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*Ceratodon purpureus*

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# Cell wall-bound ultraviolet-screening compounds explain the high ultraviolet tolerance of the Antarctic moss, *Ceratodon purpureus*

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## Summary

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• Studies of ultraviolet (UV) light-induced DNA damage in three Antarctic moss species have shown *Ceratodon purpureus* to be the most UV tolerant, despite containing lower concentrations of methanol-soluble UV-screening compounds than the co-occurring *Bryum pseudotriquetrum*.

• In this study, alkali extraction of cell wall-bound phenolics, combined with methanol extraction of soluble phenolics, was used to determine whether cell wall-bound UV screens explain the greater UV tolerance of *C. purpureus*.

• The combined pool of UV screens was similar in *B. pseudotriquetrum* and *C. purpureus*, but whilst *B. pseudotriquetrum* had almost equal concentrations of MeOH-soluble and alkali-extractable cell wall-bound UV-screening compounds, in *C. purpureus* the concentration of cell wall-bound screening compounds was six times higher than the concentration of MeOH-soluble UV screens. The Antarctic endemic *Schistidium antarctici* possessed half the combined pool of UV screens of the other species but, as in *C. purpureus*, these were predominantly cell wall bound. Confocal microscopy confirmed the localization of UV screens in each species.

• Greater investment in cell wall-bound UV screens offers *C. purpureus* a more spatially uniform, and potentially more effective, UV screen. *Schistidium antarctici* has the lowest UV-screening potential, indicating that this species may be disadvantaged under continuing springtime ozone depletion. Cell wall compounds have not previously been quantified in bryophytes but may be an important component of the UV defences of lower plants.

**Key words:** Antarctic, *Bryum pseudotriquetrum*, cell wall, *Ceratodon purpureus*, confocal microscopy, ozone depletion, *Schistidium antarctici* (*Grimmia antarctici*), UV-screening compounds.

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## Introduction

Since the late 1970s, stratospheric ozone depletion has led to increased ultraviolet-B radiation (UV-BR; 280–315 nm) at the Earth's surface. Ozone depletion is most severe over the Antarctic in the austral spring (September–November), with the 2006 ozone hole being the largest ever recorded (Cracknell & Varotsos, 2007). As ozone screens UV-BR most effectively, ozone depletion leads to both an increase in the

incident daily dose of ultraviolet-B (UV-B) as well as an increase in the ratio of UV-BR to photosynthetically active radiation (PAR) over Antarctica (Newsham *et al.*, 2002; Newsham, 2003). The combined effects of springtime ozone depletion and the approach of the annual radiation peak at the summer solstice results in continental Antarctic vegetation being exposed to an extended period of high UV-BR. Because Antarctic vegetation has historically experienced some of the lowest levels of UV-BR on Earth, it has been hypothesized

that it might show particular susceptibility to the elevated UV-BR resulting from ozone depletion. This has stimulated studies investigating the degree and mechanisms of UV tolerance in Antarctic plants.

Continental Antarctic vegetation is sparse and cryptogamic in nature, with mosses being the dominant plants. Three moss species (*Bryum pseudotriquetrum*, *Ceratodon purpureus* and *Schistidium antarctici* (formerly *Grimmia antarctici*)) co-occur in the Windmill Islands region near Casey station, East Antarctica. *Bryum pseudotriquetrum* and *C. purpureus* are both cosmopolitan moss species that occur in temperate regions as well as in the Antarctic, whereas *S. antarctici* is endemic to the Antarctic region. Previous studies have shown that *S. antarctici* is one of the few Antarctic mosses to show negative effects from current levels of UV radiation, with damage in the form of abnormal gametophyte morphology and loss of photosynthetic pigments apparent in UV-exposed plants (Robinson *et al.*, 2005). Recent studies of UV-induced DNA damage have also shown that *S. antarctici* accumulates much higher concentrations of DNA photoproducts, under elevated UV-BR, than the two co-occurring cosmopolitan species (Leslie, 2003). *Ceratodon purpureus* is found in the most exposed, driest sites (Robinson *et al.*, 2000; Wasley *et al.*, 2006) and shows the highest UV tolerance, whilst *B. pseudotriquetrum* accumulates intermediate levels of photoproducts (Leslie, 2003). However, this relative susceptibility to UV damage is at odds with measurements of methanol-soluble UV-screening compounds from the three species because *B. pseudotriquetrum* contains approx. 2–4 times higher concentrations of such compounds than either *C. purpureus* or *S. antarctici* (Lovelock & Robinson, 2002; Dunn & Robinson, 2006). Given the difference in UV-screening compound content, the mechanism by which *C. purpureus* maintains such a high UV-tolerance compared with *B. pseudotriquetrum* is unclear.

Although many studies have quantified methanol (MeOH)-soluble UV-screening compounds in Antarctic plants, to date cell wall-bound UV-screening compounds have been examined in only two plants experiencing enhanced UV-BR as a result of Antarctic ozone depletion (Ruhland & Day, 2000; Ruhland *et al.*, 2005). Ruhland & Day (2000) studied insoluble phenylpropanoids in *Deschampsia antarctica* and *Colobanthus quitensis*, the only vascular plants native to continental Antarctica, to test the hypothesis that cell wall-bound phenolics, such as ferulic acid, may constrain cell expansion, leading to the reduced leaf length observed under elevated UV-BR. A trend towards increased ferulic acid with increasing UV-B exposure was found in *D. antarctica*; however, the UV-screening capacity of cell wall-bound phenolics was not measured. Both species were found to have low epidermal transmittance of UV-BR, despite relatively low bulk-leaf concentrations of soluble UV-screening compounds, which the authors suggested may be a result of the high concentrations of cell wall-bound UV-screening compounds (Ruhland & Day, 2000). Lower concentrations of several soluble and cell wall-bound hydroxy-

cinnamic acids were also found under reduced UV-BR in *D. antarctica*, but no effect of UV-BR on flavonoid concentrations was observed (Ruhland *et al.*, 2005). Cell wall-bound UV-screening compounds were also measured in three species of the dwarf shrub *Vaccinium* at a site in north Sweden influenced by ozone depletion over the Arctic (Semerdjieva *et al.*, 2000). The three species were shown to possess contrasting responses to enhanced UV-BR, with concentrations of methanol-soluble UV-screening compounds increasing in *Vaccinium myrtillus* and *Vaccinium uliginosum*, whereas the concentration of cell wall-bound UV-screening compounds increased in *Vaccinium vitis-idaea* (Semerdjieva *et al.*, 2000). Cell wall-bound flavonoids were visualized microscopically in two moss species from central Finland, and methanol-soluble UV-screening compounds were shown to respond to seasonal changes in the radiation environment (Taipale & Huttunen, 2002). Cell wall-bound UV-screening compounds were not quantified in the latter study, but microscopic examination suggested no increase in cell wall-bound flavonoids in response to enhanced UV-BR (Taipale & Huttunen, 2002).

We used alkali extraction of cell wall-bound phenolics, in combination with methanol extraction of soluble phenolics, to determine whether cell wall-bound UV-screening compounds could explain the greater UV tolerance of *C. purpureus* compared with *B. pseudotriquetrum* and *S. antarctici*. To the best of our knowledge, this is the first study to quantify cell wall-bound UV-screening compounds in any moss species and the first to detect them in Antarctic mosses. As UV-BR is highest early in the summer season and decreases in late January (Dunn & Robinson, 2006), the concentration of UV-screening compounds was compared in samples collected early (November–December) and late (January–February) in the summer growing season to determine whether there is any association between the content of UV-screening compounds and the UV radiation environment. Confocal microscopy was also used to examine the localization of UV-screening compounds in each species. We hypothesized that *C. purpureus* would contain more cell wall-bound UV-screening compounds than the other two species.

## Materials and Methods

### Plant material

Samples of the mosses *B. pseudotriquetrum* (Hedw.) Gaertn., *C. purpureus* (Hedw.) Brid. and *S. antarctici* (Cardot) Savicz-Ljubitskaya & Smirnova (formerly *G. antarctici*) were collected by Johanna Turnbull over the summer of 2002–2003 from a site within the Antarctic Specially Protected Area (ASPA) 135 (66°16.92'S, 110°32.36'E) near Casey Station in the Windmill Islands region, East Antarctica. Samples were stored on ice for 1–3 h after collection, after which the photosynthetically active shoot tips (3–5 mm) were removed in a walk-in freezer (–20°C) and stored at –80°C before analysis in 2007. Early

season samples were collected between 9 November and 2 December 2002, and late season samples were collected between 16 January and 1 February 2003.

### Radiation data

Radiation measurements for sampling days in the two collection periods, including UV-B (280–315 nm), total UV (290–400 nm) and total solar radiation (TSR), were obtained from Australian Radiation Protection and Nuclear Safety Agency (ARPANSA) sensors at Casey Station, as described in Dunn & Robinson (2006). The total UV sensor is not calibrated to collect the shortest UV-B wavelengths (280–290 nm); however, the amount of radiation below 290 nm that reaches the Earth's surface is negligible, particularly in the Antarctic. The TSR sensor was not calibrated in 2002–2003 and therefore the values for this parameter are approximate; however, measured changes in TSR over the season are robust. Ratios of radiation parameters (total UV : TSR and UV-B : TSR) were also calculated for each sampling day. As samples were typically collected during favourable weather, radiation measures were also averaged over the 10 d before sampling to remove bias and to obtain a better representation of the radiation environment.

### Methanol extraction of soluble phenolics

Soluble phenolics were extracted from gametophyte tissue using a modified version of the method of Schnitzler *et al.* (1996). Samples were dried at 50°C to constant weight before extraction and then 20–50 mg of shoot tips from each species was frozen in liquid nitrogen and ground to a fine powder. Samples were extracted in 1 ml of MeOH for 1 h at room temperature in the dark, centrifuged (16 000 *g* for 15 min) and the supernatant was collected. The remaining cell debris was re-extracted three times with 0.5 ml of MeOH. The supernatants were pooled and made up to 3 ml with MeOH. The amount of MeOH used at each step was doubled for large samples to ensure complete extraction. Extracts were stored at 4°C over 1–2 nights before the spectrophotometric measurements were made. This length of storage time did not affect the results.

### Alkali extraction of cell wall-bound phenolics

Cell wall-bound UV-screening compounds were extracted using the method of Semerdjieva *et al.* (2000). Cell debris remaining from the methanol extraction was incubated for 20 min each in 1 M NaCl, twice in 0.5% (w/v) sodium dodecyl sulphate, then twice in chloroform/methanol (1:1, v/v). After each incubation, extracts were centrifuged at 16 000 *g* for 5 min. The pellet was washed in acetone and air-dried at room temperature before use. A 10-mg sample of crude cell wall material was incubated in 1 ml of 1 M NaOH

for 16 h in the dark (Strack *et al.*, 1988). Samples were then centrifuged at 16 000 *g* for 15 min, and 0.7 ml of supernatant was mixed with 0.7 ml of 1.5 M formic acid and centrifuged for 5 min at 16 000 *g*. The resulting supernatant was used for spectrophotometric measurements.

### Spectrophotometric measurements

Absorbance of methanol and cell wall-bound phenolic extracts in the region 250–400 nm was measured spectrophotometrically using a Shimadzu UV-1601 UV-visible spectrophotometer (Shimadzu, Sydney, Australia). Cell wall-bound phenolic extracts were blanked with distilled water, and methanol extracts were blanked with methanol. Concentrations of UV-B screening compounds were calculated as the area under the absorbance curve in the UV-B spectrum ( $AUC_{280-315}$ )  $\text{mg}^{-1}$  dry weight of tissue according to Newsham (2003). Spectra were normalized to common absorbance peaks in the three species (268 and 259 nm for methanol and alkali extracts, respectively) to compare UV-screening compound composition.

### Confocal microscopy

Moss leaves of each species were mounted in water, and auto-fluorescence was detected in an emission window of 500–530 nm using an excitation wavelength of 488 nm on a Leica DMIRBE inverted microscope coupled to a Leica TCS SP confocal system (Leica Microsystems, Sydney, Australia). Samples were then stained with 0.5% (w/v) Naturstoffreagenz A (2-aminoethyl diphenylborate; Sigma-Aldrich, Sydney, Australia, Schnitzler *et al.*, 1996) in 10 mM phosphate buffer (pH 6) containing 10% (w/v) sucrose and 2% (v/v) dimethyl sulfoxide, prepared immediately before use from a stock solution of 2.5% (w/v) Naturstoffreagenz A in ethanol (EtOH), and the fluorescence was detected as described at the start of this paragraph. Images were processed using TCS NT software (Leica Microsystems).

### Statistical analysis

The effect of collection time on the values of radiation parameters, and the effect of species and collection time on the concentration of UV-screening compounds, was tested using one-way and two-way ANOVA, respectively, performed with JMP version 5.1 (SAS Inc., Cary, NC, USA). Radiation and UV-screening compound data were transformed where necessary to satisfy the assumptions of ANOVA. Post-hoc comparisons were made using Tukey–Kramer HSD tests.

### Results

Most values for radiation parameters were similar for sampling days in the early season and late season collection periods, with the exception of the 10-d mean UV-B : 10-d mean solar

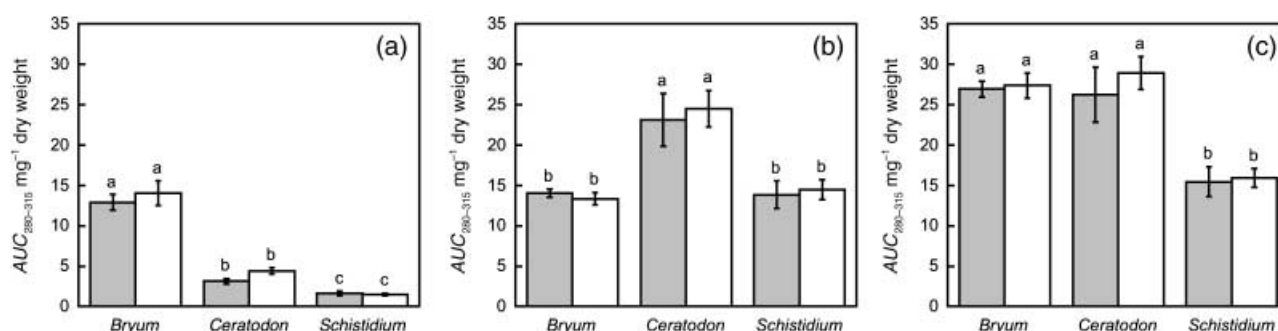
**Table 1** Radiation parameters for sampling days in the early season (9 November to 2 December 2002) and late season (16 January to 1 February 2003) collection periods at Casey station in the Windmill Islands region, East Antarctica

Radiation parameter	Early season	Late season	F value	P value
Daily mean UV	21.9	19.5	0.4	0.530
Daily mean UV-B	0.17	0.09	1.4	0.266
Daily mean solar	223.0	207.0	0.1	0.781
10-d mean UV	19.2	20.2	0.6	0.442
10-d mean UV-B	0.12	0.10	1.4	0.268
10-d mean solar	183.5	207.8	1.7	0.228
Daily mean UV : daily mean solar	0.107	0.103	0.1	0.751
Daily mean UV-B : daily mean solar	0.00064	0.00038	2.4	0.155
10-d mean UV : 10-d mean solar	0.106	0.098	3.9	0.076
10-d mean UV-B : 10-d mean solar	0.00063	0.00047	10.5	*0.009

All radiation parameters are expressed in units of  $W m^{-2}$ .

Error df = 10 in all analyses except 'Daily mean UV-B' and 'Daily mean UV-B : daily mean solar' where df = 9.

UV, ultraviolet; UV-B, ultraviolet B.



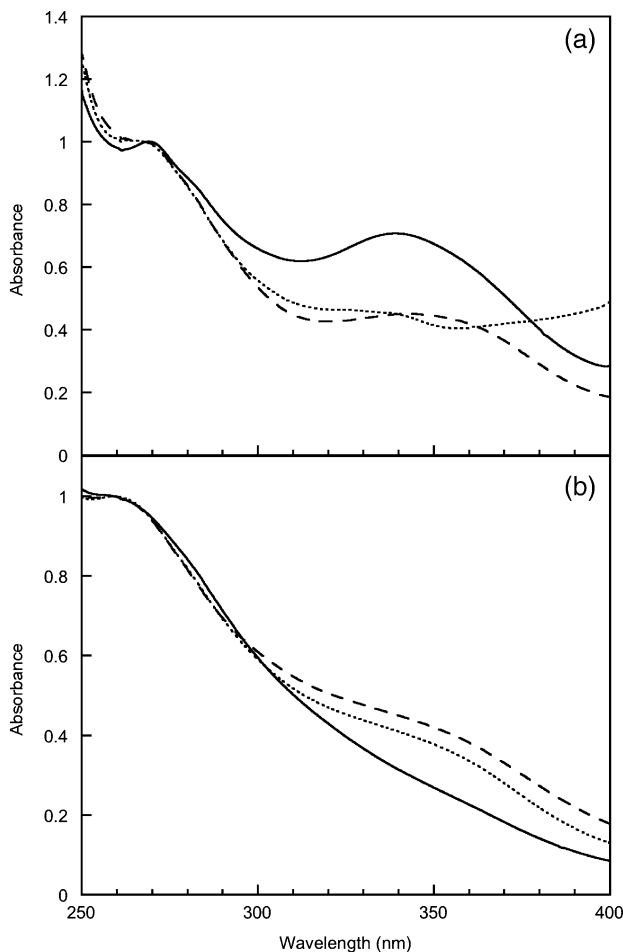
**Fig. 1** Concentrations of (a) MeOH-soluble ultraviolet (UV)-screening compounds, (b) alkali-extractable cell wall-bound UV-screening compounds and (c) combined MeOH-soluble and alkali-extractable cell wall-bound UV-screening compounds for *Bryum pseudotriquetrum*, *Ceratodon purpureus* and *Schistidium antarctici* collected early (closed bars; 9 November to 2 December 2002) and late (open bars; 16 January to 1 February 2003) in the summer growing season from the Windmill Islands region, East Antarctica. Concentrations are expressed as the area under the absorbance curve ( $AUC_{280-315}$ )  $mg^{-1}$  dry weight. Values are means  $\pm$  1 standard error ( $n = 5-8$ ). Different letters indicate means that are significantly different ( $P < 0.05$ ).

radiation ( $F_{1,10} = 10.5$ ,  $P < 0.01$ ) ratio, which was 36% higher in the early season collection period (Table 1). There was a similar, but not significant, trend for both the 10-d mean UV : 10-d mean solar radiation ratio and the daily mean UV-B : daily mean solar radiation ratio to be higher in the early season collection period.

The relative proportions of methanol-soluble and alkali-extractable cell wall-bound UV screening compounds differed for the three species. *Bryum pseudotriquetrum* possessed significantly higher levels of soluble UV-screening compounds than the other two species ( $13.3 \pm 0.8$   $AUC_{280-315}$   $mg^{-1}$ ,  $F_{5,37} = 71.9$ ,  $P < 0.0001$ ), 3.5-fold higher than *C. purpureus* and almost 9-fold higher than *S. antarctici* (Fig. 1a). *Schistidium antarctici* also possessed significantly lower concentrations of soluble UV-screening compounds than *C. purpureus*. Over the season, soluble UV-screening compounds increased (on a dry weight basis) by approx. 40% in *C. purpureus*. The apparent increase was not significant in the two-way ANOVA but was significant

when the concentration of soluble UV-screening compounds for *C. purpureus* was analysed by one-way ANOVA ( $F_{1,14} = 5.9$ ,  $P = 0.03$ ). However, no significant difference was found between early season and late season material for the other two species.

In *B. pseudotriquetrum*, the concentrations of alkali-extractable cell wall-bound and soluble UV screening compounds were similar; by contrast, the concentrations of cell wall-bound UV screening compounds were up to nine times higher than the concentrations of soluble UV-screening compounds in *C. purpureus* and *S. antarctici*. *Ceratodon purpureus* contained significantly higher concentrations (on a dry weight basis) of cell wall-bound UV screening compounds ( $24 \pm 2$   $AUC_{280-315}$   $mg^{-1}$ , Fig. 1b) than either *B. pseudotriquetrum* or *S. antarctici* ( $F_{5,36} = 9.7$ ,  $P < 0.0001$ ), which had similar concentrations of these compounds ( $13.8 \pm 0.4$  and  $14 \pm 1$   $AUC_{280-315}$   $mg^{-1}$ , respectively). There was no significant difference between the concentration of cell wall-bound UV-screening compounds in material collected early or late in the season for any species.



**Fig. 2** Absorption spectra of MeOH-soluble ultraviolet (UV)-screening compounds normalized to 268 nm (a) and alkali-extractable cell wall-bound UV-screening compounds normalized to 259 nm (b) from *Bryum pseudotriquetrum* (solid line), *Ceratodon purpureus* (dashed line) and *Schistidium antarctici* (dotted line).

Combining the concentrations of MeOH-soluble and alkali-extractable cell wall-bound UV-screening compounds for each sample showed that *B. pseudotriquetrum* and *C. purpureus* have almost twice the UV-screening potential of *S. antarctici* ( $F_{5,35} = 14.21$ ,  $P < 0.0001$ , Fig. 1c). *Bryum pseudotriquetrum* and *C. purpureus* had combined totals of  $27.1 \pm 0.8$  and  $28 \pm 2$   $AUC_{280-315} \text{ mg}^{-1}$ , respectively, significantly more than *S. antarctici* ( $16 \pm 1$   $AUC_{280-315} \text{ mg}^{-1}$ ). There was no change in the combined pool of UV-screening compounds over the season for any species.

Inspection of the normalized spectra of MeOH-soluble phenolic extracts in the range 250–400 nm revealed qualitative differences in UV-screening compounds between the three moss species. In addition to a peak at 268 nm, common to all species, *B. pseudotriquetrum* showed a well-defined maximum at 340 nm (Fig. 2a). *Ceratodon purpureus* had a similar, but

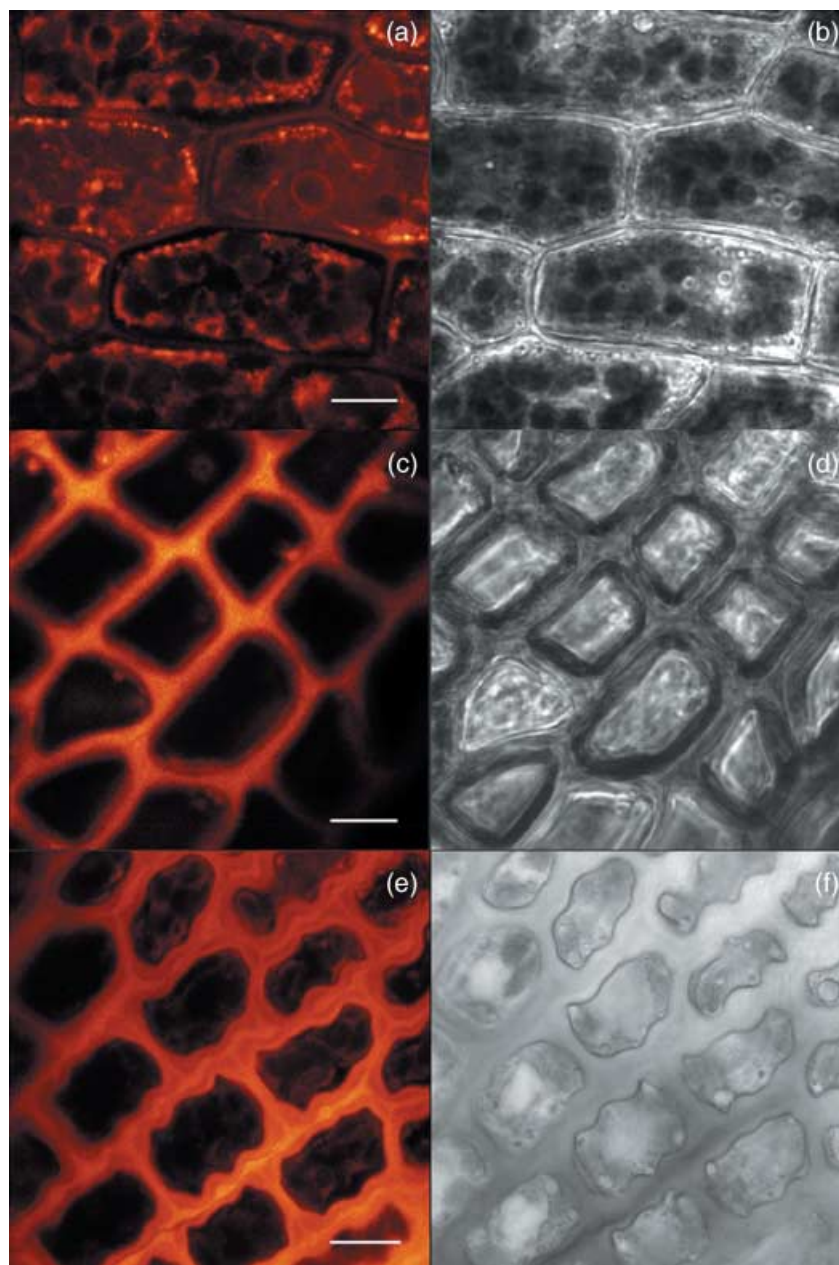
lower, peak shifted to 345 nm, whereas *S. antarctici* had a much less defined peak (c. 330 nm). Unlike the other two species, extracts from *S. antarctici* showed an increase in absorption with increasing wavelength above 355 nm. This is likely to reflect large amounts of chlorophyll, in particular chlorophyll *a*, which has an absorption maximum in MeOH near 420 nm with a shoulder extending into the UV-A region, in the extracts from this species. The spectra of the alkali-extractable cell wall-bound UV-screening compounds were similar between the three mosses, with absorption maxima at 259 nm in each species (Fig. 2b). The only substantial difference between the cell wall compound spectra was the presence of a shoulder at 350 nm in *C. purpureus* and *S. antarctici* that was absent in *B. pseudotriquetrum*.

Staining of leaves with Naturstoffreagenz A allowed the location of phenolic compounds to be visualized using confocal microscopy. These data confirmed the alternative UV-screening strategies of the three species. The leaves of *B. pseudotriquetrum* showed substantial amounts of both intracellular and cell wall-associated fluorescence (Fig. 3a), whereas the leaves of *C. purpureus* showed intense cell wall fluorescence with minimal staining of intracellular compounds (Fig. 3c). Cell walls of this species were still fluorescent after repeated methanol extraction. Like *C. purpureus*, fluorescence was associated more with the cell walls of *S. antarctici* than the cell contents; however, some intracellular fluorescence was also apparent (Fig. 3e). No species showed significant autofluorescence in the detection window (500–530 nm).

## Discussion

We found that the two cosmopolitan moss species present in the Windmill Islands region have alternative strategies for UV screening. *Bryum pseudotriquetrum* has equal proportions of MeOH-soluble and alkali-extractable cell wall-bound UV-screening compounds, whereas *C. purpureus* relies more strongly on MeOH-insoluble, alkali-extractable cell wall-bound UV-screening compounds (Fig. 1, note that salt-wash, sodium dodecyl sulphate-wash, chloroform/methanol-wash and acetone-wash fractions were disregarded). Although the Antarctic endemic *S. antarctici* also has a high ratio of cell wall-bound, alkali-extractable to MeOH-soluble UV screens, it only possesses half the combined pool of soluble and cell wall-bound UV-screens on a dry weight basis compared with the cosmopolitan species (Fig. 1). It is possible that UV screens other than the MeOH-soluble and alkali-extractable cell wall-bound compounds isolated could contribute to the total pool of UV-screening compounds in these species and similarly that some MeOH-soluble UV-screening compounds may be associated with the cell wall. However, the confocal microscopy images support the alternative UV-screening strategies, as suggested above (Fig. 3).

Previous studies of UV-induced DNA damage suggest that *S. antarctici* is the least UV-tolerant of the three moss species



**Fig. 3** Confocal and light transmission images of leaves from (a, b) *Bryum pseudotriquetrum*, (c, d) *Ceratodon purpureus* and (e, f) *Schistidium antarctici* stained with Naturstoffreagenz A to show the localization of UV-screening compounds in each species. Orange fluorescence indicates the presence of phenolic compounds, but note that differences in fluorescence intensity between images do not indicate differences in the concentration of phenolics between species. Bars, 10  $\mu\text{m}$ .

present in the Windmill Islands region, with *B. pseudotriquetrum* showing intermediate sensitivity and *C. purpureus* being the most UV tolerant (Leslie, 2003). Our results show that *S. antarctici* has the smallest pool of MeOH-soluble and alkali-extractable cell wall-bound UV-screening compounds, consistent with its ranking as the least UV-tolerant of the three moss species. *Schistidium antarctici* is negatively affected by current levels of ambient UV, with damage in the form of abnormal gametophyte morphology and chlorophyll bleaching apparent in UV-exposed plants (Robinson *et al.*, 2005). These results support the hypothesis that this species might be at a disadvantage compared with the two cosmopolitan species

under continuing springtime ozone depletion. We found no significant difference in the combined MeOH-soluble and alkali-extractable cell wall-bound UV-screening compound content between *C. purpureus* and *B. pseudotriquetrum*, and therefore these species would be expected to have similar degrees of UV tolerance. The greater UV tolerance of *C. purpureus* could arise from this species accumulating UV-screening compounds at the leaf surface, providing a more effective UV screen; however, the leaf blade in each of these moss species is only one cell thick (10–20  $\mu\text{m}$ ) and the small angle between leaf and stem and the tightly packed nature of Antarctic moss turfs (> 500 shoots  $\text{cm}^{-2}$ , Wasley *et al.*, 2006) makes it

difficult to determine whether the abaxial or adaxial leaf surface would receive a higher UV flux. Confocal microscopy also suggests that the distribution of UV-screening compounds is uniform at the leaf level in these moss species. However, the confocal microscope image of *B. pseudotriquetrum*, the species with the highest fraction of soluble UV-screening compounds, shows that the intracellular UV-screening compounds are not equally distributed throughout the cell (Fig. 3a), but are possibly contained within the vacuoles, as is the case in many plant species (Hutzler *et al.*, 1998; Cockell & Knowland, 1999; Meijkamp *et al.*, 1999; Kolb & Pfündel, 2005). The cell wall-bound UV-screening compounds, by contrast, appear to be evenly distributed throughout the cell walls in each of the three species (Fig. 3). Cell wall-bound UV-screening compounds are likely to provide a more spatially uniform, and thus more effective, UV screen for the cell contents than intracellular UV-screening compounds. This is especially the case in mosses because the leaves are composed of only a single cell layer and thus UV-screening compounds in the vacuoles will not provide screening for lower cell layers, as is the case for the epidermal layer in vascular plants. The greater investment in cell wall-bound UV-screening compounds may explain the higher UV tolerance of *C. purpureus* compared with *B. pseudotriquetrum*, despite the combined UV-screening compound content being very similar in the two species. Post (1990) found higher concentrations of anthocyanins and carotenoids in Antarctic *C. purpureus* from high-light environments compared with low-light environments. It is possible that the antioxidant properties of anthocyanins, carotenoids and flavonoids could play a role in the tolerance of *C. purpureus* and *B. pseudotriquetrum* to multiple stressors, including UV-BR, by reducing oxidative damage (Grace, 2005; Smirnov, 2005). Further work is needed to determine if intracellular compounds are more effective as antioxidants than those bound to the cell walls.

Differences in the absorption spectra of the methanol and alkali extracts between the three moss species indicate qualitative as well as quantitative differences in soluble and cell wall-bound UV-screening compounds (Fig. 2). The absorption spectra for MeOH-soluble UV-screening compounds differed between the three species, suggesting different compositions of soluble UV-screening compounds in these mosses. The peaks at *c.* 270 and 340 nm in these spectra suggest that flavonoid-type compounds may be present in these mosses, although other compounds also absorb in these regions (Harborne, 1989). Flavonoids have been previously identified in several species of the genus *Bryum* (e.g. Markham & Given, 1988; Webby *et al.*, 1996), and in sporophytes, but not in gametophytes, of *C. purpureus* (Vandekerckhove, 1978); however, recent studies by our group suggest that flavonoids are also present in gametophytes of this species (S. A. Robinson & V. Chobot, unpublished data). Absorption spectra for alkali-extractable wall-bound UV-screening compounds were more similar between species, although *B. pseudotriquetrum* absorbed less

in the UV-A region. The single absorption maximum in the spectrum of the alkali extract of *B. pseudotriquetrum* suggests that the cell walls of this species contain a relatively large proportion of simple phenolics compared to the other two species, which are less effective UV-screens than flavonoids (Harborne, 1989). The absorption spectra of the alkali-extractable cell wall-bound UV-screening compounds for this species are also similar to those observed in species of the dwarf shrub *Vaccinium* (Semerdjieva *et al.*, 2000). Cell wall-bound UV-screening compounds may be more conserved across species than MeOH-soluble UV-screening compounds. Identification of the MeOH-soluble and cell wall-bound compounds involved in UV screening in these mosses and other plant species is needed to determine whether this is the case.

Our research suggests that cell wall-bound UV-screening compounds are an important component of the defence against UV radiation in all three moss species present in the Windmill Islands region. Field studies have shown that ambient or enhanced UV-BR rarely has an effect on photosynthesis in mosses (Caldwell *et al.*, 2007). Cell wall-bound UV-screening compounds may explain the resilience of mosses to UV-BR, and moreover, such UV-screening compounds seem to be constitutive in these three species. The only seasonal difference in UV-screening compounds observed in this study was a greater concentration of MeOH-soluble UV-screening compounds in *C. purpureus* collected later in the growing season, whereas no change in alkali-extractable wall-bound UV screens was found in any species.

Although previous studies have shown the concentration of MeOH-soluble UV-screening compounds in *B. pseudotriquetrum* to be responsive to changes in the UV radiation environment (Dunn & Robinson, 2006), no change in the concentrations of soluble UV-screening compounds was found over the growing season for *B. pseudotriquetrum* in this study. We also found higher levels of MeOH-soluble UV-screening compounds in *C. purpureus* collected later in the growing season, even though UV-BR is typically higher earlier in the growing season under the influence of the ozone hole. Both results may be a consequence of the anomalously small ozone hole in the austral spring of 2002, which was both smaller in total area and broke up earlier in the season than in previous and subsequent years (Allen *et al.*, 2003; Solomon, 2004). In fact, whereas the UV-BR was higher on average in November than January for the years 1997–2004, in 2002 the UV-BR was significantly lower in November than in January (J. D. Turnbull, unpublished). The relatively low UV-BR early in the season may not have been sufficient to induce UV-screening compound production in *B. pseudotriquetrum* to the extent observed in years with more extreme ozone-hole events. It should also be noted that the study by Dunn & Robinson (2006) was carried out over a much longer period of the growing season (November–March) than the present study (November–early February) and thus encompassed a larger range of UV radiation than the present study, regardless of the size of the ozone hole.

## Conclusion

The greater investment in cell wall-bound UV-screening compounds offers *C. purpureus* a more spatially uniform and potentially more effective UV screen, and could explain the high UV tolerance of *C. purpureus* compared with *B. pseudotriquetrum*. The Antarctic endemic *S. antarctici* has only half the combined pool of MeOH-soluble and alkali-extractable cell wall-bound UV screens of the two cosmopolitan species, consistent with the hypothesis that this species may be disadvantaged under continuing springtime ozone depletion. Our research suggests that cell wall-bound compounds are an important component of the UV defences in mosses and deserve further investigation.

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