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Solar Ultraviolet-B Radiation Affects Seedling Emergence, DNA Integrity, Plant Morphology, Growth Rate, and Attractiveness to Herbivore Insects in *Datura ferox*[1]

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To study functional relationships between the effects of solar ultraviolet-B radiation (UV-B) on different aspects of the physiology of a wild plant, we carried out exclusion experiments in the field with the summer annual *Datura ferox* L. Solar UV-B incident over Buenos Aires reduced daytime seedling emergence, inhibited stem elongation and leaf expansion, and tended to reduce biomass accumulation during early growth. However, UV-B had no effect on calculated net assimilation rate. Using a monoclonal antibody specific to the cyclobutane-pyrimidine dimer (CPD), we found that plants receiving full sunlight had more CPDs per unit of DNA than plants shielded from solar UV-B, but the positive correlation between UV-B and CPD burden tended to level off at high (near solar) UV-B levels. At our field site, *Datura* plants were consumed by leaf beetles (Coleoptera), and the proportion of plants attacked by insects declined with the amount of UV-B received during growth. Field experiments showed that plant exposure to solar UV-B reduced the likelihood of leaf beetle attack by one-half. Our results highlight the complexities associated with scaling plant responses to solar UV-B, because they show: (a) a lack of correspondence between UV-B effects on net assimilation rate and whole-plant growth rate, (b) nonlinear UV-B dose-response curves, and (c) UV-B effects of plant attractiveness to natural herbivores.

Plant responses to enhanced UV-B radiation have received considerable attention during the last several years, particularly since a general erosion of the stratospheric ozone layer was documented on a global scale (for review, see World Meteorological Organization, 1995) and increased UV-B irradiances at the ground surface were measured in some locations (for refs., see Madronich et al., 1995). The current literature has been reviewed extensively by Bornman and Teramura (1993), Tevini (1993), Caldwell and Flint (1994), and Caldwell et al. (1995).

Considering the attention given to predicting the impacts of increased UV-B, it is surprising that the effects of natural, present-day UV-B irradiances on the functioning of terrestrial plants and plant communities are poorly characterized. Early studies of solar UV-B exclusion (e.g. Brodführer, 1955) have been criticized, especially with respect to the lack of appropriate controls, the possibility of differences in the thermal regime between UV-B treatments, or the optical instability of the filters employed (Caldwell, 1971). In more recent times there have been a few studies of the effects of solar UV-B exclusion on terrestrial plants. In one approach, solar UV-B is filtered through a thin layer of ozone that is passed through a cuvette of UV-B-transparent plexiglass. With this technique, Tevini and associates showed that solar UV-B radiation incident at low latitudes can affect growth and morphology of seedlings grown under controlled conditions of temperature and air humidity (see Tevini, 1993). In another approach, filters of PE, polyvinyl chloride, or other plastic polymers are used to exclude radiation in the UV region without greatly affecting longer wavelengths. Because little expense is involved, this approach is suitable for studying the impact of solar UV-B on plants or plant communities exposed to normal levels of other environmental and biotic factors.

The studies carried out by different laboratories using this approach have generally focused on UV-B impacts on plant growth and morphology, and their results have been mixed. Caldwell (1968) did not find statistically significant effects of solar UV-B exclusion on the naturally occurring plants of an alpine plant community, except for an enhanced flowering of one species. Searles et al. (1995), working in Panama, detected effects of solar UV-B on elongation in seedlings of several tree species, but did not find any evidence of UV-B effects on biomass accumulation or PSII activity. On the other hand, Tezuka et al. (1993) obtained a transient reduction in biomass accumulation in tomato plants by excluding solar UV-B radiation in field experiments carried out in Japan, whereas Becwar et al. (1982) did not detect any effects of solar UV-B exclusion at a high-elevation site in Colorado on final biomass of plants of potato, radish, and wheat.

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Abbreviations: CA, cellulose diacetate; CPD, cyclobutane pyrimidine dimer; PE, clear polyester.

Other studies reporting variable, sometimes difficult-to-interpret effects of solar UV-B exclusion on the growth of terrestrial plants (mostly cultivated species) have been discussed by Tevini and Iwanzik (1986), Bornman and Teramura (1993), and Caldwell and Flint (1993). Exclusion studies have also been performed in aquatic systems. These studies have shown that present-day levels of solar UV-B radiation at mid-latitudes can affect aspects of phytoplankton motility (e.g. Sebastian et al., 1994), primary productivity of phytoplankton and macroalgae (Cullen and Neale, 1994; Grobe and Murphy, 1994, and refs. therein), and interactions between plants and herbivores (Bothwell et al., 1994).

The lack of detailed knowledge about the ecological functions of solar UV-B contrasts with the well-developed ideas regarding the roles of other wavelengths of the electromagnetic spectrum (e.g. blue, red, and far-red) in controlling plant physiology and plant-plant interactions in terrestrial plant communities. Some of the important questions that remain unanswered about UV-B impacts on the physiology of plants in their natural environment are: (a) Is the present-day flux of solar UV-B radiation a limiting factor for plant growth? (b) Are the processes known from laboratory studies to be sensitive to UV-B radiation, such as photosynthesis and maintenance of DNA integrity, affected by solar UV-B irradiances in the plants' natural areas of distribution? (c) Does solar UV-B radiation play a significant role in influencing interactions between plants and other organisms (e.g. pathogens and herbivores)? To address these questions, we report here the results of solar UV-B exclusion experiments using the annual dicot *Datura ferox*.

MATERIALS AND METHODS

Datura ferox L. ("Chamico") is a summer annual dicot that occurs naturally in recently disturbed open places such as cultivated fields, roadsides, and waste places in subtropical and temperate areas of South America. Seed for our experiments was obtained from plants invading soybean crops in San Antonio de Areco, Buenos Aires, Argentina. Recently germinated seeds of *D. ferox* were planted at a depth of 0.5 cm in individual cones (0.3 dm^3) filled with standard topsoil. Planting was carried out in a greenhouse; the pots, containing the pregerminated seeds, were transferred to the field and seedlings were allowed to emerge under the films used to filter solar radiation. In the growth-analysis experiments, seedlings were transplanted to 1-dm^3 plastic pots and fertilized with 1 g of N per pot applied as urea 10 d after emergence. In all cases the pots were watered twice daily to maintain the soil near field capacity. The experiments were carried out at the experimental field of Ifeva, Faculty of Agronomy, University of Buenos Aires, (34°35′ S; 58°29′ W), during the summer of 1994–1995 and the spring of 1995.

Light Treatments and Measurements

Measurements of incoming solar UV-B radiation (305 nm) were obtained with a multi-band radiometer (model GUV-511, Biospherical Instruments, San Diego, CA) installed 1 km to the east of our experimental site (the edge of Buenos Aires City) as part of a UV-monitoring network. The 305-nm channel has a bandwidth of 7 ± 1 nm and the peak transmission is at 305 ± 1 nm. The light receiver has a directional response that follows a cosine response curve; according to the manufacturer, typical errors in measurement are 0 to −5% from 0 to 70° solar zenith angle. The reason for using 305 nm as a reference wavelength is that it lays within the narrow band that is most affected by changes in the ozone column, and it is close to the apparent peak of activity of several UV-induced morphological and pigmentation responses in plants (e.g. Curry et al., 1956; Ensminger, 1993; Beggs and Wellmann, 1994; Ballaré et al., 1995a, and refs. therein). Peak UV-B (305 nm) irradiance for clear-sky conditions fluctuated between approximately 2.5 $\mu W\ cm^{-2}\ nm^{-1}$ (spring) and approximately 3.5 $\mu W\ cm^{-2}\ nm^{-1}$ (summer). During the same period, peak PPFD varied between 1425 and 2200 $\mu mol\ m^{-2}\ s^{-1}$.

PE filters were used to exclude UV-B radiation (wavelength ≤ 320 nm) from sunlight without significantly affecting UV-A or visible radiation. CA filters were used as a full-spectrum control. Transmittance spectra for these materials were measured with a spectrophotometer (model 2500, Metrolab, Buenos Aires, Argentina) and are given in Figure 1. The CA film, used as a control, filters out some of the shortwave UV-B; the transmittance of the film at the reference wavelength of 305 nm is approximately 70%. Comparative measurements of UV-B under the filters at canopy level, taken with a radiometer (model IL 1700, International Light, Newburyport, MA) equipped with a broad-band, cosine-corrected UV-B detector head (SUD/240/W; $wavelength_{max}$ = 300 nm, bandwidth approxi-

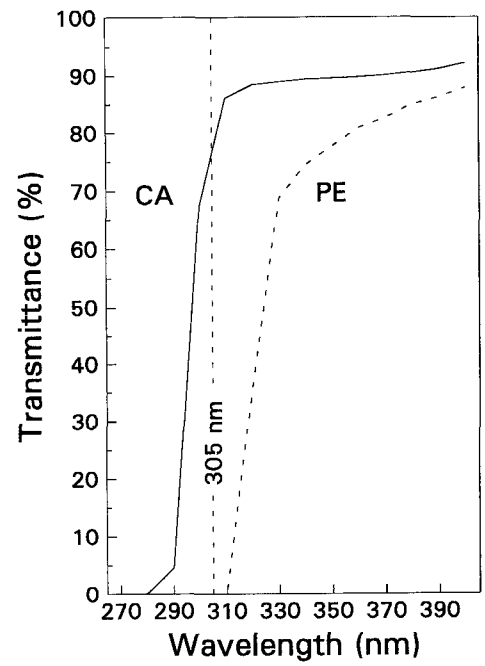

Figure 1. Transmittance spectra of filters. CA film was 0.1 mm; PE film was 0.13 mm.

mately 25 nm, International Light), indicated a 22% reduction of UV-B irradiance under the CA filters, compared to unfiltered sunlight, and more than 96% exclusion under the PE films. Therefore, it is important to point out that the effects of solar UV-B reported in this study are likely to be underestimates of the actual effects of unfiltered sunlight.

Small samples of the filters were taken every 4 d to check for changes in transmittance. Under our conditions, there were no significant reductions in the transmittance of the CA film even after 3 weeks of exposure to solar UV-B. However, because the filters tended to rip and to accumulate dust over time, they were normally replaced after 10 d of use. Sheets of film of 1.1 × 0.7 m were used to cover a canopy area of <0.5 × 0.25 m, thereby providing a generous edge area. The filters were draped over metal frames that were erected in the field and were maintained about 3 cm above the plant canopy during the course of the experiments. The sides were left open to allow air to circulate. Soil temperature under the filters was recorded using a model 21X Micrologger (Campbell Scientific, Logan, UT) or with sensors (model LM 335Z, National Semiconductor, Santa Clara, CA) attached to a chart recorder (model AR/G 35–7e Arucomp 100, Hartmann & Braun, Frankfurt, Germany). No differences between pots placed under PE or CA filters were detected. Leaf temperature was measured at midday using an IR thermometer (model Therm 2228–1/I-9628, Ahlborn, Holzkirchen, Germany). Average canopy temperatures under CA and PE filters were always within <0.5°C of each other, and in no case were consistent differences in temperature detected between filter treatments.

To obtain intermediate fluxes of UV-B radiation for the dose-response experiments, narrow strips of UV-B-absorbing PE (0.7 m long × 0.9 cm wide) were fixed parallel to each other to 1.1 × 0.7 m sheets of UV-B-transparent CA film by means of two segments of packing tape stuck to the long edges of the acetate sheet. The distance between strips was adjusted to yield 25, 50, and 75% coverage of the filter area with UV-B-absorbing PE film, i.e. to achieve a nominal UV-B flux of 75, 50, and 25% of the CA control, respectively. Comparative measurements of UV-B under the filters taken with the broad-band detector (model IL 1700, International Light) confirmed that filters were effective at creating a UV-B gradient. Relative to the CA control, broad-band UV-B irradiance (±SE) under the filters just mentioned was 70 (±7), 45 (±5), and 35 (±7)%, respectively.

Responses to Solar UV-B

Growth and Morphology

Three independent experiments were carried out during the summer of 1994–1995, two for growth analysis (each with five independent blocks) and one to study dose-response relationships (five UV-B levels with three replicates of 8 to 12 plants each). In addition, two groups of 300 pregerminated seeds were planted to study the effects of solar UV-B on the dynamics of seedling emergence during early spring and summer. Although seeds were planted in pots that were distributed under several PE or CA filters, only the two exposure times should be considered as the independent replicates of this experiment, because the temporal pattern of emergence is a population-level attribute. The pots were inspected at 9 AM and 7 PM every day, and newly emerged seedlings were marked with color-coded rings made of plastic-coated wire. The plumular hook is the first part of the *Datura* seedling that emerges from the soil; sometime later (usually a few hours), the cotyledons exit the endosperm cavity in the buried seed and emerge from the soil. In our experiments seedlings were considered to be "emerged" if they had both cotyledons completely off the ground. Stem length was measured with a ruler to the nearest millimeter in the three cohorts of plants used for the growth-analysis and dose-response experiments. Four plants per block and treatment were randomly selected from the seedling populations used in the growth-analysis experiments; all plants in the dose-response experiment were measured.

Growth-analysis parameters (e.g. relative growth rate and unit leaf rate; Hunt, 1981) were derived from two independent experiments (5 blocks, each under independent filters; 32 individual plants per block) that were conducted during the summer of 1994–1995. The leaf area was measured with an area meter (model LI 3000, Li-Cor, Lincoln, NE). Dry weight was obtained after oven drying the tissue for 48 h at 72°C. For determinations of specific leaf area, discs of 0.785 cm^2 were obtained from young leaves that had reached more than two-thirds of their maximum size. For starch determinations, leaf discs (0.283 cm^2) were collected at 3 PM from leaves 4 to 6 during the 4th week of the growth-analysis experiment, and stored at −20°C. Starch was obtained by alkaline extraction (Whistler et al., 1984) from 50 mg of leaf tissue per sample and determined with I_2/I^- reagent in saturated $CaCl_2$ (Krisman, 1962). A_{500} was determined using 10 g dm^{-3} starch solution as a standard.

DNA Damage

Leaves from 21 individual seedlings per UV-B dose were harvested at 3 PM and immediately frozen in liquid N_2. DNA was extracted from approximately 0.5 g of frozen tissue according to the method of Rogers and Bendich (1988). Quantification of DNA damage was performed using a monoclonal antibody that specifically recognizes CPD, as described by Stapleton et al. (1993). Each assay was done on three replicates per DNA sample, and the entire assay procedure was repeated a second time with similar results. The two sets of three measurements were combined, since by analysis of variance the samples were not significantly different ($P = 0.39$).

Herbivory

D. ferox plants were visited by a diverse guild of insects, which included sucking hemipterals such as *Nezara viridula* ("chinche," Hemiptera) and biting insects such as grasshoppers (*Schistocerca* spp., Orthoptera), and especially *Diabrotica speciosa* ("vaquita de San Antonio," a type of

leaf beetle) and *Epitrix* spp. ("pulguilla," Coleoptera: Chrysomelidae). Insects of the biting group left typical lesions on the host plant, which consisted of large, damaged areas (usually larger than 3 mm in diameter), in the case of grasshoppers, or small holes concentrated along the margins and the base of expanding leaves (*Epitrix*) or scattered all over the lamina (*Diabrotica*). To characterize the intensity of insect attack, we counted the number of plants with biting lesions 10 d after planting in the dose-response experiment, i.e. after approximately 7 d of exposure to the different UV-B regimes. In the 1-d herbivory experiment, we took plants grown for 7 to 26 d under UV-B-absorbing PE or UV-B-transparent CA films, marked all existing bite lesions on their leaves with permanent ink, and placed them intermingled in a 1-m^2 area at 5 PM. Twenty-four hours later, we inspected the plants and counted the number of leaves with new lesions. The experiment was repeated 13 times during late February to early March using plants of two different cohorts. Typically, three plants for each cohort and UV-B history were exposed each time, and the percentages of attack were calculated over populations of 28 to 50 leaves.

RESULTS

Solar UV-B Reduces Daytime Seedling Emergence and Inhibits Stem Elongation

To test the effects of solar UV-B on the dynamics of seedling emergence, we carried out two field experiments of UV-B exclusion. All newly emerged seedlings were assessed in the early morning and late afternoon for 5 d after planting. In early spring, with average soil temperatures (−5 cm) of 12°C (mean daily minimum) and 22°C (mean daily maximum), seedling emergence from well-watered soil under near-ambient UV-B (CA filters) was evenly distributed between daytime and nighttime (Fig. 2, early spring, UV-B). Filtering out solar UV-B with PE films increased the ratio of daytime:nighttime emergence by a factor of approximately 3 (Fig. 2, early spring, no UV-B). During the summer, with average soil temperatures of 22°C (mean daily minimum) and 35°C (mean daily maximum), seedling emergence under solar UV-B occurred predominantly during nighttime (Fig. 2, summer, UV-B). Filtering out solar UV-B caused a 70% increase in the number of seedlings that emerged during the day, resulting in an emergence pattern concentrated during the daytime (Fig. 2, summer, no UV-B). Exclusion of solar UV-B did not affect the total number of seedlings that emerged over 24-h periods (Fig. 2, inset).

Solar UV-B radiation at ground level during the summer inhibited hypocotyl elongation by as much as 20 to 29% in three independent cohorts of *Datura* seedlings (Fig. 3). The degree of inhibition increased with the UV-B dose (Fig. 3, inset). The differences in plant height caused by solar UV-B also were evident in mature plants, but the effects were not as pronounced as in young seedlings (data not shown).

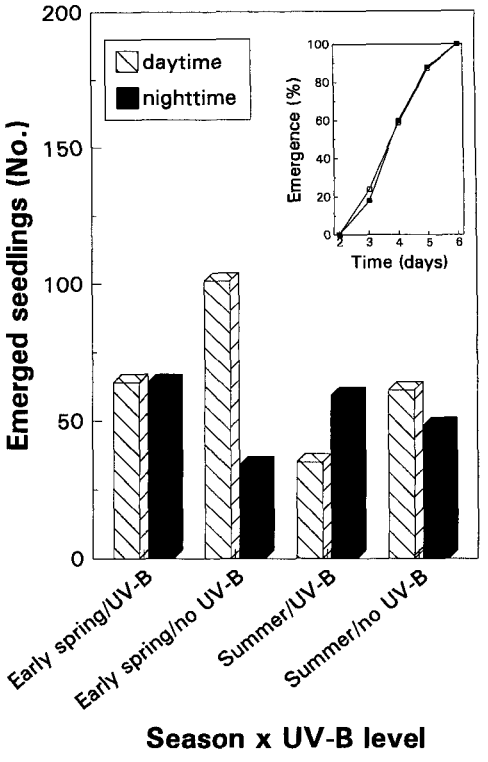

Figure 2. The effect of excluding solar UV-B on the timing of seedling emergence. Results of two independent exposures are shown. Seedlings were allowed to emerge under CA (UV-B) or PE (no UV-B) films from soil that was kept near field capacity; the bars show the number of seedlings emerging between 9 AM and 7 PM (daytime) or 7 PM and 9 AM (nighttime) during a period of 5 d after planting. Total seedling populations were 203 (summer) or 263 (early spring). The inset shows the time course of seedling emergence (accumulated daily totals over time) for the summer experiment (□, PE film; ■, CA film).

Solar UV-B Increases DNA Damage

To test whether solar UV-B radiation affects DNA integrity in *Datura* plants grown in their natural area of distribution, we extracted and purified DNA from leaves exposed for 10 d during the summer to different levels of solar UV-B. Using a sensitive antibody-based technique specific for CPD, we found that leaves that received near-ambient UV-B levels (CA filters) had about twice the number of lesions per unit of DNA than plants grown with UV-B exclusions (Fig. 4). The relationship between UV-B dose and DNA damage level was not linear, since an exponential curve fitted the data significantly ($P = 0.006$) better than a first-order polynomial. Leaf specific mass (mg cm^{-2}) increased with UV-B (Fig. 4, inset), most likely reflecting an increase in tissue thickness. Increased specific leaf mass may have contributed to reduce DNA exposure at high (i.e. near solar) UV-B fluxes, because plant tissue effectively attenuates UV-B radiation.

Solar UV-B Reduces Growth Rate but Not Unit Leaf Rate

Preliminary experiments with *Datura* and other dicots suggested that solar UV-B incident over Buenos Aires may

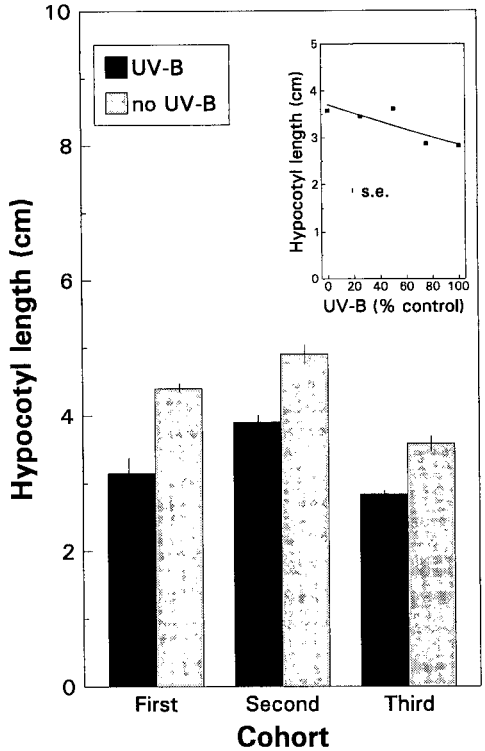

Figure 3. The effect of excluding solar UV-B radiation on hypocotyl elongation. Seedlings were measured 12 d after planting (approximately 10 d after emergence) in three independent experiments carried out during the summer of 1994–1995. Bars indicate the average of five blocks (first and second cohort; 4 seedlings per block and treatment) or three blocks (8 to 12 individual seedlings per block and treatment); thin lines show ± SE (n = 5 or 3). The inset shows the relationship between hypocotyl length 12 d after planting and UV-B dose in the third cohort; the line represents the least-squares linear fit. The different UV-B levels were obtained by superimposing narrow strips of UV-B-absorbing PE films onto CA sheets.

be effective at reducing the growth rate of field-grown plants. To characterize this effect further, two experiments were carried out during the summer of 1994–1995. The results were subjected to growth analysis (Hunt, 1981) to assess the impact of UV-B on net assimilation rate and photosynthetic area. Plants grown under UV-B-absorbing PE films accumulated more biomass than plants receiving near-ambient UV-B (CA filters) (Fig. 5, top), indicating that ambient UV-B levels can inhibit early growth of *Datura* plants in their natural area of distribution. Growth analysis showed that most of the effect of UV-B on growth rate could be accounted for by an inhibition of leaf area development (Fig. 5, middle), with no consistent effect of UV-B on net assimilation rate (i.e. unit leaf rate) (Fig. 5, bottom). Plants receiving solar UV-B (CA filters) accumulated more starch in leaf tissue than plants under PE filters (i.e. 18.2 [CA] versus 16.2 [PE] mg starch g^{-1} fresh weight in the leaf 4 of 21-d-old plants; 5.2 [CA] versus 3.7 [PE] mg starch g^{-1} fresh weight in a pooled sample of leaves 4 to 6 of 24-d-old plants). The relative increases (12–40%) in starch levels were larger than the increases in specific leaf mass caused by UV-B (usually between 6 and 10%).

Solar UV-B Affects Plant-Herbivore Interactions

For *Datura* seedlings grown in the field, there was an inverse relationship between the amount of UV-B radiation received during growth and the intensity of leaf tissue damage caused by phytophagous insects (Fig. 6). The inspection of the plant lesions indicated that damage was caused mostly by leaf beetles (Chrysomelidae). Because these observations were made on plants continuously exposed to contrasting UV-B regimes, they do not distinguish between UV-B effects on tissue attractiveness to herbivores and potential direct effects of UV-B on insect activity or behavior during the treatment period. To distinguish between these two possibilities, we evaluated the intensity of phytophagous insect attack in plants exposed to natural herbivory under a PE cover after being grown for several days under either solar UV-B (CA film) or UV-B exclusion (PE film). We reasoned that if UV-B affected plant attractiveness to herbivores, the effects were probably mediated by UV-B-induced changes in leaf appearance (e.g. changes in leaf thickness) or chemical composition (e.g. changes in

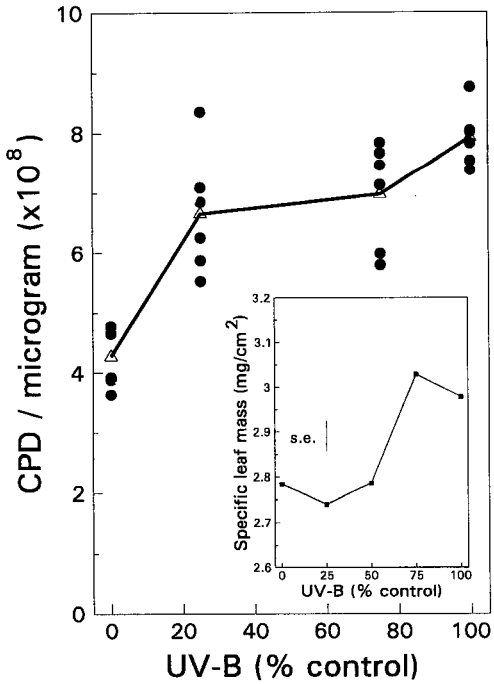

Figure 4. The effect of excluding solar UV-B radiation on DNA integrity. Seedlings were grown for 10 d at the indicated UV-B level during the summer of 1994–1995. Samples of the first and second true leaves were obtained at 3 PM and immediately frozen in liquid nitrogen; DNA was extracted and treated as indicated in "Materials and Methods." ●, Individual measurements (six per treatment); △, averages. Pairwise comparisons between treatments indicated that all sample sets were significantly different from each other ($P < 0.05$), except 25% compared to 75%, which gave $P = 0.6$). An exponential curve fitted the data significantly ($P = 0.006$) better than a linear equation (comparison of fits; Graph Pad Prism version 2.0, Graph Pad Software, San Diego, CA). The inset shows the relationship between specific leaf mass and UV-B dose in leaf number 4 (n = 20). Different UV-B levels were obtained by superimposing narrow strips of UV-B-absorbing PE films onto CA sheets.

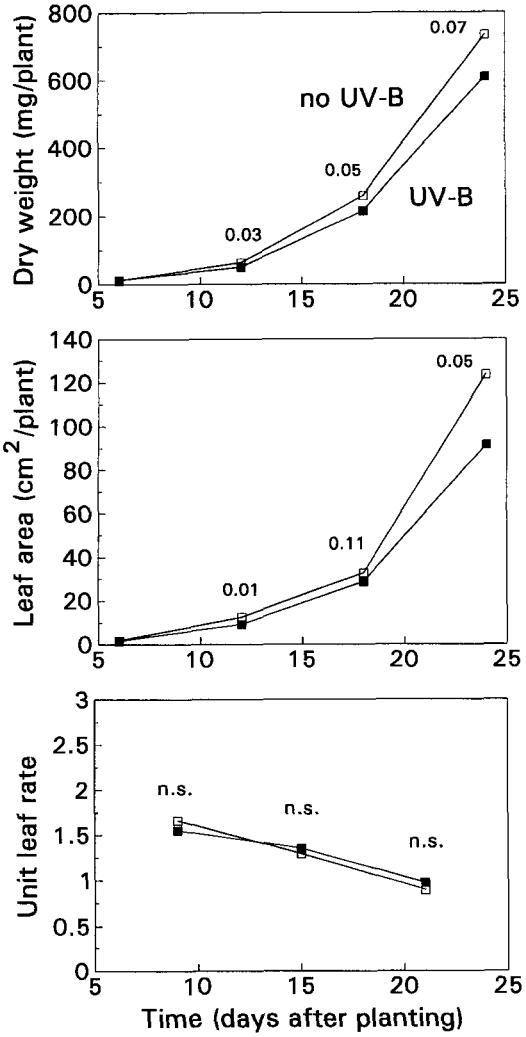

Figure 5. The effect of excluding solar UV-B radiation on biomass accumulation, leaf area expansion, and calculated unit leaf rate (net assimilation rate) of *Datura* plants grown in the field during the summer of 1994–1995. Each point is the average of 10 independent blocks (2 experiments with 5 independent blocks each; 4 individual plants per block treatment were collected on each harvest). The significance levels of the differences between treatments (one-way analysis of variance) are indicated for each sampling date. There were no significant differences between the treatments in unit leaf rate (n.s.; $P \geq 0.50$).

phenolics or N content) (Bernays and Chapman, 1994). Because chemical composition can acclimate rapidly to changes in UV-B levels, we used short-duration (24 h) exposures in an effort to maintain the relevant contrasts between UV-B- and no-UV-B-exposed plants. Leaves from plants that did not receive UV-B during growth were, on average, two times more likely to be bitten by insects than plants raised under solar UV-B (Fig. 7). In only 6 of 26 independent exposures were leaves from plants grown under solar UV-B attacked more than leaves from non-UV-B plants, giving a significant effect of UV-B on plant attractiveness to herbivores at $P = 0.0048$ (sign test; De Campos, 1979).

DISCUSSION

Our experiments with *D. ferox* show that solar UV-B radiation has several significant effects on the physiology and ecology of a wild plant, modulating both the growth rate and interactions with phytophagous insects. These findings are discussed below in connection with (a) results from previous experiments under controlled environmental conditions and (b) the problem of scaling plant responses to UV-B.

Many of the morphological responses presented by *Datura* plants to solar UV-B exclusion were similar to those observed in controlled-environment studies. Thus, inhibition of stem elongation and leaf area expansion, two of the responses we detected (Figs. 3 and 5), have been observed consistently in other species in response to artificial and solar UV-B radiation (for refs., see Bornman and Teramura, 1993; Tevini, 1993; Caldwell, et al., 1995). The origin of these responses is unclear. It has been demonstrated that UV-B can have significant effects on elongation even if biomass accumulation is not affected (Barnes et al., 1990; Ballaré et al., 1991). The action spectrum for the inhibition of elongation in cress (*Lepidium sativum*) shows increased effectiveness with decreasing wavelength in the UV range (Steinmetz and Wellmann, 1986), suggesting that the effect of UV is either a direct consequence of damage to proteins

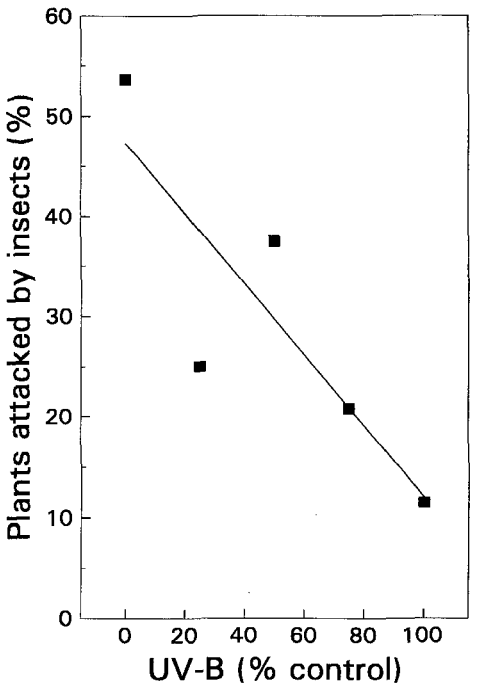

Figure 6. The effect of excluding solar UV-B radiation on the proportion of plants attacked by phytophagous insects during the summer of 1994–1995. Seedlings emerged and grew for 7 d under the indicated UV-B treatment. Herbivore attack was diagnosed on d 7 based on the presence of biting lesions on leaf tissue. The percentage of attack was calculated on the basis of 24 to 28 seedlings, resulting from the combination of three independent replicates per treatment; the line represents the least-squares linear fit. The different UV-B levels were obtained by superimposing narrow strips of UV-B-absorbing PE films onto CA sheets.

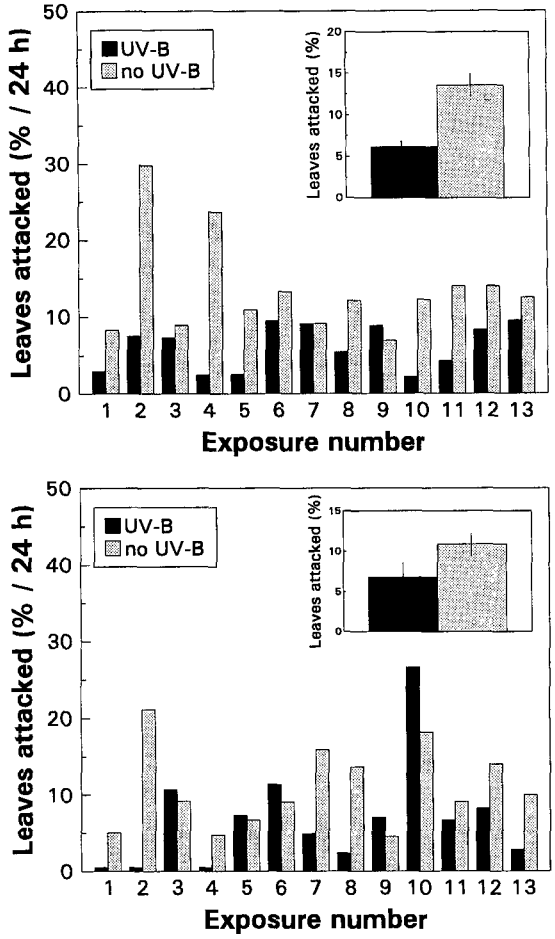

Figure 7. The effect of excluding solar UV-B radiation during growth on the proportion of leaves attacked by phytophagous insects during 24 h of exposure. Plants of two cohorts were grown during the summer of 1994–1995 for 7 to 26 d under near-ambient UV-B levels (CA filters) or under UV-B exclusion (PE filters). After that period, plants grown with and without UV-B were intermingled in a common area under a PE filter and inspected 24 h later for signs of insect attack (the presence of biting lesions on leaf tissue). Typically, three plants for each cohort and UV-B history were exposed each time, and the percentages of attack shown are calculated over populations of 28 to 50 leaves. The results for each cohort are shown in different panels. The insets show the average intensity of insect herbivory for each cohort ± SE (n = 13). The effect of UV-B on plant attractiveness to herbivores was significant at P = 0.0048 (n = 26 exposures; sign test; De Campos, 1979).

or is somehow elicited by cellular signals derived from DNA damage (e.g. Walker, 1984; see also Beggs et al., 1985) or oxidative stress (e.g. Strid, 1993; Malanga and Puntarullo, 1995).

In contrast, in tomato, inhibition of hypocotyl elongation has an apparent maximum around 300 nm, and physiological experiments suggest that the response is triggered by a specific UV-B receptor (Ballaré et al., 1995a). Whatever the mechanism, the UV-B-induced inhibition of stem elongation and leaf area expansion may have adaptive consequences by affecting the degree to which sensitive cellular targets are exposed to UV-B. Thus, it has been suggested (Ballaré et al., 1995b) that the inhibition of hypocotyl elongation and retardation of plumular hook opening in emerging seedlings allows time for the accumulation of UV-absorbing compounds in the epidermis, thereby reducing the damaging impact of UV-B when the seedling eventually is exposed to full sunlight. Similarly, the reduced leaf area expansion and increased specific leaf mass induced by solar UV-B may lengthen the optical path between the leaf epidermis and sensitive cellular sites in the mesophyll (see below).

An interesting finding is that ambient levels of UV-B radiation can affect DNA integrity in a wild plant grown within its normal area of distribution. Results from previous studies with cabbage leaves (Beggs and Wellmann, 1994) and cotyledons of etiolated mustard seedlings (Buchholz et al., 1995) have suggested that solar UV-B irradiances are unlikely to increase the CPD burden as long as photoreactivation is not disturbed (e.g. by low temperatures). Other studies that used the same protocol employed here to detect CPD indicated that the steady-state CPD damage level in maize plants grown in California under normal temperatures increases slightly over the course of a day, but there is no increase in steady-state DNA damage levels over the course of the growing season (A.E. Stapleton, unpublished data).

At variance with the results of short-term laboratory experiments (e.g. Quaite et al., 1992; Stapleton and Walbot, 1994; Takayanagi et al., 1994; Buchholz et al., 1995), our experiments (Fig. 4) show a nonlinear relationship between UV-B irradiance and CPD numbers, suggesting that screening or repair mechanisms are activated at high, near-solar UV-B irradiances. There is good evidence that photolyase is induced by UV-B (Pang and Hays, 1991), and accumulation of UV-B screening compounds in epidermal tissue is potentiated by exposure to solar UV-B radiation in field-grown plants (Robberecht and Caldwell, 1986). Although there is no information from field experiments on fluence-response relationships for these effects of UV-B, both of them could have contributed to the amelioration of DNA damage at high, near-solar UV-B fluxes in our experiments. For example, Takayanagi et al. (1994) showed that outdoor-grown alfalfa seedlings photorepair pyrimidine dimers more rapidly than plants grown in a growth chamber under UV-free radiation, and Stapleton and Walbot (1994) demonstrated that flavonoids can offer some protection against induction of CPDs by UV-B in maize sheaths. Perhaps the simplest explanation for the nonlinear relationship between UV-B dose and DNA damage is that specific leaf mass (and presumably leaf thickness) increased at high UV-B doses (Fig. 4, inset), thus lengthening the optical path between the leaf surface and the majority of tissue DNA in the interior cells.

The impact of UV-B radiation on photosynthesis has traditionally received great attention. Studies have been conducted to identify molecular targets and to characterize the effects of UV-B on gas exchange and the associated diffusion and carboxylation resistances (see reviews by Bornman and Teramura, 1993; Strid et al., 1994; Teramura and Sullivan, 1994). It has been known for some time that plants grown in controlled environments are much more

sensitive to UV-B (in terms of photosynthesis inhibition) than field-grown plants. Beyschlag et al. (1988) were unable to detect any effect of experimental UV-B supplements, which simulated ozone depletion, on gas exchange of field-grown wheat and wild oat plants. It is interesting, however, that the same doses of UV-B were effective in altering the balance of competition between the two species when grown in mixtures via differential effects on morphological development (Ryel et al., 1990). In a high-PPFD, controlled-environment study with cucumber seedlings, Adamse and Britz (1992) found that high UV-B doses greatly inhibited leaf expansion but that net photosynthesis was not affected. Field experiments using UV-B exclusion and supplementation have generally shown little or no effect of UV-B on PSII function evaluated using chlorophyll *a* fluorescence (see Naidu et al., 1993; Tevini, 1993; Caldwell et al., 1994; Searles et al., 1995). However, experiments using the ozone cuvette technique have indicated inhibitory effects of solar UV-B (in Portugal) on net photosynthesis in some cultivated species (see refs. in Tevini, 1993). Our exclusion experiments with *Datura* show that solar UV-B can limit the growth rate of field-grown plants (Fig. 5, top). This UV-B-induced growth inhibition is unlikely to result from effects of UV-B on photosynthesis per unit of leaf area, because calculated net assimilation rate was not affected by solar UV-B (Fig. 5, bottom). UV-B effects on leaf area expansion appear to have a greater impact on limitation of growth rate. The hypothesis that solar UV-B has a greater effect on growth and carbohydrate utilization than on photosynthesis in field-grown *Datura* plants also is supported by the data showing increased starch accumulation in plants receiving solar UV-B compared with plants under PE exclusions. Britz and Adamse (1994) also reported increased starch accumulation in leaves of cucumber plants receiving high UV-B doses, especially when plants received low blue-light irradiances in controlled-environment experiments. They suggested that the UV-B-induced increases in starch levels reflected accumulation of assimilates not utilized for growth of developing sinks such as young leaves.

A limited number of controlled-environment studies have demonstrated that the treatment of plant tissue with UV-B radiation can influence the growth, the survivorship, and the feeding patterns of phytophagous insects. McCloud and Berenbaum (1994) demonstrated that caterpillars reared on leaves of *Citrus* plants in the absence of UV-B grow better and suffer lower mortality than their counterparts reared on leaves exposed to UV-B radiation. They proposed that the effect of UV-B on the insects was mediated by UV-B-induced accumulation of furanocoumarins in leaf tissue and potentiation of this phototoxin by UV-B. Hatcher and Paul (1994) reported that the consumption rate of pea leaf tissue by moth larvae was reduced by previous exposure of the plants to UV-B. They suggested that UV-B-induced increases in N concentration in leaf tissue resulted in an increase in the efficiency with which larvae utilized their food, leading to a concomitant reduction of the quantity of leaf material consumed. To our knowledge, the data in Figures 6 and 7 provide the first evidence that present-day solar UV-B radiation can alter the attractiveness of field-grown terrestrial plants to the insects that naturally feed upon them. Bothwell et al. (1994) recently have shown marked effects of excluding solar UV-B on interactions between diatoms and herbivore larvae (Diptera:Chironomidae) in experimental freshwater systems. Most of the impact of UV-B on the algae/consumer interaction in their experiments was apparently the result of deleterious effects of UV-B on the herbivores themselves, rather than mediated through changes in the algae.

Implications for Scaling

Although our experiments were simple and of relatively short duration, we found multiple influences of solar UV-B on the physiology and ecology of field-grown *Datura* plants. Three points warrant further attention: (a) the multilevel impact of UV-B and the nonlinearity of some responses to UV-B dose; (b) the lack of correspondence between effects of UV-B at different scales (e.g. assimilation rate per unit of leaf area versus whole-plant growth rate); and (c) the marked effect of UV-B on interactions between plants and consumers.

General statements about the biological consequences of increased UV-B radiation are often made based on the spectral sensitivity of a particular UV-induced response and the predicted changes in sunlight spectral composition under ozone depletion. The observation of multiple effects of present-day solar UV-B on plant biology (see Figs. 4–7) suggests that caution should be used when making inferences about "biological impacts" of changes in UV-B. But even if we ignore such complexities as species interactions and interactions between UV-B and other environmental factors, it is important to realize that it is not only the wavelength-dependence of quantum effectiveness that is important when predicting the impact of an alteration in UV—the actual slope of the dose-response relationships also needs to be considered. This point is important for two reasons. First, different responses can have different fluence dependencies (cf. Figs. 3 and 4), even if they have similar action spectra. Second, the fluence-response relationships can be nonlinear at high, ambient UV-B fluxes and when time is allowed for acclimation of the responding biological system (Fig. 4). Nonlinearity in fluence-response curves precludes extrapolation beyond the fluence range used to construct the action spectra, and plant acclimation violates the time × fluence-rate reciprocity that is normally assumed in extrapolations from laboratory results. Other problems associated with upscaling spectral responses have been discussed recently (Caldwell et al., 1994).

Another limitation for upscaling physiological results is that the effects of UV-B on specific rates (e.g. rates per unit area or dry weight) can be poor predictors of the effects on the corresponding whole-plant rates. For instance, our experiments show that solar UV-B does reduce whole-plant growth rate, even though net assimilation rate (an estimate of the rate of net photosynthesis per unit of leaf area) is not consistently affected (Fig. 5; see also Deckmyn and Impens, 1995).

A significant limitation for upscaling is suggested by the observation that UV-B, although reducing plant growth and affecting physiological parameters, appears to reduce the attractiveness of plant tissue to natural herbivores, at least in *Datura* (Figs. 6 and 7). Plant-herbivore interactions are normally completely excluded in controlled-environment experiments and in field trials oriented to evaluate the impacts of UV-B radiation on the physiology and yield of agricultural species. Currently, we do not have the estimates of the quantitative impact of herbivory on *Datura* growth, nor do we know the effects of UV-B-induced changes in *Datura* tissue quality on the fate of its natural insect grazers. However, our demonstration of effects of solar UV-B on herbivory under field conditions strongly supports recent claims for "top-down," ecosystem-level approaches to study the biological impacts of solar UV-B (e.g. Scientific Committee on Problems of the Environment, 1993; Bothwell et al., 1994; Caldwell et al., 1995; Johanson et al., 1995).

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