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Functional acclimation to solar UV-B radiation in *Gunnera magellanica*, a native plant species of southernmost Patagonia

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ABSTRACT

The ecosystems of Tierra del Fuego (in southern Patagonia, Argentina) are seasonally exposed to elevated levels of ultraviolet-B radiation (UV-B: 280–315 nm), due to the passage of the 'ozone hole' over this region. In the experiments reported in this article the effects of solar UV-B and UV-A (315–400 nm) on two UV-B defence-related processes: the accumulation of protective UV-absorbing compounds and DNA repair, were tested. It was found that the accumulation of UV-absorbing sunscreens in *Gunnera magellanica* leaves was not affected by plant exposure to ambient UV radiation. Photorepair was the predominant mechanism of cyclobutane-pyrimidine dimer (CPD) removal in *G. magellanica*. Plants exposed to solar UV had higher CPD repair capacity under optimal conditions of temperature (25 °C) than plants grown under attenuated UV. There was no measurable repair at 8 °C. The rates of CPD repair in *G. magellanica* plants were modest in comparison with other species and, under equivalent conditions, were about 50% lower than the repair rates of *Arabidopsis thaliana* (L*er* ecotype). Collectively our results suggest that the susceptibility of *G. magellanica* plants to current ambient levels of solar UV-B in southern Patagonia may be related to a low DNA repair capacity.

Key-words: cyclobutane-pyrimidine dimer photorepair; ozone depletion; UV-absorbing compounds; UV-B.

INTRODUCTION

A reduction of the stratospheric ozone layer has taken place over the last two decades in response to emissions of halogen-containing compounds of anthropogenic origin (Madronich *et al*. 1998). The main consequence of stratospheric ozone depletion is an increase in the levels of solar ultraviolet-B radiation (UV-B: 280–315 nm) that reach the Earth's surface (Frederick *et al*. 1994; McKenzie, Connor & Bodeker 1999; Díaz *et al*. 2000). Ozone depletion is particularly severe over the Antarctic continent, where a dynamically isolated air mass cools down to extremely low temperatures during the austral winter, facilitating ozone photo-destruction and leading to the formation of the so-called springtime 'ozone hole' (Solomon 1990). Parcels of ozone depleted air reach the southern part of Patagonia (in southernmost South America), exposing the native ecosystems of the region to elevated levels of UV-B during the period of active vegetation growth (Frederick *et al*. 1994; Kirchhoff, Casiccia & Zamora 1997; Rousseaux *et al*. 1999)

UV-B radiation produces several detrimental effects on plant cells such as damage to proteins, membrane lipids, and DNA (Strid, Chow & Anderson 1994; Jordan 2002). At the whole-plant level, these effects can depress CO_2 assimilation and cause a general reduction of growth and alteration of morphological development (Teramura & Sullivan 1994; Jansen, Gaba & Greenberg 1998). UV radiation also triggers protective responses in plants, which help reduce UV-B penetration into plant tissue, scavenge free radicals, and repair UV-B-induced DNA damage (Rozema *et al*. 1997; Jordan 2002).

Direct effects of solar UV-B on plants from the terrestrial ecosystems of southernmost Patagonia have been detected in field studies carried out in the Tierra del Fuego National Park (20 km west of Ushuaia, Tierra del Fuego, Argentina, latitude 55° S) (for a review, see Ballaré *et al*. 2001). The UV-B component of solar radiation caused measurable DNA damage in the perennial herb *Gunnera magellanica,* and the damage load (CPD density) was well correlated with ambient UV-B levels during the austral spring (Rousseaux *et al*. 1999). *Gunnera magellanica* leaf expansion was consistently reduced by near-ambient solar UV-B when compared with attenuated UV-B (Rousseaux *et al*. 2001). Inhibitory effects of ambient solar UV-B were also found in other herbaceous species in this field location, whereas woody perennials appeared to be mostly unaffected (Ballaré *et al*. 2001; Rousseaux *et al*. 2001; Searles *et al*. 2002). Strong inhibition of shoot growth by ambient UV-B has been reported for the two species of vascular plants (*Deschampsia antarctica* and *Colobanthus quitensis*) that occur along the Antarctic peninsula, across the Drake Passage from Tierra del Fuego, where ozone depletion has been

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more pronounced than over southern Patagonia (Day *et al*. 1999; Xiong & Day 2001; Day, Ruhland & Xiong 2001).

Acclimation responses to UV-B have been studied in some detail in laboratory and indoor-exposure experiments (Jordan 2002; and references therein). However, our understanding of the functional regulation of these protective responses under natural conditions is very limited. One of the best documented adaptive responses to UV-B appears to be the increased accumulation of UV-absorbing compounds in the vacuoles of epidermal cells (Caldwell & Flint 1994; Mazza *et al*. 2000; Searles, Flint & Caldwell 2001). Effects of solar UV-B on epidermal sunscreens have been documented (Robberecht & Caldwell 1986; Meijkamp *et al*. 1999; Mazza *et al*. 2000), although recent field studies with agricultural plants suggest that the solar wavelengths involved in the photoregulation of phenolic sunscreens might be different for different species (Mazza *et al*. 2000; Kolb *et al*. 2001). Previous work in Tierra del Fuego has indicated that for most of the herbaceous species investigated, solar UV-B does not regulate the accumulation of protective UV-absorbing compounds (Rousseaux *et al*. 1998; Barnes *et al*. 2000; Rousseaux *et al*. 2001), although there were some exceptions (Searles *et al*. 2002). The importance of ultraviolet-A radiation (UV-A: 315–400 nm) has not been investigated.

Another defence response that has been studied in several species under controlled environmental conditions is the repair of UV-B-induced DNA damage. UV-B produces two major classes of aberrant photoproducts in DNA: cyclobutane-pyrimidine dimers (CPDs) and pyrimidine (6-4)-pyrimidinone dimers (6-4 photoproducts). Plants repair these photoproducts by two basic mechanisms: photorepair (or photoreactivation) and excision repair (Britt 2002). Photorepair, which is catalysed by product-specific photolyase enzymes, and energized by blue and UV-A photons absorbed by the photolyase chromophores, appears to be the most important mechanism of DNA repair in plants (Taylor *et al*. 1996; Dany *et al*. 2001). Photolyases of 6-4 photoproducts are expressed constitutively in etiolated seedlings (Chen, Mitchell & Britt 1994; Hada *et al*. 1999), whereas the expression of CPD photolyases is frequently regulated by light. Studies in various plant species have shown that this photoregulation may involve phytochromes (Langer & Wellmann 1990; Buchholz, Ehmann & Wellmann 1995), blue/UV-A- (Ahmad *et al*. 1997; Hada *et al*. 1999), visible light- (Ahmad *et al*. 1997; Kang, Hidema & Kumagai 1998), and UV-B-receptors (Pang & Hays 1991; Ries *et al*. 2000). However, there is virtually no information on the photocontrol of photolyase activity under natural conditions, where all these photoreceptors are simultaneously excited.

The native species of Tierra del Fuego provide an opportunity to investigate the physiological impacts of already enhanced levels of solar UV-B on field-grown plants. In the experiments reported in this paper we used the native herb *G. magellanica* to investigate the regulation of sunscreen accumulation and DNA repair capacity by the UV-B and UV-A components of solar radiation under natural conditions. We carried out a manipulative field experiment where we selectively attenuated UV-B or UV-B + UV-A radiation, and tested the effects of these wavelengths on the accumulation of UV-absorbing compounds and the capacity to repair of UV-B-induced DNA damage. Sunscreen accumulation was evaluated using UV absorbance of crude extracts and chlorophyll fluorescence imaging. DNA repair capacity was evaluated by following the kinetics of CPD disappearance under standardized environmental conditions. Because DNA repair is an enzymatic process regulated by temperature, we measured CPD disappearance at two temperatures (8 and 25 °C), which were chosen to represent the range of daytime temperatures experienced by *G. magellanica* during the spring in Tierra del Fuego. Finally, in order to gain perspective on the capacity of *G. magellanica* to repair UV-B-induced damage, we compared the repair kinetics of *G. magellanica* with those of the model plant *Arabidopsis thaliana* in a controlled-environment experiment where both species were grown under identical conditions.

MATERIALS AND METHODS

Field site, experimental design and sampling

The field experiments were established in the Tierra del Fuego National Park (Province of Tierra del Fuego, Argentina, 54°04′ S, 68°35′ W). The plots were located in a plant community dominated by the evergreen shrub *Chiliotrichum diffusum* (Foster f) O. Kuntze. The area is surrounded by a *Nothofagus* spp. deciduous forest and the shrub interspaces are covered by a herbaceous layer, which is dominated by the creeping perennial herb *Gunnera magellanica* Lam., the fern *Blechnum penna-marina* (Poiret) Khun, and a few species of grasses [see Ballaré *et al*. (2001) and Rousseaux *et al*. (2001) for a detailed description of the field site].

At the beginning of the spring of 1999 (October 1999), 21 plots (70 cm × 70 cm) were delimited in open places with abundant cover of *G. magellanica*. The plots were covered with clear plastic filters, which were kept 15 cm above the canopy by means of metal frames. The filters had a very high transmittance in the visible region of the daylight spectrum, and selectively attenuated different wave-bands in the UV region (Fig. 1a). The following materials were used: Aclar polyfluorine film (38 μm thick; type 22 A; Allied Signal, Pottsvile, PA, USA) for the near-ambient UV treatment (control), clear polyester plastic (100 μm thick, Extra Clear Polyester; JCS Plastic, Lemirada, CA, USA) for the reduced UV-B treatment (–UVB), and Lexan sheets (3 mm thick, Lexan MR5; General Electric, Fairfield, CT, USA) for the reduced UV treatment (–UVB/–UVA). The filters of the control and –UVB treatments were perforated to allow the natural rainfall pass through. It proved impractical to perforate the Lexan sheets; therefore, the –UVB/–UVA plots were watered periodically to approximately match natural rainfall. Approximately 20% of the plant-weighted, biologically effective UV-B radiation ($UV\text{-}B_{be}$) (Caldwell 1971),

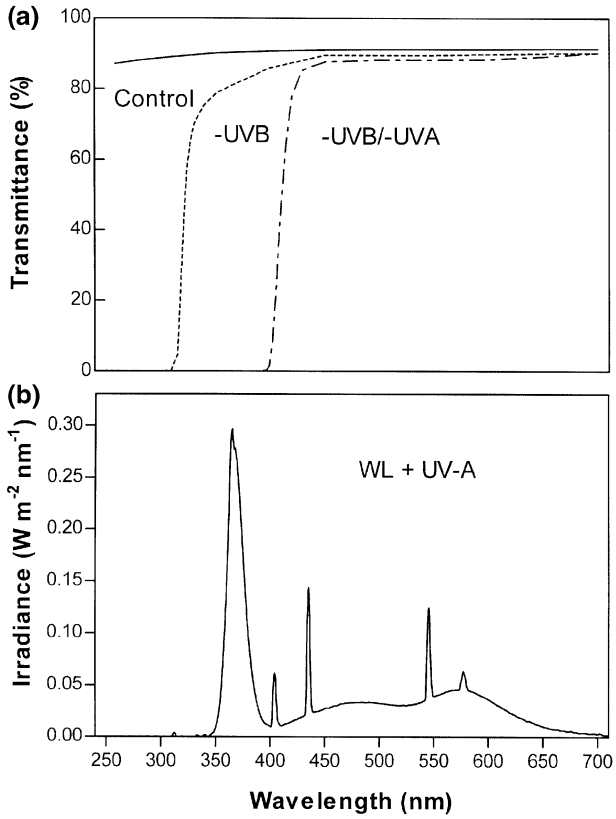

Figure 1. (a), Transmittance spectra of the filters placed over the plots to produce the different UV treatments: (——), Aclar film 38 μm; (- - - -), Mylar film 100 μm; (— - —) Lexan sheet 3 mm. (b), Spectral energy distribution of the light source used for the CPD photorepair experiments (WL + UV-A). WL, white light, 400–700 nm.

present in sunlight passed through the perforated polyester film (–UVB); the perforated Aclar plastic (control) allowed 90% transmission of the solar UV-B_{be} (Searles *et al*. 1999). Previous experiments using similar types of filters in this field site have shown that neither the temperature regime nor soil moisture are differentially affected by the –UVB (polyester) films, compared with the control (Aclar) filters (Searles *et al*. 1999, 2002; Rousseaux *et al*. 2001). The lack of differential effects on temperature was confirmed by our own temperature records (see below).

The experimental layout followed a completely randomized block design. The 21 plots were delimited in seven different areas (blocks) of the field site, and the treatments (control, –UVB, –UVB/–UVA) were assigned at random to the plots inside each block. All the field experiments described in this paper were performed during November and December of 1999. Leaf samples for analyses of phenolics, epidermal transmittance, and CPD repair experiments were always collected around local noon.

Microclimatology and meteorological data

Air temperature records for the nearby city of Ushuaia (20 km to the east of the National Park) were kindly provided by Mr Rodolfo Iturraspe, from the meteorological station of the Centro Austral de Investigaciones Científicas (CADIC). During the experimental period, in the spring, canopy air temperature was measured inside the plots (approximately 10 cm above the soil surface) using custom-made thermocouples shielded by aluminium guards, and attached to a 21x Campbell datalogger (Campbell Scientific Inc., Logan, UT, USA). These records confirmed that there were no significant differences in air temperature among the UV treatments ($P = 0.23$, $n = 54$ d, data not shown).

UV-B levels over Ushuaia were recorded by the National Science Foundation spectroradiometer located at CADIC; UV-B_{be} daily doses [calculated using the generalized plant action spectrum (Caldwell 1971), normalized at 300 nm] were kindly provided by Ing. Susana Díaz. Ozone levels were obtained from the Earth Probe TOMS (Total Ozone Mapping Spectrometer) satellite data, available on line at http://jwocky.gsfc.nasa.gov.

Measurement of UV-B absorbing compounds

Acidified methanol extraction

Leaf discs (0.65 cm diameter; three discs per plot) were placed in 4 mL of 99 : 1 methanol : HCl and extracted for at least 48 h at –20 °C. Absorbance was read at 305 nm (Rousseaux *et al*. 2001).

Epidermal transmittance to UV-B

We estimated the overall transmittance to UV-B of the adaxial epidermis of fully expanded leaves using chlorophyll fluorescence imaging, essentially as described by Mazza *et al*. (2000); UV emission of the lamps ranged between 290 and 320 nm; λ_{max}, 302 nm.

In vivo CPD photorepair experiments

DNA repair experiments

Gunnera magellanica leaves (approximately three-quarters of their final size) were harvested from the experimental plots, placed in plastic boxes on cotton wool saturated with tap water, and transferred from the field site to the laboratory in darkness at low temperature (< 4 °C; shipping time 40 min). Leaves collected from the control (near ambient UV-B) plots had between 30 and 50% more CPD than leaves from the –UVB, –UVB/–UVA treatments (data not shown), which is in agreement with previous results for sampling dates with normal ozone levels (Rousseaux *et al*. 1999 and M. C. Rousseaux, C. V. Giordano and C. L. Ballaré, unpublished results). In the laboratory, the leaves from the different UV treatments (control, –UVB, –UVB/–UVA) were allowed to photorepair for 30 min at 25 °C under photoreactivating light (see below for details). After this period, leaves were given a 20-min UV-B pulse (UV-B_{be} dose: 1.10 kJ m^{-2}) to induce a similar initial level of DNA damage in leaves from the three treatments. Obtaining similar initial damage levels was thought to be impor-

tant, since the initial CPD load may influence the type of DNA repair mechanism used by plant cells, and thereby affect the repair kinetics (Quaite *et al*. 1994). Although it is technically not possible to establish a direct comparison between CPD loads induced by the pre-conditioning UV-B pulse and the steady-state levels of CPD in the field (because these sets of samples were run in different dot-blot experiments and analysed using different quantification protocols) comparisons to common standards indicate that CPD loads after the UV-B pulse approximately doubled those present at the time of harvest in leaves of our control treatment. Therefore, we estimate that the CPD loads induced by the pre-conditioning pulse are roughly representative of those found in *G. magellanica* plants exposed to enhanced UV-B during ozone depletion events, which can increase CPD levels by 50–100% (Rousseaux *et al*. 1999). At the end of the pulse irradiation period, leaves were placed at either 25 or 8 °C to follow the dynamics of CPD repair under photoreactivating light (see below) or in darkness. During all these manipulations the leaves were kept in the plastic containers wrapped with UV-transparent film to preserve leaf water content. The leaves used for the dark repair experiments were kept in plastic containers wrapped with aluminium foil in the same chamber used for the photorepair experiments. Leaves were harvested at different times of the repair period and immediately frozen in liquid nitrogen. Due to restrictions imposed by the size of the repair cabinet, only three field blocks (from the original seven blocks delimited in the field site) were sampled for the repair experiments (i.e. $n = 3$).

For the comparative experiments with *Arabidopsis thaliana* (Landsberg *erecta* ecotype) we used *G. magellanica* cuttings brought from the field site and *A. thaliana* seedlings, which were propagated in a common glasshouse in Buenos Aires before the start of the repair experiments. During the propagation period, average daily temperature and PAR in the glasshouse were 25 °C and 26 mol m^{-2} d^{-1}, respectively. For the DNA repair experiments, leaves were harvested and exposed to a UV-B pulse that provided 0.6130 kJ m^{-2} (*G. magellanica*) or 0.2454 kJ m^{-2} (*Arabidopsis*) of UV-B_{be}; the different UV-B doses were obtaining by varying the irradiation time, and were selected based on preliminary experiments to obtain similar initial CPD loads in leaves of the two species. After this initial irradiation the leaves were subjected to the same repair treatments used for the field samples.

Radiation sources and dosimetry

UV-B pulses to establish initial damage levels for the photorepair assays (both in Ushuaia and Buenos Aires) were applied using six FS20TS UV-B lamps (Solarc Systems Inc., Barrie, Ontario, Canada) placed 20 cm above the leaves. The radiation emitted by the bulbs was filtered through one layer of 100-μm thick cellulose di-acetate film, to filter out UV-C radiation; the filter was changed at the end of each irradiation period. Photoreactivating light (WL + UV-A, Fig. 1b) in the repair cabinet was provided by a panel of two LT 18 W daylight lamps (Narva, Brand-Erbisdorf, Germany) and two TD/L 18 W/08 UV-A lamps (Philips, The Netherlands) suspended approximately 15 cm above the leaves. Irradiance measurements were obtained using an IL-1700 double-monochromator spectroradiometer (International Light, Newburyport, MA, USA) in the UV range, and a microspectroradiometer (S2000; Ocean Optics, Dunedin, FL, USA) in the visible region of the spectrum. The radiometers were calibrated against a standard lamp (OL-40; Optronic, Orlando, FL, USA) in the short-wavelength range and a calibrator for wavelengths >320 nm (Model 1800; Li-Cor Inc., Lincoln, NE, USA). Wavelength accuracy was checked using a germicidal UV-C lamp. UV-B_{be} doses were calculated based on the generalized plant action spectrum (Caldwell 1971) normalized at 300 nm.

Isolation of DNA and CPD detection

DNA extraction and quantification

Frozen leaves (0.15 g) were ground in liquid nitrogen with mortar and pestle. DNA was extracted using a DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA, USA). Concentration of total genomic DNA was determined with Hoeschst 33258 Dye (Amersham Pharmacia Biotech UK Limited, Chalfont St Giles, Bucks., UK). The fluorescence of the DNA-dye complex was measured with a Versa Fluor fluorometer (Bio-Rad Laboratories Inc., Hercules, CA, USA); calf thymus DNA (Sigma, St Louis, MO, USA) was used as a concentration standard.

CPD detection and quantification

DNA samples (typically 1 μg) were alkali-denatured and bound to a positively charged nylon membrane (Zeta Probe; Bio-Rad) using a dot blotter (Bio-Rad). CPDs were detected with TDM-2 monoclonal antibody (Mori *et al*. 1991) and a secondary antibody bound to alkaline phosphatase (Bio-Rad). CDP-Star (Tropix, Bedford, MA, USA) was used as the chemiluminescent substrate and the light signal was detected in a Fluor-S Multi-Imager (Bio-Rad). Quantification of the total emission from each dot (which is linearly related to the content of CPDs in the sample) was performed with the 'Volume' tool of the MULTI ANALYST/PC version 1.1 software, and corrected by local background subtraction. Each sample was spotted in quadruplicate.

Statistical analysis

Differences among UV treatments in accumulation of UV-absorbing compounds and epidermal transmittance to UV-B were determined by means of analysis of variance (ANOVA), using PROC GLM of the SAS package (version 6.12, SAS Institute, Cary, NC, USA). The time courses of CPD repair (data in Figs 3 & 4) were analysed by a repeated measures ANOVA, using PROC GLM. The

univariate approach was used to test the hypothesis of within-subjects effects (time) and interactions between time and the rest of the effects tested. When the sphericity assumption was not met, adjusted *P*-values for the correction factor Huynh–Feldt epsilon were used. In the field experiment, differences among treatments in CPD content at time =0 and in numbers of CPDs photorepaired after 2 h were tested by ANOVA; mean separation was carried out by a Duncan's multiple range test. In the greenhouse experiment, comparisons between *A. thaliana* and *G. magellanica* were performed by a *t*-test.

RESULTS AND DISCUSSION

Previous work in the Tierra del Fuego National Park has shown that present-day levels of solar UV-B can have inhibitory effects on *G. magellanica*, reducing leaf growth in the spring and increasing the load of aberrant CPD photoproducts in DNA (Rousseaux *et al*. 1999, 2001). In the present experiments we have studied acclimation responses elicited by solar UV-B and UV-A in field-grown plants of *G. magellanica*.

Microclimatology and weather characteristics

Records of air temperature obtained at the CADIC meteorological station for the period 1998–2000 (Fig. 2) displayed two salient features: (1) a very high level of day-to-day fluctuation, which reflects the unstable weather of the island; and (2), a relatively low seasonality in the temperature pattern. Mean monthly temperature increased only 2 °C from October to December (from 6 to 8 °C). This is in marked contrast with the pattern observed in northern locations, even those that are under the buffering influence of the Atlantic ocean. For example, in Buenos Aires (34°S), the mean monthly temperature is 6 °C higher in December than in October (FAO 1985).

When the temperature profiles are combined with the UV-B record, it is clear that conditions of relatively high UV-B levels, associated with ozone depletion, can occur in coincidence with low air temperatures (approximately 10 °C) throughout the spring (October to December) (Fig. 2). It may be argued that daytime temperatures are more relevant than mean daily temperatures in terms of their potential influence on enzymatic repair of UV-B-

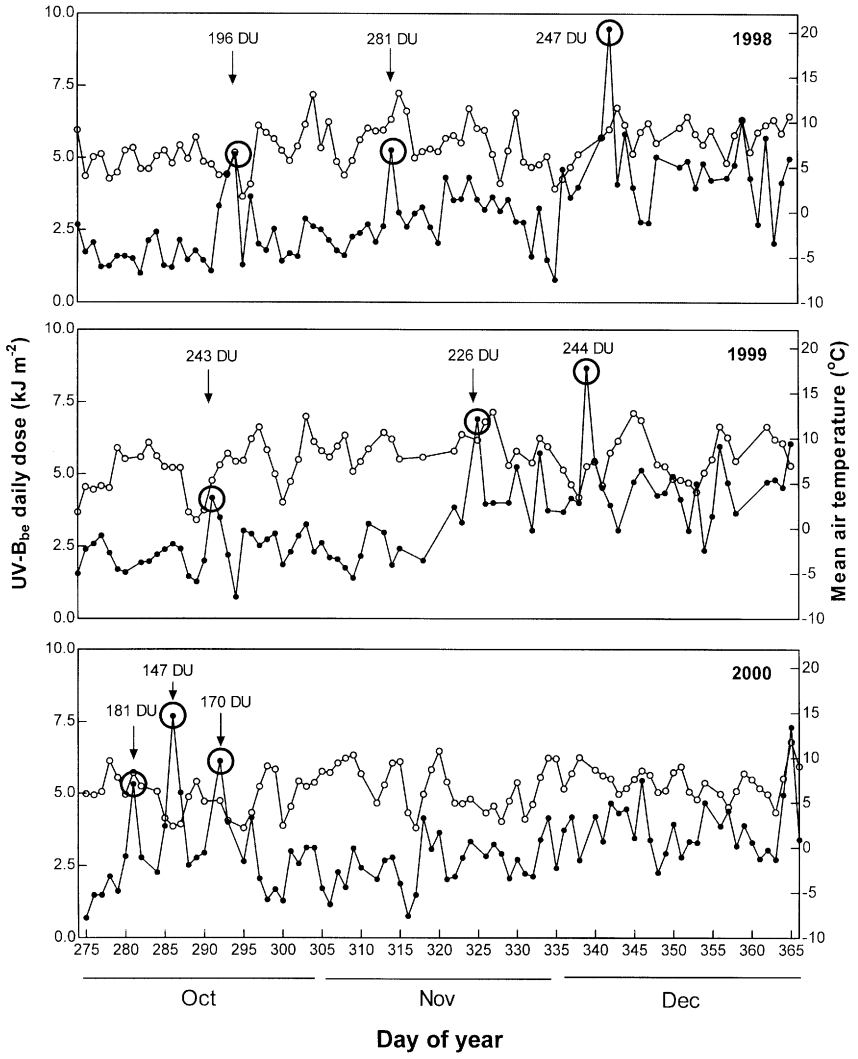

Figure 2. Mean daily air temperatures (○) (CADIC meteorological station data), and daily UV-B_{be} doses over Ushuaia (NSF UV Monitoring Network data) (●), recorded from October to December in 1998, 1999 and 2000. Days of relative high UV-B_{be} doses associated with the passage of the ozone hole, and the corresponding ozone levels (in DU), are indicated in each panel. DU, Dobson unit (1 DU is 2.7×10^{16} ozone molecules cm^{-2}).

induced damage. Using maximum daily temperatures for the months of October, November and December during period 1998–2000, we found that 82% of the days with UV-B_{be} dose >4 kJ m^{-2} d^{-1} had peak temperatures ≤ 15 °C (18% at ≤ 8 °C). Maximum daily temperatures during this period never exceeded 20 °C. It is important to point out, however, that on the microsite scale, the peak daytime temperatures are frequently warmer than at the meteorological shelter. Thus, during the period covered by our plot measurements, daytime maximum temperatures on sunny days were, on average, 8 °C higher at the *G. magellanica* canopy level than at the meteorological station, and canopy air temperatures >20 °C were common around local noon (approximately one out of three days between November and December; data not shown). On cloudy days the difference between canopy and meteorological shelter temperatures was much less pronounced. Several factors contributed to the daytime warming effect on sunny days. The experimental plots were on a north-oriented slope, which received direct sunlight during most of the photoperiod at this southern hemisphere location. Also, the *G. magellanica* patches (canopy height <20 cm) are surrounded by shrubs (canopy height >60 cm), which increase the boundary layer and thereby limit heat transfer between the herbaceous canopy and the cool air above the shrub layer.

Effect of solar UV-B and UV-A on UV-absorbing sunscreens

Work on model plants such as *Arabidopsis*, and several cultivated and native species from temperate regions, has shown that one of the most common effects of UV-B is to increase the accumulation of UV-absorbing phenylpropanoid derivatives (Robberecht & Caldwell 1986; Bornman & Vogelmann 1991; Lois & Buchanan 1994; Tosserams, Pais de Sa & Rozema 1996; Mazza *et al*. 2000; Rozema *et al*. 2002) and epicuticular waxes (Steinmüller & Tevini 1985), which reduce the exposure of sensitive tissue to UV-B photons. In our experiments, no effects of solar UV-B were detected on the accumulation of methanol-soluble, UV-B-absorbing compounds per unit leaf dry mass ($P = 0.63$, Table 1), or per unit leaf area ($P = 0.50$, data not shown). This result is in agreement with the observations of Rousseaux *et al*. (1998, 2001). We were also unable to detect a measurable effect of UV-A attenuation (Table 1), suggesting that UV receptors are not involved in controlling the accumulation of UV-B-absorbing sunscreens in *G. magellanica*. As the absorbance at 305 nm of crude leaf extracts is a very coarse estimate of UV-B protection, and does not take into account the spatial and tissue distribution of UV-B-absorbing compounds, we used chlorophyll fluorescence imaging to obtain more detailed information of UV-B penetration to the photosynthetic leaf parenchyma (Mazza *et al*. 2000). The UV-attenuation treatments did not affect the intensity of chlorophyll fluorescence excited by blue light (RF_b; $P = 0.16$, data not shown). Therefore, the intensity of chlorophyll fluorescence excited by UV-B (RF_{uvb}) was used to estimate epidermal transmittance to UV-B (Mazza *et al*. 2000). Using this approach we were unable to detect any effects of UV-B or UV-B + UV-A attenuation on epidermal screening of the adaxial leaf surface ($P = 0.74$, Table 1), which is directly exposed to solar radiation. Barnes *et al*. (2000), using chlorophyll fluorescence analysis, found that solar UV-B actually appeared to enhance the calculated epidermal transmittance to UV-B in *G. magellanica* (instead of decreasing it). They concluded that this apparent increment in UV epidermal transmittance was the result of a reduction in the intensity of blue/green-excited chlorophyll fluorescence (used as a reference in chlorophyll fluorescence analysis), caused by accumulation of anthocyanins in leaves of the UV-B treatment. Taken together, these results suggest that *G. magellanica*, which constitutively has low epidermal UV transmittance (< 10%, Barnes *et al*. 2000), fails to react to solar UV exposure with induced sunscreen responses that have been well characterized in other plant species of temperate and tropical origin (Searles *et al*. 2001).

Table 1. Accumulation of UV-absorbing compounds in *Gunnera magellanica* leaves from the various UV attenuation treatments. Absorbance data are expressed as absorbance units per milligram of dry mass diluted in 1 ml of extractant. Epidermal transmittance to UV-B was assessed by the quantification of RF_{uvb} (higher RF_{uvb} indicates higher epidermal transmittance to UV-B) (Mazza et al. 2000). SE indicates the standard error derived from the ANOVA ($n = 7$ blocks)

Treatment	Absorbance at 305 nm mg^{-1}	RF_{uvb} (mean counts)
Control	11.70	355
–UVB	12.02	318
–UVB/–UVA	11.80	324
SE	0.11	23.35
Treatment effect, *P*	0.63	0.74

Environmental regulation of DNA repair capacity

A critical component of the mechanisms that allow plants to tolerate UV-B is the ability to repair UV-B-induced DNA damage. Photorepair, catalysed by product-specific photolyases, is thought to be the predominant mechanism of CPD repair in plants (Taylor *et al*. 1996; Dany *et al*. 2001). We studied the impacts of temperature and solar UV attenuation on CPD repair in *G. magellanica*. Leaves collected in the experimental field plots were allowed to repair DNA under controlled conditions, after pre-treatments that generated similar initial levels of CPDs. Repair experiments were carried out at 8 and 25 °C, to cover a significant fraction of the range of temperatures that *G. magellanica* plants may experience during the spring in their natural environment (see above).

At 25 °C, and under WL + UV-A, the CPD repair kinetics measured in leaves collected from the different UV-attenuation treatments were distinctly different (Fig. 3a, open symbols). The overall analysis of CPD repair over time indicated a significant time × treatment effect

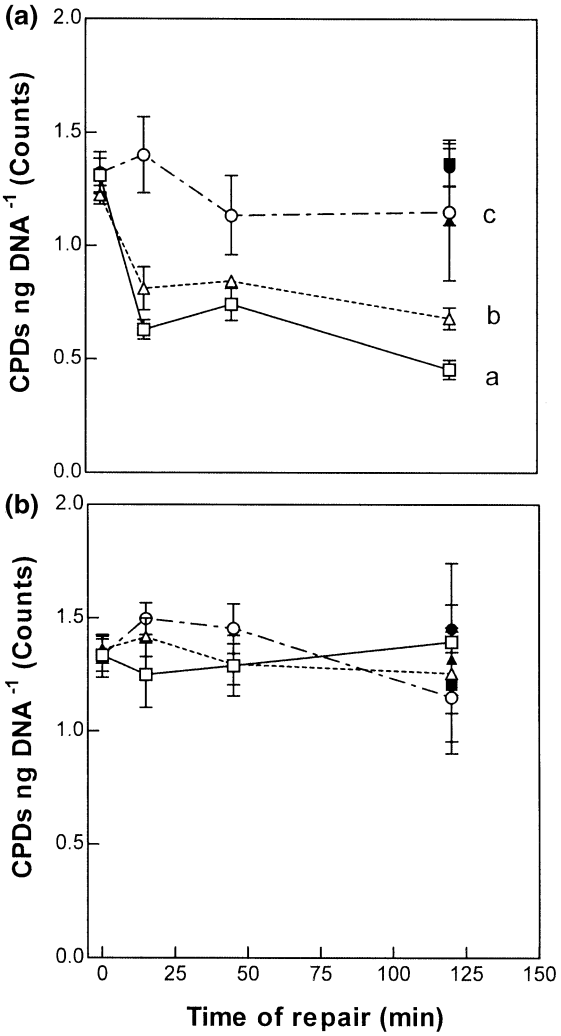

Figure 3. Time course of CPD repair in *G. magellanica* leaves collected from the near-ambient UV (control, squares), –UVB (triangles) and –UVB/–UVA (circles) treatments and incubated under standard conditions of temperature [(a), 25 °C and (b) 8 °C] and irradiation (open symbols: WL + UV-A; closed symbols: darkness). A repeated measures ANOVA indicated a significant time × treatment effect ($P < 0.0001$) at 25 °C, and no time ($P = 0.26$) and no treatment effect ($P = 0.96$) at 8 °C. In both panels, initial CPD content did not differ among treatments (ANOVA, $P = 0.45$ at 25 °C; $P = 0.44$ at 8 °C). In (a), different letters indicate significant differences between UV-attenuation treatments in the number of CPD repaired after 2 h of incubation under WL + UV-A ($P < 0.05$), according to Duncan's test. CPDs are expressed as chemiluminescent counts per ng DNA, adjusted by local background subtraction (see Materials and methods for details). Vertical bars are standard error of the means.

($P < 0.0001$). Comparison of numbers of CPDs removed in the 2 h time period indicated that plants from the near-ambient UV treatment had the fastest rates of CPD repair. No changes in CPD numbers were detected in plants incubated for 2 h in complete darkness (Fig. 3, closed symbols), suggesting that photorepair is the principal type of CPD repair in *G. magellanica*.

At 8 °C, there was no detectable DNA repair, regardless of origin of the leaves (i.e. UV attenuation treatment) or repair conditions (WL + UV-A or darkness) (Fig. 3b; no time and no treatment effect in a repeated measures ANOVA).

There is relatively little information on temperature effects on photorepair rates in higher plants, and none of this information comes from studies with field-grown plants. *In vivo* CPD photorepair in *Arabidopsis* plants grown in a growth chamber declined between 22 and 37 °C (Pang & Hays 1991). In etiolated cotyledons of cucumber seedlings, artificially irradiated with UV-B, CPD photorepair was maximal at 25 to 30 °C, and declined at lower (15–20 °C) or higher (35 °C) temperatures (Takeuchi *et al.* 1996). In suspension-cultured tobacco cells, a high CPD removal was maintained at 24 and 12 °C, but no CPD photorepair was detected at 0 °C (Li, Paulsson & Björn 2002). Our experiments revealed a marked temperature dependency in *G. magellanica*, which is likely to be ecologically significant. The combination of low daily mean temperatures and relatively high UV-B levels is not an uncommon occurrence during the first half of the growing season (October to December), particularly when the site is directly under the influence of the Antarctic ozone hole. Under such conditions, the very limited CPD repair capacity might result in rapid accumulation of DNA lesions in *G. magellanica* tissues.

The expression of CPD photolyase activity has been shown to be regulated by light of different wavelengths in controlled-environment studies with seedlings of various cultivated species (Langer & Wellmann 1990; Pang & Hays 1991; Buchholz *et al.* 1995; Ahmad *et al.* 1997; Kang *et al.* 1998; Hada *et al.* 1999; Ries *et al.* 2000). However, the effects of solar UV-B had not been investigated hitherto. Our results indicated that exposure to solar UV increases the ability of the leaf cells to photorepair CPDs at optimal temperature (25 °C) (Fig. 3a). Interestingly, attenuating the UV-B component alone, which had a negligible impact on the quantum flux density received by the plants (less than 0.6% attenuation of the total UV + visible flux), reduced the CPD photorepair capacity to a measurable extent (Fig. 3a). This result suggests that the regulation of photolyase activity in *G. magellanica* is influenced by the shortest wavelengths of the solar spectrum. In this regard the photoregulation of DNA photorepair capacity in *G. magellanica* appears to stand in marked contrast with the regulation of methanol-soluble, UV-absorbing sunscreens in this species, which is not affected by exposure to solar UV-B (Rousseaux *et al.* 2001; Table 1, this paper).

Although no comparative surveys are available in the case of terrestrial plants, variation appears to exist in DNA repair capacity among animal species that live in contrasting UV environments (Blaustein *et al.* 1994; Malloy *et al.* 1997; Smith, Kapron & Berrill 2000). Even under optimal conditions (25 °C, WL + UV-A), the CPD repair rates displayed by *G. magellanica* leaves appeared to be modest in comparison with published repair kinetics for other species (Pang & Hays 1991; Quaite *et al.* 1994; Sutherland *et al.*

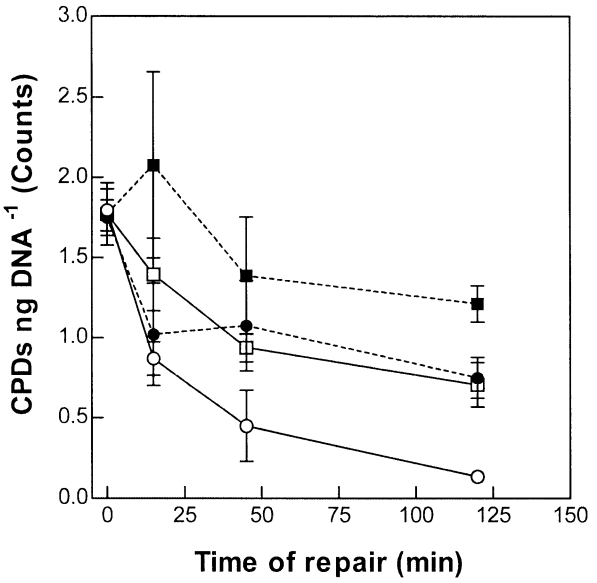

Figure 4. Time course of CPD repair in leaves of *G. magellanica* (squares) and *A. thaliana* (circles) collected from plants grown in a greenhouse in Buenos Aires and incubated under standard conditions of temperature (25 °C) and irradiation (open symbols: WL + UV-A; closed symbols: darkness). Repeated measures ANOVA indicated a significant time × repair treatment (WL + UV-A or darkness) effect ($P = 0.05$) and time ×species effect ($P = 0.01$, Huynh–Feldt epsilon correction). The amount of CPDs at the beginning of the experiment did not differ between species (*t*-test, $P = 0.91$). After 2 h of incubation, *A. thaliana* repaired more CPDs than *G. magellanica*, both under WL + UV-A and in darkness ($P < 0.05$). Vertical bars are standard errors of the mean.

1996; Taylor *et al*. 1996; Takeuchi *et al*. 1996; Hidema *et al*. 1997), particularly when the results from experiments with field-grown plants are included in the comparison (Takayanagi *et al*. 1994; Kang *et al*. 1998). To obtain a valid comparison under equivalent environmental conditions, we carried out parallel repair experiments with *G. magellanica* and *Arabidopsis* leaves. The results indicated that CPD photorepair was much faster in *Arabidopsis* than in *G. magellanica*, both in darkness and under photoreactivating light (Fig. 4). The leaves from greenhouse-grown *G. magellanica* plants used in this experiment appeared to have a higher dark repair rate than those collected directly form the field site in Tierra del Fuego. This is likely to reflect differences in physiological status that influence DNA repair or synthesis of new DNA.

CONCLUSIONS

Taken collectively, our results suggest that *G. magellanica* is not particularly well equipped to respond to the current springtime levels of UV-B in Tierra del Fuego, which is consistent with the observation that present-day ambient UV-B has growth inhibitory effects in this species (Rousseaux *et al*. 1998, 2001). The lack of UV-induced screening responses (Table 1), the limited DNA repair capacity (Fig. 4), and the modest acclimation response induced by UV-B exposure (Fig. 3a), suggest that increments of UV-B levels (caused by ozone reduction) would produce mainly damaging effects in *G. magellanica*, without sufficient enhancement of protective mechanisms. Under the current UV climate, the $UV\text{-}B_{be}$ dose at ground level can increase by a factor of three in response to springtime ozone depletion (Rousseaux *et al*. 1999). The low DNA repair capacity, particularly at low temperatures (Fig. 3b), may be one of the reasons why the midday CPD load in naturally occurring plants of *G. magellanica* was linearly correlated with the pre-midday UV-B dose (Rousseaux *et al*. 1999). Growth-inhibitory effects of ambient UV-B have been detected in nearly all the herbaceous species of the Tierra del Fuego National Park (Ballaré *et al*. 2001), and also in the two species of vascular plants that occur in the Antarctic peninsula (Day *et al*. 2001). It would be interesting to find out whether a low DNA repair capacity is a general feature of all the species of this high-latitude region, which have evolved under low levels of ambient UV-B.

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