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1 **Impact of changes in natural UV radiation on pigment composition,**
2 **physiological and morphological characteristics of the Antarctic moss,**
3 ***Grimmia antarctici*.**

4 **RUNNING TITLE: Impact of UV radiation on an Antarctic moss**

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19 **Keywords:** anthocyanins, β -carotene, chlorophyll, chlorophyll fluorescence, leaf morphology,
20 surface reflectance, UV-B absorbing pigments, xanthophyll cycle pigments.

21 **Abbreviations:** A, antheraxanthin; $D1_{RE}$, 1st derivative of red edge; ETR, electron transport
22 rate; NIR, near infrared; REP, red edge position; TWC, turf water content; UV-B, ultraviolet-
23 B; V, Violaxanthin; WC, water content; Z, zeaxanthin.

1 Abstract

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

1 **Introduction**

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

9 The plant life on continental Antarctica is limited to bryophytes and other cryptogams. These
10 organisms are desiccation and freezing tolerant, and able to survive frozen beneath snow
11 during the long polar winter. The emergence from snow and the start of the short, growing
12 season currently coincides with an extended period of elevated UV-B radiation due to ozone
13 depletion. In general, organisms which are native to habitats with naturally high levels of
14 UV-B exposure (e.g. alpine and tropical areas) tend to have better developed mechanisms for
15 UV-B tolerance than natives of lower UV environments (Barnes *et al.* 1987; Caldwell *et al.*
16 1982; Ziska *et al.* 1992). Over recent decades, Antarctic plants have been exposed to the
17 largest relative increase in UV-B exposure as a result of ozone depletion (Madronich *et al.*
18 1995). Given that they have historically been exposed to low background UV-B radiation,
19 endemic Antarctic plants may be particularly sensitive to these relatively large increases.

20 Plant responses to elevated UV-B radiation have been well documented in recent years,
21 especially in relation to agricultural and model plants and in response to elevated UV-B.
22 More recently the results of long term studies of plants in high latitudes have considered the
23 impact of natural increases in UV-B as a result of ozone depletion (Robson *et al.* 2003;
24 Searles *et al.* 1999). These studies have demonstrated responses from the molecular to the
25 ecosystem level (Caldwell *et al.* 2003; Frohnmeyer & Staiger 2003; Jansen *et al.* 1998). At

1 the plant level UV-B appears to affect growth, morphology and secondary metabolism rather
2 than processes such as photosynthesis (Allen *et al.* 1998; Pancotto *et al.* 2003; Searles *et al.*
3 2001). Modest reductions in herbaceous plant growth are a common impact of UV-B
4 radiation at mid latitudes (Ballare 2003) and have also been shown for a number of species at
5 high latitudes (Robson *et al.* 2003; Rousseaux *et al.* 2001; Searles *et al.* 1999; Searles *et al.*
6 2002). For Antarctic plants, most studies have found little effect on photosynthesis, but
7 growth was affected by exposure to UV-B radiation especially in the two vascular species. In
8 both *Deschampsia antarctica* and *Colobanthus quitensis*, shorter, thicker leaves, reduced
9 branching and fewer leaves per shoot were observed with increasing exposure to UV-B
10 radiation (Day *et al.* 2001; Xiong & Day 2001). In a study of the Antarctic moss *Sanionia*
11 *uncinata*, shoot biomass and length were unaffected but shoots had less branching, after 2
12 years growth under reduced UV-B radiation (Lud *et al.* 2002).

13 Although the accumulation of UV-B absorbing compounds, such as flavonoids, is one of the
14 best documented responses to solar UV-B (Caldwell & Flint 1994), it may not be as
15 widespread in high latitude herbaceous plants. In *D. antarctica* and *C. quitensis*, although
16 UV-B absorbing pigments were higher after 4 months under ambient rather than reduced UV-
17 B radiation, this trend was not sustained in longer term studies (Lud *et al.* 2001; Ruhland &
18 Day 2000; Xiong & Day 2001). Long term studies in Tierra del Fuego have also shown that
19 for the majority of herbaceous species studied, solar UV-B does not regulate the accumulation
20 of UV-absorbing compounds (Giordano *et al.* 2003; Phoenix *et al.* 2002; Rousseaux *et al.*
21 1998; Rousseaux *et al.* 2001).

22 Mosses are generally assumed to have a low capacity to produce flavonoids (Gwynn-Jones *et*
23 *al.* 1999). Two of the five Antarctic bryophytes studied, *Grimmia antarctici* and *Ceratodon*
24 *purpureus*, support this observation, showing low levels of UV-B absorbing compounds and
25 no evidence of stimulation in response to elevated UV-B levels (Dunn 2000; Lovelock &
26 Robinson 2002). However, four Antarctic bryophytes *Bryum pseudotriquetrum*, *S. uncinata*,

1 *Andreaea regularis* and *Cephaloziella varians* showed positive correlations between
2 concentrations of UV-B pigments and levels of natural solar UV-B (Dunn 2000; Lovelock &
3 Robinson 2002; Newsham *et al.* [in press](#); Newsham *et al.* 2002); confirming that some
4 bryophytes can produce screening pigments (Markham & Given 1988).

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5 Studies have also shown that the ratio of total carotenoids to chlorophyll increases in UV-B
6 exposed Antarctic vascular plants and bryophytes (Newsham *et al.* 2002; Ruhland & Day
7 2000; Xiong & Day 2001). In particular, high levels of photoprotective and antioxidant
8 carotenoids have been observed in some Antarctic mosses, and it appears that species with
9 low levels of UV-B absorbing pigments accumulate higher levels of carotenoids, suggesting
10 the possibility that bryophytes may use alternative mechanisms of UV protection (Lovelock &
11 Robinson 2002).

12 The aim of this field study was to investigate the impact of ambient UV-B radiation on the
13 Antarctic endemic bryophyte *G. antarctici*. We predicted that this species might be less
14 tolerant to the increasing UV-B radiation that has resulted from ozone depletion, by virtue of
15 its historically low UV-B environment, and its apparent low levels of UV-B screening
16 pigments (Lovelock & Robinson 2002). The study was performed in the Windmill Islands
17 region of Antarctica where extensive beds of this species are found. We investigated the
18 effect of reduced UV and visible radiation on the pigment concentrations, surface reflectance
19 and physiological and morphological characteristics of the moss. Four screen combinations
20 were used to investigate the impact of both a decrease in ambient UV flux and a 50%
21 decrease in total radiation. The latter were used to separate UV photoprotective from visible
22 photoprotective strategies, since these bryophytes are growing in relatively high light
23 environments compared to many mosses.

1 **Materials and methods**

2 *Study site and experimental treatments*

3 The Windmill Islands region of East Antarctica (66°17'S, 110°32'E), consists of a series of
4 low-lying peninsulas and islands, which become partially ice-free during the summer melt
5 period. The ice-free habitat occurs in a 35 x 15 km area, supporting four bryophyte species
6 including the endemic moss species *Grimmia antarctici* Card. (\approx *Schistidium antarctici*,
7 Card.) used in this study. Moss turves have a distinctive undulating microtopography caused
8 by cryo-perturbation of the moss beds.

9 Screening treatments were established on two peninsulas approximately 20 km apart. One
10 site was directly adjacent to the accommodation building at the Australian Casey Station (Red
11 Shed site, 66.283° S, 110.527° E), while the other site was 20 km west at Robinson Ridge
12 (66.368° S, 110.587° E). These sites were chosen because they support extensive
13 communities of *G. antarctici*, and they differ in moisture availability and exposure. Robinson
14 Ridge is more xeric, with lower water availability, cooler, and greater exposure to both
15 sunlight and wind, compared to the more mesic Red Shed site (Melick & Seppelt 1997). The
16 Red Shed site was directly adjacent to a large summer melt lake and remains moist
17 throughout the summer. The Robinson Ridge site was adjacent to a melt stream that flows
18 early in the summer and provides intermittent water later in the season.

19 At each site, four sets of open sided, treatment screens were applied. The screens (30 x 30
20 cm) were composed of 4 mm plexiglass that either transmitted (near-ambient UV; Plexiglass
21 GS 2458, Plastral Pty Ltd, Sydney, Australia) or attenuated (reduced UV; Plexiglass G 233)
22 UV radiation, in addition 50% shade cloth was also applied to half the screens. The screens
23 transmitted 90% of incident radiation from 400-700 nm. The UV-blocking screens then
24 blocked transmittance of almost all wavelengths below 350 nm, whilst the UV-transmitting
25 screens allowed all UV-B and UV-A wavelengths to pass (cutoff 255 nm; see Fig 1). Mean

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

13 Since most of the water available to these moss beds occurs as melt water, screens had little
14 effect on water availability. Observations over the 14 months of the study showed that snow
15 accumulation under screens was also similar to surrounding areas (Robinson 1999). The
16 absence of sides promoted blowing snow and also air movement thus reducing the potential
17 for fungal contamination (Huiskes *et al.* 2001).

18 Temperatures of the moss turf were assessed over several overcast to full sun days, using an
19 infrared thermometer (Scotchtrack T Heat tracer IR1600L; 3M, Austin, Texas) held 15-20 cm
20 from the surface. Moss temperatures were compared under UV and shade treatment screens,
21 beside the screens and over microtopographic ridges and valleys formed through cycles of
22 freezing and thawing (Kappen *et al.* 1989). As expected, moss under shaded screens and in
23 microtopographic valleys had lower temps than that under clear screens or on
24 microtopographic ridges ($3.5\text{ }^{\circ}\text{C}$ and $1.5\text{ }^{\circ}\text{C}$ respectively) but UV treatment screens did not

1 affect temperature compared with unscreened control turf. Temperature differences between
2 shaded and clear screens were most pronounced on sunny days and least on overcast days.
3 In January 1999, after 14 months growth under the screens, samples (2 cm^2) were collected
4 from separate ridge and valley areas of turf beneath each screen (32 samples for each of the
5 Robinson Ridge and Red shed sites). Additional samples were also collected from ridges and
6 valleys adjacent to each frame in areas which were not impacted by the screens (8 samples per
7 site). Samples were returned to the laboratory at Casey where each sample was split into two
8 with one half used for measurement of surface reflectance and subsequently frozen for
9 pigment analysis (chlorophylls, carotenoids and anthocyanins), whilst the other half was used
10 for measurement of photosynthetic parameters and then oven dried for determination of UV
11 absorbing pigments.

12 *Measuring surface reflectance*

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

22 *Physiological characteristics (photosynthetic parameters and turf water content)*

23 After dark adaptation for 20 mins, the chlorophyll fluorescence parameter F_v/F_m was measured
24 for all samples using a PAM 2000 chlorophyll fluorometer (H. Walz, Effeltrich, Germany).
25 Electron transport rates (ETR) were then determined during the programmed light response

1 curve of the PAM 2000 with a maximum light level of 550 $\mu\text{mol m}^{-2}\text{s}^{-1}$ PAR. The ETR values
2 shown are the maximum achieved during each light response curve, but may not necessarily
3 be the same as maximum light saturated rates.

4 Turf water content (TWC) of these samples was then determined after oven drying at 80 °C to
5 stable weight. Turf water content is expressed as $\text{gH}_2\text{O g}^{-1}$ dry weight as described in Robinson
6 *et al.* (2000). In higher plants, where tissue is bound by an epidermis, water content (WC) of
7 tissues can be expressed relative to the WC at full turgor. Determination of the WC at full
8 turgor is difficult in intact mosses (Fowbert 1996) because mosses store much of their water
9 externally, in capillary spaces, or in the cell wall apoplastic spaces. We have chosen to
10 rename it as TWC to recognise both the inclusion of external water in these measurements
11 and that this is a measure of the water holding capacity of the turf as a whole, rather than
12 individual gametophytes.

13 *Pigment content of moss samples*

14 After the reflectance spectra were measured, the photosynthetically active apices of the moss
15 were removed using a razor blade and the tissue was frozen in liquid nitrogen. Samples were
16 returned to Australia in liquid nitrogen and then stored at -80 °C in a freezer prior to pigment
17 analysis. For chlorophyll and carotenoid determination, samples (50-100 mg fresh weight)
18 were ground with liquid nitrogen and sand in a mortar and pestle and then extracted in 100%
19 acetone, followed by a second extraction in 80% acetone after Dunn *et al.* (2004).

20 Chlorophylls and carotenoids were quantified by HPLC using a method adapted from
21 Gilmore and Yamamoto (1991) as described in Dunn *et al.* (2004).

22 Anthocyanin concentrations were determined using the differential pH method (Francis 1982)
23 as modified by Lovelock and Robinson (2002). UV-B absorbing pigments were extracted in
24 1.5 ml of acidified methanol (methanol- H_2O -HCl; 79:20:1) as described in Lovelock and
25 Robinson (2002).

1 *Morphological measurements*

2 UV-B radiation can affect both plant growth and morphology (Barnes *et al.* 1990). Therefore,
3 gametophyte leaf density and length of photosynthetic tissue were measured and the
4 occurrence of malformed leaves within each sample was noted. In addition, qualitative
5 determinations of gametophyte colour were made during these microscopic inspections.
6 Samples were stored at -20°C prior to measurement and were defrosted and rehydrated in
7 water prior to measurement. Five single gametophytes were randomly selected from each
8 sample for measurement, with juvenile, atypical and/or dead gametophytes excluded from the
9 selection. Measurements were conducted using a binocular microscope (Leica Wild M3C,
10 [Leica Microsystems, Gladesville, NSW, Australia](#)). The mean value of the five gametophytes
11 was used in subsequent analyses.

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

18 *Data analysis*

19 Tests of the influence of UV-B exposure, shading, site and microtopography on reflectance
20 parameters, pigments and photosynthetic and physiological characteristics were performed
21 using analysis of variance (ANOVA; Data Desk 6.1, Data Descriptions, Ithaca, NY, USA).
22 The adequacy of ANOVA models were assessed by inspecting residual plots. To achieve
23 homogeneity of variance and normality the data were transformed as follows; chlorophyll,
24 anthocyanin, chlorophyll a/b, Z/VAZ, TWC (square root); AZ/VAZ (arcsin); R320 (log);
25 R850, (squared); $D1_{RE}$ (cubed) transformation. Results for statistical tests are shown where

1 P<0.1. Morphological parameters were assessed by Chi squared tests and results shown
2 where P<0.05.

3

4 **Results**

5 *Biochemical and physiological responses*

6 **Effect of reduced incident UV radiation.** *Grimmia antarctici* growing under near ambient
7 UV radiation had lower concentrations of total chlorophyll and correspondingly higher
8 relative concentrations of carotenoids than moss growing under reduced UV radiation (Fig 2).
9 For the photoprotective pigments, the relative concentration of β -carotene was higher under
10 near ambient UV radiation (Fig 2C). Whilst the photoprotective zeaxanthin (Z) comprised a
11 larger fraction of the xanthophyll cycle (VAZ) pool in moss exposed to near ambient UV
12 radiation on unshaded ridges (Fig 2D, Table 1; UVscreen*shade interaction, P=0.03). The
13 total carotenoid and neoxanthin concentration relative to chlorophyll also tended to be higher
14 under near ambient UV radiation (Fig 2B, D). However, anthocyanin and UV-B absorbing
15 pigment concentrations were not affected by variation in UV exposure.
16 Despite little change in UV-B absorbing pigments, turf-surface reflectance in the UV region
17 declined under reduced UV radiation, whilst reflectance of green and near infrared (NIR)
18 wavelengths increased compared to moss exposed to near ambient UV radiation (Fig. 3). The
19 $D1_{RE}$ increased by 12.5% ($F_{1,31} = 6.10$, P=0.017) and the REP shifted from 700.09 to 700.79
20 nm when UV radiation was reduced (Fig. 3 inset; $F_{1,31} = 6.10$, P=0.017).
21 None of the physiological characteristics measured (Fv/Fm, ETR and TWC) were altered as a
22 result of reduced UV exposure.

23 **Incident solar radiation.** Shading of moss led to an increase in anthocyanins and UV-B
24 absorbing pigments (Fig 4H, I). In contrast there was a decrease in the xanthophyll cycle

1 pigments relative to chlorophyll and to the proportion of this pool present as antheraxanthin
2 (A) and Z in shaded moss (Fig 4G). Lutein concentration relative to chlorophyll declined
3 4.5% under shade treatments. Often the effects of shading were dependent on the site (Table
4 1; shade*site interactions for Tchl, $P=0.007$; VAZ/Tchl, $P=0.02$; L/Tchl, $P=0.01$) with
5 shading having a more pronounced effect on moss at the Robinson Ridge than at the Red
6 Shed site. The effects of shading were also dependent on both site and topography for several
7 pigments (shade*site*topography interactions; Tcar/Tchl, $P=0.001$; β -carotene/Tchl $P < 0.001$;
8 Z/VAZ; $P=0.006$). These pigments were all highest in moss on unshaded ridges at the
9 Robinson Ridge site than under other screen treatments.

10 Reduction of total radiation through shading of moss had less effect on the individual
11 reflectance parameters although visible reflectance was 10% lower (R_{526} ; $F_{1,31} = 3.35$,
12 $P=0.073$) and the REP was 0.64 nm longer under shaded screens ($F_{1,31} = 5.43$, $P=0.024$).

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

Comment [SR1]: This should be in capitals

15 **Effects of microtopography.** Moss from valleys showed similar, but stronger, responses to
16 those seen for shaded treatments (Fig 4A-F). Total chlorophyll, anthocyanin and UV-B
17 absorbing pigments were all found in higher concentrations in moss growing in valleys than
18 that found on ridges (Fig 4A, E-F). However, carotenoid concentrations were generally
19 higher relative to chlorophyll in ridge moss, particularly the carotenoids involved in
20 photoprotection. The relative size of the VAZ pool, and the proportion present in the
21 photoprotective forms A and Z, were 24% and 42% higher respectively, whilst β -carotene
22 was 113% higher on ridges than in valleys (Fig 4C-D). The relative concentration of lutein
23 was also significantly higher on ridges but the difference was only 7% ($P=0.002$).

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

1 Max ETR and Fv/Fm were higher in valleys than on ridges (Table 2). Shading by screens
2 also increased the TWC of moss growing on ridges compared to that under unshaded screens
3 (shade*topography interaction term; P=0.05).

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

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

15 Turf water content was twice as high at the Red Shed site whilst ETR was greater at the
16 Robinson Ridge site (Table 3).

17 *Morphological characteristics*

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

24 Density of leaves on individual gametophytes was 7% higher in gametophytes growing under
25 near ambient UV compared with those growing under reduced UV radiation (8.83 ± 0.51 and

1 8.23±0.41 leaves mm⁻¹ respectively; $F_{1,31} = 3.82$, $P=0.056$). In addition, leaf density was 60%
2 higher on ridges than in valleys (Table 2). Leaf density was reduced by shading treatments at
3 Robinson Ridge but not at the Red Shed site (Table 1; shade*site interaction, $P=0.026$).
4 The proportion of leaves with blunt-tip morphology decreased from 26% under near ambient
5 UV, to 12% under reduced UV exposure (Plate 1; $P<0.01$). Leaves with atypical morphology
6 were more common on ridges than in valleys (30% compared to 8%, $P<0.0001$) and in moss
7 growing under unshaded rather than shaded screens (26% compared to 11%, $P<0.001$).

8 *Effects of screening moss*

9 Comparison of samples from outside the screened area with samples under the UV
10 transparent screens show enhanced reflectance at shorter wavelengths under screens
11 compared to unscreened moss (30% higher at R320; $P=0.005$ and 16% higher at R526;
12 $P=0.098$). Pigment concentrations were largely unaffected by the presence of screens with
13 the exception of a 40% decrease in anthocyanin concentration under screens ($P=0.08$). The
14 presence of screens affected maximum ETR but not Fv/Fm. Maximum ETR was reduced
15 from 81.5 ± 5.0 to 58.8 ± 5.5 $\mu\text{mol m}^{-2}\text{s}^{-1}$ in moss under screens ($P = 0.014$) although this
16 effect was modified by topography (screen*topography interaction, $P=0.07$) with ETR
17 reduced only for mosses on ridges under screens, whilst moss in valleys had similar ETRs in
18 both screened and unscreened samples.

1 **Discussion**

2 *Reduction in UV-B exposure*

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

9 Chlorophyll concentration increased by 27% in moss grown under reduced UV radiation
10 whilst β -carotene/Tchl. and the proportion of the VAZ pool present as Z decreased by 25%
11 and 21% respectively. Other significant changes to pigments, reflectance parameters or
12 morphology were smaller in magnitude (2-15%). UV radiation therefore had a significant
13 destructive effect on the main light absorbing pigments whilst increasing the relative
14 proportions of pigments involved in photoprotection, such as β -carotene and zeaxanthin.
15 Interestingly, there was no evidence of accumulation of screening pigments in moss exposed
16 to near ambient UV radiation levels. The reflectance data support this, with moss exposed to
17 near ambient UV having higher reflectance in the UV region and lower reflectance in the
18 visible and NIR consistent with reductions in concentration of the dominant chlorophyll
19 pigments. The shift of the REP to longer wavelengths under reduced UV radiation is also
20 indicative of increased absorption by chlorophyll. These results show that protection from
21 UV-B is unlikely to occur through screening or reflectance of UV-B radiation. However,
22 increased levels of antioxidant carotenoids such as β -carotene and Z might ameliorate some of
23 the damage associated with UV-B exposure. Increased ability to scavenge reactive oxygen
24 species has been shown to improve plant tolerance to UV-B radiation (Fujibe *et al.* 2004;
25 Mazza *et al.* 1999; Middleton & Teramura 1993).

1 Increased accumulation of UV-B screening pigments or epicuticular waxes is one of most
2 common effects of UV-B on temperate and tropical plants (Bornman & Vogelmann 1991;
3 Krause *et al.* 2003; Robberecht *et al.* 1980). However, recent studies suggest that
4 accumulation of UV-B absorbing pigments may be a less common response in high latitude
5 plants. Three out of five species studied in a long term experiment in Terra del Fuego,
6 showed no response of UV-B absorbing pigments to UV-B exposure whilst the results for the
7 remaining two species were inconclusive (Giordano *et al.* 2003; Rousseaux *et al.* 1998;
8 Rousseaux *et al.* 2001). In a similar sub arctic study, two out of the three species showed
9 increased UV-B absorbing pigments under reduced UV-B (Phoenix *et al.* 2002). Similarly
10 conflicting results have been observed for vascular plants on the Antarctic peninsula with
11 higher concentrations of UV-B absorbing pigments observed in short term but not in longer
12 term studies (Lud *et al.* 2001; Ruhland & Day 2000; Xiong & Day 2001).

13 Although mosses have been reported as generally lacking inducible UV-B screening pigments
14 (Gehrke 1998; Gehrke 1999; Gwynn-Jones *et al.* 1999; Niemi *et al.* 2002a; Niemi *et al.*
15 2002b; Searles *et al.* 1999) recent studies suggest that many Antarctic mosses do produce
16 UV-B screening pigments in response to natural exposure to UV-B radiation (Dunn 2000;
17 Lovelock & Robinson 2002; Newsham 2003; Newsham *et al.* 2002; Robinson *et al.* 2003).

18 Interestingly, tests of sensitivity to UV-B responses in moss species to date have not involved
19 Antarctic endemics, and we believe that this study of *G. antarctici* represents the first
20 endemic species tested under variable UV radiation.

21 Changes to chlorophyll have been observed in some species but are not a consistent response
22 to natural variations in UV-B exposure, although they have previously been observed in
23 Arctic bryophytes in response to enhanced UV-B radiation (Caldwell *et al.* 2003; Gehrke
24 1999; Searles *et al.* 2001). No change in chlorophyll concentration was observed as a result
25 of seasonal changes in UV-B radiation in either the South American *Sphagnum magellanicum*
26 (Searles *et al.* 2002) or two Antarctic bryophytes studied by Newsham *et al.* (2002). This

1 destruction of pigments under ambient UV radiation is thus unusual and again may indicate
2 that *G. antarctici* is less tolerant of UV exposure than other moss species studied. UV-
3 induced increases in the carotenoid to chlorophyll ratio are however relatively common in
4 Antarctic plants and algae (Newsham 2003; Newsham *et al.* 2002; Post & Larkum 1993;
5 Ruhland & Day 2000).

6 The morphological changes also indicate that UV exposure may be having negative effects on
7 this species. The increased leaf density per gametophyte is likely due to reduced
8 gametophytic growth under ambient UV radiation, producing shorter internodes. Increased
9 leaf density was also observed in *Polytrichum splendens* under elevated UV-B radiation
10 (Gehrke 1999). Reductions in internode length and increases in leaf thickness are highly
11 correlated with reduced growth rates over a wide range of species (Saverimuttu & Westoby
12 1996), and thus it is likely that high levels of UV radiation are reducing growth in *G.*
13 *antarctici*. Similar morphological changes have been shown in response to water and
14 temperature stress in Arctic and Antarctic bryophytes (Dorrepaal *et al.* 2003; Fowbert 1996;
15 Heegard 1997; Wilson 1990). Increased numbers of leaves displaying atypical morphology in
16 the near ambient UV treatment could be the result of UV-B damage to tissues. Growth
17 measurements of Antarctic mosses are extremely difficult due to the slowness of growth
18 (estimate for this species 0.6 mm y^{-1} ; Melick & Seppelt 1997) and long term studies would be
19 needed to confirm any negative impact on growth.

20 Studies of the moss *S. uncinata*, performed over similar periods on the warmer Antarctic
21 peninsula, failed to detect alterations in growth rates by UV-B radiation but similar to this
22 study, moss also showed morphological alterations (Lud *et al.* 2002). Although the height of
23 *S. magellanicum* appeared unaffected by ambient UV-B in the first season of a study in Tierra
24 del Fuego, results after 3 and 6 years showed that growth was reduced by 9%. These studies
25 also demonstrate the need for long term experiments, since interannual variation was high,
26 and significant annual reductions were only observed in one of the six years of the study

1 (Robson *et al.* 2003; Searles *et al.* 1999; Searles *et al.* 2002). Effects of UV-B on plant growth
2 may be evident within shorter time intervals where UV-B is elevated above ambient levels.
3 Reductions in growth were observed in three sub arctic bryophytes grown under supplemental
4 UV-B lamp treatments for 3 years (Gehrke 1998; Gehrke 1999).
5 Similar to other plant species, changes in UV radiation altered pigment composition and
6 morphology of *G. antarctici* while photosynthesis appears to be relatively robust to such
7 changes (Allen *et al.* 1998; Searles *et al.* 2001). With UV-B radiation predicted to remain at
8 elevated levels in the Antarctic, potential reductions in growth rates of *G. antarctici* with
9 higher UV-B, particularly in exposed conditions, could lead to alterations in bryophyte
10 community composition.

11 *Shading and Topographical differences*

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

19 Topography had a significant effect on many of the parameters investigated. This study
20 confirms our previous findings that topographical location is a key factor in determining the
21 physiological and biochemical characteristics of this species (Lovelock & Robinson 2002).
22 Lower chlorophyll and higher protective pigment concentrations on ridges compared to
23 valleys confirm that ridges are stressful environments. However, increased photoprotection on
24 ridges was not accompanied by increases in pigments that potentially screen UV-B, since the

1 levels of anthocyanins and UV-B absorbing pigments were higher in moss growing in valleys
2 than on ridges.

3 Although the changes in chlorophylls and carotenoids may reflect acclimation of
4 photosynthetic pigments to the light levels experienced by the moss, it is possible that these
5 results are in part explained by photooxidation of pigments and could explain the reduction in
6 anthocyanins and chlorophyll on ridges and under clear screens. In sub-arctic shrubs,
7 reductions in UV-B radiation resulted in increased levels of UV-B absorbing compounds
8 (Phoenix *et al.* 2002) which was attributed to either increased secondary metabolism in the
9 absence of UV-B radiation or UV photo-oxidation of these pigments. In this study, higher
10 levels of anthocyanins were observed when UV was reduced (albeit not significant), under
11 shade and most significantly in moss growing in valleys rather than on ridges. Taken together
12 this is supportive of photodestruction of pigments occurring in the higher radiation
13 environments. In addition, given that the shading treatment only increased chlorophyll
14 content by 13% (less than half that observed for the reduced UV treatment) we can be
15 confident that there is a UV radiation component to this photodestruction of photosynthetic
16 pigments.

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

1 *Site effects*

2 Moss growing in the two sites varied considerably in reflectance parameters, pigment content,
3 physiologically and morphologically. We initially chose the sites as representative of xeric
4 (Robinson Ridge) and mesic (Red Shed) habitats. Our results indicate that despite the more
5 xeric habitat at Robinson Ridge, pigment concentrations, ETR and length of photosynthetic
6 tissue were generally greater than at the Red Shed site, and there were also only small
7 differences in parameters associated with stress. The proportion of the VAZ pool present as
8 AZ was higher at Red Shed but it is probable that this reflects the proximity of this site to the
9 lab and therefore a faster freezing of samples after collection. Other photoprotective
10 carotenoids such as β -carotene and the total VAZ pool did not differ between sites, and Fv/Fm
11 did not change. Site strongly influenced the effects of the various screen treatments (Table 1).
12 In particular shading was far more significant a treatment at Robinson Ridge than at the Red
13 Shed, perhaps pointing to a strong effect of exposure at the latter site which was ameliorated
14 by the screens. Moss at the Red Shed site is covered by water for most of the season whereas
15 moss at the Robinson Ridge site is saturated for only a short period during snow melt and has
16 a more seasonal water supply. The increased exposure at the Robinson Ridge site also leads
17 to more frost heaving and hence more pronounced microtopographic differences. The
18 combination of light stress with intermittent water stress could explain the stronger response
19 to shading at Robinson Ridge. Due to its increased exposure and cooler temperatures we had
20 assumed that the Robinson Ridge site is harsher and less favorable for moss growth.
21 However, our results suggest that the Red Shed site is less favorable than previously thought,
22 possibly due to anaerobic conditions when moss is submerged for long periods during the
23 summer. It maybe that when water is available, the Robinson Ridge site is more favorable for
24 growth but that increased exposure at this site increases the variability in this response and
25 increases the probability of microtopograpy influencing treatment responses.

1 **Conclusions**

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

10 Since UV-B absorbing pigments showed variation in response to reduced total radiation (in
11 shaded or valley moss) and were closely correlated with chlorophyll concentrations, this
12 suggests that they may not act as UV specific screens in this species. Other photoprotective
13 pigments would appear to be more important for survival of this endemic species under
14 increased UV-B radiation. Current studies, similar to those described by Rousseaux *et al.*
15 (1999), will investigate the relationship between capacity for UV-screening and
16 photoprotection and DNA damage in this species.

17 Our results show that ambient UV radiation has the potential to adversely affect an endemic,
18 Antarctic bryophyte and confirms that this species has low potential for UV-B
19 photoprotection. Indications of slower growth under UV-B as well as in the more stressful
20 environments (ridges) may suggest lowered competitive advantage under high UV-B,
21 possibly leading to changes in the balance of species in these extreme environments. Since
22 other cosmopolitan mosses that co-occur with this species possess greater potential for UV-B
23 screening and appear to be more desiccation tolerant than this endemic species (Dunn 2000;
24 Lovelock & Robinson; Robinson *et al.* 2000) *G. antarctici* may lose its dominant position
25 within the Windmill bryoflora.

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1 **Table 1.** ANOVA summary table showing significant effects of UV exposure, shading, site
 2 and microtopography on surface reflectance characteristics, pigment concentrations,
 3 physiological and morphological parameters of *Grimmia antarctici*. Interactions are described
 4 in the table footnote. “XX” and “X” indicate significant effect at $p < 0.05$ and $p < 0.10$
 5 respectively.

		UV	Shade	Topography	Site
Reflectance parameters	R ₃₂₀	XX		XX	
	R ₅₂₆ ^C	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 Chl. ^A	XX		XX	XX
	Chl a:b				XX
	Tcar./Tchl. ^E	X	X	XX	
	VAZ/TChl ^A		X	XX	
	%(AZ/VAZ)		XX	XX	XX
	%(Z/VAZ) ^{D,E}	XX	XX	XX	XX
	L/Tchl ^A		XX	XX	X
	N/Tchl	X			XX
B-carotene/Tchl ^E	XX		XX		
Physiological parameter	ETR			XX	XX
	Fv/Fm			XX	
	TWC ^B			X	XX
Morphological parameter	Length of photosynthetic tissue			XX	XX
	Leaf density ^A	XX		XX	XX

6 Interaction terms:

7 ^AShade*site; ^BShade*topography; ^CTopography*site; ^DUVscreen*shade; ^EShade*site*topography.

1 **Table 2.** Comparison of surface reflectance, physiological and morphological parameters for
 2 *Grimmia antarctici* moss growing in ridges or valleys under the screen treatments. Data are mean
 3 values \pm SEM (n=32).

	Ridge	Valley	F	P
Reflectance parameter				
R ₃₂₀	0.0085 \pm 0.0006	0.0066 \pm 0.0004	13.15	0.001
REP (nm)	699.79 \pm 0.216	701.09 \pm 0.192	22.6	<0.0001
Physiological parameters				
ETR max ($\mu\text{mol m}^{-2}\text{s}^{-1}$)	68.42 \pm 3.87	80.91 \pm 4.61	5.24	0.027
Fv/Fm	0.691 \pm 0.004	0.714 \pm 0.004	10.82	0.002
TWC ($\text{g}^{-1}\text{H}_2\text{O g}^{-1}\text{dwt}$, n=26,28)	5.71 \pm 0.62	4.8 \pm 0.73	3.26	0.079
Morphological characteristics				
Length of photosynthetic tissue (mm)	1.95 \pm 0.12	4.05 \pm 0.36	42.15	<0.0001
Leaf density (leaves mm^{-1})	10.6 \pm 0.36	6.52 \pm 0.18	135.34	<0.0001

1 **Table 3.** Comparison of surface reflectance parameters, pigment concentrations,
 2 physiological and morphological parameters for *Grimmia antarctici* moss growing under
 3 screen treatments at the Robinson Ridge and Red Shed sites. Data are mean values \pm SEM
 4 (n=32).

	Robinson Ridge	Redshed	F	P
Reflectance parameter				
R ₅₂₆	0.0305 \pm 0.0014	0.0261 \pm 0.0014	5.64	0.022
R ₈₅₀	0.4097 \pm 0.0158	0.3309 \pm 0.0189	10.48	0.002
D1 _{RE}	0.0092 \pm 0.0004	0.0074 \pm 0.0005	6.66	0.013
Pigments				
Anthocyanins (A ₅₂₆ diff g ⁻¹ fwt)	1.82 \pm 0.20	1.07 \pm 0.20	7.61	0.008
UV-B absorbing pigments (Mean A ₃₂₀₋₂₈₀ g ⁻¹ dwt)	0.187 \pm 0.005	0.157 \pm 0.005	16.068	0.000
Total Chlorophyll (nmol g ⁻¹ fwt)	622 \pm 52	469 \pm 50	4.65	0.036
Chlorophyll a/b	3.32 \pm 0.09	2.98 \pm 0.09	7.86	0.007
AZ/VAZ (%)	24.1 \pm 1.66	31.1 \pm 1.60	19.26	<0.0001
Z/VAZ (%)	21.4 \pm 1.79	23.8 \pm 1.70	6.28	0.016
L/Tchl (mmol mol ⁻¹)	179 \pm 3.17	172 \pm 3.07	3.48	0.069
N/Tchl (mmol mol ⁻¹)	74.1 \pm 2.32	60.8 \pm 2.28	17.51	0.000
Physiological parameters				
ETR max (μ mol m ⁻² s ⁻¹)	88.2 \pm 4.04	61.1 \pm 3.26	25.032	<0.0001
TWC (g ⁻¹ H ₂ O g ⁻¹ dwt, n=28, 26)	3.47 \pm 0.24	7.15 \pm 0.82	29.19	<0.0001
Morphological characteristics				
Length of photosynthetic tissue (mm)	3.61 \pm 0.385	2.39 \pm 0.22	13.58	0.006
Leaf density (leaves mm ⁻¹)	8.09 \pm 0.42	8.97 \pm 0.49	7.7079	0.008

Comment [SR2]: This is italicised incorrectly in the proofs

1 *Figure Legends*

2 **Figure 1.** Transmittance spectra of near-ambient (solid line, Plexiglass GS 2458) or reduced
3 (dotted line, Plexiglass G 233) UV-B screens measured in a spectrophotometer.

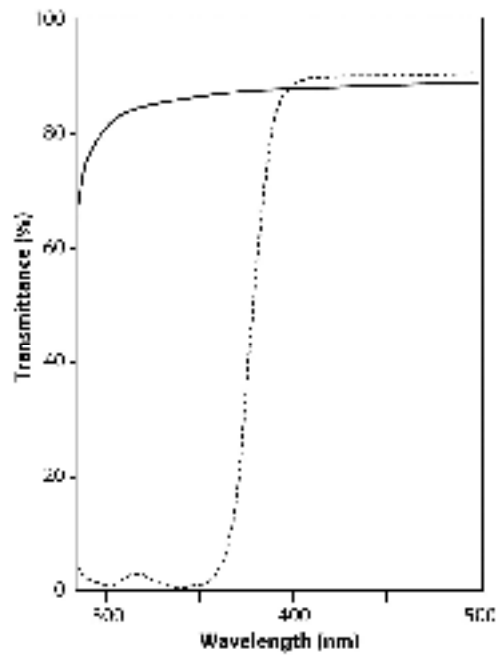
4
5 **Figure 2.** The effect of reducing UV radiation on pigment concentrations of *Grimmia*
6 *antarctica*. Total chlorophyll (A), total carotenoid/chlorophyll (B), β -carotene/chlorophyll
7 (C), neoxanthin/chlorophyll (D) and xanthophyll cycle pigments, violaxanthin,
8 antheraxanthin, zeaxanthin (E). Data are means \pm SEM, n=32.

9
10 **Figure 3.** The effect of reducing UV radiation on reflectance spectra of turf of *Grimmia*
11 *antarctica*. Turf exposed to near-ambient (solid line) or reduced (dotted line) UV radiation.
12 Reflectance 280-850 nm (main graph) and 280-700 nm (inset). Data are means \pm SEM
13 (n=32). Mean reflectance levels are significantly different at R_{320^*} ($P < 0.01^{***}$), R_{526} & R_{850}
14 ($P < 0.05^{**}$), R_{526} ($P = 0.06$).

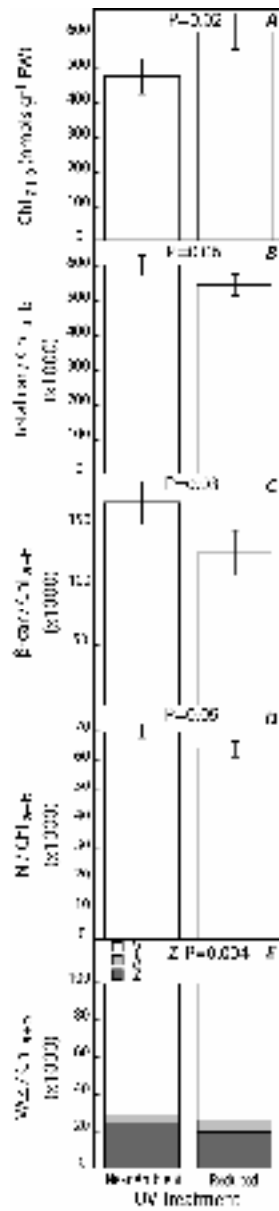
15
16 **Figure 4.** The effect of shading by screens (G-I) or topographical position within the turf (A-
17 F) on pigment concentrations of *Grimmia antarctica*. Total chlorophyll (A), total
18 carotenoid/chlorophyll (B), β -carotene/chlorophyll (C), xanthophyll cycle pigments,
19 violaxanthin, antheraxanthin, zeaxanthin (D, G), anthocyanins (E, H) and UV-B absorbing
20 pigments (F, I). Data are means \pm SEM, n=32.

21
22 **Plate 1.** Comparison of gametophytes of *Grimmia antarctica* showing the normal leaf
23 morphology and green colouration (c-d) and atypical leaf morphology and yellow colouration
24 (a-b), enlarged (e). Atypical leaves were characterized by short length and blunted leaf tips.

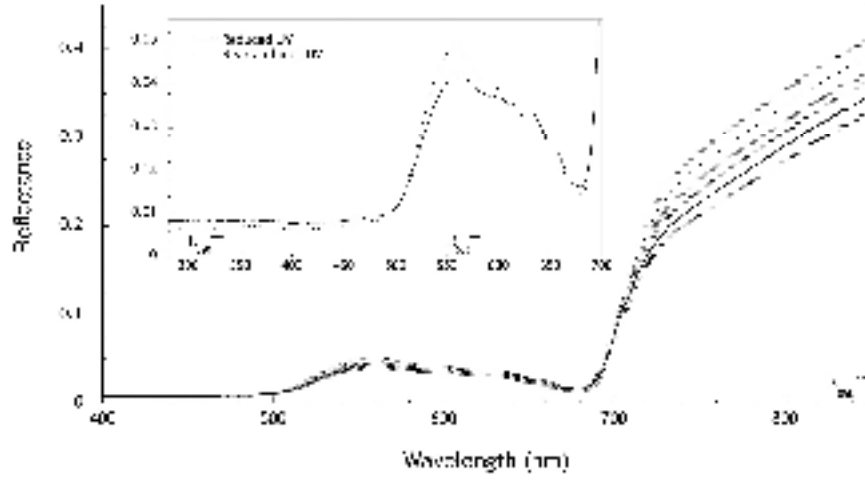
-- Figure 1 --



-- Figure 2 --



-- Figure 3 --



-- Figure 4 --

