosmopolitan species, Ceratodon purpureus (Hedw.) Brid. and Bryum pseudotriquetrum (Hedw.) Gaertn., B.Mey. and Scherb, were protected from DNA damage when desiccated, with accumulation of cyclobutane pyrimidine dimers reduced by at least 60% relative to hydrated moss. The endemic Schistidium antarctici (Cardot) L.I. Savicz and Smirnova accumulated more DNA damage than the other species and desiccation was not protective in this species. The cosmopolitan species remarkable ability to tolerate high ultraviolet-B exposure, especially in the desiccated state, suggests they may be better able to tolerate continued elevated ultraviolet-B radiation than the endemic species.

Keywords

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Desiccation protects two Antarctic mosses from ultraviolet-B induced DNA damage.

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Abstract

Living in a frozen desert, Antarctic mosses are characterised by the ability to survive desiccation and can tolerate multiple desiccation-rehydration events over the summer growing season. As a result of recent ozone depletion, such mosses may also be exposed to ultraviolet-B radiation whilst desiccated. The ultraviolet-B susceptibility of Antarctic moss species was examined in a laboratory experiment that tested whether desiccated or hydrated mosses accumulated more DNA damage under enhanced ultraviolet-B radiation. Accumulation of cyclobutane pyrimidine dimers and pyrimidine (6-4) pyrimidone dimers was measured in moss samples collected from the field and then exposed to ultraviolet-B radiation in either a desiccated or hydrated state. Two cosmopolitan species, *Ceratodon purpureus* (Hedw.) Brid. and *Bryum pseudotriquetrum* (Hedw.) Gaertn., B. Mey. & Scherb, were protected from DNA damage when desiccated, with accumulation of cyclobutane pyrimidine dimers reduced by at least 60% relative to hydrated moss. The endemic *Schistidium antarctici* (Cardot) L.I. Savicz & Smirnova accumulated more DNA damage than the other species and desiccation was not protective in this species. The cosmopolitan species remarkable ability to tolerate high ultraviolet-B exposure, especially in the desiccated state, suggests they may be better able to tolerate continued elevated ultraviolet-B radiation than the endemic species.

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Introduction

Antarctic plants, of which mosses are a dominant component, have been exposed to large increases in springtime UV-B radiation (UV-BR) over the last three decades as a result of austral ozone depletion (McKenzie *et al.* 2007). Living in a frozen desert, Antarctic mosses are characterised by the ability to survive both desiccation and freezing and can tolerate multiple freeze-thaw and desiccation-rehydration events over the summer growing season (Lovelock *et al.* 1995). As a result of recent ozone depletion, these mosses are currently exposed to multiple stressors, in particular the combination of UV-BR and water stress. Since full recovery of the ozone layer is not expected until after 2060 and the largest ozone holes have occurred in the last decade (McKenzie *et al.* 2007) it is important to understand how these combined stressors impact on Antarctic mosses.

UV-B radiation is damaging to biological molecules including DNA, proteins, lipids and photosynthetic pigments. Plants can protect themselves from UV-B induced damage by screening UV-BR before it reaches these molecules (Cockell and Knowland 1999) or by repairing damage once it has occurred (Britt 2004). Pyrimidine dimers are the most common type of UV-B induced DNA damage, with cyclobutane pyrimidine dimers (CPDs) forming the bulk of these photoproducts (approximately 75%) whilst pyrimidine (6-4) pyrimidone dimers [(6-4) photoproducts] account for the remainder (Britt 2004). These photoproducts distort the structure of DNA, blocking transcription and replication and are potentially cytotoxic and mutagenic (Jiang *et al.* 1997; Taylor *et al.* 1997; Britt 2004). DNA photoproducts that form in mature plant cells are repaired by photoreactivation, a light-dependent process that requires UV-inducible enzymes (Britt 2004). Nucleotide excision repair is a light-independent process that is prevalent in proliferating cells (Kimura *et al.* 2004). Since both of these repair methods are enzymatic processes, their effectiveness could be limited when plants are exposed to low temperatures (MacFadyen *et al.* 2004) or desiccation (Buffoni-Hall *et al.* 2003).

UV-induced CPD accumulation has been measured in a few terrestrial polar organisms including seven mosses (Lud *et al.* 2002; Boelen *et al.* 2006; Turnbull and Robinson 2009), the alga *Prasiola crispa* ssp. *antarctica* (Kützing Knebel (Lud *et al.* 2001) and the Patagonian herb, *Gunnera magellanica* Lam., (Rousseaux *et al.* 1999).

Ambient UV-B radiation failed to produce significant levels of DNA damage in four of the Antarctic mosses (Lud *et al.* 2002; Boelen *et al.* 2006) but damage was detected in *Ceratodon purpureus, Bryum pseudotriquetrum, Schistidium antarctici*, and in both the alga and the herb (Rousseaux *et al.* 1999; Lud *et al.* 2001; Giordano *et al.* 2003; Turnbull and Robinson 2009). Supplementing the UV-B dose 10-fold resulted in measurable DNA damage in *Sanionia uncinata* (Hedw.) Loeske (Lud *et al.* 2002) but not in *Chorisodontium aciphyllum* (Hook. f. & Wilson) Broth, *Warnstorfia sarmentosa* (Wahlenb.) Hedenas or *Polytrichum strictum* Menzies ex Brid. and all such damage to mosses was repaired overnight (Boelen *et al.* 2006).

Studies of several plant species have demonstrated links between drought and UV-B tolerance, with UV-BR exposure alleviating drought symptoms in several higher plants (Petropoulou *et al.* 1995; Manetas *et al.* 1997; Allen *et al.* 1999; Nogues and Baker 2000). Bryophytes however, have a fundamentally different strategy for drought tolerance and many are desiccation tolerant. To our knowledge the relationship between CPD accumulation and desiccation has only been investigated in the lichen *Cladonia arbuscula* (Wallr.) Flot ssp. *mitis* (Sandst.) Ruoss, with more DNA damage accumulating in desiccated samples, presumably due to decreased photoreactivation (Buffoni-Hall *et al.* 2003).Whilst desiccation tolerant bryophytes often show a higher tolerance of both photosynthetically active radiation (PAR) and UV-BR, than desiccation sensitive species (Seel *et al.* 1992a; Tákacs *et al.* 1999; Csintalan *et al.* 2001) we do not know if the desiccated state confers protection from UV-BR induced DNA damage in these plants. Since the accumulation of DNA photoproducts represents the balance of damage and repair, and desiccation is likely to reduce the capacity for enzymatic repair, mosses could be particularly vulnerable to UV-BR in the desiccated state. However, if the process of desiccation confers greater stability on DNA molecules or the dehydration process results in an increased concentration of UV-B screening compounds, this could confer greater resilience to UV-BR in the desiccated state. If desiccation tolerance predicts UV-BR tolerance we would expect that *C. purpureus* and *B. pseudotriquetrum,* two cosmopolitan species found in East Antarctica, would be more tolerant of UV-BR than the co-occurring endemic, *S. antarctici,* due to their demonstrated higher tolerance of desiccation (Robinson *et al.* 2000; Wasley *et al.* 2006).

Several Antarctic moss species have been shown to accumulate UV-B absorbing compounds (Lovelock and Robinson 2002; Newsham *et al.* 2002; Newsham 2003; Dunn and Robinson 2006) and concentrations of these compounds correlate positively with exposure to UV-BR in some species (Newsham 2003; Newsham *et al.* 2005; Arróniz-Crespo *et al.* 2006; Dunn and Robinson 2006; Lappalainen *et al.* 2008). For the three co-occurring Antarctic mosses mentioned above, concentrations of UV absorbing compounds also appear to be positively associated with desiccation tolerance as *B. pseudotriquetrum* and *C. purpureus* accumulate two-fold higher concentrations of total UV-B absorbing compounds than *S. antarctici* (Lovelock and Robinson 2002; Dunn and Robinson 2006; Clarke and Robinson 2008). In addition, there was a negative association between UV-B absorbing compounds and turf water content in *B. pseudotriquetrum,* and a positive association between anthocyanins and wind speed in *C. purpureus,* suggesting higher concentrations of potentially protective compounds in desiccated mosses (Dunn and Robinson 2006). In contrast there was no evidence of changes in concentrations of UV-B absorbing compounds in *S. antarctici*, and damage under ambient UV-BR, in the form of abnormal morphology and loss of photosynthetic pigments, has also been reported for this species (Robinson *et al.* 2005).

We measured UV-BR induced DNA damage, as accumulation of DNA photoproducts, in three moss species from the Windmill Islands region, East Antarctica. Samples of field-collected moss were subjected to elevated UV-BR in a laboratory experiment designed to test the relative resilience of the three species to the effects of UV-B irradiation, as well as the UV-BR resilience of hydrated versus desiccated moss. Our hypotheses were that 1) *S. antarctici* would accumulate higher concentrations of photoproducts due to its lower capacity to screen UV-BR at the cellular level and 2) that desiccated mosses would accumulate more photoproducts than hydrated mosses, particularly if repair processes were more important than screening ability in these species.

Materials and Methods

We measured UV-B induced DNA damage as accumulation of two types of photoproduct; CPDs and (6-4) photoproducts using an enzyme linked immunosorbent assay (ELISA). Preliminary experiments were conducted with

Schistidium antarctici (Cardot) L.I. Savicz & Smirnova (formerly *Grimmia antarctici*) to establish an appropriate radiation dose. An experiment to test whether desiccated and hydrated mosses responded differently in their accumulation of UV-B induced DNA damage, as well as to test the relative resilience of the three species, was then conducted in Antarctica. Samples of field-collected moss were first subjected to either a desiccation or hydration treatment and then to artificial UV-BR in a light box.

Preliminary experiments

Dose responses of CPD accumulation to artificial UV-BR were examined in *S. antarctici*. Samples were collected in Antarctica and kept frozen at -20˚C for several months before measurement. Prior to the experiment moss samples were thawed in a fridge, rehydrated and maintained moist overnight to restore physiological activity (determined as constant, high maximum quantum yield, F_v/F_m). Two light boxes were used, to allow simultaneous irradiation at different doses, with moss samples placed at different heights to alter the UV-BR dose. Moss samples (50 mg green gametophyte tips, n=4) were floated on a thin layer of distilled water in metal bottle caps and irradiated in the light boxes for 4 h at one of five light treatments (0, 2, 4, 6, 8 W m^{-2} UV-BR supplied by 4 x 2ft conditioned UV-B lights; Phillips FL20SE; Davis Ultra Violet, Melbourne, Australia). Samples were placed under UV-B permeable screens (cellulose acetate and plexiglass GS2458; Plastral Pty Ltd, Australia) to remove UV-C radiation. Radiation was measured using a compact radiometer (RM21, Dr Gröbel; UV Electronik GmbH, Ettlingen, Germany) with IP65 UV-B (280-315 nm) and UV-A (315-400 nm) sensors. To maintain normal physiological temperatures (between 0 and 5˚C) the metal bottle caps containing the moss were placed in containers of packed ice and the light box was placed in a 4˚C cold room. Following irradiation samples were frozen in liquid N_2 and stored at -80˚C for subsequent DNA extraction and analysis of DNA photoproducts.

Study sites and sampling

The Windmill Islands (centred at 66˚22' S, 110˚30' E) is a region of ice-free islands and peninsulas on the eastern coastline of Antarctica. Three moss species are found in the region; *S. antarctici* is endemic to the Antarctic continent, whilst both *Bryum pseudotriquetrum* (Hedw.) Gaertn., B. Mey. & Scherb and *Ceratodon purpureus* (Hedw.) Brid. have cosmopolitan distributions. Species distribution within the

bryophyte community generally follows the moisture gradient, with *S. antarctici* associated with wetter areas, *C. purpureus* more common in drier areas and *B. pseudotriquetrum* co-occurring with both. This distribution relates to the desiccation tolerance of each of the three species (Robinson *et al.* 2000; Wasley *et al.* 2006). Samples were collected from within Antarctic Specially Protected Area (ASPA) 135 on Bailey Peninsula from the site designated ASPA 1 in Dunn & Robinson (2006). A total of 72 individual 1 cm^2 samples (24 per species) were collected at midday on 27th December 2002 (a sunny day with a maximum air temperature of 2.5˚C and overhead ozone 337 Dobson Units). Care was taken not to shade moss during sampling and samples were immediately transported on ice to the Station Science Building.

Desiccation and hydration pre-treatments

Immediately on return to the laboratory, gametophyte tips were cut from each moss sample, placed on pre-weighed filter paper (2.5 cm diameter, Whatman, Grade 1, http://www.whatman.com) and weighed to determine fresh weight. Half of the samples (n=12 for each species) were then allowed to desiccate whilst the remaining samples were maintained in a fully hydrated state. Desiccated samples (D) were allowed to dry in a relative humidity of 22% for 6 h and then maintained in the presence of silica gel until constant weight was achieved (6 h). Hydrated samples (H) were maintained during this period by adding filtered water from melted snow to each sample until moss and filter paper were saturated. Hydrated samples were kept in a sealed, clear plastic container to maintain high humidity. All samples were maintained under low light (~10 µmol m^{-2} s^{-1}) at 18˚C.

UV-irradiation treatment

Four samples of each species, from each water pre-treatment (desiccated or hydrated), were irradiated with 8.5 W m^{-2} UV-B and 2.8 W m^{-2} UV-A for 4 h in the light box. Based on the distance between the lamps and the plant material and the radiation spectrum of the lamps measured under similar conditions, we estimate that 8.5 W m^{-2} treatment is equivalent to a UV-B$_{BE}$ dose of 12 kJ m^{-2} (Caldwell 1971) over the 4h. This approximates a two-fold increase in the maximum daily UV-BR measured at various Antarctic stations (Seckmeyer *et al.* 1995). Throughout the light treatment desiccated moss samples were maintained in metal bottle caps on dry filter paper and were separated from hydrated samples, and surrounded by silica gel to

prevent rehydration. Hydrated moss samples were maintained in metal caps, floating on a thin layer of melted snow. To maintain physiological temperatures (between 0 and 5°C) during the radiation treatment the light box was placed in the cold porch of the Science Building. Following irradiation, samples were frozen in liquid N_2 and transported to Australia for DNA extraction and analysis of DNA photoproducts.

To ensure that all photoproduct accumulation measured resulted from UV-BR a series of controls were also included. Pre-irradiation controls were sampled direct from the field, and samples were also taken after the 12 h hydration and desiccation treatments. Additional samples were included in the light box, with UV-BR reduced by 98% (UVA controls) with UV-B-blocking plexiglass screens (GS233 Plastral Pty Ltd, Australia) or light eliminated totally by foil covers (dark controls), to test whether DNA damage occurred specifically as a result of UV-BR. Samples are designated by water treatment, either hydrated or desiccated (H and D respectively), followed by light treatment, either with (+) or without (-) UV-B radiation.

DNA extraction

DNA was extracted from all samples using a modification of the method of Mason & Schmidt (2002) as described in Turnbull and Robinson (2009). DNA concentration and purity for each sample were determined spectrophotometrically (UV-1601 UV visible spectrophotometer, Shimadzu, Melbourne, Australia).

Quantification of DNA photoproducts by ELISA

The concentration of CPDs and (6-4) photoproducts was quantified by ELISA in a method modified from Taylor *et al*. (1996) by substituting an Amplex red detection system (Turnbull and Robinson 2009). The ELISA method differed from that described in Turnbull and Robinson (2009) only in that two monoclonal, primary antibodies, specific for CPDs (TDM-2) and (6-4) photoproducts (64M-2; O. Nikaido, Kanazawa University, Kanazawa, Japan; Mori *et al*. 1991) were used in separate ELISAs. DNA (4 ng μl^{-1}) from each individual moss sample was plated into four wells each of a 96 well plate for CPD or (6-4) photoproduct determinations. Standards containing pre-irradiated and un-irradiated calf thymus DNA (0.6 ng DNA) were plated onto each ELISA plate and used as positive and zero internal controls, respectively. The relative fluorescence value for each moss sample was calculated by normalising to the pre-irradiated and unirradiated calf thymus controls

on the respective plates. The relative standard deviation was 7% of the mean for irradiated calf thymus values for both photoproducts (Turnbull and Robinson 2009).

Statistical analysis

The accumulation of CPDs and (6-4) photoproducts for each independent moss sample was calculated as the mean relative fluorescence of its replicate, plated DNA samples. The mean for each treatment was then calculated from these individual sample means. Differences between means were examined using ANOVA. Initially a two-way ANOVA was conducted to determine if either water pre-treatments (hydration and desiccation) or control irradiation treatments (dark or UV-A only) caused increased photoproduct accumulation relative to field samples. For each species a two-way ANOVA then tested if irradiation (UV-B + UV-A versus UV-A only) or water status (hydrated versus desiccated moss samples) or the interaction term affected accumulation of each photoproduct. Differences between species were also explored in a two-way ANOVA of photoproduct accumulation using only UV-B irradiated samples (hydrated versus desiccated moss samples). Photoproduct data were fourth root transformed to satisfy the assumptions of the ANOVA. Where significant effects ($P<0.05$) were observed *post hoc* Tukey-HSD tests were performed. Statistical analyses were conducted using JMP 7 (SAS Institute, Cary, NC, USA) computer package.

Results

Preliminary experiments were conducted to determine the UV-BR dose response of CPD accumulation in Antarctic collected *S. antarctici*. There was no increase in CPD accumulation damage in *S. antarctici* exposed to 2 W m^{-2} UV-BR (Fig. 1). Above 2 W m^{-2} there was a linear relationship between CPD accumulation and UV-BR dose in this species. In a similar experiment, using cultured, Antarctic *Ceratodon purpureus*, 4 h irradiation with 8 W m^{-2} UV-BR was required to achieve significant accumulation of CPDs and (6-4) photoproducts (Venturini 2003).
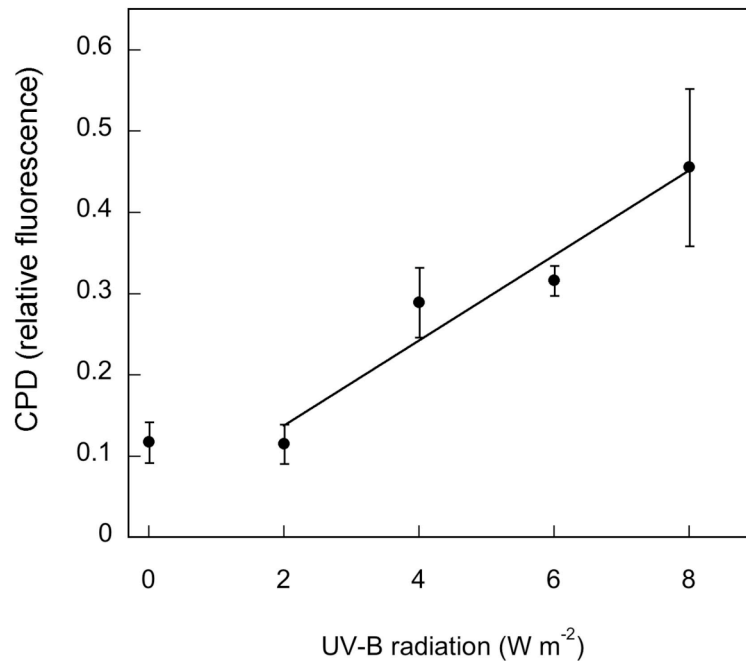
Figure 1: The effect of irradiation with five different doses of UV-B radiation on CPD accumulation (relative fluorescence) in the moss *Schistidium antarctici*. Data are means ± SEM (n = 4-8) Equation: CPD = 0.032 + 0.052 UV-BR dose, R^2adj= 0.94 *P*<0.03. The zero values were excluded from analysis, as there was no significant change in CPDs below UV-BR fluxes of 2 W m^{-2}.

Samples of the three moss species collected from the field showed similar levels of CPDs and (6-4) photoproducts regardless of species (Table 1; Turnbull and Robinson 2009). There was no significant change in concentration of either photoproduct as a result of the initial desiccation/hydration treatments. Although CPDs increased (72%) during the pre-treatments in *S. antarctici*, this increase was not significant and applied equally to hydrated and desiccated treated samples (Table 1). The dark and UV-A radiation treatments did not cause significant accumulation above pre-treatment levels in any species (c.f. Table 1 with Figures 2 & 3). Significant photoproduct accumulation was thus restricted to samples that received UV-B irradiation (Table 2). When CPD accumulation in UV-B irradiated, hydrated samples (H+ treatment) was compared with that in the corresponding UV-AR controls (H-treatment) there was a 3-fold increase in *C. purpureus* and more than a 6-fold increase in both *B. pseudotriquetrum* and *S. antarctici* (Fig. 2). Similarly, (6-4) photoproduct accumulation in H+ samples was approximately 2-fold higher in *C.*

purpureus and *B. pseudotriquetrum* and 4-fold higher for *S. antarctici* than the corresponding H- control samples (Fig. 3).

Table 1 CPD and (6-4) photoproduct concentration in three species of Antarctic moss, *Ceratodon purpureus, Bryum pseudotriquetrum* and *Schistidium antarctici;* on collection in the field, after water (desiccation/hydration) pre-treatment and following the dark treatment in the light box. CPDs and (6-4) photoproducts are reported in units relative to 4 h irradiated calf thymus DNA and values for different photoproducts are not directly comparable. No significant differences in photoproducts were observed either between species in the field, or within species as a result of desiccation/hydration pre-treatment or radiation controls.

Photoproduct and species	Field samples (Mean fluorescence ± SEM, n=4)	Water pre-treatment (Mean fluorescence ± SEM, n=8)	Dark treatment (Mean fluorescence ± SEM, n=8)
CPDs			
B. pseudotriquetrum	0.034±0.006	0.034±0.014	0.039±0.005
C. purpureus	0.041±0.005	0.030±0.006	0.040±0.003
S. antarctici	0.036±0.005	0.062±0.009	0.116±0.011
(6-4) photoproducts			
B. pseudotriquetrum	0.700±0.030	0.689±0.029	0.553±0.031
C. purpureus	0.556±0.170	0.752±0.087	0.553±0.049
S. antarctici	0.630±0.073	0.737±0.043	0.663±0.087

CPDs, cyclobutane pyrimidine dimers; (6-4) photoproducts, pyrimidine (6-4) pyrimidone dimers.
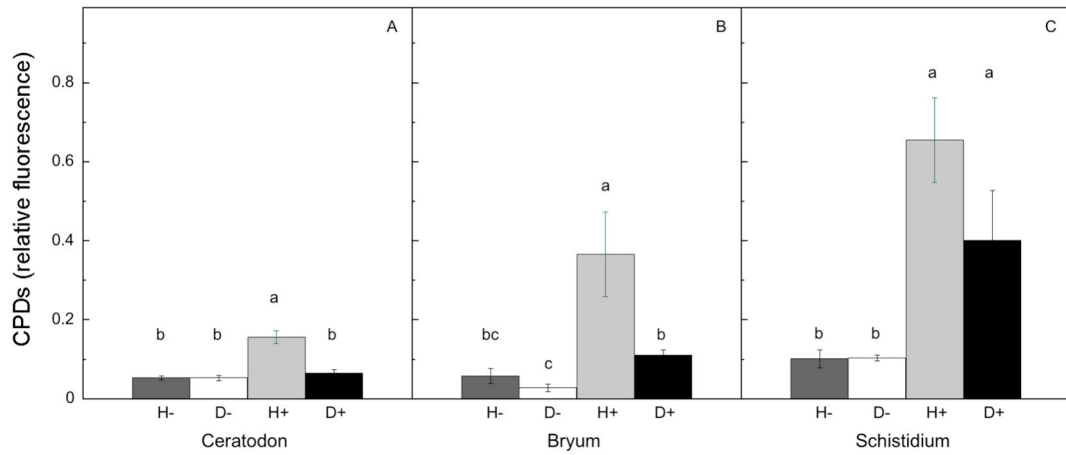
Figure 2: Accumulation of CPDs in three species of Antarctic moss, *Ceratodon purpureus* (A)*, Bryum pseudotriquetrum* (B) and *Schistidium antarctici* (C) after 4 h exposure to radiation with (+) or without (-) UV-B wavelengths. Moss samples were irradiated in either a hydrated (H) or desiccated (D) state. Data are means for individual moss samples ± SEM (n = 4). CPDs are reported in units relative to 4 h irradiated calf thymus DNA. Different letters indicate mean photoproducts are significantly different within species at *P*<0.05.
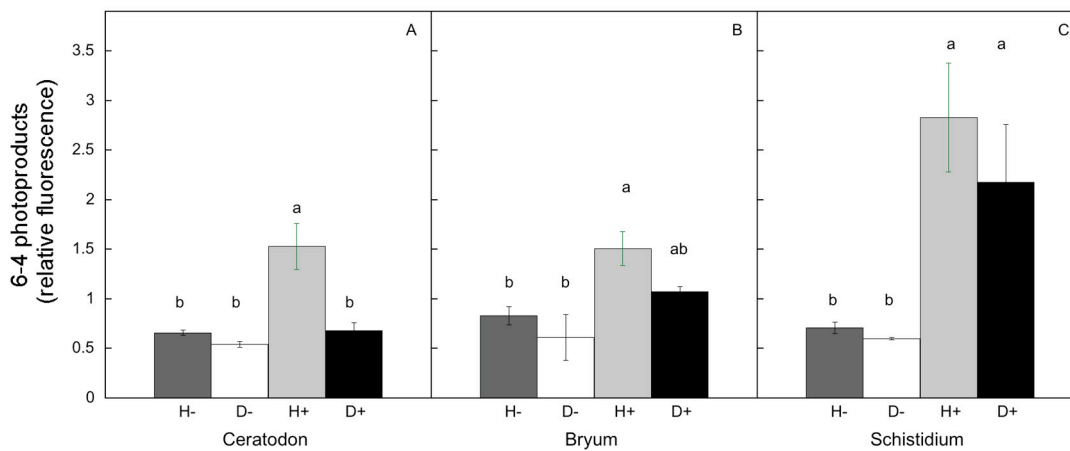

Figure 3: Accumulation of (6-4) photoproducts in three species of Antarctic moss, *Ceratodon purpureus* (A)*, Bryum pseudotriquetrum* (B) and *Schistidium antarctici* (C) after 4 h exposure to radiation with (+) or without (-) UV-B wavelengths. Moss samples were irradiated in either a hydrated (H) or desiccated (D) state. Data are means for individual moss samples ± SEM (n = 4) except for *S. antarctici* H+ where n=3. Photoproducts are reported in units relative to 4 h irradiated calf thymus DNA. Different letters indicate mean photoproducts are significantly different within species at *P*<0.05.

Table 2 Results of two-way ANOVA to test the effects of irradiation (UV-B + UVA or UVA alone) and water status (hydrated or desiccated) treatments on CPD and (6-4) photoproduct accumulation in Antarctic mosses. ANOVA were conducted separately on three species of Antarctic moss, *Ceratodon purpureus, Bryum pseudotriquetrum* and *Schistidium antarctici.* Mean values are shown in Figure 2 (CPDs) and Figure 3 [(6-4) photoproducts].

Species and factors	CPDs		(6-4) photoproducts	
	F stat	P-value	F stat	P-value
Ceratodon purpureus				
Light	33.0	<0.0001	22.9	0.0004
Water	18.0	0.001	21.6	0.0006
Light * water	17.4	0.001	8.97	0.011
Bryum pseudotriquetrum				
Light	31.1	0.0001	15.1	0.002
Water	10.3	0.007	4.98	0.044
Light * water	1.34	0.269	0.65	0.433
Schistidium antarctici				
Light	50.5	<0.0001	45.4	<0.0001
Water	1.87	0.196	1.58	0.234
Light * water	2.69	0.127	0.36	0.561

CPDs, cyclobutane pyrimidine dimers; (6-4) photoproducts, pyrimidine (6-4) pyrimidone dimers.

When desiccated and hydrated samples of the three species were irradiated with enhanced UV-BR, desiccation conferred significant protection from UV-B induced DNA damage in *C. purpureus* and *B. pseudotriquetrum*, but not *S. antarctici* (Figures 2 & 3).

For *C. purpureus,* only hydrated samples exposed to UV-B radiation (H+ treatment, Figures 2A & 3A) accumulated significant photoproducts. For both CPDs and (6-4) photoproducts there were significant light * water treatment interaction (Table 2). When desiccated *C. purpureus* was treated with UV-B radiation (D+ treatment) there

was no increase in either photoproduct above the level in the UV-A irradiated control samples (H- and D- treatments; Figures 2A & 3A).

For *B. pseudotriquetrum,* UV-B irradiation caused significant accumulation of CPDs and (6-4) photoproducts (light treatment, Table 2, Figures 2B & 3B). There was also a significant effect of water treatment (Table 2) with hydrated samples showing higher levels of both photoproducts than desiccated samples. Desiccation protected *B. pseudotriquetrum* from DNA damage, with desiccated samples (D+) accumulating only 26% of the CPDs in hydrated samples (H+; Fig. 2B). Protection from UV-BR by desiccation was less pronounced for (6-4) photoproducts, however, with desiccated samples accumulating almost 70% of the photoproducts measured in the hydrated samples (c.f. D+ with H+), a non-significant decline (Fig. 3B).

Whilst UV-B irradiation caused significant accumulation of CPDs and (6-4) photoproducts in *S. antarctici* (light treatment, Table 2, Figures 2C & 3C), desiccation did not provide significant protection against DNA damage in this species. Desiccated samples accumulated 54% of the CPDs and 74% of the (6-4) photoproducts found in the respective H+ treatments, but variance was high and the decline was not significant (Table 2; Figures 2C & 3C).

Photoproduct accumulation in response to enhanced UV-BR differed between the three species. *Schistidium antarctici* accumulated significantly more CPDs and (6-4) photoproducts than the other two species (CPDs; $F_{2,21}=28.0$, P<0.0001; (6-4) photoproducts; $F_{2,20}=28.0$, P=0.0004). *Ceratodon purpureus* also accumulated significantly fewer CPDs than *B. pseudotriquetrum* (Fig. 2) but (6-4) photoproduct accumulation was similar in the two cosmopolitan species (Fig. 3).

Discussion

The main findings of this study are that 1) desiccation confers protection from UV-BR induced DNA damage in two cosmopolitan species of Antarctic moss and 2) whilst all the species in this study have relatively high tolerance to enhanced UV-BR, the endemic *S. antarctici* is the least tolerant species.

Desiccation confers protection from UV-B induced DNA damage in two moss species

Although tolerance of solar radiation and either drought stress or desiccation are associated in many plants, this study is the first to demonstrate that bryophytes are

protected from UV-BR induced DNA damage in the desiccated state and this finding was unexpected, since a previous study had shown that lichen thalli accumulate more damage when desiccated (Buffoni-Hall *et al.* 2003). Desiccation tolerant mosses and lichens can often tolerate exposure to both high PAR and UV-BR (Seel *et al.* 1992a; Seel *et al.* 1992b; Tákacs *et al.* 1999; Heber *et al.* 2000) and this tolerance can manifest differently in the hydrated and desiccated state. For example, the photosynthetic apparatus of the desiccation tolerant moss species, *Tortula ruralis*, is more tolerant of photoinhibition when the moss is desiccated than when it is hydrated (Seel *et al.* 1992a; Seel *et al.* 1992b), but even when hydrated this species was able to tolerate elevated UV-BR for 8 days with no significant decline in F_v/F_m (Tákacs *et al.* 1999). In the Antarctic mosses studied here, both the tolerance to UV-B induced DNA damage, and the extent to which desiccation is protective, fits with the degree of desiccation tolerance and hence the hydrological habitat of each species (Robinson *et al.* 2000; Wasley *et al.* 2006), as well as with its relative accumulation of UV-B absorbing compounds (Lovelock and Robinson 2002; Dunn and Robinson 2006; Clarke and Robinson 2008).

The fact that these mosses are so well protected when dry is suggestive of passive protection, as enzymatic repair processes are unlikely to be active in desiccated organisms (Buffoni-Hall *et al.* 2003). Passive protection mechanisms would also be effective when these mosses are frozen and could thus be particularly beneficial to polar and alpine plants. Protection from UV-BR when desiccated could be due to morphological changes upon drying, which reduce light levels in the cell. Desiccation tolerant plants typically reduce exposed leaf area when dry by folding or curling of leaves (Davey and Ellis-Evans 1996; Proctor and Tuba 2002). This reduces transmission of PAR into the cell by between 40-60% in a range of moss species including *B. pseudotriquetrum* and *T. ruralis* (Seel *et al.* 1992a). UV-BR is likely to be similarly reduced which would contribute considerable protection at the molecular level. Based on relative turf densities, desiccation of these three mosses results in reductions in size ranging from 25% in *S. antarctici* to 40 to 50% in *B. pseudotriquetrum* and *C. purpureus* respectively (Wasley *et al.* 2006). When moss cells shrink upon desiccation, cytoplasm volume is reduced, concentrating cellular contents including UV-B screening compounds and possibly increasing the attenuation of UV-BR.

In most ecosystems, periods of high insolation (and associated UV-BR stress) cause desiccation in bryophytes as they equilibrate leaf turgor with that of their surroundings (Gehrke 1999). The reverse is true in the Antarctic environment however, where the major water source is snow-melt, which is maximal during periods of high insolation, and can coincide with elevated UV-BR as a result of ozone depletion. Thus if desiccation is a major strategy for protection from UV-BR these plants may still be at risk from high UV-BR during ozone depletion, especially when this coincides with spring melt.

UV-B irradiation induces more DNA damage in an Antarctic endemic than two cosmopolitan moss species

This research confirms an earlier field study that showed *S. antarctici* was sensitive to ambient UV-BR (Robinson *et al.* 2005). The difference in tolerance between the three species could be the result of a number of factors since these species vary in their desiccation tolerance, morphology and concentrations of UV-B absorbing compounds and may differ in their ability to repair DNA damage. Whilst the morphology of the three species is different; the leaves of *C. purpureus* and *B. pseudotriquetrum* adhere to the 'stem' whereas the leaves of *S. antarctici* are flat and more exposed (Robinson *et al.* 2000), UV-B reflectance is uniformly low in all three species and so reflectance is less likely to be an important factor in protection (Lovelock and Robinson 2002).

Higher concentrations of UV absorbing compounds could explain the decreased DNA damage in the two cosmopolitan species relative to *S. antarctici* since the latter species contains the lowest concentration of these screens. The location of UV-B absorbing compounds also differs, with *B. pseudotriquetrum* accumulating more of these compounds inside the cell compared to *C. purpureus* and *S. antarctici*, in which the bulk of these compounds are bound to the cell walls (Lovelock and Robinson 2002; Dunn and Robinson 2006; Clarke and Robinson 2008). If cell wall-bound UV-B absorbing compounds make more effective screens this could also explain the particularly high resilience of *C. purpureus* to UV-BR induced damage.

In a related field study, DNA damage from ambient UV-BR was relatively low for all three species but there were indications that repair of DNA damage might be enhanced by warmer, wetter conditions, especially in *C. purpureus* (Turnbull and

Robinson 2009). If the three species differ in their ability to repair DNA damage this might also explain the difference in UV sensitivity of the hydrated samples.

Limitations of the study

The UV-BR dose used in this experiment was artificially high almost double that currently experienced by plants anywhere at the Earth's surface (Kinzie *et al.* 1998). This dose was chosen because; preliminary experiments showed that below this dose no significant DNA damage was observed in cultured, Antarctic *C. purpureus* (Venturini 2003) and, due to restrictions on the number of samples that can be collected in the Antarctic, multiple UV-BR doses could not be justified. Our preliminary study using *S. antarctici* showed that DNA photoproducts did not accumulate at or below 2 W m^{-2} (UV$_{BE}$ dose approximately 3 kJ m^{-2}), suggesting a threshold for damage accumulation, but a linear relationship with UV-BR from 4 to 8 W m^{-2} was observed. This is similar to experiments using Antarctic *Sanionia uncinata,* where ambient UV-BR of 2.1 W m^{-2} failed to produce measurable CPD accumulation, and a 10-fold increase in UV$_{BE}$ dose was required to induce significant photoproduct accumulation (Lud *et al.* 2002).

Repair of DNA photoproducts occurs predominantly via photolyases in plants. These enzymes are induced by visible light and require blue or UV-A light for photoreactivation to occur (Kimura *et al.* 2004). The induction of UV-absorbing compounds can also require prior exposure to solar radiation. Since these plants were collected from the field during mid summer, they are likely to have induced photolyases and protective compounds. However, their capacity for repair of DNA damage during the experiment could have been limited by the relatively low levels of photoreactivating light, leading to a possible overestimate of photoproduct accumulation in hydrated samples.

Conclusion

Although the high UV-BR dose limits extrapolation of these results to the field situation, our study has highlighted the remarkable tolerance of the three species whilst in the desiccated state. The high resilience of desiccated mosses to DNA damage suggests that passive screening maybe more important than repair in these species. Differences in UV-BR tolerance between the three species match their desiccation tolerance, with *C. purpureus* most tolerant of both stressors, *B.*

pseudotriquetrum intermediate and *S. antarctici* the least. The finding that the two cosmopolitan species are likely to be more resilient in the face of continued ozone depletion raises biodiversity concerns for the endemic species *S. antarctici.*

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