Effect of Solar Ultraviolet-B Radiation during Springtime Ozone Depletion on Photosynthesis and Biomass Production of Antarctic Vascular Plants¹

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We assessed the influence of springtime solar UV-B radiation that was naturally enhanced during several days due to ozone depletion on biomass production and photosynthesis of vascular plants along the Antarctic Peninsula. Naturally growing plants of *Colobanthus quitensis* (Kunth) Bartl. and *Deschampsia antarctica* Desv. were potted and grown under filters that absorbed or transmitted most solar UV-B. Plants exposed to solar UV-B from mid-October to early January produced 11% to 22% less total, as well as above ground biomass, and 24% to 31% less total leaf area. These growth reductions did not appear to be associated with reductions in photosynthesis per se: Although rates of photosynthetic O₂ evolution were reduced on a chlorophyll and a dry-mass basis, on a leaf area basis they were not affected by UV-B exposure. Leaves on plants exposed to UV-B were denser, probably thicker, and had higher concentrations of photosynthetic and UV-B absorbing pigments. We suspect that the development of thicker leaves containing more photosynthetic and screening pigments allowed these plants to maintain their photosynthetic rates per unit leaf area. Exposure to UV-B led to reductions in quantum yield of photosystem II, based on fluorescence measurements of adaxial leaf surfaces, and we suspect that UV-B impaired photosynthesis in the upper mesophyll of leaves. Because the ratio of variable to maximal fluorescence, as well as the initial slope of the photosynthetic light response, were unaffected by UV-B exposure, we suggest that impairments in photosynthesis in the upper mesophyll were associated with light-independent enzymatic, rather than photosystem II, limitations.

Increases in solar UV-B radiation (280–315 nm) reaching the Earth’s surface due to stratospheric ozone depletion (Madronich et al., 1998) have raised concerns about UV-B impacts on plants (Caldwell et al., 1998). The influence of UV-B on Antarctic organisms is of particular relevance since ozone depletion and corresponding enhancements in solar UV-B are most pronounced in Antarctica (Madronich et al., 1998). For example, ozone concentrations over Antarctica can decline by one-half during austral spring and lead to a doubling in levels of solar UV-B (Frederick and Lubin, 1994; Booth et al., 1998a).

Few studies have examined the influence of solar UV-B on Antarctic biota, and the vast majority of these have focused on marine phytoplankton; solar UV-B levels in Antarctica can depress photosynthesis in these microorganisms, resulting in reductions in marine productivity of 5% to 20% (Smith et al., 1992; Prézelin et al., 1994). Few studies have examined the influence of UV-B on Antarctic terrestrial plants. Regarding the two vascular plant species native to Antarctica (*Colobanthus quitensis* [Kunth] Bartl. and *Deschampsia antarctica* Desv.), Day et al. (1999) and Ruhland and Day (2000) excluded UV-B over naturally growing plants near Palmer Station and found that ambient UV-B levels reduced the vegetative growth of both species. However, it is unknown whether ambient UV-B levels also reduce photosynthesis in these species, and whether growth reductions are correlated with reductions in photosynthesis.

Generalizations about how ecologically realistic UV-B levels affect photosynthesis and whether this in turn affects plant growth under field conditions have been tenuous. Plant exposure to UV-B indoors can impair all major processes in photosynthesis including photochemical reactions in thylakoid membranes, enzymatic processes in the Calvin cycle, and stomatal limitations to CO₂ diffusion (Bormann, 1989; Allen et al., 1998). Several studies have shown that photosystem II (PSII) is often sensitive to UV-B and it has often been assumed to be the most sensitive photosynthetic target for UV-B (Bornman, 1989; Melis et al., 1992). However, UV-B-induced reductions in CO₂ assimilation can occur prior to, or in the absence of, depressions in PSII function and may more likely involve impairments in the Calvin cycle, possibly mediated by Rubisco (Nogués and Baker, 1995; Lesser and Neale, 1996; Allen et al., 1999). Most studies examining the influence of UV-B on photosynthesis have been conducted in growth chambers or greenhouses and the low background levels of UV-A radiation (315–400 nm) and visible or photosynthetically active radiation (PAR; 400–700 nm) in these indoor studies typically exaggerate UV-B responses, compared with those found in more spectrally realistic outdoor studies (Caldwell et al., 1994).

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Hence, it is unclear not only as to what photosynthetic target is most sensitive to UV-B, but also whether photosynthesis is even responsive to UV-B under ecologically realistic outdoor spectral regimes. In this study we first examined the relationship between atmospheric ozone content and UV-B levels during spring along the Antarctic Peninsula to determine whether ozone depletion appeared responsible for enhanced levels of UV-B. We also placed UV-B exclusion filters over vascular plants growing along the Peninsula to assess whether they were responsive to solar UV-B. We compared plants growing under UV-B transparent filters with those under UV-B absorbing filters with respect to their: (a) photosynthetic performance, which we assessed by measuring leaf chlorophyll fluorescence parameters and photosynthetic oxygen evolution rates, (b) ability to recover from high PAR and UV-B-induced impairments in photosynthesis, (c) concentrations of leaf soluble UV-B-absorbing compounds, chlorophyll, and carotenoids, and (d) biomass and leaf area production.

RESULTS AND DISCUSSION

Treatment Microclimate

Analysis of the microclimatic data from the UV-B treatments revealed that diurnal (PAR > 100 μmol m⁻² s⁻¹) mean daily UV-B BE (biologically effective UV-B based on Caldwell’s [1971] generalized plant damage action spectrum) under near-ambient UV-B (Aclar filter) and reduced UV-B (Mylar filter) treatments averaged 83% and 13%, respectively, of ambient levels over the course of the experiment (October 17–November 30, 1998). Mean daily UV-A irradiance and PAR under both UV-B treatments averaged 80% and 90%, respectively, of ambient levels. Mean diurnal and diel canopy air temperatures in both UV-B treatments were elevated approximately 5°C and 3.5°C, respectively, above ambient.

Solar UV-B Is Negatively Correlated with Ozone Column Content

During the experiment there were three periods of relatively severe ozone depletion (130–210 DU [Dobson units]) that occurred in mid-October, early November, and early December (Fig. 1A). The average ozone column content over the experimental period was 281 DU, which translates into a 20% ozone depletion, assuming an unperturbed ozone column of 350 DU (Lubin et al., 1992; Frederick and Lubin, 1994). Linear least-squares correlation/regression analyses of ozone concentrations and midday UV-B BE revealed a significant negative correlation between these variables (P < 0.01; r² = 0.31; Fig. 1E, inset). Because of possible non-linearity, as well as uncertainties as to whether these data met normality assumptions, we also examined this relationship using Spearman’s rank correlation analysis (Sokal and Rohlf, 1981). This analysis also showed a significant negative correlation between ozone column content and midday UV-B BE (P < 0.01; r = −0.50). To take into account some of the variability imposed on UV-B BE by cloud cover and solar angle, we also examined trends in UV-B BE by using the ratio of UV-B BE:PAR. Linear least-squares regression analysis of ozone column content and midday UV-B BE:PAR showed an even stronger, significant negative relationship between these variables (P < 0.01; r² = 0.68), as did Spearman’s rank correlation analysis (P < 0.01; r = −0.80; Fig. 1E).

The significant negative correlations between ozone column content and midday levels of UV-B BE (r² = 0.31) and UV-B BE:PAR (r² = 0.68) over the course of the experiment strongly suggest that higher UV-B BE levels and ratios were at least partly attributable to ozone depletion. The ratios of UV-B-to-PAR during our experiment were high compared with values from lower latitudes. For example, the ratio of integrated (not biologically effective) UV-B-to-PAR measured under clear skies at midday in the summer in Logan, UT (Caldwell et al., 1994) and Neuherberg, Germany (Thiel et al., 1996) was 0.0056 and 0.0037, respectively, whereas this ratio averaged 0.0080 (maximum 0.0126) at midday over the course of our experiment.

UV-B Exposure Reduces Biomass and Leaf Area Production

Over the 85-d growth period (October 17, 1998–January 10, 1999), D. antarctica and C. quitensis plants produced 22% and 11% less total biomass, respectively, under near-ambient UV-B than under reduced UV-B (P < 0.05; Fig. 2, A and B). Treatment effects were more pronounced on above ground than below ground biomass production in both species, and D. antarctica and C. quitensis produced 18% and 11% less above ground biomass, respectively, under near-ambient UV-B (P < 0.05). In contrast, there was no significant UV-B treatment effect on root mass production in C. quitensis and only a tendency (P < 0.10) for less root mass under near-ambient UV-B in D. antarctica. Not surprisingly, root-to-shoot ratios tended to be higher under near-ambient UV-B in both species (P < 0.10; data not shown). Cushion diameters of C. quitensis plants under near-ambient UV-B were 9% smaller and tillers of D. antarctica were 15% shorter than those under reduced UV-B (P < 0.05; Fig. 2, C and D). In addition, C. quitensis and D. antarctica plants under near-ambient UV-B produced 24% and 31% less total leaf area than those under reduced UV-B (Fig. 2, E and F; P < 0.05). Day et al. (1999) and Ruhland and Day (2000) filtered UV-B over naturally growing plants for whole growing seasons (early November–early March) in previous years at other sites near Palmer Station and also...
found that ambient UV-B reduced vegetative growth of these species. Taken collectively, these results indicate that solar UV-B along the Peninsula represents an environmental stress that may consistently limit the performance of vascular plants.

UV-B Exposure Increases Specific Leaf Mass (SLM) and Pigment Concentrations

In both species plants exposed to near-ambient UV-B had substantially greater SLM ($P < 0.01$). SLM of *C. quitensis* and *D. antarctica* under near-ambient UV-B was 30% and 25% greater, respectively, than under reduced UV-B (Fig. 3, A and B). In both species, plants exposed to near-ambient UV-B also had higher leaf concentrations of UV-B absorbing compounds on an area basis ($P < 0.05$; Fig. 4, inset), and there was a tendency for this trend on a dry-mass basis as well ($P < 0.10$, data not shown). This was true whether we assessed concentrations by measuring absorbance at 300 or 330 nm. Higher concentrations of UV-absorbing compounds were also apparent in the absorbance spectra of methanol extracts that we used to assess photosynthetic pigment concentrations (Fig. 4). Concentrations of carotenoids on a leaf-areas basis were also significantly higher in

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**Figure 1.** Trends in total ozone column content (A), midday biologically effective UV-B (UV-B$_{B_{et}}$, B), midday PAR (C), and midday ratio of UV-B$_{B_{et}}$-to-PAR (D) at Palmer Station, Antarctica during the experiment. Arrows indicate when plants were placed under UV-B treatments (October 17, 1998) and when they were harvested at the end of the experiment (January 10, 1999). Ozone column content was measured with the National Aeronautical and Space Administration Total Ozone Mapping Spectrometer. Midday UV-B$_{B_{et}}$ was taken as the mean of five measurements made at 15-min intervals between 12 noon and 1:15 pm by the SUV-100 spectroradiometer at Palmer Station that is part of the U.S. National Science Foundation’s Polar UV Monitoring Network. PAR was measured with quantum sensors. The midday ratio of UV-B$_{B_{et}}$-to-PAR was calculated in units of radiant flux density (e.g. $\mu$W cm$^{-2}$), after converting PAR data from units of photon flux density to radiant flux density by assuming an average wavelength of 550 nm. There were significant negative correlations between ozone column content and UV-B$_{B_{et}}$-to-PAR (E) and UV-B$_{B_{et}}$ (E, inset).
both species under near-ambient UV-B (Fig. 4; \( P < 0.05 \)). Total chlorophyll concentrations tended to be higher on a leaf-area basis in both species under near-ambient UV-B (\( P < 0.10 \); Fig. 4, inset), but not on a dry-mass basis (data not shown). There were no significant UV-B treatment effects on the ratio of chlorophyll \( a/b \) in either species (data not shown).

**UV-B Exposure Does Not Affect Leaf Area-Based Photosynthetic Rates**

Light-saturated rates of POE (photosynthetic \( \text{O}_2 \) evolution) on a leaf-area basis were not affected by UV-B treatment in either species on any of the four sampling dates (Fig. 5A). However, on a total chlorophyll-concentration basis, POE was significantly lower under near-ambient UV-B in both species on three of the four sampling dates (\( P < 0.05 \)). Averaging the means from the four sampling dates, POE per dry mass was 21% (\( C. \) quitensis) and 26% (\( D. \) antarctica) lower in plants under near-ambient UV-B (Fig. 5C).

To help distinguish UV-B effects on photochemical versus enzymatic reactions of photosynthesis we determined the POE light-response curves on plants collected at midday on two dates. On a leaf-area basis neither the initial slope of the light-response curve nor maximal rates of POE (at high PAR) were affected by UV-B treatment in either species on either date (Fig. 6, A and B). On a chlorophyll basis there were no treatment effects on the initial slopes of the light-response curves in either species (Fig. 6, C and D). However, maximal rates of POE (at high PAR) on a chlorophyll basis were significantly lower in plants of both species under near-ambient UV-B on both sampling dates, in agreement with our previous measurements of light-saturated POE.

Reductions in vegetative growth and biomass production have been detected in other species in ambient UV-B filter-exclusion studies (Ballaré et al., 1996; Krizek et al., 1997, 1998; Mazza et al., 1999). Several mechanisms have been proffered to explain these growth reductions. One candidate is reduced photosynthetic rate per unit leaf area, although there is little evidence for this mechanism in field studies. Although none of the above exclusion studies assessed UV-B effects on photosynthesis, Ballaré et al. (1996) suggested that the reductions in biomass production they observed were not due to impairments in photosynthesis, since the growth analysis parameter net assimilation rate (dry mass produced per leaf area per time) was not affected by UV-B level. In other UV-B studies, reductions in vegetative growth or changes in canopy architecture due to UV-B often occur in the absence of changes in photosynthetic rates per unit leaf area (Beyschlag et al., 1988; Barnes et al., 1990; Adamse and Britz, 1992; Searles et al., 1995; González et al., 1996, 1998; Allen et al., 1998;
Hunt and McNeil (1998), and Fiscus and Booker (1995) and Allen et al. (1998) concluded that photosynthesis in acclimated plants growing outdoors does not appear at risk from UV-B. Consistent with this we found that exposure to substantial, natural increases in UV-B had no effect on leaf area-based rates of POE.

Although there appears to be little evidence that exposure to UV-B outdoors can reduce rates of POE or CO₂ uptake on a leaf-area basis, we did detect reductions in rates of POE on a chlorophyll and dry-mass basis in plants growing under near-ambient UV-B. These plants not only had higher SLM, suggesting that UV-B exposure led to denser, probably thicker, leaves but also had higher leaf carotenoid concentrations and tended to have higher chlorophyll concentrations. This could explain why POE per leaf area was not affected by UV-B exposure; plants responded to higher UV-B levels by producing thicker leaves that contained more photosynthetic pigments per area, thereby maintaining photosynthetic gas-exchange rates on an area basis. Higher SLM and/or thicker leaves, along with higher concentrations of soluble UV-B-absorbing compounds, have been found in other species in response to UV-B (Day and Vogelmann, 1995; Johanson et al., 1995; Searles et al., 1995; Ballaré et al., 1996). Both responses could reduce damage to targets in the mesophyll by attenuating and increasing the pathlength of UV-B, thereby reducing fluxes in the mesophyll. The costs associated with producing thicker leaves containing more photosynthetic and UV-B-absorbing pigments per unit leaf area are difficult to quantify. However, this would certainly involve allocating more resources to the construction of new leaf area. Over the course of the growing season, the additional resources required for construction of new photosyn-

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**Figure 4.** Representative absorption spectra of leaf methanol extracts from *C. quitensis* (A) and *D. antarctica* (B), illustrating the higher levels of UV-B absorbing compounds in plants under near-ambient (UV-B) than reduced UV-B (~UV-B). The absorption spectra were normalized so that A at 660 nm were equal. The inset shows leaf concentrations of total chlorophyll (Chl), carotenoids (Car), and soluble UV-B-absorbing compounds (A₃₉₀nm) under each treatment, using extractions in methanol, or acidified methanol for UV-B-absorbing compounds. Leaves were collected on December 16, 1998. Values are means from three frames per treatment (n = 3), with three plants of each species sampled per frame, ± 1 s.e. Double asterisks indicate significant treatment effect at the P < 0.05 level.

**Figure 5.** Midday light-saturated rate of POE on a leaf area (A), chlorophyll concentration (B), leaf dry-mass (C) basis, Ð′ (D), and the ratio of F₄/F₅ (E) in *C. quitensis* and *D. antarctica* under near-ambient (UV-B) or reduced UV-B (~UV-B). Values are means of the averages from four sunny sampling dates (n = 4), ± 1 s.e. Double asterisks indicate significant treatment effect at the P < 0.05 level.
thentic leaf area due to UV-B exposure could be impressive, and the limitation this imposes on production of new leaf area and subsequent whole-plant photosynthesis may ultimately explain the reductions in vegetative growth and biomass production we found attributable to solar UV-B.

UV-B Effects on Photosynthesis May Be Associated with Enzymatic Rather than PSII Limitations

Plants of both species under near-ambient UV-B had significantly lower midday \( \Phi_{\text{PSII}} \) (quantum yield of PSII) than those under reduced UV-B on three of the four sunny sampling dates \((P < 0.05)\). When we averaged means from all four sampling dates, midday \( \Phi_{\text{PSII}} \) was 8\% \((C. \ quitensis)\) and 16\% \((D. \ antarctica)\) lower in plants under near-ambient UV-B \((\text{Fig. 5D})\). These reductions in \( \Phi_{\text{PSII}} \) under near-ambient UV-B over the four sampling dates ranged from 4\% to 14\% in \( C. \ quitensis \) and 8\% to 21\% in \( D. \ antarctica \). The ratio of variable to maximal fluorescence \( (F_{\text{v}}/F_{\text{m}}) \) was not affected by UV-B treatment in \( C. \ quitensis \) on any sampling date \((\text{Fig. 5E})\). Values were lower in \( D. \ antarctica \) under near-ambient UV-B on one of the four sampling dates \((P < 0.05)\), but when the means from all four sampling dates were averaged, there was no significant treatment effect.

UV-B treatment also altered the patterns of chlorophyll fluorescence induction curves in both species. \( F_{\text{m}} \) was lower and the time taken for fluorescence yield to reach one-half of maximum \( (t_{1/2}) \) was faster in plants exposed to near-ambient UV-B on all three sampling dates \((\text{Fig. 7})\). Averaging the means of the three sampling dates under near-ambient UV-B, \( F_{\text{m}} \), and \( t_{1/2} \) were 46\% \((\text{range } 35\% - 58\%)\) and 27\% \((\text{range } 19\% - 32\%)\) lower, respectively, in \( C. \ quitensis \), and 44\% \((\text{range } 29\% - 53%)\) and 27\% \((17\% - 38\%)\) lower, respectively, in \( D. \ antarctica \). In addition, plants of both species exhibited substantially lower M-peaks under near-ambient UV-B \((\text{Fig. 7})\). The faster \( t_{1/2} \) in both species suggests a smaller plastoquinone pool \((\text{Anderson et al., 1988})\) in our UV-B-exposed plants. A similar shortening of \( t_{1/2} \) was observed in diatoms following exposure to enhanced UV-B \((\text{Nilawati et al., 1997})\). Pfündel et al. \((1992)\) reported that violaxanthin deepoxidation was inhibited when plants were exposed to enhanced UV-B, which might increase risks for PAR-induced photoinhibition. Exposure to UV-B may have led to a smaller plastoquinone pool, which may have promoted photoinhibition via over-oxidation of electron carriers around PSII, especially under high PAR.

Although we did not detect any reductions in POE on a leaf-area basis in plants exposed to near-ambient UV-B, we suspect that much of this apparent photosynthetic insensitivity to UV-B may be the result of increases in leaf thickness and chlorophyll concentrations that mitigated any reductions in leaf-area based photosynthetic gas-exchange rates. We did detect reductions in some chlorophyll fluorescence parameters and these data provide information on how UV-B exposure may have affected the photosynthetic apparatus, at least in the upper mesophyll of leaves. Although midday \( \Phi_{\text{PSII}} \) was lower in \( C. \ quitensis \) and \( D. \ antarctica \) plants under near-ambient UV-B, midday \( F_{\text{v}}/F_{\text{m}} \) was unaffected by UV-B treatment. This greater sensitivity of \( \Phi_{\text{PSII}} \) than \( F_{\text{v}}/F_{\text{m}} \) to UV-B has previously been observed \((\text{Figueroa et al., 1997; Le- vall and Bornman, 2000})\). Nogués and Baker \((1995)\) found that supplemental UV-B lowered the light-saturated CO\(_2\) assimilation rate in the absence of any significant impairments in \( F_{\text{v}}/F_{\text{m}} \) in pea. Lesser and Neale \((1996)\) found that although there were no significant differences in \( F_{\text{v}}/F_{\text{m}} \) between UV-B exposed and UV-B filtered Antarctic diatoms, the concentrations of large subunits of Rubisco were 20\% lower in the former, and appeared well correlated with a 22\%
reduction in CO₂ assimilation rates. Consistent with this, we found that UV-B exposure led to lower \( F_{PSII} \), as well as POE per unit chlorophyll and dry mass, but had no affect on \( F_v/F_m \) or the initial slope of the light response curves, suggesting that impairment of photosynthesis was associated with light-independent enzymatic limitations, rather than structural damage or photochemical dysfunction of PSII.

A corollary to this idea is that most of the chlorophyll fluorescence signal emitted from the leaf surface originates in the outer 50 μm of leaves (Bornman et al., 1991). Hence, we suspect that leaf surface chlorophyll fluorescence signals may overestimate reductions in whole-leaf photosynthetic rates because they focus on the status of the surface layers of the mesophyll and do not take into account the status of photosynthetic machinery deeper in the mesophyll (Day and Vogelmann, 1995). This bias could be particularly evident in the case of UV-B because damage would likely be most pronounced in surface layers of the mesophyll, and leaves tend to thicken with UV-B exposure such that the contribution of deeper layers of the mesophyll to whole-leaf photosynthesis would probably increase (Day and Vogelmann, 1995), but would go undetected with surface fluorescence measurements. This may explain the discrepancies between rates of leaf-area based POE, which were unaffected by UV-B exposure, and rates of \( \Phi_{PSII} \), which were consistently reduced by UV-B.

\( \Phi_{PSII} \) and \( F_v/F_m \) Appear More Sensitive to PAR than UV-B

Both species showed similar diurnal patterns in \( \Phi_{PSII} \), which were characterized by a midday depression and recovery beginning in late afternoon. These midday depressions were evident for both species under both UV-B treatments on both sunny and cloudy days. Figure 8 shows diurnal patterns for a sunny (November 19) and cloudy (November 21) day, and are representative for patterns on the other two pairs of sunny/cloudy days. Several points are apparent from these patterns. First, the midday reductions in \( \Phi_{PSII} \) tended to be more pronounced under near-ambient UV-B than under reduced UV-B, particularly in *D. antarctica*. On all six sampling dates, \( \Phi_{PSII} \) in *D. antarctica* was significantly lower at midday (1 PM; \( P < 0.05 \)) and tended to be significantly lower in mid-afternoon (4 PM; \( P < 0.10 \)) under near-ambient UV-B than under reduced UV-B. In *C. quitensis*, \( \Phi_{PSII} \) tended to be lower (\( P < 0.10 \)) at midday under near-ambient UV-B on two of the six sampling dates. Although UV-B treatments had a significant effect on midday \( \Phi_{PSII} \), at least in *D. antarctica*, the depressions in midday \( \Phi_{PSII} \) appeared much more attributable to the PAR/UV-A wavebands than UV-B. For example, averaging across all six sampling days, we found that from early morning (8 AM) to midday (1 PM), \( \Phi_{PSII} \) in *D. antarctica* dropped by 21% in plants under reduced UV-B. This reduction in \( \Phi_{PSII} \) was 28% in plants under near-ambient UV-B, suggesting that the addition of UV-B contributed to a further reduction in \( \Phi_{PSII} \) of only 7%, on average. This corresponds to 25% (7/28) of the midday depression in \( \Phi_{PSII} \) being attributable to UV-B. In a similar manner, in *C. quitensis* the midday depression in \( \Phi_{PSII} \) was 21% in plants under reduced UV-B and increased to 25% in plants under near-ambient UV-B, suggesting that 16% (4/25) of the midday depression in \( \Phi_{PSII} \) was attributable to UV-B. Also, the midday depressions were more pronounced in both species on sunny than on cloudy days, implying that high irradiance was at least partly responsible for these depressions in \( \Phi_{PSII} \). For example, midday \( \Phi_{PSII} \) in *D. antarctica* averaged only 0.51 on the three sunny days, compared with 0.66 on the three cloudy days. In *C. quitensis*, midday \( \Phi_{PSII} \) averaged 0.55 on the sunny days compared with 0.64 on the cloudy days. Last, both species showed relatively fast recovery from these midday depressions.
under outdoor conditions. Averaging the means from all six sampling dates we found that on average, \( \Phi_{PSII} \) had recovered 74% (\textit{C. quitensis}) and 67% (\textit{D. antarctica}) of its midday depression by 7:30 PM.

Although there were no significant UV-B treatment effects on midday \( \Phi_{V/Fm} \) in either species, our diurnal measurements confirmed that midday values were depressed in both species, and these depressions were more pronounced on sunny than cloudy days. For example, on the three sunny sampling dates, mean midday \( F_v/F_m \) in \textit{D. antarctica} and \textit{C. quitensis} declined by 16% and 14%, respectively, of their early morning values, whereas on cloudy days, midday \( F_v/F_m \) in \textit{D. antarctica} and \textit{C. quitensis} declined by 8% and 6%, respectively, of their early morning values (diurnal data not shown).

Our findings support the idea that PSII is more sensitive to high visible (or UV-A) irradiance than UV-B (Allen et al., 1999). Only 16% to 25% of the midday depressions we observed in \( \Phi_{PSII} \) appeared attributable to UV-B, and furthermore, the midday depressions in \( F_v/F_m \) were not affected by UV-B exposure. Krause et al. (1999) found that exposure of two tropical species to sunlight resulted in substantial midday depressions in \( F_v/F_m \), but these depressions were still very evident when UV-B was excluded, and we estimate that <15% of the reductions they observed were attributable to UV-B. In a similar manner, only about 5% to 12% of the midday depressions in photosynthesis in marine algae appear attributable to UV-B (Figuerola et al., 1997; Herrmann et al., 1997; Gómez et al., 1998).

### Appreciable Recovery of \( \Phi_{PSII} \) at Low Temperatures

Because low temperatures can impede the recovery from photoinhibition following exposure to high irradiance, at least in temperate and tropical species (Gong and Nilsen, 1989; Sukhvibul et al., 2000), we assessed the effect of temperature on recovery of \( \Phi_{PSII} \) by removing plants from midday sunlight and placing them in incubators under low visible irradiance at a temperature of 4°C or 12°C. In both species, recovery from midday depression of \( \Phi_{PSII} \) was faster in plants at 12°C than 4°C (Fig. 9). At the higher temperature, \( \Phi_{PSII} \) had recovered 86% (\textit{C. quitensis}) and 81% (\textit{D. antarctica}) of its depression from early morning (8 AM) values after 8 h indoors. However, recovery was appreciable even at the lower temperature, and \( \Phi_{PSII} \) had recovered 60% (\textit{C. quitensis}) and 55% (\textit{D. antarctica}) of its early morning values after 8 h at 4°C, which is impressive considering that recovery in temperate and tropical species is much slower or eliminated at 3°C to 8°C (Gong and Nilsen, 1989; Sukhvibul et al., 2000).
and daily UV-B BE dose. The largest photosynthetic
rates measured outdoors in the early morning (8 AM). Values are means (n = 6
plants), ±1 SE.

Figure 9. Recovery of \( \Phi_{\text{PSII}} \) from midday depression in C. quitensis
and D. antarctica under near-ambient UV-B. Plants were brought
indoors at midday (1 PM) and placed under low PAR (250 \( \mu \)mol m\(^{-2}\)
\( s^{-1} \)) at 4°C or 12°C. After 10 min (time = 0), and at 1- to 2-h intervals
thereafter for 8 h, we monitored \( \Phi_{\text{PSII}} \). The shaded circle in the upper
left of A and B shows the rate of \( \Phi_{\text{PSII}} \) measured out-
doors in the early morning (8 AM). Values are means (n = 6
plants), ±1 SE.

Were Plants Responsive to Day-to-Day Variations in
Solar UV-B Levels?

A relevant, although rarely tested, question is
whether plants are responsive to variations in ambient
UV-B levels from day to day. Some plant re-
sponses such as DNA damage (Stapleton and Wal-
bot, 1994; Kang et al., 1998), D1 protein degradation
(Jansen et al., 1996), and anthocyanin synthesis (Hada
et al., 1996) can be approximately linearly related to
UV-B dose in short-term laboratory experiments.
However, whether plant responses are significantly
correlated with fluctuations in natural UV-B levels
outdoors over periods of several days or weeks has
rarely been tested. Ballaré et al. (1996) found that
transmission of greater percentages of ambient UV-B
led to corresponding increases in leaf DNA damage
levels in a summer annual. In one of the few studies
to examine the relationship between natural tempo-
ral fluctuations in UV-B levels and plant response
Rousseaux et al. (1999) found that fluctuations in
ambient UV-B\(_{\text{BE}}\) dose explained a large proportion
(68%) of the variation in leaf DNA damage levels
over 14 sampling dates during springtime in south-
er Argentina. We found no significant correlations
between any of the photosynthetic variables we mea-
sured and several UV-B parameters we examined,
including midday UV-B\(_{\text{BE}}\), midday UV-B\(_{\text{BE}}\)-to-PAR,
and daily UV-B\(_{\text{BE}}\) dose. The largest photosynthetic
data set we had for this analysis was midday \( \Phi_{\text{PSII}} \) (10
d) and regardless of whether we expressed this in
terms of percentage of inhibition (near-ambient/re-
duced UV-B treatment) or absolute \( \Phi_{\text{PSII}} \) rates under
near-ambient UV-B, the relationships were weak
(\( P > 0.20; r^2 < 0.25 \)). Examination of these correla-
tions with a non-linear test (Spearman’s rank corre-
alation) also failed to detect any significant correla-
tions. The lack of correlation between UV-B level and
photosynthetic inhibition could be due to several
factors, including: (a) Inhibition was saturated by
relatively low levels of ambient UV-B, (b) response to
other environmental factors such as air temperature
(Xiong et al., 1999, 2000) may have confound or
overshadowed their photosynthetic responses to
UV-B, (c) acclimation or protective responses might
have occurred over the season, as well as during
short periods of high UV-B levels, or simply (d) the
relatively small sample size of our data set.

CONCLUSIONS

We provide evidence that ozone depletion was at
least partly responsible for enhanced levels of UV-B
along the Antarctic Peninsula during this experi-
ment. Furthermore, exposure of native vascular
plants to these UV-B levels led to appreciable reduc-
tions in biomass production and leaf area. Rates of
photosynthetic gas exchange, on a leaf area basis,
were not affected by exposure to UV-B, and cannot
explain these reductions in growth. Leaves on plants
exposed to UV-B were denser, probably thicker, and
had higher concentrations of photosynthetic and UV-
B-absorbing pigments. We suspect that the develop-
ment of thicker leaves containing more photosyn-
thetic pigments allowed these plants to maintain
their photosynthetic rates per unit leaf area at rates
similar to plants under reduced UV-B levels. How-
ever, the additional resources required for construc-
tion of leaf area, and subsequent reductions in whole-
plant photosynthetic surface area over the course of
the season might ultimately explain the reductions in
growth and biomass reductions we found attribut-
able to solar UV-B. Exposure to solar UV-B did re-
duce \( \Phi_{\text{PSII}} \), although \( F_v/F_m \) was unaffected, suggest-
ing that UV-B did impair photosynthesis, at least in
the upper mesophyll of leaves, and that this was
associated with light-independent enzymatic limita-
tions, rather than direct damage to PSII.

MATERIALS AND METHODS

Plant Material and UV-B Treatments

Naturally growing plants of Colobanthus quitensis
(Kunth) Bartl. (a small cushion-forming plant) and Des-
champsia antarctica Desv. (a small tussock grass) were col-
clected on October 13 and 14, 1998, from the eastern island
of Stepping Stones (64°47'S, 64°00'W), a group of three
small islands 3 km east-southeast of Palmer Station, Anvers
Island along the west coast of the Antarctic Peninsula. Over
90% of the area covered by plant communities containing
these species on the island was snow free at this time and
plants were beginning to produce new leaves. The site is
described in more detail in Day et al. (1999). The climate is
maritime Antarctic with a mean annual air temperature at
Palmer Station of −2.3°C (Smith et al., 1996). Mean monthly air temperatures during the experiment ranged from −1.6°C in October 1998 to 2.5°C in January 1999, with monthly precipitation (melted) ranging from 27 to 79 mm. Plants with a 2.5- to 3.0-cm cushion diameter (C. quitensis) or 5 to 6 green tillers (approximately 2.0 cm in length; D. antarctica) were excavated with native soil, placed in square plastic pots (800 cm³), watered, and transported to Palmer Station.

On October 17, the plants were assigned to one of two UV-B treatments on Gamage Point, adjacent to Palmer Station. UV-B treatments were effected by placing plants under frames holding filters that transmitted most UV-B (transmission > 90% across the UV-B waveband; Aclar Type 22A, ProPlastics, Linden, NJ) or absorbed most UV-B (sharp transmission cutoff below 325 nm; Mylar-type Cadco clear polyester, Cadillac Plastic and Chemical, Phoenix). The Aclar-filtered frames are referred to as the “near-ambient UV-B” treatment, whereas the Mylar-filtered frames are referred to as the “reduced UV-B” treatment.

The wedge-shaped frames were constructed of 2.5 cm³ wood and were 90 cm long and 80 cm wide at the base. The rear of the frames was 65 cm high. The wedge-shaped front of the frames sloped 30° and faced north. The bottoms of the frames were nailed to a piece of plywood placed on the ground. The frames were covered with filters except for the bottom one-half of the rear panel to facilitate air circulation, and filters were replaced every 12 d. There were three replicate frames for each UV-B treatment, and 25 randomly-chosen plants of each species were placed under the center of each frame. Plants were watered every other day and fertilized once a month with Miracle-Gro Fertilizer (Marysville, OH).

To characterize differences between our treatments we measured several microclimate variables under the center of one Aclar-filtered frame, one Mylar-filtered frame, and an adjacent open or ambient area every 30 s and averaged these hourly with dataloggers (21X, Campbell Scientific Inc., Logan, UT) from October 17, 1998, through January 10, 1999. Canopy air temperature (2-cm height) and soil temperature in a pot (2-cm depth) at the center of each frame were measured with shielded fine-wire copper-constantan thermocouples, whereas PAR, UV-A, and UV-B were measured with quantum sensors (LI-190SA, LI-COR, Lincoln, NE), and broadband UV-A (SKU420, Skye, Powys, UK) and UV-B (SKU430, Skye) dosimeters, respectively. To calculate an integrated dose from the output of these broadband UV dosimeters, we calibrated them by comparing their midday output under ambient (non-filtered) conditions to simultaneous spectral measurements made with a scanning spectroradiometer (SUV-100, Biospherical Instruments, San Diego; Booth et al., 1998b) following Day et al. (1999). There was a strong linear correlation ($r^2 > 0.98$) between the output of the SKU420 sensors and the integrated UV-A irradiance measured by the SUV-100. There was also a strong linear correlation ($r^2 > 0.97$) between the output of the Skye UV-B (SKU430) sensors and biologically effective UV-B (UV-B$_{BE}$) measured by the SUV-100, using the generalized plant damage action spectrum (Caldwell, 1971) normalized to 300 nm.

**Ozone Depletion and Solar UV Radiation**

We used daily satellite images collected by the National Aeronautical and Space Administration Total Ozone Mapping Spectrometer to characterize ozone column content and depletion, and ground-based UV spectral irradiance data collected every 15 min by the SUV-100 scanning spectroradiometer to characterize the ambient solar UV regime during the experiment. The SUV-100 spectroradiometer is permanently housed at Palmer Station as part of the U.S. National Science Foundation’s Polar UV Monitoring Network (Booth et al., 1998b).

**Biomass and Leaf Area Production**

At the beginning of the experiment on October 17 we measured the cushion diameter (C. quitensis) and average tiller length (D. antarctica), above and below ground biomass, and total leaf area during an initial harvest of nine randomly chosen plants of each species. At the end of the experiment on January 10, this was repeated on nine plants of each species per treatment (three plants per frame). Plants were then separated into above and below ground parts and oven dried at 60°C for 72 h. Prior to drying, the leaf area of an above-ground subsample (approximately one-fourth of the canopy) was measured with an area meter (CI-202, CID, Vancouver, WA) to allow us to estimate total one-sided leaf area from above-ground biomass.

**SLM and Pigment Concentrations**

In conjunction with photosynthetic gas-exchange measurements (see below), we assessed SLM and chlorophyll concentrations of the leaf samples used in the former measurements. In addition, a last set of leaf samples were collected near the end of the experiment on December 16 for assessment of SLM and concentrations of chlorophyll and carotenoids, as well as soluble UV-B-absorbing compounds. For these measurements we collected two shoots (C. quitensis) or four tillers (D. antarctica) from nine plants of each treatment (three plants per frame). The shoots or tillers were placed in scintillation vials containing distilled water and placed on ice. Within 2 h, three samples of 0.8 to 1.0 cm² one-sided leaf areas from each plant were measured with the area meter. One sample was used for extracting chlorophyll and carotenoids, another for UV-B-absorbing compounds, and the third was oven dried (60°C) for 72 h to determine SLM and allow concentrations to be expressed on a dry-mass basis. Pigments were extracted by grinding leaf tissue in methanol (chlorophyll and carotenoids), or acidified methanol (MeOH:HCl:H₂O, 90:1:1 [v/v]), soluble UV-B-absorbing compounds), stirring for 15 min at 60°C, and filtering through 90-μm screens. The absorption spectrum of the methanol extract was measured with a UV/visible spectrophotometer (Lambda4, Perkin-Elmer, Norwalk, CT), and chlorophyll $a$ and $b$, and carot-
enoid concentrations were estimated using the extinction coefficients of Lichtenthaler and Wellburn (1983). Concentrations of soluble UV-B-absorbing compounds were estimated by measuring absorbance at 300 and 330 nm.

**Midday Chlorophyll Fluorescence and POE**

We measured midday (1–2 pm) leaf chlorophyll a fluorescence yield and light-saturated POE rates on four sunny days beginning 20 d after plants were placed in their respective UV-B treatments (November 6 and 10, and December 3 and 4). During these four days there was considerable ozone depletion (>30%; see Fig. 1) with ozone concentrations between 190 and 245 DU. Midday UV-B BE ranked from 4.4 to 11.2 μW cm⁻², whereas PAR ranged from 1,070 to 1,460 μmol m⁻² s⁻¹.

In situ measurements of chlorophyll fluorescence were made on adaxial leaf surfaces using a pulse amplitude modulated fluorometer (OS-500, Opti-Sciences Inc., Havertown, MA) as described by Xiong et al. (1999). The Fv and the Fm were measured using 20-min dark-adapted leaves and the potential efficiency of PSII was estimated with the ratio of variable (Fv = Fm – Fd) to Fm according to Schreiber et al. (1986). The Fv/Fm was measured using light-adapted plants and calculated as (Fv / Fm') (Genty et al., 1989). Mean values of Fv/Fm, and POE were obtained by averaging measurements of six plants of each species per frame from the three frames per treatment. Along with these measurements, on the latter three of these sampling dates we also assessed chlorophyll fluorescence induction to determine τ₁/₂ and characterize the M-peak (Sivak and Walker, 1985).

Rates of POE were measured with a liquid-phase Clark-type O₂ electrode (YSI, Yellow Springs, OH) in a 5-mL glass reaction cuvette. We collected four to five shoots of *C. quitensis* and four to six tillers of *D. antarctica* from five plants per frame at midday (1–2 pm) and placed them in distilled water at 4°C in the dark. Leaves were separated from each sample, their one-sided areas were measured, and POE was measured within 3 h of collection. For *C. quitensis*, leaf segments (approximately 0.1 g fresh weight) were immersed in Pi buffer (0.2 M, pH 7.8) in the reaction cuvette, whereas for *D. antarctica* the leaves were transversely cut into 1.5-mm-wide slices (approximately 0.07 g fresh weight) before immersing in the buffer. We added 0.2 mL of NaHCO₃ solution (0.625 g) to the cuvette to provide 25 mM of HCO₃-. Visible light from a halogen lamp (80V/300W, USHIO Inc., Tokyo) filtered through a 2.5% CuSO₄ solution was adjusted to provide 860 μmol m⁻² s⁻¹ PAR at the cuvette surface, which was saturating for POE based on preliminary measurements. During measurements, leaves were stirred and temperature inside the cuvette was maintained at 15°C by circulating water from a water bath through the cuvette jacket. Preliminary measurements demonstrated that leaves of both species exhibited maximal light-saturated POE at this temperature. After measurements each sample was removed from the cuvette and its chlorophyll concentration was determined to allow rates to be expressed on a leaf-area and chlorophyll-concentration basis.

In addition to rates of light-saturated POE we assessed the photosynthetic light response of these samples on two of the four sampling dates (November 10 and December 3). Leaf samples were pre-illuminated at 860 μmol m⁻² s⁻¹ for 5 min in the cuvette and POE was measured at nine PAR levels starting from low (48 μmol m⁻² s⁻¹) to high (1,785 μmol m⁻² s⁻¹) PAR. Visible irradiance at the cuvette surface was measured with the quantum sensor and was adjusted with neutral-density filters, whereas temperature inside the cuvette was maintained at 15°C.

**Diurnal Patterns of ΦPSII and Fv/Fm**

During our midday measurements, we noticed that values of ΦPSII and Fv/Fm were relatively low. To further characterize these suspected midday depressions and to assess the relative contributions of high PAR (and UV-A) versus UV-B wavebands in these depressions, we monitored diurnal patterns of ΦPSII and Fv/Fm on three pairs of sunny and cloudy days (November 19/November 21, November 24/November 27, and December 8/December 10, sunny/cloudy). Midday PAR ranged from 1,320 to 1,486 μmol m⁻² s⁻¹ on the sunny days and 680 to 870 μmol m⁻² s⁻¹ on the cloudy days, whereas canopy air temperatures ranged from 11°C to 22°C on the sunny days and 8°C to 12°C on the cloudy days. We measured the same six plants of each species (two from each frame) per treatment on each sampling date. Measurements began at 7:30 to 8 AM and continued at 2- to 3-h intervals until 7:30 PM.

**Influence of Temperature on Recovery of ΦPSII**

We observed appreciable midday depressions in ΦPSII during our diurnal measurements on sunny days. Because low temperatures can impede the recovery of photoinhibition following exposure to high irradiance, at least in temperate and tropical species (Gong and Nilsen, 1989; Sukhvibul et al., 2000), we assessed the influence of temperature on recovery of ΦPSII by removing plants from midday sunlight and placing them in incubators under low visible irradiance at a temperature of 4°C or 12°C. On December 7, a sunny day (midday PAR = 1,560 μmol m⁻² s⁻¹; UV – BBE = 11 μW cm⁻²), we measured early morning (8 AM) and midday (1 PM) ΦPSII of 12 plants under the near-ambient UV-B (Aclar-filtered) frames (four from each frame), and then brought plants indoors. Six plants were placed in an incubator at 4°C, whereas the other six were placed in an incubator at 12°C. White fluorescent lights in each incubator provided a visible irradiance of 250 μmol m⁻² s⁻¹ PAR at plant height. After 10 min, and at 1- to 2-h intervals thereafter for 8 h, we measured ΦPSII of each plant.

**Statistical Analyses**

UV-B treatment and block or frame effects were tested using a two-way ANOVA, and the UV-B treatment means
were compared using the LSD test. There were no significant frame effects. Treatment means and errors shown in figures were compiled by taking the mean of the averages from each of the three frames within a UV-B treatment (n = 3), except in cases where we pooled several dates and used the mean of the averages from each date. Linear least-squares correlation and regression analyses, as well as a non-parametric test (Spearman’s coefficient of rank correlation; Sokal and Rohlf, 1981), were used to examine correlations between ozone column content and solar irradiance. Unless otherwise specified we considered treatment effects and correlations significant at the P < 0.05 level.

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LITERATURE CITED


Ruhland CT, Day TA (2000) Effects of ultraviolet-B radiation on leaf elongation, production and phenylpropanoid concentrations of *Deschampsia antarctica* and *Colobanthus quitensis* in Antarctica. Physiol Plant 109: 244–251


Smith RC, Stammerjohn SE, Baker KS (1996) Surface air temperature variations in the western Antarctic Peninsula region. In RM Ross, EE Hofman, LB Quetin, eds,


