Impact of changes in natural ultraviolet radiation on pigment composition, physiological and morphological characteristics of the Antarctic moss, *Grimmia antarctici*

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Abstract

The impact of ambient ultraviolet (UV)-B radiation on the endemic bryophyte, *Grimmia antarctici*, was studied over 14 months in East Antarctica. Over recent decades, Antarctic plants have been exposed to the largest relative increase in UV-B exposure as a result of ozone depletion. We investigated the effect of reduced UV and visible radiation on the pigment concentrations, surface reflectance and physiological and morphological parameters of this moss. Plexiglass screens were used to provide both reduced UV levels (77%) and a 50% decrease in total radiation. The screen combinations were used to separate UV photoprotective from visible photoprotective strategies, because these bryophytes are growing in relatively high light environments compared with many mosses. *G. antarctici* was affected negatively by ambient levels of UV radiation. Chlorophyll content was significantly lower in plants grown under near-ambient UV, while the relative proportions of photoprotective carotenoids, especially *β*-carotene and zeaxanthin, increased. However, no evidence for the accumulation of UV-B-absorbing pigments in response to UV radiation was observed. Although photosynthetic rates were not affected, there was evidence of UV effects on morphology. Plants that were shaded showed fewer treatment responses and these were similar to the natural variation observed between moss growing on exposed microtopographical ridges and in more sheltered valleys within the turf. Given that other Antarctic bryophytes possess UV-B-absorbing pigments which should offer better protection under ambient UV-B radiation, these findings suggest that *G. antarctici* may be disadvantaged in some settings under a climate with continuing high levels of springtime UV-B radiation.

Nomenclature

A = antheraxanthin
$D1_{RE}$ = 1st derivative of red edge
ETR = electron transport rate
NIR = near infrared
REP = red edge position
TWC = turf water content
$UV\text{-}B$ = ultraviolet-B
V = violaxanthin
WC = water content
Z = zeaxanthin

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Introduction

Depletion of stratospheric ozone, resulting from anthropogenic, atmospheric pollution has led to increased ultraviolet (UV) radiation at the Earth's surface. The increase in UV-B is most pronounced and consistent over Antarctica, with record levels of ozone depletion reported recently and predicted to continue for several decades (NASA, 2004). Increased UV-B levels as a result of the 'ozone hole' have been reported as far north as New Zealand, with the most biologically damaging UV-B wavelengths (280–320 nm) increasing by 12% over the past decade (McKenzie et al., 1999).

The plant life on continental Antarctica is limited to bryophytes and other cryptogams. These organisms are desiccation and freezing tolerant, and able to survive frozen beneath snow during the long polar winter. The emergence from snow and the start of the short, growing season currently coincides with an extended period of elevated UV-B radiation due to ozone depletion. In general, organisms which are native to habitats with naturally high levels of UV-B exposure (e.g. alpine and tropical areas) tend to have better developed mechanisms for UV-B tolerance than natives of lower UV environments (Caldwell et al., 1982; Barnes et al., 1987; Ziska et al., 1992). Over recent decades, Antarctic plants have been exposed to the largest relative increase in UV-B exposure as a result of ozone depletion (Madronich et al., 1995). Given that they have historically been exposed to low background UV-B radiation, endemic Antarctic plants may be particularly sensitive to these relatively large increases.

Plant responses to elevated UV-B radiation have been well documented in recent years, especially in relation to agricultural and model plants and in response to elevated UV-B. More recently the results of long-term studies of plants in high latitudes have considered the impact of natural increases in UV-B as a result of ozone depletion (Searles et al., 1999; Robson et al., 2003). These studies have demonstrated responses from the molecular to the ecosystem level (Jansen et al., 1998; Caldwell et al., 2003; Frohnmeyer & Staiger, 2003). At the plant level, UV-B appears to affect growth, morphology and secondary metabolism rather than processes such as photosynthesis (Allen et al., 1998; Searles et al., 2001; Pancotto et al., 2003). Modest reductions in herbaceous plant growth are a common impact of UV-B radiation at midlatitudes (Ballare, 2003) and have also been shown for a number of species at high latitudes (Searles et al., 1999, 2002; Rousseaux et al., 2001; Robson et al., 2003). For Antarctic plants, most studies have found little effect on photosynthesis, but growth was affected by exposure to UV-B radiation especially in the two vascular species. In both

Deschampsia antarctica and Colabanthus quitensis, shorter, thicker leaves, reduced branching and fewer leaves per shoot were observed with increasing exposure to UV-B radiation (Day et al., 2001; Xiong & Day, 2001). In a study of the Antarctic moss Sanionia uncinata, shoot biomass and length were unaffected but shoots had less branching, after 2 years growth under reduced UV-B radiation (Lud et al., 2002).

Although the accumulation of UV-B-absorbing compounds, such as flavonoids, is one of the best-documented responses to solar UV-B (Caldwell & Flint, 1994), it may not be as widespread in high-latitude herbaceous plants. In D. antarctica and C. quitensis, although UV-B-absorbing pigments were higher after 4 months under ambient rather than reduced UV-B radiation, this trend was not sustained in longer term studies (Ruhland & Day, 2000; Lud et al., 2001; Xiong & Day, 2001). Long-term studies in Tierra del Fuego have also shown that for the majority of herbaceous species studied, solar UV-B does not regulate the accumulation of UV-absorbing compounds (Rousseaux et al., 1998; Rousseaux et al., 2001; Phoenix et al., 2002; Giordano et al., 2003).

Mosses are generally assumed to have a low capacity to produce flavonoids (Gwynn-Jones et al., 1999). Two of the five Antarctic bryophytes studied, Grimmia antarctici and Ceratodon purpureus, support this observation, showing low levels of UV-B-absorbing compounds and no evidence of stimulation in response to elevated UV-B levels (Dunn, 2000; Lovelock & Robinson, 2002). However, four Antarctic bryophytes Bryum pseudotriquetrum, S. uncinata, Andreaea regularis and Cephaloziella varians showed positive correlations between concentrations of UV-B pigments and levels of natural solar UV-B (Dunn, 2000; Lovelock & Robinson, 2002; Newsham et al., in press; Newsham et al., 2002); confirming that some bryophytes can produce screening pigments (Markham & Given, 1988).

Studies have also shown that the ratio of total carotenoids to chlorophyll increases in UV-B-exposed Antarctic vascular plants and bryophytes (Ruhland & Day, 2000; Xiong & Day, 2001; Newsham et al., 2002). In particular, high levels of photoprotective and antioxidant carotenoids have been observed in some Antarctic mosses, and it appears that species with low levels of UV-B-absorbing pigments accumulate higher levels of carotenoids, suggesting the possibility that bryophytes may use alternative mechanisms of UV protection (Lovelock & Robinson, 2002).

The aim of this field study was to investigate the impact of ambient UV-B radiation on the Antarctic endemic bryophyte G. antarctici. We predicted that this species might be less tolerant to the increasing UV-B radiation that has resulted from ozone depletion, by virtue of its historically low UV-B environment, and its

apparent low levels of UV-B-screening pigments (Lovelock & Robinson, 2002). The study was performed in the Windmill Islands region of Antarctica where extensive beds of this species are found. We investigated the effect of reduced UV and visible radiation on the pigment concentrations, surface reflectance and physiological and morphological characteristics of the moss. Four screen combinations were used to investigate the impact of both a decrease in ambient UV flux and a 50% decrease in total radiation. The latter were used to separate UV photoprotective from visible photoprotective strategies, because these bryophytes are growing in relatively high light environments compared with many mosses.

Materials and methods

Study site and experimental treatments

The Windmill Islands region of East Antarctica (66°17′S, 110°32′E), consists of a series of low-lying peninsulas and islands, which become partially ice-free during the summer melt period. The ice-free habitat occurs in a $35 \times 15 \, \text{km}^2$ area, supporting four bryophyte species including the endemic moss species *G. antarctici* Card. ($\approx$ *Schistidium antarctici,* Card.) used in this study. Moss turves have a distinctive undulating microtopography caused by cryoperturbation of the moss beds.

Screening treatments were established on two peninsulas approximately 20 km apart. One site was directly adjacent to the accommodation building at the Australian Casey Station (Red Shed site, 66.283°S, 110.527°E), while the other site was 20 km west at Robinson Ridge (66.368°S, 110.587°E). These sites were chosen because they support extensive communities of *G. antarctici*, and they differ in moisture availability and exposure. Robinson Ridge is more xeric, with lower water availability, cooler, and greater exposure to both sunlight and wind, compared with the more mesic Red Shed site (Melick & Seppelt, 1997). The Red Shed site was directly adjacent to a large summer melt lake and remains moist throughout the summer. The Robinson Ridge site was adjacent to a melt stream that flows early in the summer and provides intermittent water later in the season.

At each site, four sets of open-sided, treatment screens were applied. The screens ($30 \times 30 \, \text{cm}^2$) were composed of 4 mm Plexiglass that either transmitted (near-ambient UV; Plexiglass GS 2458, Plastral Pty Ltd, Sydney, Australia) or attenuated (reduced UV; Plexiglass G 233) UV radiation, in addition 50% shade cloth was also applied to half the screens. The screens transmitted 90% of incident radiation from 400 to 700 nm. The UV-blocking screens then blocked transmittance of almost all wavelengths below 350 nm, while the UV-transmitting screens allowed all UV-B and UV-
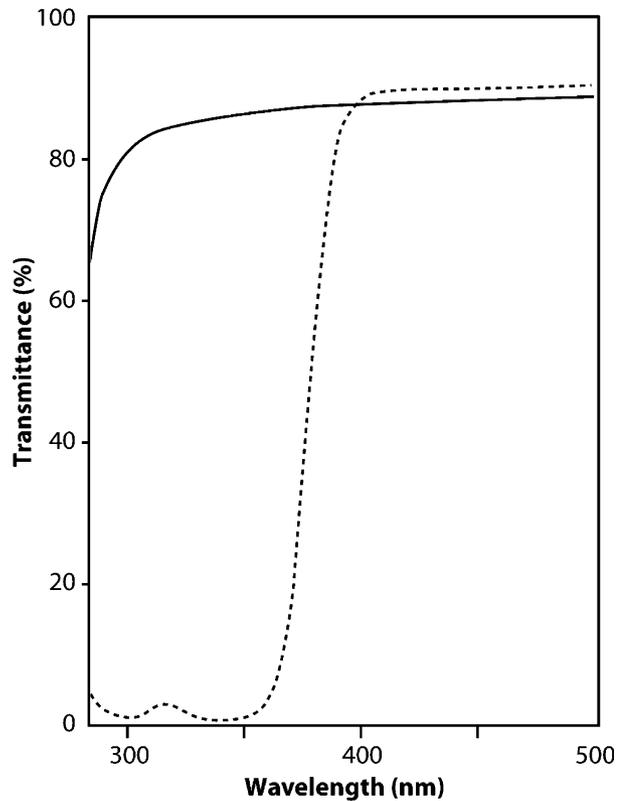
Fig. 1 Transmittance spectra of near-ambient (solid line, Plexiglass GS 2458) or reduced (dotted line, Plexiglass G 233) ultraviolet screens measured in a spectrophotometer.

A wavelengths to pass (cutoff 255 nm; see Fig. 1). Mean transmittance of UV radiation in the field was $81 \pm 3\%$ of incident radiation for the near-ambient UV treatment and $16 \pm 2\%$ for the reduced UV treatment (measured using a compact radiometer; RM21, and IP65 UV-B sensor; 280–315 nm (Dr Grobel, UV-Electronic, Ettingen, Germany). The shade cloth treatment was uniform across all wavelengths measured. Four replicates of each of the four treatment screens (near-ambient UV, near-ambient UV + shade, reduced UV and reduced UV + shade) were arranged randomly, within aluminium frames placed over *G. antarctici* turf, at each of the two sites. Screens were oriented along an east–west axis and frames were angled down on the northern face so as to minimize direct, mid-day solar radiation bypassing the screens. The height of the screens was 5–20 cm from the moss on the northern face, and 10–25 cm on the southern face. Frames were secured using guy wires in early December 1997. Measurements of UV transmission of the Plexiglass at the end of the experiment revealed no photodegradation had occurred.

As most of the water available to these moss beds occurs as melt water, screens had little effect on water availability. Observations over the 14 months of the

study showed that snow accumulation under screens was also similar to surrounding areas (Robinson, 1999). The absence of sides promoted blowing snow and also air movement thus reducing the potential for fungal contamination (Huiskes et al., 2001).

Temperatures of the moss turf were assessed over several overcast to full sun days, using an infrared thermometer (Scotchtrack T Heat tracer IR1600L; 3M, Austin, TX, USA) held 15–20 cm from the surface. Moss temperatures were compared under UV and shade treatment screens, beside the screens and over microtopograpic ridges and valleys formed through cycles of freezing and thawing (Kappen et al., 1989). As expected, moss under shaded screens and in microtopographic valleys had lower temperatures than that under clear screens or on microtopographic ridges (3.5 and 1.5 °C, respectively) but UV treatment screens did not affect temperature compared with unscreened control turf. Temperature differences between shaded and clear screens were most pronounced on sunny days and least on overcast days.

In January 1999, after 14 months growth under the screens, samples ($2 \, cm^2$) were collected from separate ridge and valley areas of turf beneath each screen (32 samples for each of the Robinson Ridge and Red shed sites). Additional samples were also collected from ridges and valleys adjacent to each frame in areas that were not impacted by the screens (eight samples per site). Samples were returned to the laboratory at Casey where each sample was split into two with one-half used for measurement of surface reflectance and subsequently frozen for pigment analysis (chlorophylls, carotenoids and anthocyanins), while the other half was used for measurement of photosynthetic parameters and then oven dried for determination of UV-absorbing pigments.

Measuring surface reflectance

Changes in surface reflectance characteristics reflect both morphological and physiological changes in plant communities (Field et al., 1994) and have been shown to change with altitude and UV-B exposure (Filella & Penuelas, 1999). We used an integrating sphere fitted to a scanning spectrophotometer (GBC UV-Vis 918, GBC, Dandenong, VIC, Australia) to measure spectral reflectance of the turf surface between 200 and 900 nm as described in Lovelock & Robinson (2002). From the entire reflectance spectra, we present reflectance at key wavelengths and also the first derivative of the reflectance change at the red-edge ($D1_{RE}$) and the position of the red-edge (REP) which has been found to differ between moss species (Lovelock & Robinson, 2002).

Physiological characteristics (photosynthetic parameters and turf water content)

After dark adaptation for 20 min, the chlorophyll fluorescence parameter F_v/F_m was measured for all samples using a PAM 2000 chlorophyll fluorometer (H. Walz, Effeltrech, Germany). Electron transport rates (ETRs) were then determined during the programmed light response curve of the PAM 2000 with a maximum light level of $550 \, \mu mols \, m^{-2} \, s^{-1}$ PAR. The ETR values shown are the maximum achieved during each light response curve, but may not necessarily be the same as maximum light saturated rates.

Turf water content (TWC) of these samples was then determined after oven drying at 80 °C to stable weight. TWC is expressed as $g \, H_2O \, g^{-1}$ dry weight as described in Robinson et al. (2000). In higher plants, where tissue is bound by an epidermis, water content (WC) of tissues can be expressed relative to the WC at full turgor. Determination of the WC at full turgor is difficult in intact mosses (Fowbert, 1996) because mosses store much of their water externally, in capillary spaces, or in the cell wall apoplastic spaces. We have chosen to rename it as TWC to recognize both the inclusion of external water in these measurements and that this is a measure of the water-holding capacity of the turf as a whole, rather than individual gametophytes.

Pigment content of moss samples

After the reflectance spectra were measured, the photosynthetically active apices of the moss were removed using a razor blade and the tissue was frozen in liquid nitrogen. Samples were returned to Australia in liquid nitrogen and then stored at −80 °C in a freezer prior to pigment analysis. For chlorophyll and carotenoid determination, samples (50–100 mg fresh weight) were ground with liquid nitrogen and sand in a mortar and pestle and then extracted in 100% acetone, followed by a second extraction in 80% acetone after Dunn et al. (2004). Chlorophylls and carotenoids were quantified by high-performance liquid chromatography using a method adapted from Gilmore & Yamamoto (1991) as described in Dunn et al. (2004).

Anthocyanin concentrations were determined using the differential pH method (Francis, 1982) as modified by Lovelock & Robinson (2002). UV-B-absorbing pigments were extracted in 1.5 mL of acidified methanol (methanol–H_2O–HCl: 79:20:1) as described in Lovelock & Robinson (2002).

Morphological measurements

UV-B radiation can affect both plant growth and morphology (Barnes et al., 1990). Therefore, gametophyte

leaf density and length of photosynthetic tissue were measured and the occurrence of malformed leaves within each sample was noted. In addition, qualitative determinations of gametophyte colour were made during these microscopic inspections. Samples were stored at $-20\,^{\circ}\mathrm{C}$ prior to measurement and were defrosted and rehydrated in water prior to measurement. Five single gametophytes were randomly selected from each sample for measurement, with juvenile, atypical and/or dead gametophytes excluded from the selection. Measurements were conducted using a binocular microscope (Leica Wild, Leica Microsystems, Gladesville, NSW, Australia M3C). The mean value of the five gametophytes was used in subsequent analyses.

The length of photosynthetic tissue (green-yellow section) was measured for each gametophyte, with the colour of the tip scored as either green or yellow (Plate 1). Where branching occurred the length of the thickest gametophyte was measured. To determine the density of leaves, each gametophyte was cut 3.5 mm from the tip and all leaves were carefully dissected from this section and counted. Entire samples were also scored for presence or absence of leaves with altered morphology at the gametophyte tip (see Fig. 2).

Data analysis

Tests of the influence of UV-B exposure, shading, site and microtopography on reflectance parameters, pigments and photosynthetic and physiological characteristics were performed using analysis of variance (ANOVA; Data Desk 6.1, Data Descriptions, Ithaca, NY, USA). The adequacy of ANOVA models were assessed by inspecting residual plots. To achieve homogeneity of variance and normality the data were transformed as follows; chlorophyll, anthocyanin, chlorophyll a/b, Z/VAZ, TWC (square root); AZ/VAZ (arcsin); R_{320} (log); R_{850}, (squared); $D1_{RE}$ (cubed) transformation. Results for statistical tests are shown where $P < 0.1$. Morphological parameters were assessed by χ^2 tests and results shown where $P < 0.05$.

Results

#### Biochemical and physiological responses

Effect of reduced incident UV radiation. G. antarctici growing under near-ambient UV radiation had lower concentrations of total chlorophyll and correspondingly higher relative concentrations of carotenoids than moss growing under reduced UV radiation (Fig. 3). For the photoprotective pigments, the relative concentration of β-carotene was higher under near-ambient UV radiation (Fig. 3c). While the photoprotective zeaxanthin (Z) comprised a larger fraction of the xanthophyll cycle (VAZ) pool in moss exposed to near-ambient UV radiation on unshaded ridges (Fig. 3d, Table 1; UVscreen × shade interaction, $P = 0.03$). The total carotenoid and neoxanthin concentrations relative to chlorophyll also tended to be higher under near-ambient UV radiation (Fig. 3b, d). However, anthocyanin and UV-B-absorbing pigment concentrations were not affected by variation in UV exposure.

Despite little change in UV-B-absorbing pigments, turf-surface reflectance in the UV region declined under
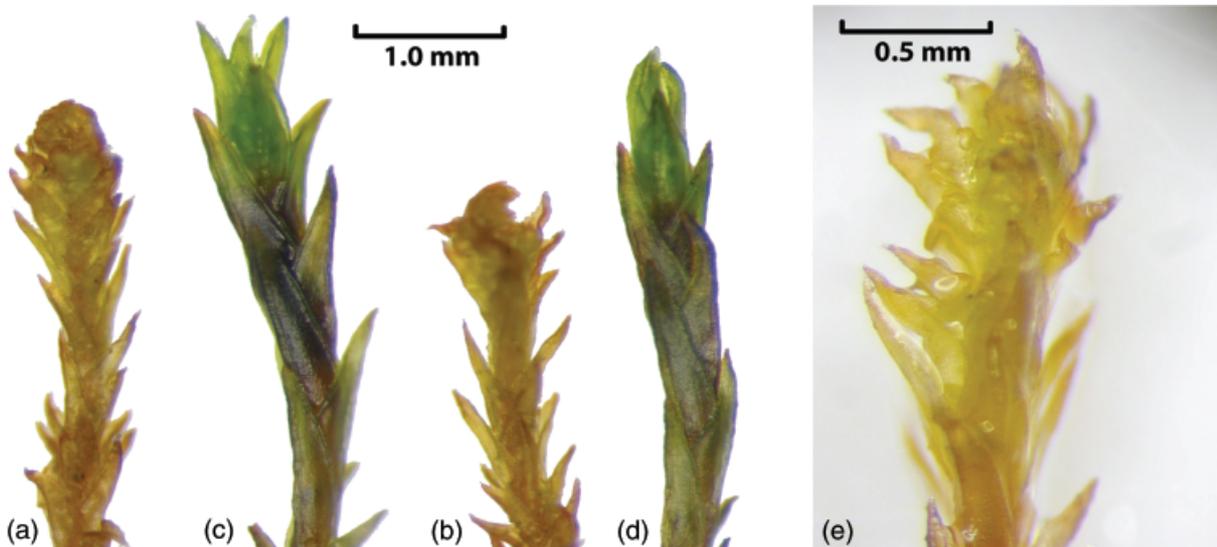
Fig. 2 Comparison of gametophytes of *Grimmia antarctici* showing the normal leaf morphology and green colouration (c–d) and atypical leaf morphology and yellow colouration (a–b), enlarged (e). Atypical leaves were characterized by short length and blunted leaf tips.
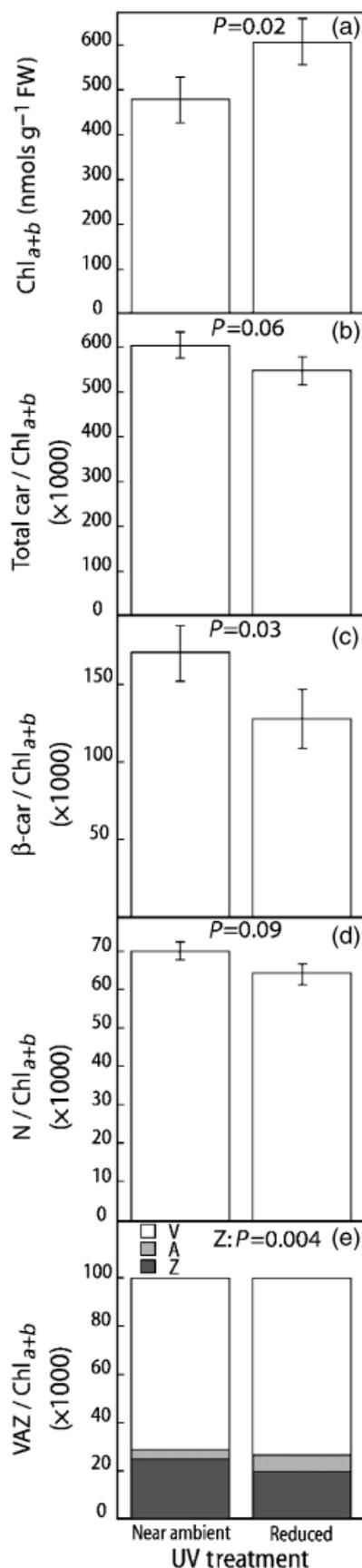
reduced UV radiation, while reflectance of green and near-infrared (NIR) wavelengths increased compared with moss exposed to near-ambient UV radiation (Fig. 4). The $D1_{RE}$ increased by 12.5% ($F_{1,31} = 6.10$, $P = 0.017$) and the REP shifted from 700.09 to 700.79 nm when UV radiation was reduced (Fig. 4 inset; $F_{1,31} = 6.10$, $P = 0.017$).

None of the physiological characteristics measured (F_v/F_m, ETR and TWC) were altered as a result of reduced UV exposure.

Incident solar radiation. Shading of moss led to an increase in anthocyanins and UV-B-absorbing pigments (Fig. 5h, i). In contrast there was a decrease in the xanthophyll cycle pigments relative to chlorophyll and to the proportion of this pool present as antheraxanthin (A) and Z in shaded moss (Fig. 5g). Lutein concentration relative to chlorophyll declined by 4.5% under shade treatments. Often the effects of shading were dependent on the site (Table 1; shade × site interactions for T_{chl}, $P = 0.007$; VAZ/T_{chl}, $P = 0.02$; L/T_{chl}, $P = 0.01$) with shading having a more pronounced effect on moss at the Robinson Ridge than at the Red Shed site. The effects of shading were also dependent on both site and topography for several pigments (shade × site × topography interactions; T_{car}/T_{chl}, $P = 0.001$; β-carotene/T_{chl} $P < 0.001$; Z/VAZ; $P = 0.006$). These pigments were all highest in moss on unshaded ridges at the Robinson Ridge site than under other screen treatments.

Reduction of total radiation through shading of moss had less effect on the individual reflectance parameters although visible reflectance was 10% lower (R_{526}; $F_{1,31} = 3.35$, $P = 0.073$) and the REP was 0.64 nm longer under shaded screens ($F_{1,31} = 5.43$, $P = 0.024$).

None of the physiological characteristics measured were altered as a result of shading treatments.

Effects of microtopography. Moss from valleys showed similar, but stronger, responses to those seen for shaded treatments (Fig. 5a–f). Total chlorophyll, anthocyanin and UV-B-absorbing pigments were all found in higher concentrations in moss growing in valleys than that found on ridges (Fig. 5a, e–f). However, carotenoid concentrations were generally higher relative to chlorophyll in ridge moss, particularly the carotenoids involved in photoprotection. The relative size of the VAZ pool, and the proportion present in the photoprotective forms A and Z, were 24% and 42%

Fig. 3 The effect of reducing ultraviolet (UV) radiation on pigment concentrations of *Grimmia antarctici*. Total chlorophyll (a), total carotenoid/chlorophyll (b), β-carotene/chlorophyll (c), neoxanthin/chlorophyll (d) and xanthophyll cycle pigments, violaxanthin, antheraxanthin, zeaxanthin (e). Data are means ± SEM, $n = 32$.
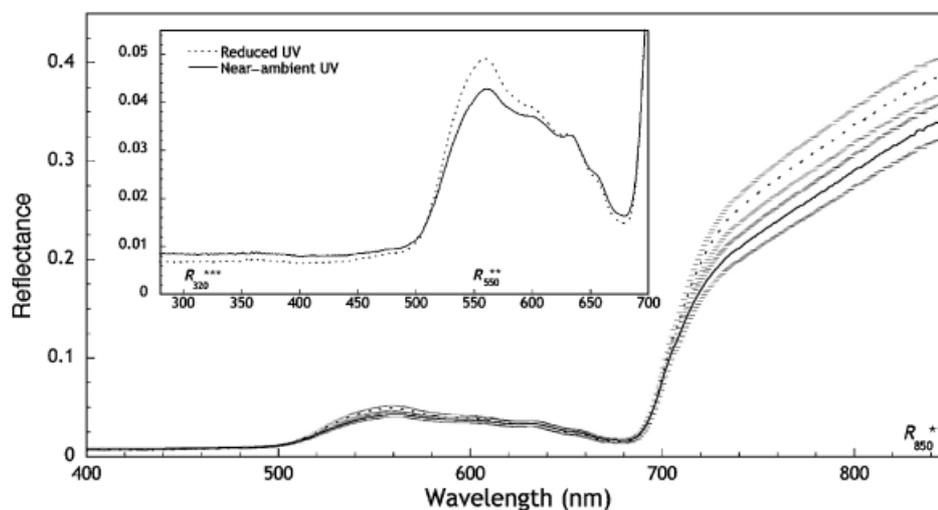
Fig. 4 The effect of reducing ultraviolet (UV) radiation on reflectance spectra of turf of *Grimmia antarctici*. Turf exposed to near-ambient (solid line) or reduced (dotted line) UV radiation. Reflectance 280–850 nm (main graph) and 280–700 nm (inset). Data are means $\pm$ SEM ($n = 32$). Mean reflectance levels are significantly different at R_{320}, ($P < 0.01$***), R_{526} and R_{850} ($P < 0.05$**), R_{526} ($P = 0.06$).

Table 1 ANOVA summary table showing significant effects of UV exposure, shading, site and microtopography on surface reflectance characteristics, pigment concentrations, physiological and morphological parameters of *Grimmia antarctici*

		UV	Shade	Topography	Site
Reflectance parameters	R_{320}	XX	–	XX	–
	R_{526}*	X	X	–	XX
	R_{850}	XX	–	–	XX
	REP	XX	XX	XX	–
	D1$_{RE}$	XX	–	–	XX
Pigment content	Anthocyanins	–	X	XX	XX
	UV-B absorbing pigments	–	XX	XX	XX
	Total chlorophyll[†]	XX	–	XX	XX
	Chlorophyll $a:b$	–	–	–	XX
	$T_{car.}/T_{chl}$[‡]	X	X	XX	–
	VAZ/T_{chl}[+]	–	X	XX	–
	AZ/VAZ	–	XX	XX	XX
	Z/VAZ[‡,§]	XX	XX	XX	XX
	L/T_{chl}[†]	–	XX	XX	X
	N/T_{chl}	X	–	–	XX
	β-carotene/T_{chl}[‡]	XX	–	XX	–
Physiological parameter	ETR	–	–	XX	XX
	F_v/F_m	–	–	XX	–
	TWC[¶]	–	–	X	XX
Morphological parameter	Length of photosynthetic tissue	–	–	XX	XX
	Leaf density[†]	XX	–	XX	XX

''XX'' and ''X'' indicate significant effect at $P < 0.05$ and $P < 0.10$, respectively.

TWC, turf water content; REP, red edge position; D1$_{RE}$, 1st derivative of red edge; UV-B, ultraviolet B; ETR, electron transport rate.

Interaction terms:

*Topography $\times$ site.

[†]Shade $\times$ site.

[‡]Shade $\times$ site $\times$ topography.

[§]UVscreen $\times$ shade.

[¶]Shade $\times$ topography.

Table 2 Comparison of surface reflectance, physiological and morphological parameters for *Grimmia antarctici* moss growing in ridges or valleys under the screen treatments

	Ridge	Valley	F	P
Reflectance parameter				
R_{320}	0.0085 ± 0.0006	0.0066 ± 0.0004	13.15	0.001
REP (nm)	699.79 ± 0.216	701.09 ± 0.192	22.6	<0.0001
Physiological parameters				
ETR_{max} ($\mu mol\,m^{-2}\,s^{-1}$)	68.42 ± 3.87	80.91 ± 4.61	5.24	0.027
F_v/F_m	0.691 ± 0.004	0.714 ± 0.004	10.82	0.002
TWC ($g^{-1}\,H_2O\,g^{-1}\,dwt$, $n = 26, 28$)	5.71 ± 0.62	4.8 ± 0.73	3.26	0.079
Morphological characteristics				
Length of photosynthetic tissue (mm)	1.95 ± 0.12	4.05 ± 0.36	42.15	<0.0001
Leaf density (leaves mm^{-1})	10.6 ± 0.36	6.52 ± 0.18	135.34	<0.0001

Data are mean values $\pm$ SEM ($n = 32$).

REP, red edge position; TWC, turf water content; ETR, electron transport rate.

higher, respectively, while β-carotene was 113% higher on ridges than in valleys (Fig. 5c–d). The relative concentration of lutein was also significantly higher on ridges but the difference was only 7% ($P = 0.002$).

Although reflectance in the UV region was lower in valleys than ridges, reflectance across the visible and NIR wavelengths was similar across turf topography. The REP was over a nanometre longer for moss growing in valleys than ridges (Table 2).

Max ETR and F_v/F_m were higher in valleys than on ridges (Table 2). Shading by screens also increased the TWC of moss growing on ridges compared with that under unshaded screens (shade $\times$ topography interaction term; $P = 0.05$).

Site differences. Site effects were common with moss pigment concentrations generally higher at Robinson Ridge than at the Red Shed (Table 3; total chlorophyll, anthocyanins, UV-B-absorbing pigments). The proportion of lutein and neoxanthin relative to chlorophyll and the chlorophyll a/b ratio were also higher in moss from the Robinson Ridge site. Conversely, the proportion of the VAZ pool present as A and Z was higher in moss from the Red Shed site. Many of these site differences were complicated by interactions with shade treatments or topographical differences (Table 1). In general, differences over treatments were most pronounced at the Robinson Ridge site, particularly in unshaded moss.

Moss from Robinson Ridge showed higher visible and NIR reflectance than moss from the Red Shed site (Table 3). This site effect was not apparent in the UV region. The $D1_{RE}$ was greater in moss from the Robinson Ridge site.

TWC was twice as high at the Red Shed site while ETR was greater at the Robinson Ridge site (Table 3).

Morphological characteristics

The length of photosynthetic tissue was greater in moss growing in valleys than on ridges, and in moss growing at Robinson Ridge compared with the Red Shed site (Tables 2 and 3). Moss growing in valleys was visually entirely green while 54% of gametophytes scored from ridge-grown moss were classified as yellow ($P < 0.0001$). Shading had a similar effect, with more than 80% of moss growing under shade screens scored as green, compared with 65% of moss growing under unshaded screens ($P < 0.01$).

Density of leaves on individual gametophytes was 7% higher in gametophytes growing under near-ambient UV compared with those growing under reduced UV radiation (8.83 ± 0.51 and 8.23 ± 0.41 leaves mm^{-1}, respectively; $F_{1,31} = 3.82$, $P = 0.056$). In addition, leaf density was 60% higher on ridges than in valleys (Table 2). Leaf density was reduced by shading treatments at Robinson Ridge but not at the Red Shed site (Table 1; shade $\times$ site interaction, $P = 0.026$).

The proportion of leaves with blunt-tip morphology decreased from 26% under near-ambient UV, to 12% under reduced UV exposure (Fig. 2; $P < 0.01$). Leaves with atypical morphology were more common on ridges than in valleys (30% compared with 8%, $P < 0.0001$) and in moss growing under unshaded rather than shaded screens (26% compared with 11%, $P < 0.001$).

Effects of screening moss

Comparison of samples from outside the screened area with samples under the UV transparent screens show enhanced reflectance at shorter wavelengths under screens compared with unscreened moss (30% higher

Table 3 Comparison of surface reflectance parameters, pigment concentrations, physiological and morphological parameters for *Grimmia antarctici* moss growing under screen treatments at the Robinson Ridge and Red Shed sites

	Robinson Ridge	Redshed	F	P
Reflectance parameter				
R_{526}	0.0305 ± 0.0014	0.0261 ± 0.0014	5.64	0.022
R_{850}	0.4097 ± 0.0158	0.3309 ± 0.0189	10.48	0.002
$D1_{RE}$	0.0092 ± 0.0004	0.0074 ± 0.0005	6.66	0.013
Pigments				
Anthocyanins (A_{526}diff g^{-1} fwt)	1.82 ± 0.20	1.07 ± 0.20	7.61	0.008
UV-B absorbing pigments				
(Mean $A_{320-280}$ g^{-1} dwt)	0.187 ± 0.005	0.157 ± 0.005	16.068	0.000
Total chlorophyll (nmol g^{-1} fwt)	622 ± 52	469 ± 50	4.65	0.036
Chlorophyll a/b	3.32 ± 0.09	2.98 ± 0.09	7.86	0.007
AZ/VAZ (%)	24.1 ± 1.66	31.1 ± 1.60	19.26	<0.0001
Z/VAZ (%)	21.4 ± 1.79	23.8 ± 1.70	6.28	0.016
L/T_{chl} (mmol mol^{-1})	179 ± 3.17	172 ± 3.07	3.48	0.069
N/T_{chl} (mmol mol^{-1})	74.1 ± 2.32	60.8 ± 2.28	17.51	0.000
Physiological parameters				
ETR$_{max}$ (μmol m^{-2} s^{-1})	88.2 ± 4.04	61.1 ± 3.26	25.032	<0.0001
TWC (g^{-1} H$_2$O g^{-1} dwt, $n = 28, 26$)	3.47 ± 0.24	7.15 ± 0.82	29.19	<0.0001
Morphological characteristics				
Length of photosynthetic tissue (mm)	3.61 ± 0.385	2.39 ± 0.22	13.58	0.006
Leaf density (leaves mm^{-1})	8.09 ± 0.42	8.97 ± 0.49	7.7079	0.008

Data are mean values $\pm$ SEM ($n = 32$).
TWC, turf water content; ETR, electron transport rate.

at R_{320}; $P = 0.005$ and 16% higher at R_{526}; $P = 0.098$). Pigment concentrations were largely unaffected by the presence of screens with the exception of a 40% decrease in anthocyanin concentration under screens ($P = 0.08$). The presence of screens affected maximum ETR but not F_v/F_m. Maximum ETR was reduced from 81.5 ± 5.0 to 58.8 ± 5.5 μmol m^{-2} s^{-1} in moss under screens ($P = 0.014$) although this effect was modified by topography (screen × topography interaction, $P = 0.07$) with ETR reduced only for mosses on ridges under screens, while moss in valleys had similar ETRs in both screened and unscreened samples.

Discussion

Reduction in UV-B exposure

As stratospheric ozone depletion has increased the UV-B radiation incident on mosses growing in Antarctica, this study compared near-ambient UV (the current situation) with reduced UV radiation. Although the reduction (77%) imposed by the screening treatment is more extreme than the change which has occurred in recent decades, this study provides indications of the likely impact of natural changes in UV-B radiation on the Antarctic endemic *G. antarctici*.

Chlorophyll concentration increased by 27% in moss grown under reduced UV radiation while β-carotene/T_{chl} and the proportion of the VAZ pool present as Z decreased by 25% and 21%, respectively. Other significant changes to pigments, reflectance parameters or morphology were smaller in magnitude (2–15%). UV radiation therefore had a significant destructive effect on the main light-absorbing pigments while increasing the relative proportions of pigments involved in photoprotection, such as β-carotene and zeaxanthin. Interestingly, there was no evidence of accumulation of screening pigments in moss exposed to near-ambient UV radiation levels. The reflectance data support this, with moss exposed to near-ambient UV having higher reflectance in the UV region and lower reflectance in the visible and NIR consistent with reductions in concentration of the dominant chlorophyll pigments. The shift of the REP to longer wavelengths under reduced UV radiation is also indicative of increased absorption by chlorophyll. These results show that protection from UV-B is unlikely to occur through screening or reflectance of UV-B radiation. However, increased levels of antioxidant carotenoids such as β-carotene and Z might ameliorate some of the damage associated with UV-B exposure. Increased ability to scavenge reactive oxygen species has been shown to improve
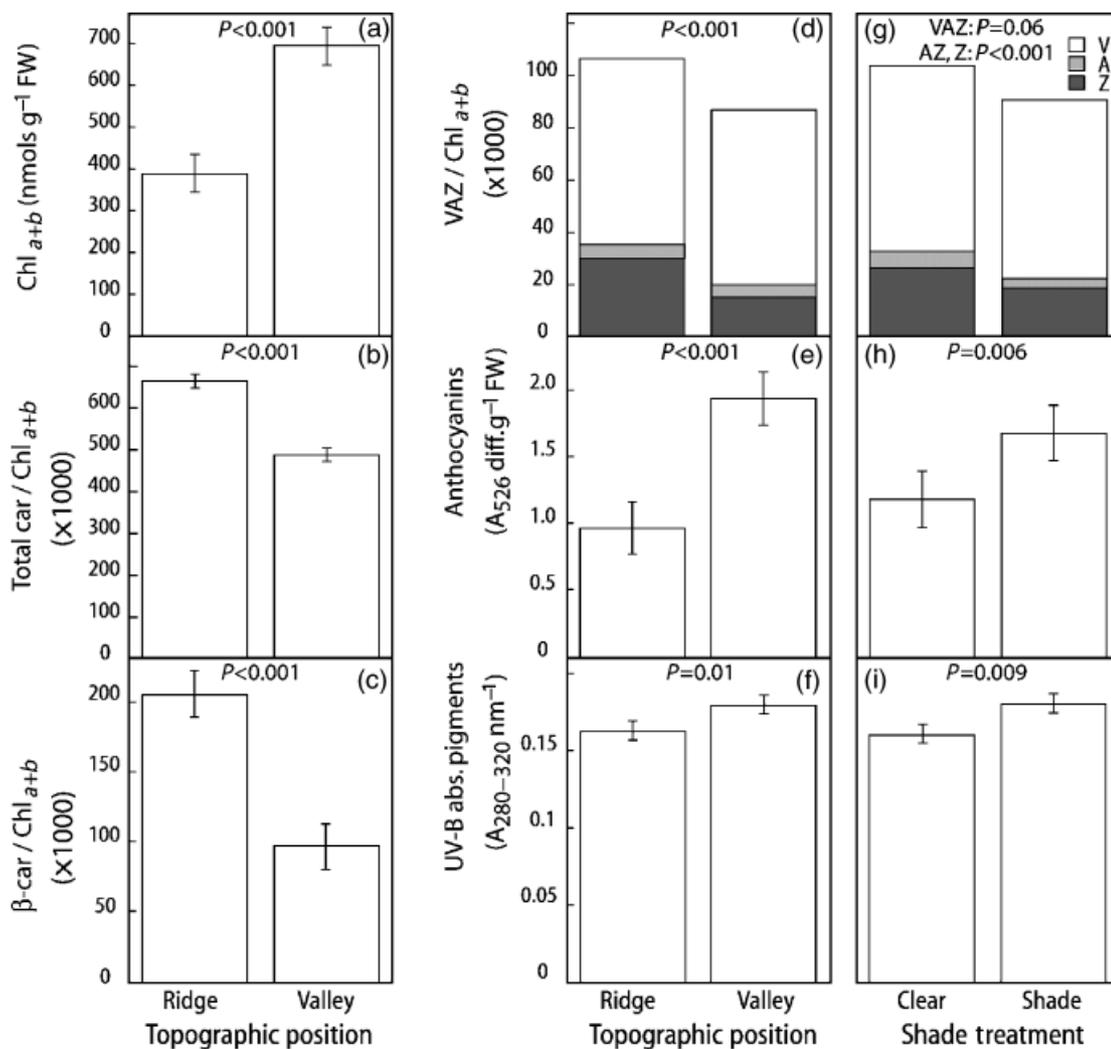
Fig. 5 The effect of shading by screens g–i) or topographical position within the turf (a–f) on pigment concentrations of *Grimmia antarctici*. Total chlorophyll (a), total carotenoid/chlorophyll (b), β-carotene/chlorophyll (c), xanthophyll cycle pigments, violaxanthin, antheraxanthin, zeaxanthin (d, g), anthocyanins (e, h) and UV-B-absorbing pigments (f, i). Data are means ± SEM, $n = 32$.

plant tolerance to UV-B radiation (Middleton & Teramura, 1993; Mazza et al., 1999; Fujibe et al., 2004).

Increased accumulation of UV-B-screening pigments or epicuticular waxes is one of most common effects of UV-B on temperate and tropical plants (Robberecht et al., 1980; Bornman & Vogelmann, 1991; Krause et al., 2003). However, recent studies suggest that accumulation of UV-B-absorbing pigments may be a less common response in high-latitude plants. Three out of five species studied in a long-term experiment in Terra del Fuego, showed no response of UV-B-absorbing pigments to UV-B exposure while the results for the remaining two species were inconclusive (Rousseaux et al., 1998, 2001; Giordano et al., 2003). In a similar subarctic study, two out of the three species showed increased UV-B-absorbing pigments under reduced

UV-B (Phoenix et al., 2002). Similarly conflicting results have been observed for vascular plants on the Antarctic peninsula with higher concentrations of UV-B-absorbing pigments observed in short-term but not in long-term studies (Ruhland & Day, 2000; Lud et al., 2001; Xiong & Day, 2001).

Although mosses have been reported as generally lacking inducible UV-B-screening pigments (Gehrke, 1998, 1999; Gwynn-Jones et al., 1999; Searles et al., 1999; Niemi et al., 2002a, b) recent studies suggest that many Antarctic mosses do produce UV-B-screening pigments in response to natural exposure to UV-B radiation (Dunn, 2000; Lovelock & Robinson, 2002; Newsham et al., 2002; Newsham, 2003; Robinson et al., 2003). Interestingly, tests of sensitivity to UV-B responses in moss species to date have not involved Antarctic

endemics, and we believe that this study of *G. antarctici* represents the first endemic species tested under variable UV radiation.

Changes to chlorophyll have been observed in some species but are not a consistent response to natural variations in UV-B exposure, although they have previously been observed in Arctic bryophytes in response to enhanced UV-B radiation (Gehrke, 1999; Searles *et al.*, 2001; Caldwell *et al.*, 2003). No change in chlorophyll concentration was observed as a result of seasonal changes in UV-B radiation in either the South American *Sphagnum magellanicum* (Searles *et al.*, 2002) or two Antarctic bryophytes studied by Newsham *et al.* (2002). This destruction of pigments under ambient UV radiation is thus unusual and again may indicate that *G. antarctici* is less tolerant of UV exposure than other moss species studied. UV-induced increases in the carotenoid to chlorophyll ratio are however relatively common in Antarctic plants and algae (Post & Larkum, 1993; Ruhland & Day, 2000; Newsham *et al.*, 2002; Newsham, 2003).

The morphological changes also indicate that UV exposure may be having negative effects on this species. The increased leaf density per gametophyte is likely because of reduced gametophytic growth under ambient UV radiation, producing shorter internodes. Increased leaf density was also observed in *Polytrichum splendens* under elevated UV-B radiation (Gehrke, 1999). Reductions in internode length and increases in leaf thickness are highly correlated with reduced growth rates over a wide range of species (Saverimuttu & Westoby, 1996), and thus it is likely that high levels of UV radiation are reducing growth in *G. antarctici*. Similar morphological changes have been shown in response to water and temperature stress in Arctic and Antarctic bryophytes (Wilson, 1990; Fowbert, 1996; Heegard, 1997; Dorrepaal *et al.*, 2003). Increased numbers of leaves displaying atypical morphology in the near-ambient UV treatment could be the result of UV-B damage to tissues. Growth measurements of Antarctic mosses are extremely difficult because of the slowness of growth (estimate for this species $0.6 \, \text{mm} \, \text{yr}^{-1}$; Melick & Seppelt, 1997) and long-term studies would be needed to confirm any negative impact on growth.

Studies of the moss *S. uncinata*, performed over similar periods on the warmer Antarctic peninsula, failed to detect alterations in growth rates by UV-B radiation but similar to this study, moss also showed morphological alterations (Lud *et al.*, 2002). Although the height of *S. magellanicum* appeared unaffected by ambient UV-B in the first season of a study in Tierra del Fuego, results after 3 and 6 years showed that growth was reduced by 9%. These studies also demonstrate the need for long-term experiments, as interannual varia-

tion was high, and significant annual reductions were only observed in one of the 6 years of the study (Searles *et al.*, 1999, 2002; Robson *et al.*, 2003). Effects of UV-B on plant growth may be evident within shorter time intervals where UV-B is elevated above ambient levels. Reductions in growth were observed in three subarctic bryophytes grown under supplemental UV-B lamp treatments for 3 years (Gehrke, 1998, 1999).

Similar to other plant species, changes in UV radiation altered pigment composition and morphology of *G. antarctici* while photosynthesis appears to be relatively robust to such changes (Allen *et al.*, 1998; Searles *et al.*, 2001). With UV-B radiation predicted to remain at elevated levels in the Antarctic, potential reductions in growth rates of *G. antarctici* with higher UV-B, particularly in exposed conditions, could lead to alterations in bryophyte community composition.

Shading and topographical differences

The impact of shading on moss turves was also investigated in order to differentiate the effects of reduced UV radiation with light reduction in general. Shading did affect the pigment content and reflectance properties of the moss and, in many ways, reproduced the natural differences in light environment produced by topography (Table 1). However, the differences produced by shading were less pronounced than those found between moss growing on ridges and valleys and were also less significant than the changes induced by reducing UV radiation alone.

Topography had a significant effect on many of the parameters investigated. This study confirms our previous findings that topographical location is a key factor in determining the physiological and biochemical characteristics of this species (Lovelock & Robinson, 2002). Lower chlorophyll and higher protective pigment concentrations on ridges compared with valleys confirm that ridges are stressful environments. However, increased photoprotection on ridges was not accompanied by increases in pigments that potentially screen UV-B, as the levels of anthocyanins and UV-B-absorbing pigments were higher in moss growing in valleys than on ridges.

Although the changes in chlorophylls and carotenoids may reflect acclimation of photosynthetic pigments to the light levels experienced by the moss, it is possible that these results are in part explained by photo-oxidation of pigments and could explain the reduction in anthocyanins and chlorophyll on ridges and under clear screens. In subarctic shrubs, reductions in UV-B radiation resulted in increased levels of UV-B-absorbing compounds (Phoenix *et al.*, 2002) which was attributed to either increased secondary metabolism in

the absence of UV-B radiation or UV photo-oxidation of these pigments. In this study, higher levels of anthocyanins were observed when UV was reduced (albeit not significant), under shade and most significantly in moss growing in valleys rather than on ridges. Taken together this is supportive of photodestruction of pigments occurring in the higher radiation environments. In addition, given that the shading treatment only increased chlorophyll content by 13% (less than half that observed for the reduced UV treatment) we can be confident that there is a UV radiation component to this photodestruction of photosynthetic pigments.

Topographical changes in reflectance data are largely those associated with increases in chlorophyll content, such as the shifting of the REP to longer wavelengths in moss growing in valleys compared with ridges. The physiological parameters also indicate that moss in the valleys is healthier and probably more productive with a higher F_v/F_m and an 18% increase in ETR compared with moss growing on ridges. These differences are presumably mainly as a result of the more benign light and exposure conditions associated with valleys as the WC was slightly lower in valleys than on ridges. The increased length of photosynthetic tissue in valley moss confirms that growth conditions are improved compared with that on ridges. Moss growing in valleys also had a lower leaf density than moss on ridges, which is consistent with leaf density being positively correlated with reduced growth.

Site effects

Moss growing in the two sites varied considerably in reflectance parameters, pigment content, physiologically and morphologically. We initially chose the sites as representative of xeric (Robinson Ridge) and mesic (Red Shed) habitats. Our results indicate that despite the more xeric habitat at Robinson Ridge, pigment concentrations, ETR and length of photosynthetic tissue were generally greater than at the Red Shed site, and there were also only small differences in parameters associated with stress. The proportion of the VAZ pool present as AZ was higher at Red Shed but it is probable that this reflects the proximity of this site to the lab and therefore a faster freezing of samples after collection. Other photoprotective carotenoids such as β-carotene and the total VAZ pool did not differ between sites, and F_v/F_m did not change. Site strongly influenced the effects of the various screen treatments (Table 1). In particular shading was far more significant a treatment at Robinson Ridge than at the Red Shed, perhaps pointing to a strong effect of exposure at the latter site which was ameliorated by the screens. Moss at the Red Shed site is covered by water for most of the season whereas moss at the Robinson Ridge

site is saturated for only a short period during snow melt and has a more seasonal water supply. The increased exposure at the Robinson Ridge site also leads to more frost heaving and hence more pronounced microtopographic differences. The combination of light stress with intermittent water stress could explain the stronger response to shading at Robinson Ridge. Because of its increased exposure and cooler temperatures we had assumed that the Robinson Ridge site is harsher and less favourable for moss growth. However, our results suggest that the Red Shed site is less favourable than previously thought, possibly because of anaerobic conditions when moss is submerged for long periods during the summer. It maybe that when water is available, the Robinson Ridge site is more favourable for growth but that increased exposure at this site increases the variability in this response and increases the probability of microtopography influencing treatment responses.

Conclusions

This study provides evidence that the endemic, Antarctic moss G. antarctici is negatively affected by UV radiation with reductions in light harvesting pigments and an increased reliance on photoprotective and antioxidant pigments when grown under near-ambient UV. Although the physiological parameters were not affected there was evidence that the plants might be suffering morphological damage or reduced growth as a result of UV exposure. Levels of UV-B-absorbing and anthocyanin pigments are known to be low in this species (Lovelock & Robinson, 2002) and there is no indication from the current study that they change in response to natural UV flux.

As UV-B-absorbing pigments showed variation in response to reduced total radiation (in shaded or valley moss) and were closely correlated with chlorophyll concentrations, this suggests that they may not act as UV-specific screens in this species. Other photoprotective pigments would appear to be more important for survival of this endemic species under increased UV-B radiation. Current studies, similar to those described by Rousseaux et al. (1999), will investigate the relationship between capacity for UV-screening and photoprotection and DNA damage in this species.

Our results show that ambient UV radiation has the potential to adversely affect an endemic, Antarctic bryophyte and confirms that this species has low potential for UV-B photoprotection. Indications of slower growth under UV-B as well as in the more stressful environments (ridges) may suggest lowered competitive advantage under high UV-B, possibly leading to changes in the balance of species in these extreme environments. As other cosmopolitan mosses that co-occur with this

species possess greater potential for UV-B screening and appear to be more desiccation tolerant than this endemic species (Dunn, 2000; Robinson *et al.*, 2000; Lovelock & Robinson, 2002) *G. antarctici* may lose its dominant position within the Windmill bryoflora.

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