

Responses to solar ultraviolet-B radiation in a shrub-dominated natural ecosystem of Tierra del Fuego (southern Argentina)

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Abstract

A study was made of the effects of solar ultraviolet-B radiation (UV-B) on the growth of the dominant plant species of a shrub-dominated ecosystem in Tierra del Fuego. This part of southern Argentina can be under the direct influence of the Antarctic 'ozone hole' during the austral spring and lingering ozone-depleted air during the summer. The plant community is dominated by an evergreen shrub (*Chilotrimum diffusum*) with an herbaceous layer of *Gunnera magellanica* and *Blechnum penna-marina* in the interspaces between the shrubs. Inspections of ozone trends indicate that the springtime and summertime ozone column over Tierra del Fuego has decreased by 10–13% from 1978/9 to 1998/9. In a set of well-replicated field plots, solar UV-B was reduced to approximately 15–20% of the ambient UV-B using plastic films. Polyester films were used to attenuate UV-B radiation and UV-transparent films (~90% UV-B transmission) were used as control. Treatments were imposed during the growing season beginning in 1996 and continued for three complete growing seasons. Stem elongation of the shrub *C. diffusum* was not affected by UV-B attenuation in any of the three seasons studied. However, frond length of *B. penna-marina* under attenuated UV-B was significantly greater than that under near-ambient UV-B in all three seasons. Attenuation of solar UV-B also promoted the expansion of *G. magellanica* leaves in two of the growing seasons. Differences between treatments in leaf or frond length in *B. penna-marina* and *G. magellanica* did not exceed 12%. Another significant effect of UV-B attenuation was a promotion of insect herbivory in *G. magellanica*, with a 25–75% increase in the leaf area consumed. Changes in plant phenology or relative species cover were not detected within the time frame of this study. The results suggest that the increase in UV-B radiation associated with the erosion of the ozone layer might be affecting the functioning of this ecosystem to some degree, particularly by inhibiting the growth of some plant species and by altering plant–insect interactions.

Keywords: *Blechnum penna-marina*, *Chilotrimum diffusum*, *Gunnera magellanica*, herbivory, ozone hole, UV-B

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Introduction

The study of the biological effects of ultraviolet-B radiation (UV-B; 280–315 nm) has attracted considerable attention during the last two decades because the thinning of the stratospheric ozone layer leads to

elevated solar UV-B at ground level (e.g. Frederick *et al.* 1994; Bojkov *et al.* 1995; McKenzie *et al.* 1999). Ozone depletion is mainly a result of the interaction between reactive chemical species derived from human-produced halocarbons and ozone molecules in the stratosphere (WMO 1998; Stolarski 1999). Because some of the critical reactions that lead to ozone depletion occur at the surface

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of polar stratospheric clouds, which form under the extreme cold temperatures of the Antarctic stratosphere during the winter and spring, ozone depletion is particularly pronounced at high latitudes in the Southern Hemisphere (Solomon 1990; Madronich *et al.* 1998; Newman 1999).

The springtime Antarctic ozone 'hole' was first documented in the early 1980s (Farman *et al.* 1985). According to satellite data, which are available for the last two decades, the area of the ozone hole has increased from $\sim 2 \times 10^6$ km² in the early 80s to $\sim 25 \times 10^6$ km² in the late 90s (Newman 1999). Consequently, the area of influence of the hole now includes the southern tip of South America. The ozone hole typically has an elongated shape, centred about the South Pole, that slowly rotates clockwise. Such rotation results in quasi-periodic passages of extremely low ozone amounts over sites on the edge of the hole, such as the Antarctic peninsula and the southern tip of South America. Therefore, the thickness of the springtime ozone layer above southern Argentina and Chile can fluctuate between its normal level (around 330 DU) to almost half of this value within a time interval of less than two days (Bojkov *et al.* 1995; Kirchhoff *et al.* 1997; Rousseaux *et al.* 1999).

From an ecological standpoint, the fluctuations in ozone and UV-B levels that occur during the spring and summer are particularly important. This is because in mid- and high-latitude ecosystems, biological activity is concentrated within a short growing season in the warmer months of the year. The historical, baseline UV-B level during the growing season, as well as the increase in UV-B caused by ozone depletion, are higher in the Southern Hemisphere than at similar latitudes in the Northern Hemisphere. There are factors other than ozone depletion that contribute to the higher effective UV-B levels in southern mid-latitudes: a closer Earth-Sun distance during the summer, lower tropospheric aerosol loading and pollution, and a period of ozone depletion that extends into the summer months (Madronich *et al.* 1998; McKenzie *et al.* 1999). A recent study has reported a 12% increase in peak summertime (erythemal) UV radiation received in Lauder, New Zealand, for the period 1988/89–1998/99, which showed a good correspondence with the predictions based on the observed trends in stratospheric ozone levels for the region (McKenzie *et al.* 1999).

Field studies with plants from agricultural and other ecosystems managed by humans have shown that solar UV-B or solar UV-B enhanced with UV-B from lamp systems can reduce the growth and economic yield of certain species (e.g. Sullivan & Teramura 1992; Sullivan *et al.* 1994; Ballaré *et al.* 1996; Mepsted *et al.* 1996; Saile-Mark & Tevini 1997; Mazza *et al.* 1999a). In a number of

other cases, however, no growth responses to ambient or enhanced UV-B have been detected (Kim *et al.* 1996; Allen *et al.* 1999; Norton *et al.* 1999; Papadopoulos *et al.* 2000). Regarding natural ecosystems, reductions in the growth of native plants in response to UV-B have been documented in field and garden studies that used either UV-B attenuation in areas of the globe that are already affected by ozone depletion (Rousseaux *et al.* 1998; Day *et al.* 1999), or UV-B supplementation (Johanson *et al.* 1995; Musil 1995; Gehrke 1998, 1999). The most dramatic effects of solar UV-B have been observed in the studies carried out by Day *et al.* (1999) in the Antarctic peninsula with the grass *Deschampsia antarctica*, where UV-B exclusion resulted in a 55% increase in leaf number and a 32% increase in average leaf length. In other field studies at high latitudes, the magnitude of the effects of UV-B manipulations (supplementation or exclusion) on the growth of vascular plants has been much less pronounced (Johanson *et al.* 1995; Tosserams *et al.* 1996; Gwynn-Jones *et al.* 1997; Weih *et al.* 1998; Mendez *et al.* 1999; Searles *et al.* 1999). In some cases the response to UV-B manipulations in a given ecosystem varied among species. For example, in a dwarf shrub community near Abisko, Sweden, the relative stem growth of *Vaccinium vitis-idaea* and *Empetrum hermafroditum* was reduced by enhanced UV-B, whereas other shrub components of the ecosystem were apparently not affected (*V. myrtillus* and *V. uliginosum*) (Johanson *et al.* 1995). At least part of the variation in the response that is apparent among the field studies may be related to the relatively subtle nature of the effects of UV-B on plant growth and the high natural variability that is inherent to ecological experiments. These factors combine to generate a low signal-to-noise ratio in the dataset that makes it difficult to draw unequivocal conclusions, particularly if the statistical power of the experiment is limited by the intensity of replication, which is commonly low in field studies with natural systems.

UV-B can affect not only plants but also organisms of other trophic levels, including insects, pathogens, decomposers, and symbiotic organisms (reviewed by Paul *et al.* 1997, 1999; Ballaré *et al.* 1999; van de Staaij *et al.* 1999). In Tierra del Fuego, attenuating solar UV-B has been shown to increase the number of lesions inflicted by chewing insect larvae on leaves of *Gunnera magellanica* (Rousseaux *et al.* 1998). This effect might have direct consequences on the growth rate of this species, if the increase in the intensity of herbivory reduces the photosynthetic leaf area. The observation of increased insect herbivory under filters that exclude the UV-B component of sunlight is consistent with the results obtained in UV-B filtration experiments in cultivated plant communities (Ballaré *et al.* 1996; Mazza *et al.* 1999a). Similarly, other workers have reported reduced

herbivory or insect densities under enhanced UV-B conditions in the field (Gwynn-Jones *et al.* 1997; Salt *et al.* 1998), although in at least one plant species of the Swedish Lapland (*Vaccinium myrtillus*), UV-B supplementation resulted in increased herbivory (Gwynn-Jones *et al.* 1997). Moreover, increased leaf consumption in leaves exposed to UV-B in a controlled-environment experiment was reported by Lavola *et al.* (1998), who studied the impacts of UV-B irradiation on silver birch consumption by winter moth larvae. Lindroth *et al.* (2000) also reported variability in the effects of UV-B on herbivory, in this case between populations of white clover. The fact that solar UV-B can affect individual species components of the same plant community differently may have consequences for plant competitive balance and community composition.

The present paper reports the results of a three-year UV-B-attenuation study carried out in a shrub-dominated ecosystem located in the Tierra del Fuego National Park (southern Argentina). During this period large and well replicated field plots were used to quantify the effects of solar UV-B attenuation on the growth, sunscreen accumulation, phenology, and herbivory levels in three principal plant components of this ecosystem: the shrub *Chiliodendron diffusum*, and the herbaceous perennials *Gunnera magellanica*, and *Blechnum penna-marina*.

Materials and methods

Experimental site and ozone trends

The experiments were established in a natural field site in the Tierra del Fuego National Park (Province of Tierra del Fuego, Argentina, 54°4' 23' S, 68°35' 77' W), approximately 20 km to the west of the city of Ushuaia. The principal vegetation zones of Tierra del Fuego are the Patagonian steppe, the deciduous forest, the evergreen forest, and the Magellanic moorland (see Moore 1983). The National Park includes mostly deciduous forest (dominated by *Nothofagus pumilio* and *N. antarctica*), some evergreen forest (dominated by *Nothofagus betuloides*), bogs dominated by *Sphagnum magellanicum*, and scrub communities. The experimental plots are located in a scrub community (total plant cover ~100%), dominated by the evergreen shrub *Chiliodendron diffusum* (Forster f.) O. Kuntze ('mata negra') (Fig. 1). The site is surrounded by a *Nothofagus* spp. deciduous forest and there are also several small, scattered individuals of *N. antarctica* and *Empetrum rubrum* between the plots that cover ~10% of the area. The interspaces between *C. diffusum* shrubs are dominated by a perennial herb, *Gunnera magellanica* Lam. ('Frutilla del Diablo', Haloragaceae), and by the fern

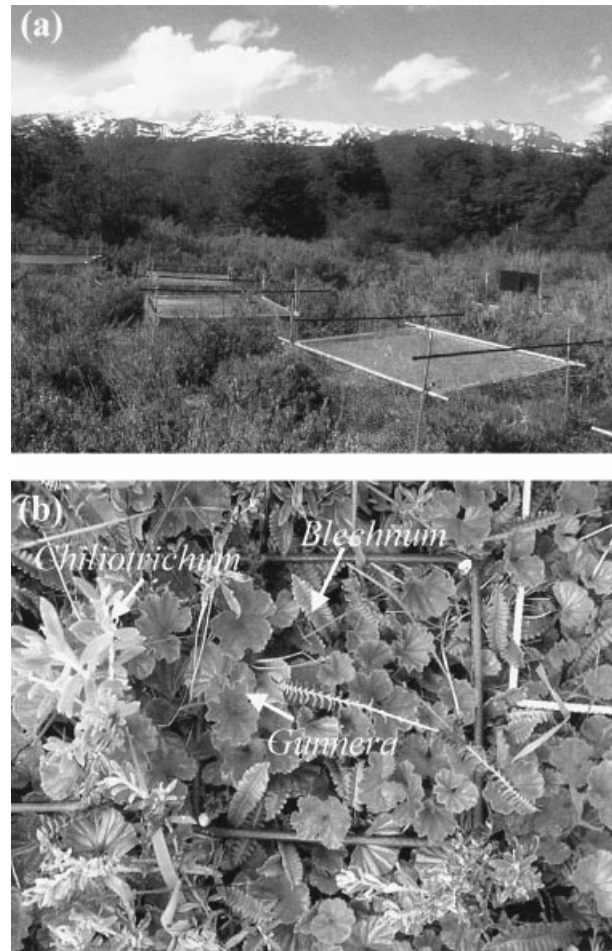


Fig. 1 Overview of the field site in Tierra del Fuego (a) and a detail of the herbaceous layer in the spaces between shrubs (b).

Blechnum penna-marina (Poiret) Kuhn along with a few species of grasses (Fig. 1). The study site receives an annual precipitation of ~520 mm; overcast skies are frequent all year, and precipitation during the growing season can be of virtually any type from light rain to heavy snowstorms. Mean annual temperature is 5.5°C. Tierra del Fuego can be under the direct influence of the Antarctic ozone hole during the spring. Ozone data reported in this paper were obtained from the NASA total ozone mapping spectrometer (TOMS) (<http://jwocky.gsfc.nasa.gov/ozone/ozone01.html>; "Overpass Data" for the city of Ushuaia).

Experimental setup and treatments

A randomized complete block design with 10 replicate plots of 1.2 × 1.2 m was used for each treatment. The plots were located in interspaces between large *C. diffusum* shrubs and included patches of *G. magellanica* and *B. penna-marina* as well as small *C. diffusum* plants.

Two treatments were applied to plots: attenuated UV-B and near-ambient UV-B. Solar UV-B was attenuated (uvb-) using clear polyester film (0.10 mm, optically equivalent to 'Mylar-D' films, Dupont Co., Wilmington, DE, USA). The near-ambient UV-B (uvb+) treatment used Aclar type 22 A plastic films (0.038 mm, Honeywell; formerly Allied Signal, Pottsville, PA, USA), which allow near-ambient UV to pass. Filters were installed in the spring (October) and removed in the autumn of each year (mid-March). Precipitation was allowed to pass through the filters by perforating them using an electric hot-wire melter that formed a perpendicular pattern of louvers (louver size: 2 × 30-mm, spaced at 10 mm) (Searles *et al.* 1999). In this paper data are reported for three consecutive growing seasons, from the beginning of the experiment in October 1996 until March 1999.

The spectral climate under the films was determined using a SUV 100 scanning, double-monochromator spectroradiometer (Biospherical Instruments, San Diego, CA) located on the roof of the Centro Austral de Investigaciones Científicas (for detailed description of the methodology see Searles *et al.* 1999). These measurements were conducted using filters of the same size and shape as those used in the field. The distance between the sensor and the filter was the same as the height of the filter over the vegetation in the experimental plots. Between 15 and 20% of the biologically effective UV-B radiation (weighted by plant action spectrum; Caldwell 1971) passed through the perforated polyester, while ~ 90% was transmitted through the Aclar film (Searles *et al.* 1999 and Searles, unpubl. results). The effectiveness of UV attenuation in the field was corroborated using a broad band UV sensor (YMT, Interscience Technologies, Silver Spring, MD). Transmission of solar radiation within UV-A waveband was 50% at 330 nm and 80% at 380 nm for the perforated polyester, and nearly 90% for the perforated Aclar plastic. Both plastics transmitted nearly 90% of the photosynthetic photon flux density. This figure was corroborated in the experimental plots using a quantum sensor (Li-Cor Inc., Lincoln, NE). When plastic filters were damaged by wind we replaced them within hours; individual filters never remained in the field for periods longer than two months. Measurements of UV transmission of the used plastics revealed no significant shifts in the optical properties of either the polyester or the Aclar films under our experimental conditions.

Air temperatures under the plastic films were not substantially different from the temperatures recorded at equivalent heights outside the filters (for details see Searles *et al.* 1999). Monthly wind speeds average 5 m s⁻¹ during the spring and summer in Ushuaia, which reduces the potential effects of the filters on air temperature.

In order to test the effects of the filters on water regime, the soil volumetric water content of the upper 15 cm of the soil profile was measured using time-domain reflectometry (TDR; Trase System I, Soil Moisture Equipment Corp.) in three locations inside and outside each plot in three periods: January 1997, November 1997, and January 1998. No differences were found either between filter types in any of these periods ($P=0.75$, $P=0.84$, $P=0.25$, respectively; $n=10$), or between filter types when the water content of the top 5 cm of the soil was measured gravimetrically in November 1997 ($P=0.72$). Further TDR measurements taken by other research group members during the 1998–99 and 1999–2000 growing seasons (using 10-cm and 15-cm deep probes) have confirmed these results (Verónica Pancotto, IFEVA, pers. comm. 2000). The soil water content inside the plots was 14.5% and 12% lower than outside ($P < 0.05$; $n=20$) in November 1997 and January 1998, respectively. The opposite situation (soil under the filters slightly moister than outside) was observed in January 1997.

A central quadrat of 0.8 × 0.8 m was delimited within each plot in which all the plant measurements and samplings were concentrated in order to reduce the influence of edge effects on the response variables measured.

Plant morphology

Plant morphological measurements were taken in January and March in each plot. In general there was very little plant growth between January and March; therefore, all the morphological data reported in this paper are from the January measurements. For each plot, leaf radii of 40 randomly selected leaves of *G. magellanica*, frond lengths on 40 randomly selected fronds of *B. penna-marina*, and lengths of new stems on 20 randomly selected branches of *C. diffusum*, were measured to the nearest millimetre. Measurements were performed on fully expanded leaves with no signs of insect herbivory. Randomization was obtained by generating random coordinates in a frame that had a 12 × 12-cm grid. Specific leaf mass (SLM) was determined using five leaf disks (0.5 cm diameter) in the case of *G. magellanica*, five pinnae in the case of *B. penna-marina*, and three leaves in the case of *C. diffusum* from each plot. Dry mass was determined after keeping the samples for at least 3 days at 70 °C.

Methanol extractable-phenolics

Levels of crude phenolics were determined on the same samples used to estimate SLM after extraction in 3 mL 99:1 methanol:HCL for at least 48 h at -20 °C (Beggs & Wellmann 1985). Absorbance was measured at 290, 305,

310, 330, 360, 450, and 530 nm. Samples for SLM and phenolic determinations were taken at the end of January. During the first season absorbance was measured at 305 nm only.

Reproductive phenology

The total number of reproductive *B. penna-marina* fronds was counted in the central 0.8×0.8 -m quadrat of each plot in mid-January, during the second and third growing seasons. *Gunnera magellanica* is a dioecious plant that tends to form clonal, single-gender patches. Sixty percent of the permanent plots had female plants and the remaining 40% were exclusively male. Because male flowers are conspicuous and their phenological development can be followed easily without disturbing the plants, an additional set of 20 (10 polyester + 10 Aclar) plastic filters was installed over small plots (0.7×0.7 m) containing patches of male plants at the beginning of October 1996 to study the effects of UV-B on flowering phenology. The number of flowers was counted every 2–5 days during the first season and the data were corrected taking into account the local leaf cover of *G. magellanica* in each plot.

Plant cover

Three 0.15×0.15 m subplots with high *G. magellanica* and *B. penna-marina* cover were identified inside each permanent plot. Plant aerial cover was measured during the summer (January) in each of these subplots (second and third growing seasons). Pictures from each subplot were taken with a digital camera (DL300, Olympus America, Melville, NY), the lens of which was held nearly parallel to the soil surface; direct sunlight impinging on the plots was avoided by using shade screens. The percentage areas of each subplot covered with *G. magellanica* or *B. penna-marina* were calculated using Adobe Photoshop 4.0. All the leaves of the target species present in the subplot picture were traced, and the areas of the leaves and of the total subplot were calculated using the number of pixels as the measurement unit. The number of pixels was then transformed into units of area using the dimensions of the subplot ($15 \text{ cm} \times 15 \text{ cm}$) as a reference.

Herbivory on Gunnera magellanica

The same 15×15 -cm subplots described above were used to estimate the degree of herbivory on *G. magellanica* leaves. In the field, the silhouette of the area removed by chewing insects from all *G. magellanica* leaves present in the subplot was drawn on sheets of clear acetate, and later quantified in the laboratory using an area-meter (LI-

3000, Li-Cor Inc., Lincoln, NE). The leaf area consumed was expressed relative to the total leaf area of *G. magellanica* for each individual subplot. The measurements of herbivory were taken in mid-summer (January) during the second and third growing seasons.

Statistical analysis

Ozone trends over the period 1978/9–1998/9 were assessed using the significance of the slope of the linear regression (total ozone vs. time, for October, December and February); a similar analysis for November, January and March yielded similar results (data not shown). Differences between UV-B treatments were analysed using analysis of variance in a randomized complete block design ($n = 10$ independent blocks). Transformation of data was applied when normality or homocedasticity assumptions were not met.

Results

Ozone trends over Ushuaia

During the last 20 years a diminishing stratospheric ozone layer has been documented on a global basis. Over Tierra del Fuego, this trend has been accentuated relative to similar high latitudes in the Northern hemisphere, because the springtime Antarctic ozone hole frequently passes directly overhead and ozone-depleted air lingers after the ozone hole has dissipated in the late spring and summer. Inspection of the TOMS ozone data available for the period 1978/9–1998/9 shows a downward trend for the month of October (Fig. 2a), when the Antarctic ozone hole reaches its peak size. The large interannual variability reflects the fact that the ozone levels prevailing over Ushuaia in October are highly dependent on the overall size and shape of the Antarctic vortex, which contains the ozone hole. Interestingly, a clear and highly significant downward trend for column ozone over Ushuaia is also evident for the summer months (Fig. 2b,c), even though the ozone levels over the Antarctic continent itself increase dramatically from October to February, as the polar stratosphere warms to relatively high temperatures and the ozone hole breaks up. This is likely attributed to ozone-depleted air that lingers and migrates northward after the vortex has dissipated. The scattering of the actual ozone data around the fitted linear model is much lower in late summer than in early spring (Fig. 2c,cf. Figure 2a), because in the summer, the day-to-day variation of the ozone hole is not a factor. It is noteworthy that the slope of the change in ozone column over the years is similar for the spring and summer months.

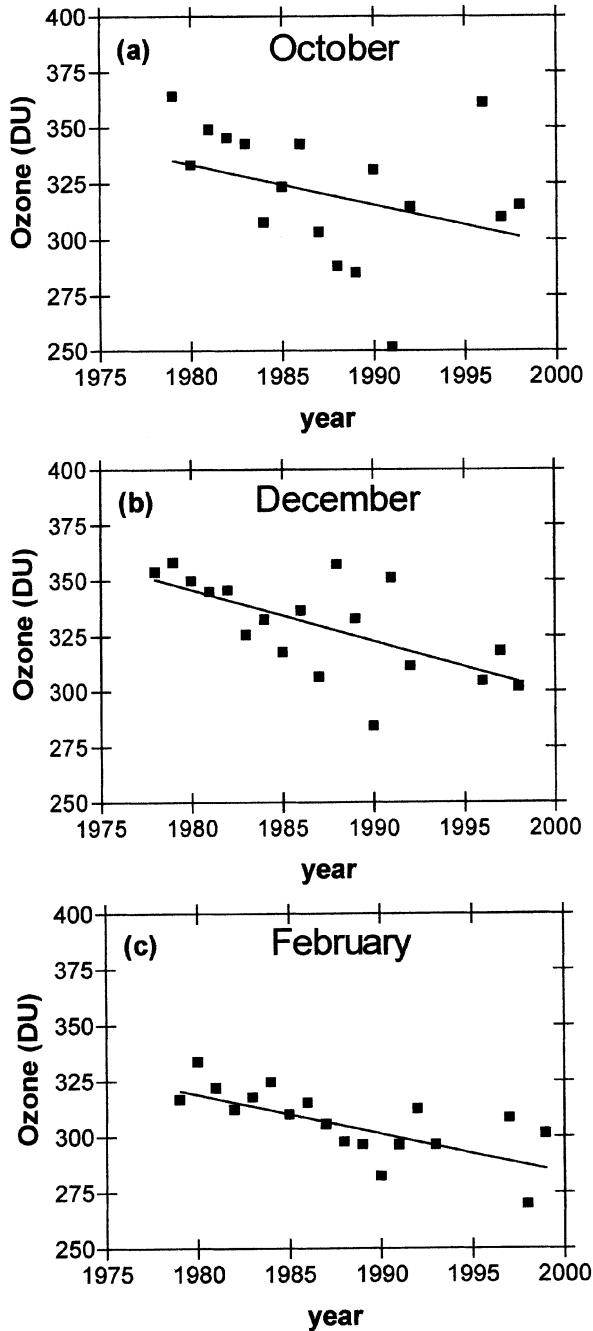


Fig. 2 Total ozone monthly averages over Ushuaia, Argentina, for the period 1978/9–1998/9; missing data in the graph indicate that no records are available in the TOMS database (see <http://jwocjy.gsfc.nasa.gov>). The thickness of the ozone layer is measured in Dobson units (DU).

Plant morphological responses to UV-B attenuation

The effect of UV-B attenuation on plant growth varied among plant species. Although stem elongation in *C. diffusum* was not affected by UV-B in any of the three seasons studied (Fig. 3a), frond length in *B. penna-marina*

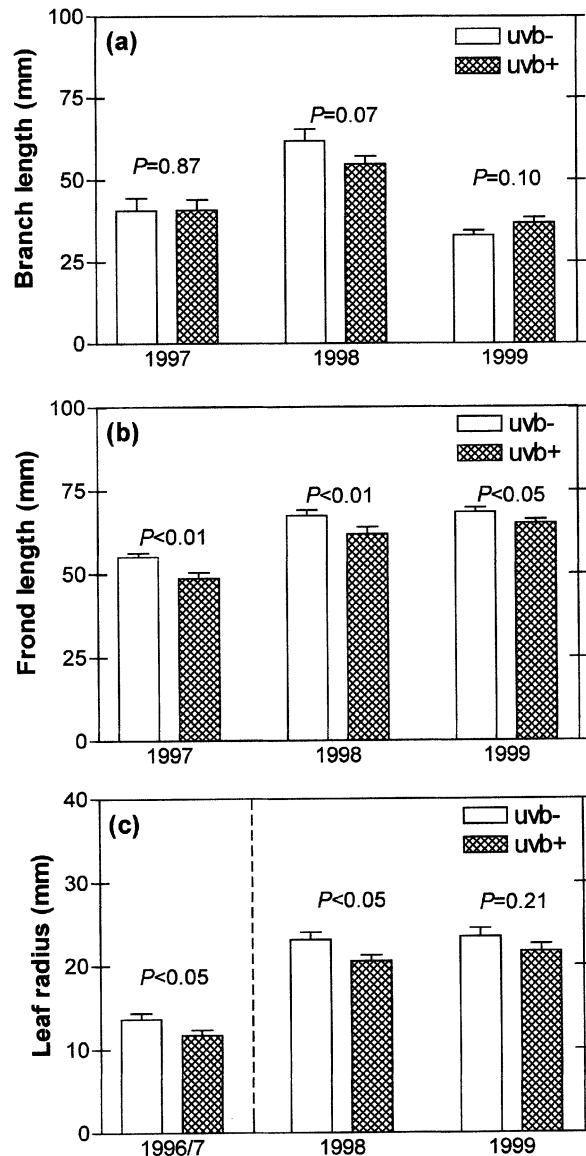


Fig. 3 Effects of solar UV-B on (a) stem length of *Chilotrichum diffusum*, (b) frond length of *Blechnum penna-marina*, and (c) leaf radius of *Gunnera magellanica* for the three seasons of this study. Measurements were performed in mid-summer on 40 randomly selected leaves (*G. magellanica* and *B. penna-marina*) or 20 randomly selected branches (*C. diffusum*) per plot. Bars represent the average ($n = 10$ independent plots) and thin lines indicate the standard error of the mean. The P -value corresponds to the ANOVA performed for each species and year. The UV-B effect on *G. magellanica* leaf expansion during the first season was studied in a different set of plots; see Rousseau *et al.* (1998). UV-B significantly affected the leaf growth of *G. magellanica* ($P < 0.05$) and *B. penna-marina* ($P < 0.01$) over the three-year period (results of a repeated measurements analysis), but not the branch length of *C. diffusum* ($P = 0.48$).

was significantly greater in plots receiving attenuated UV-B than in the controls that received near-ambient UV-B, and the difference was consistent over the three

Table 1 Effects of UV-B level on specific leaf mass (SLM, g m^{-2}) and absorbance at 305 nm of methanol-extractable phenolic compounds for the three dominant plant species of the scrub community. Values represent the average ($n=10$) and the numbers in italics the standard error of the mean. No significant differences between treatments for SLM were found for any year in any plant species. Treatment differences in methanol-extractable UV-absorbing compounds were only observed during the first season in *C. diffusum* leaf extracts. Determinations were done during January. Absorbance data are expressed as absorbance units per mg of dry mass diluted in 1 mL of extractant

		<i>C. diffusum</i>			<i>B. penna-marina</i>			<i>G. magellanica</i>		
		1997	1998	1999	1997	1998	1999	1997	1998	1999
SLM (g m^{-2})	uvb-	74.6	67.4	74.3	69.9	51.4	58.6	32.1	26.0	27.2
		<i>3.6</i>	<i>3.0</i>	<i>3.9</i>	<i>2.6</i>	<i>5.5</i>	<i>1.7</i>	<i>0.9</i>	<i>1.1</i>	<i>0.6</i>
	uvb+	79.0	69.9	75.0	70.4	57.6	61.1	32.0	26.2	26.7
		<i>4.3</i>	<i>2.7</i>	<i>2.4</i>	<i>1.6</i>	<i>1.3</i>	<i>1.9</i>	<i>0.9</i>	<i>0.7</i>	<i>0.5</i>
Absorbance at 305 nm mg^{-1}	uvb-	10.5	10.5	15.8	9.0	12.2	12.0	6.8	10.0	11.4
		<i>0.6</i>	<i>0.8</i>	<i>0.9</i>	<i>0.6</i>	<i>1.0</i>	<i>0.5</i>	<i>0.3</i>	<i>0.5</i>	<i>0.4</i>
	uvb+	15.0	10.5	14.5	8.4	11.7	11.7	6.5	9.2	11.6
		<i>1.3</i>	<i>0.7</i>	<i>0.8</i>	<i>0.2</i>	<i>0.4</i>	<i>0.1</i>	<i>0.1</i>	<i>0.7</i>	<i>0.5</i>

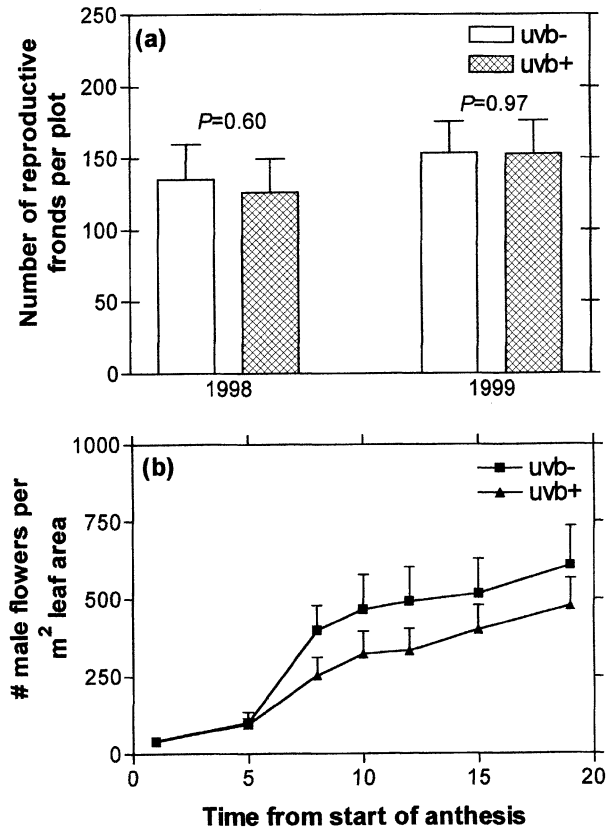


Fig. 4 Effects of solar UV-B on reproductive variables. (a) Number of reproductive fronds of *Blechnum penna-marina* measured in January of the second (1998) and third (1999) growing seasons. Bars represent the average and the thin lines the standard error of the mean ($n=10$). (b) Number of *Gunnera magellanica* male flowers (corrected by *G. magellanica* cover) produced during the 20 days after the beginning of anthesis. Measurements were performed during the spring of the first growing season (1996–7) counting all the flowers present in small (0.70×0.70 m) plots ($n=10$).

seasons (Fig. 3b). UV-B attenuation also increased leaf size in *G. magellanica* during the first and second seasons. Although no significant differences could be detected during the third season, the direction of the UV-B effect was the same as in previous years (Fig. 3c). The average effect of UV-B attenuation on leaf or frond size in *B. penna-marina* and *G. magellanica* was about 12%. Solar UV-B did not affect SLM in any of the species studied (Table 1). However, differences in SLM among species were found, with leaves of *G. magellanica* having roughly about half the SLM of those of *B. penna-marina* and *C. diffusum* (Table 1).

Effects of UV-B on protective sunscreen compounds

Levels of methanol-extractable UV-absorbing compounds in the leaves of all three species were measured during the middle of the summer in each of the three field seasons. With the exception of *C. diffusum* (first season), UV-B-absorbing compounds were not influenced by the attenuation of solar UV-B (Table 1). Also, there were no differences between UV-B treatments at wavelengths other than 305 nm (data not shown). Differences among species were apparent, and the absorbance at 305 nm (per mg and ml) in *C. diffusum* leaves was ~25% higher than in *B. penna-marina* pinnae, and ~45% higher than in *G. magellanica* leaves.

Effects of UV-B on reproductive phenology

Of the three dominant plant species present in the main plots, it was only possible to study reproductive phenology of the fern, as few reproductive shrubs were included under the filters and not all the blocks had *G. magellanica* plants of the same sex. No differences were

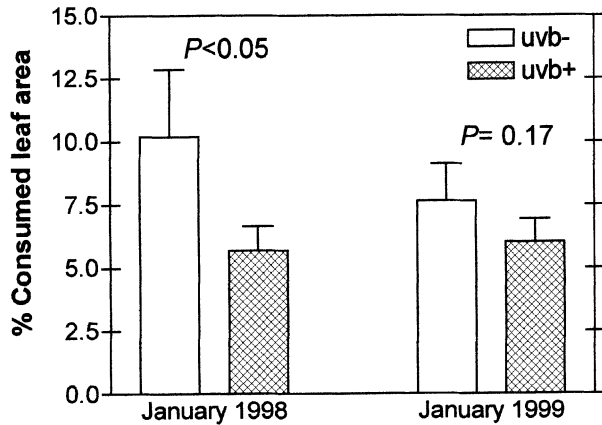


Fig. 5 Effects of solar UV-B on leaf herbivory in *Gunnera magellanica*. The surface area removed by chewing insects was measured in January of the second and third seasons for leaves grown under uvb- and uvb+ treatments. Measurements were carried out in small (0.15 × 0.15 m) subplots that had high *G. magellanica* cover; for details, see Materials and methods.

found between near-ambient UV-B and attenuated UV-B treatments on the number of reproductive fronds during the two seasons analysed (1997–98 and 1998–99; Fig. 4a). The production of *G. magellanica* male flowers was studied in a set of ancillary plots laid out in 1996–97 (see Materials and Methods); again it was not possible to detect differences between UV-B treatments in the total number of flowers ($P = 0.34$) nor in the time required to reach 75% anthesis ($P = 0.40$; Fig. 4b).

Effects of UV-B on herbivory and plant cover

Solar UV-B attenuation increased the intensity of leaf herbivory on *G. magellanica* (Fig. 5). During the 1997–98 growing season, phytophagous insects consumed 75% more *G. magellanica* leaf area under the attenuated-UV-B treatment than in the control plots under near-ambient UV-B. During the 1998–99 growing season, differences between treatments were less marked (Fig. 5).

In mid-summer (January), each of the herbaceous species covered between 20 and 35% of the ground area in the mini-plots used to study herbivory and canopy structure. It was not possible to detect a consistent effect of solar UV-B attenuation on changes in percentage cover by *G. magellanica* or *B. penna-marina* during the time frame of our study (Fig. 6).

Discussion

The attenuation of solar UV-B radiation by filters or enhancement of solar UV-B using UV-B fluorescent lamp systems are the two most commonly used approaches to study the effects of UV-B in field experiments evaluating

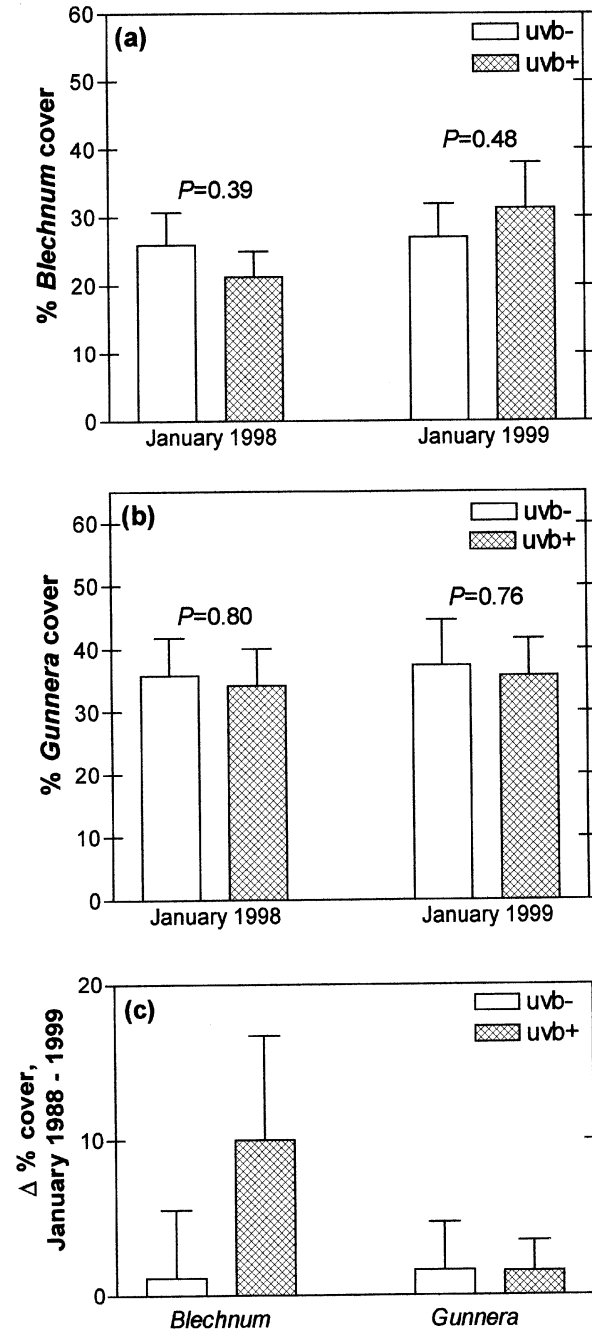


Fig. 6 Percentage of ground area covered in mid-summer by *Blechnum penna-marina* (a) and *Gunnera magellanica* (b) in the interspaces between *C. diffusum* shrubs, and changes in percentage cover between the second and third growing seasons of the experiment (c). Measurements were carried out on the same small (0.15 × 0.15 m) subplots used for the herbivory determinations; for details, see Materials and methods.

the influence of ozone reduction. The attenuation of solar UV-B using filters is particularly useful in regions already exposed to enhanced UV-B resulting from

stratospheric ozone depletion, such as Antarctica and southern South America. A clear reduction in the thickness of the ozone column over Ushuaia has been observed since TOMS measurements began in 1978/9 (Fig. 2). This ozone reduction is evident for every month of the growing season (October to February), indicating that even after the dissipation of the Antarctic ozone hole in late spring, ozone-depleted air remains over the region throughout the summer months. On the basis of TOMS ozone data and local UV-B measurements for 1997, Rousseaux *et al.* (1999) have shown that, despite frequent cloud cover during the spring, there was a significant inverse correlation between the daily ozone levels over Ushuaia and the doses of biologically effective UV-B received at the ground surface. Filters were used here to attenuate UV-B in order to explore the effect of the current, enhanced UV-B levels, and this paper is the first to report on the impacts of a long-term (3-year) UV-B attenuation experiment on a natural shrubland ecosystem of southern South America.

Solar UV-B (near-ambient level) reduced foliage growth in *B. penna-marina* and *G. magellanica* by as much as 12%, in comparison with the UV-B attenuation treatment (Fig. 3). Reductions of growth caused by UV-B in native species of high-latitude ecosystems have been documented in other studies that used either solar UV-B attenuation (Rousseaux *et al.* 1998; Day *et al.* 1999) or UV-B enhancement treatments (Johanson *et al.* 1995; Gehrke 1998, 1999). The magnitude of the effects of UV-B measured in the attenuation experiments herein were smaller than those observed in other experiments using lamps enhancing the solar UV-B to simulate an ozone depletion of $\approx 15\%$ on clear days. For example, results of these UV-B-enhancement studies in high-latitude natural ecosystems include: 15% decrease in leaf growth of *Vaccinium myrtillus*, 27% and 33% reduction in stem growth of *V. vitis-idaea* and *Empetrum* sp., respectively (Johanson *et al.* 1995), and 25% decrease in *Sphagnum fuscum* height (Gehrke 1998). These UV-B lamp systems that do not adjust themselves with cloud cover can yield very high UV-B: photosynthetic-photon-flux density ratios under overcast conditions (McLeod 1997). Under these conditions an overestimation of the UV-B effect could be expected. The effects of UV-B attenuation on leaf growth found in the present experiment were smaller than those observed in Antarctica, where Day *et al.* (1999) reported a 32% increase in *Deschampsia antarctica* leaf length and a 55% increase in leaf number in response to UV-B exclusion. However, ozone depletion has been less severe in Tierra del Fuego than in Antarctica, where a $\sim 50\%$ reduction in ozone column has taken place during the last two decades for the month of October (Madronich *et al.* 1998; WMO 1998; data available in: <http://jwocky.gsfc.nasa.gov/ozone/ozone01.html>).

Therefore, a smaller effect of attenuating ambient UV-B is to be expected in Tierra del Fuego than in Antarctica.

Solar UV-B causes measurable levels of DNA damage in naturally occurring plants of *G. magellanica* (Rousseaux *et al.* 1999), one of the species included in the present study. The steady-state levels of DNA damage measured at mid-day in the field correlated well with the UV-B dose received at ground level during the morning hours, and the level of DNA damage was greatly reduced under the filters that attenuated solar UV-B (Rousseaux *et al.* 1999). Increased DNA damage may have been responsible for the detrimental effects of solar UV-B on growth. For example, studies with *Arabidopsis* mutants that are deficient in DNA repair have shown clearly that the growth of these mutants is extremely sensitive to UV-B radiation (Jiang *et al.* 1997; Landry *et al.* 1997). Moreover, other field studies that reported deleterious effects of solar UV-B radiation on plant productivity have also found that these effects were accompanied by increased levels of DNA damage (Ballaré *et al.* 1996; Mazza *et al.* 1999a). However, it is important to bear in mind that the growth-inhibiting effects of solar UV-B may be triggered by an array of primary events and signals, which may include damage to DNA and also direct damage to proteins, indirect effects of elevated levels of reactive oxygen species, excitation of specific UV-B receptors, etc.

Not all the species of the scrub ecosystem exhibited growth reduction in response to solar UV-B. Shoots of the dominant shrub (*C. diffusum*) did not respond to solar UV-B attenuation with changes in elongation rate (Fig. 3a). Variability in the growth and morphological responses to UV-B among species has been observed previously (Barnes *et al.* 1990; Musil 1995; Cybulski & Peterjohn 1999), even among different species of the same genus (Johanson *et al.* 1995). Differences in the response to UV-B among plant species could be the result of variations in the mechanisms that allow plants to reduce or tolerate the damage inflicted by UV-B. One such protective mechanism consists of minimising the penetration of UV-B radiation. The presence of leaf hairs (Karabourniotis *et al.* 1992), a high content of epidermal UV-B screening compounds (Day 1993; Li *et al.* 1993; Lois & Buchanan 1994; Mazza *et al.* 2000), and/or the production of thick leaves (i.e. a high SLM) reduce the penetration of UV-B to the mesophyll. The difference in growth response to solar UV-B between the herbaceous (*B. penna-marina* and *G. magellanica*) and woody species might be attributed to differences in UV-B screening between the two groups of species (Day *et al.* 1992). In agreement with this hypothesis the present data show that *C. diffusum* had higher SLM and constitutive levels of UV-absorbing phenolics than *B. penna-marina* and *G. magellanica* (Table 1). Barnes *et al.* (2000) have recently reported that adaxial epidermal transmittance of UV-B is

higher in *G. magellanica* than in *B. penna-marina* or *C. diffusum*; however, in *C. diffusum* it is the abaxial surface that is most exposed to sunlight in young leaves, and this abaxial surface is extremely protected from radiation by a dense pubescence.

Evidence of a cumulative effect of UV-B on the growth of the study species was not found. For perennial plants, it has been proposed that the effects of UV-B on growth might accumulate over the years (e.g. Sullivan & Teramura 1992; Johanson *et al.* 1995; Björn *et al.* 1997; Day *et al.* 1999). However, in many cases two or three years of data are not sufficient to distinguish between a cumulative treatment effect and the yearly variation in environmental factors that might affect the response to UV-B. Also, in some cases it is difficult to distinguish among an increase in the statistical detection of treatment differences through time, which might be related to decreased sample variability, a larger magnitude of UV effect in particular years, or a truly cumulative effect. The latter would imply an increase in the percentage growth reduction caused by UV-B over the years investigated.

Solar UV-B radiation reduced natural herbivory on *G. magellanica* (Fig. 4), which is consistent with the results of previous UV-B attenuation studies in this (Rousseaux *et al.* 1998) and other systems (Ballaré *et al.* 1996; Mazza *et al.* 1999b). In previous work with *G. magellanica*, the response variable was the number of lesions inflicted by phytophagous insect larvae. It was assumed that this variable reflected differences in host attractiveness, although it did not necessarily reflect differences in total food consumption (Rousseaux *et al.* 1998). The earlier experiments also clearly demonstrated that the effect was the result of changes in the host plant tissues rather than a direct effect on the insects. The present data demonstrate that exposure to near-ambient UV-B levels indeed reduces the amount of leaf area that *G. magellanica* loses to phytophagous insects during the course of the growing season in its natural environment.

Within the time frame of this study (growing seasons of 1997–98 and 1998–99) no significant effects of UV-B attenuation leading to changes in plant cover in the mixed patches of *G. magellanica* and *B. penna-marina* were detected (Fig. 6). The fact that UV-B attenuation slightly increased the growth of both these herbaceous species (Fig. 3) could make it difficult to detect changes in competitive balance between them over a short time period. In *G. magellanica* a 12% increase in leaf radius caused by partial UV-B exclusion (Fig. 3c; January 1998) was accompanied by a significant increase in leaf herbivory in one season (Fig. 5; January 1998), suggesting that the effect of reducing UV-B on plant–insect interactions could more than compensate for the increase in growth. In an Arctic system, enhanced UV-B increased herbivory by 70% in one of the species under study

(*Vaccinium myrtillus*) whereas no effects on growth were detected (Gwynn-Jones *et al.* 1997). Therefore, the limited information available at present suggests that the principal impact of UV-B on plant competitive balance could be expected to be indirect, and involve alterations in trophic level interactions.

Is it possible to assess the significance of the impact of ozone depletion on this ecosystem? Obviously, such an assessment involves more assumptions than certainties. All other things being equal, the $\approx 11\%$ reduction in average summertime ozone levels that occurred over Ushuaia during the last two decades (Fig. 2) should have resulted in $\approx 22\%$ increase in biologically effective UV-B (assuming a radiation amplification factor of 2; see Rousseaux *et al.* 1999). The present experiments show that current UV-B levels have measurable inhibitory effects on the growth of the dominant herbaceous species in this natural ecosystem (Fig. 3). Rousseaux *et al.* (1999) showed a linear correspondence between UV-B dose in the field and the steady-state level of DNA damage in *G. magellanica*. Ballaré *et al.* (1996) reported a quasi-linear relationship between solar UV-B and hypocotyl growth inhibition in field-grown *Datura ferox*. Assuming that this linear correspondence between UV-B dose and biological response holds over longer time-scales (which clearly may be not the case), simple calculations suggest that the additional UV-B dose that reaches the ground as a consequence of ozone depletion over the last two decades would be responsible for approximately one fourth of the total growth inhibitory effect (Fig. 3) of the present UV-B+ treatment. This may be a wildly speculative exercise, because acclimation, feed-backs and nonlinearities are common in biological systems, but it does afford some perspective on the predicted magnitude of the impacts of ozone depletion on plant growth in this region. The impacts on ecosystem functioning are obviously much harder to predict. However, two experimental results suggest that these impacts can be significant: (i) there are large effects of ambient UV-B on plant–insect interactions for at least some plant species of Tierra del Fuego (Rousseaux *et al.* 1998; Fig. 5, this paper; and Rousseaux *et al.* 2000, unpublished data), and (ii), in other field studies, the intensity of the herbivory response was found to be proportional to the dose of UV-B, in the range between near-zero and full ambient UV-B levels (Ballaré *et al.* 1996, 1999).

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